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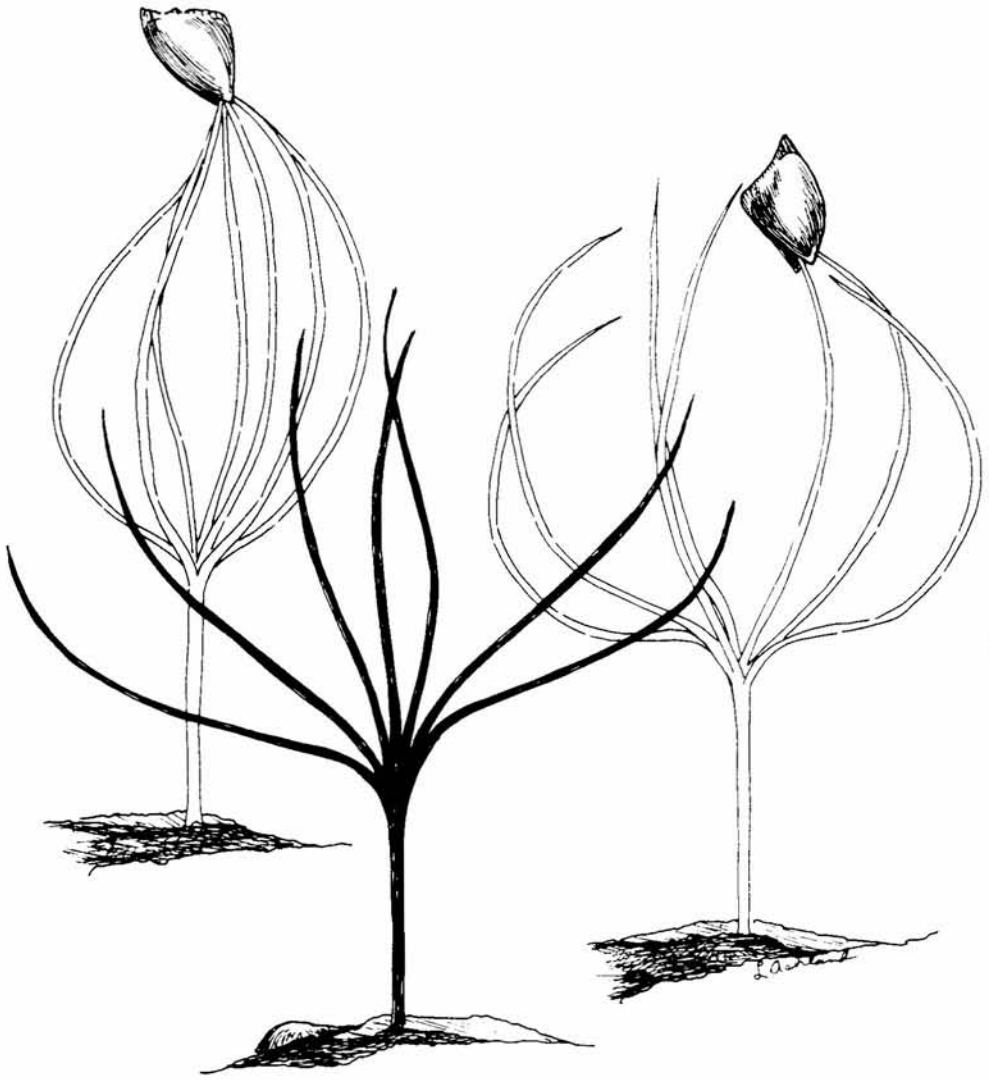

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by R.L. James, R.K. Dumroese, C.J. Gilligan, and D.L. Wenny

Abstract

Twenty *Fusarium* isolates from Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) seed and seedlings were evaluated for their effects on Douglas-fir seed germination, post-emergence damping-off, and seedling root disease. Inoculum consisting of perlite:cornmeal colonized with fungal isolates was mixed into peat:vermiculite growing medium then seed was sown or seedlings transplanted into the mixture. A wide range of virulence occurred in populations of *Fusarium* isolated from Douglas-fir. Several isolates were consistently the more virulent. As a group, *F. oxysporum* isolates were more virulent than *F. "roseum"* isolates, although some *F. oxysporum* isolates were non-pathogenic and some *F. "roseum"* isolates were quite virulent. Test isolates of *F. sambucinum* were not virulent. Both source of isolation (healthy versus diseased seed or seedlings) and species of *Fusarium* were unreliable predictors of potential virulence.

Introduction

Production of conifer seedlings in containers has steadily increased in the northern Rocky Mountains during the past several years. New nurseries with container facilities have begun production, and established nurseries have increased their output. With increased production, diseases have become more troublesome, especially those caused by *Fusarium* spp. Because very little is known about the behavior of *Fusarium* on containerized conifer seedlings, investigations were conducted to elucidate epidemiological characteristics of this group of pathogens. Douglas-fir (*Psuedotsuga menziesii* var. *glauca* [Beissn.] Franco) was studied since experience indicates this species is often severely damaged by *Fusarium*-associated diseases (James 1985, 1986, 1987).

We previously investigated inoculum sources, seedling infection, symptom production, and disease progression within greenhouses (James et al. 1987). During those investigations, we obtained several hundred isolates of *Fusarium*. These isolates were systematically identified and characterized by colony characteristics, spore morphology, and growth rates and are described in another report (James et al. 1989).

One of the most difficult questions arising from our epidemiological investigations concerned the potential of different *Fusarium* isolates to cause disease in seedlings. Previous studies of conifer seedlings (James and Gilligan 1988a, 1988b) show *Fusarium* on seedling roots does not necessarily lead to disease. In addition, the presence of *Fusarium* on the roots of diseased seedlings does not necessarily mean *Fusarium* caused the disease symptoms. These uncertainties are best resolved by subjecting representative isolates to Koch's postulates (pathogenicity tests).

Briefly, Koch's postulates require that organisms isolated from diseased plants be introduced onto healthy plants, cause the same type of disease symptoms previously found, and be re-isolated from the inoculated plants. In this way, we can conclude the isolated organism(s) indeed caused the disease symptoms. Such techniques are laborious, especially if many isolates are screened. However, this is the only accepted way of proving organisms are pathogens.

To determine the ability of *Fusarium* isolates obtained from Douglas-fir seed and seedlings to elicit disease symptoms, pathogenicity tests were conducted on representative examples of several *Fusarium* species.

Materials and Methods

Fusarium Isolates

Twenty *Fusarium* isolates obtained from Douglas-fir seed or seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho, or the University of Idaho Forest Research Nursery, Moscow, Idaho, were tested for pathogenicity (table 1). Isolates were obtained from diseased or healthy seed or from roots of diseased (including damped-off) or non-diseased seedlings. They were maintained on PDA slants at about 15°C for several months prior to the tests.

Ten isolates were classified as *F. oxysporum* Schlecht. based on colony morphology, growth rate, pigmentation, and types of sporulation (Nelson et al. 1983). The other 10 isolates were placed in the *"roseum"* group because they produced carmine red pigment on potato dextrose agar (PDA). Seven of the *"roseum"* isolates were identified as *F. acuminatum* Ell. & Ev. because of their needle-thin macroconidia, lack of microconidia, and abundant

production of chlamydospores (Nelson et al. 1983). One isolate was classified as *F. avenaceum* (Fr.) Sacc. because, even though it had spores resembling those of *F. acuminatum*, it failed to produce chlamydospores in culture after a long incubation. The other two isolates were identified as *F. sambucinum* Fuckel based on their macroconidial morphology, chlamydospore production, and cultural characteristics.

Inoculum **Preparation**

Inoculum for all pathogenicity tests was prepared using the techniques of Miles and Wilcoxson (1984). This inoculum type was effective in previous pathogenicity tests on conifer seedlings (James and Gilligan 1984). Inoculum was produced in galvanized metal pans (5 cm x 25 cm x 35 cm) lined with a double layer of aluminum foil. Perlite, an inert, inorganic, siliceous rock of volcanic origin commonly used in potting mixtures, was the matrix for fungal growth. In each metal pan, 150 g of yellow cornmeal was moistened with 300 ml warm 1% PDA and left standing for 15 minutes before 75 g of perlite was thoroughly mixed in. The pans were covered with aluminum foil and autoclaved for 60 minutes at 121°C. After cooling, the perlite-cornmeal cake was inoculated with 1 cm^2 pieces of mycelium from 14-day-old *Fusarium* cultures grown on PDA. Pieces from two culture plates were mixed with the perlite-cornmeal cake with a sterile knife, 50 ml of sterile distilled water was added, and the pan was sealed. Sealed pans were incubated in the dark at about 24°C for 24 days. Following incubation, fungal cake mixtures were air dried on a tabletop for 3 days and stored in plastic bags at 8°C until needed.

Prior to each pathogenicity test, inoculum was tested for viability by placing pieces on plates of PDA and incubating under cool fluorescent light at about 22°C for 5-7 days. All prepared inoculum remained viable and uncontaminated for several months and was in this condition prior to use in pathogenicity tests.

Metal trays (38 cm x 23 cm x 5 cm) were filled with a standard, pre-mixed 1:1 peat:vermiculite growing medium (W. R. Grace & Co., Portland, Oregon) used to grow containerized seedlings. Trays with soil mix were autoclaved (121°C for 60 min.) and allowed to cool. Perlite-

Table 1. Characteristics of *Fusarium* isolates evaluated for pathogenicity on Douglas-fir seed and seedlings.[1]

			Isolation Information		
Isolate	*Fusarium* **Species**	**Nursery[2]**	**Seedlot**	**Host type[3]**	**Date isolated (1986)**
23C	*oxysporum*	CDA	4010	DOR	March 26
26C	*oxysporum*	CDA	2682	DS	March 31
27B	*oxysporum*	CDA	2682	NDR	March 31
29G	*oxysporum*	UI	FN	NDS	April 11
43B	*oxysporum*	CDA	4010	NDR	June 6
46H	*oxysporum*	CDA	4486	NDR	June 9
49S	*oxysporum*	CDA	6070	DR	June 9
56M	*oxysporum*	UI	FN	NDR	July 14
75B	*oxysporum*	CDA	2741	DR	September 30
75C	*oxysporum*	CDA	6070	DR	September 30
5E	*acuminatum*	CDA	4486	NDS	March 4
23G	*acuminatum*	CDA	4010	DOR	March 27
26E	*acuminatum*	CDA	2682	DOR	March 31
28G	*acuminatum*	CDA	2741	NDR	April 4
30F	*sambucinum*	UI	DAW	NDS	April 11
39F	*acuminatum*	UI	DAW	DR	May 14
46E	*avenaceum*	CDA	4486	NDR	June 9
50A	*acuminatum*	CDA	2682	NDR	June 9
52F	*acuminatum*	CDA	6070	DR	June 30
69B	*sambucinum*	UI	IDL	NDR	August 8

[1] All isolates obtained from Douglas-fir seed or seedling roots and maintained on potato dextrose agar for several months prior to use in pathogenicity tests.

[2] CDA = USDA Forest Service Nursery, Coeur d'Alene, Idaho.
UI = University of Idaho Forest Research Nursery, Moscow.

[3] DOR = Roots from damped-off seedling.
DS = From decayed seed.
DR = Roots from a seedling with root disease symptoms.
NDS = From seed that appeared non-diseased and lacked evidence of decay.
NDR = Roots from a seedling that lacked root disease symptoms.

cornmeal inoculum was mixed thoroughly into the autoclaved growing medium at two concentrations: 1:25 and 1:50 w/w basis. The inoculum-growing medium mixture was then moistened with sterile distilled water.

Damping-off Test

Seed for this test came from lot FN of the University of Idaho Forest Research Nursery. Seed were collected in 1981 near Bovill, Idaho, (1,000 m elevation) and had been in refrigerated storage at 2°C prior to sowing.

Seed were treated using the nursery's standard bleach (sodium hypochlorite) treatment (Wenny and Dumroese 1987) then immersed 3 minutes in a 70 % v/v solution of ethanol. Next, treated seed were placed in a running water rinse for 48 hours. After 28 days of cold stratification at 2°C, 200 seed were sown in each tray containing an inoculum-growing medium mixture and covered with a 0.5-cm layer of grit. Trays were watered when needed but not fertilized. Controls consisted of autoclaved growing medium amended with uninoculated perlite at the same two treatment concentrations. Therefore, the experimental design was for 20 isolates, each tested at two inoculum concentrations, and two controls tested at two non-inoculated perlite concentrations for a total 44 trays. All experiments were conducted inside a greenhouse with standard seedling growing regimes.

Germination and emergence of seedlings 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 by toothpicks (plate 1). The seedlings were washed carefully and placed on a selective medium for *Fusarium* isolation (Komada 1975). Plates were incubated under cool fluorescent light at about 22°C for 7-10 days. Emerging fusaria were compared with inoculated isolates to confirm they were the same. The experiment was terminated when germination was considered complete (27 days after sowing).

Upon completion of the experiment, 10 non-diseased seedlings were collected from each tray for root isolations. In some cases, 10 non-diseased seedlings were not available, either because the tested isolate had killed all emerged seedlings or because less than 10 non-diseased seedlings had emerged. Roots of non-diseased seedlings were washed thoroughly to remove adhering soil particles, surface sterilized in 0.525 % sodium hypochlorite, rinsed with sterile distilled water, plated on Komada's medium, and incubated as described previously. Emerging fusaria were compared with inoculated isolates to determine if the isolates had invaded the non-diseased seedlings. Also, 10 ungerminated seed were carefully removed from each tray and plated directly onto Komada's medium to determine if seed decay or pre-emergence damping-off had occurred. Emerging fusaria were again compared with inoculated isolates.

After seedlings and seed were removed from the trays, the inoculum-growing medium mixture was passed through screens to remove large pieces of perlite, peat, and vermiculite. The resulting fine growing medium was mixed with sterile distilled water, and 1 ml of the solution was placed on Komada's medium. Emerging fusaria were compared with the inoculated isolates to determine if these organisms had colonized the fine growing medium particles.

Plate 1. Damping-off test 27 days after sowing (isolate 75B). Toothpicks show where emerged seedlings damped-off.

Young Seedling Test

All isolates were tested for their ability to elicit root disease symptoms on young seedlings. Seedlings from lot FN had been grown in Spencer-LaMeire Root Trainers® for 7 weeks prior to inoculation. Inoculum was prepared and mixed with growing medium (1:50 w/w) as previously described. Seedlings were carefully removed from their containers to minimize root damage. Seedling roots were washed thoroughly to remove adhering particles of growing medium, and the seedlings were repotted into Ray Leach® pine cells (66 cm^3) with the inoculum-growing medium mixture. Twenty-five seedlings were repotted per isolate. Controls consisted of repotted seedlings in an uninoculated perlite-soil mixture. Seedlings were watered with fine-mist, overhead irrigation as needed.

Severity of root disease symptoms was rated using a 5-point system describing extent of foliar chlorosis and necrosis (table 2). When seedlings were considered dead, they were carefully removed from containers. Their roots were thoroughly washed to remove growing medi-

Table 2. Rating system for severity of foliar symptoms resulting from infection of Douglas-fir seedling roots with *Fusarium*.

Rating	Description
1	Seedling appeared healthy; no foliar chlorosis or necrosis.
2	Foliage chlorotic over 50% of the crown area.
3	Foliage chlorotic over more than 50% of the crown area *or* foliage necrotic over less than 50% of the crown area.
4	Foliage necrotic over more than 50% of the crown area, but some foliage still alive.
5	Seedling dead.

um and aseptically dissected into at least 10 2-3-mm-long pieces. As many root tips as possible were included in each sample. Root pieces were placed on Komada's medium and incubated as previously described. Emerging fusaria were compared with inoculated isolates to confirm they were the same.

The experiment was 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 Seedling Test

A final test was conducted to evaluate isolate pathogenicity on 5-month-old seedlings. Inoculum was prepared and mixed with growing medium (1:50 w/w) as described previously. Test seedlings were carefully removed from their pine cell containers and their roots thoroughly washed to remove as much growing medium as possible. They were repotted into Ray Leach® super cells (144 cm^3) with the inoculum-growing medium mixture. Ten seedlings per isolate were repotted. Controls consisted of repotted seedlings in uninoculated perlite-soil mixture.

Seedlings were watered and monitored for foliar indications of root disease as described in the young seedling test (table 2). This experiment was terminated after 68 days; this was the end of the growing season and most seedlings were dormant. During this test, only one seedling died. Its roots were washed, dissected, and incubated on Komada's medium as described for the young seedling test. All other seedlings were rated for extent of disease symptoms and carefully removed from their containers. From each, 10 lateral roots were randomly selected and 2-3-mm-long tips were aseptically severed and incubated on Komada's medium as previously described. Emerging fusaria were compared with inoculated isolates to confirm root infection by test isolates.

Data Analysis

Comparisons of germination and post-emergence damping-off between the two inoculum concentrations were made using a paired "t" test. Statistical tests were conducted separately for each group of isolates.

Results

Damping-off Test

The two inoculum concentrations of *F. oxysporum* isolates were significantly ($P = 0.05$) different in their effects on percent seed germination but not on post-emergence damping off (table 3). The higher concentration (1:25) resulted in less seed germination. For the *F. "roseum"* isolates (table 4), no significant differences in either germination or post-emergence damping-off were noted when comparing inoculum concentrations.

There were large differences in the ability of various isolates to cause disease of seed and recently emerged seedlings (tables 3 and 4). All isolates successfully colonized the roots of surviving non-diseased seedlings (table 5); most also colonized seed and were probably important in limiting seed germination.

A few "control" seedlings became diseased (tables 3 and 4). Isolations from diseased control seedlings indicated that many were colonized with *F. oxysporum* by the end of the experiment.

Isolations from fine, growing medium particles at the conclusion of this experiment commonly yielded inoculated isolates. Apparently the inoculated fusaria completely colonized all organic matter within the growing medium.

Table 3. Effects of *Fusarium oxysporum* isolates on germination of Douglas-fir seed and post-emergence damping-off of young seedlings.

Isolate[1]	Inoculum concentration	Germination (%) n = 200)	Damping-off (%)
23C	1:25	41.5	16.9
	1:50	53.5	8.4
26C	1:25	48.0	9.4
	1:50	40.0	18.8
27B	1:25	61.0	4.1
	1:50	57.5	4.3
29G	1:25	15.0	96.7
	1:50	15.5	96.7
43B	1:25	62.5	9.6
	1:50	63.0	12.7
46H	1:25	17.0	47.1
	1:50	45.5	19.8
49S	1:25	25.5	33.3
	1:50	43.5	32.2
56M	1:25	6.0	100.0
	1:50	17.5	94.3
75B	1:25	28.5	35.1
	1:50	24.0	45.8
75C	1:25	38.0	11.8
	1:50	49.5	12.1
All Isolates	1:25	34.3[2]	20.8[3]
	1:50	40.0	22.9
	Average	37.6	22.0
Control	1:25	71.8[3]	0.7[3]
	1:50	68.8	1.1
	Average	70.2	0.9

[1] See table 1 for isolate descriptions.
[2] Difference between means for inoculum concentrations is significant using a paired "t" test (P = 0.05).
[3] Difference between means for inoculum concentrations is not significant using a paired "t" test (P = 0.05).

Table 4. Effects of selected *Fusarium "roseum"* isolates on germination of Douglas-fir seed and post-emergence damping-off of young seedlings.

Isolate[1]	Inoculum concentration	Germination (%) (n = 200)	Damping-off (%)
5E	1:25	25.0	50.0
	1:50	29.0	72.4
23G	1:25	35.0	71.4
	1:50	16.0	96.9
26E	1:25	50.0	7.0
	1:50	52.5	4.8
28G	1:25	35.5	49.3
	1:50	40.0	60.0
30F	1:25	62.0	2.4
	1:50	62.5	4.0
39F	1:25	24.5	28.6
	1:50	50.5	9.9
46E	1:25	11.0	90.1
	1:50	23.0	95.6
50A	1:25	70.0	2.9
	1:50	62.0	6.4
52F	1:25	64.5	4.6
	1:50	60.0	0.8
69B	1:25	63.5	3.1
	1:50	70.0	0.7
All Isolates	1:25	44.1[2]	19.0[2]
	1:50	46.5	20.9
	Average	45.3	20.0
Control	1:25	71.8[2]	0.7[2]
	1:50	68.8	1.1
	Average	70.2	0.9

[1] See table 1 for isolate descriptions.
[2] Difference between means for inoculum concentrations is not significant using a paired "t" test (P = 0.05).

Table 5. Isolation results from non-diseased Douglas-fir seedlings and non-germinated seed inoculated with *Fusarium* (damping-off test).

Isolate[1]	Non-diseased seedlings tested (number)	Non-diseased seedlings infected[2] (%)	Non-germinated seed infected[2] (%) (n = 10)
F. Oxysporum			
23C	10	100	100
26C	10	100	100
27B	10	100	70
29G	1	100	100
43B	10	100	100
46H	10	100	100
49S	10	100	100
56M	1	100	100
75B	10	100	100
75C	10	100	75
F. "roseum"			
5E	10	100	100
23G	6	100	100
26E	10	100	90
28G	10	100	100
30F	10	100	100
39F	10	100	85
46E	5	100	100
50A	10	100	100
52F	10	100	100
69B	10	100	100
Controls	20	85[3]	68[3]

[1] See table 1 for isolate descriptions.
[2] Infected with inoculated isolates.
[3] Infected with *Fusarium* spp.

Plate 2. Young seedlings 87 days after inoculation with isolate 29G. Seedlings display various levels of foliar root disease symptoms.

Table 6. Effects of selected *Fusarium* isolates on disease severity in young, inoculated Douglas-fir seedlings.

Isolate[1]	Mortality[2] (%)	Average disease rating[3]	Surviving seedlings[4] percent root colonization
F. oxysporum			
23C	0	1.4	98
26C	12	2.6	100
27B	0	2.0	100
29G	92	4.7	100
43B	0	2.2	80
46H	0	1.6	78
49S	24	2.8	100
56M	96	4.9	100
75B	32	3.0	100
75C	4	2.1	100
Average	26.1	2.7	93.7
F. "roseum"			
5E	20	2.3	100
23G	40	3.1	100
26E	0	1.8	99
28G	0	1.9	98
30F	0	1.9	100
39F	0	1.9	67
46E	56	3.8	96
50A	0	1.8	100
52F	0	2.0	100
69B	0	2.3	68
Average	11.6	2.2	92.2
Average (All isolates)	18.8	2.4	92.9
Controls	0	1.6	0

[1] See table 1 for isolate descriptions.
[2] Percent mortality among 25 inoculated seedlings per isolate within 84 days of inoculation.
[3] See table 2 for description of disease severity rating system.
[4] Seedlings not killed by *Fusarium* within 84 days of inoculation. Root colonization is the percent of sampled root system colonized by inoculated isolates.

Young Seedling Test

Two *F. oxysporum* isolates, 29G and 56 M, caused 92% and 96% seedling mortality, respectively (table 6 and plate 2). Average disease ratings of seedlings inoculated with these two isolates were also much higher than those of seedlings inoculated with other isolates.

Isolates 5E, 23G, and 46E were the only *F. "roseum"* isolates that killed young seedlings (table 6). However, most isolates in this group colonized the roots of non-diseased seedlings and were easily recovered at the end of the experiment.

In general, *F. oxysporum* isolates were slightly more virulent than the *F. "roseum"* isolates, i.e., they caused greater mortality and overall higher disease ratings among young Douglas-fir seedlings.

Table 7. Effects of selected *Fusarium* isolates on disease severity in older inoculated Douglas-fir seedlings.

Isolate[1]	Average disease rating[2]	Surviving Seedlings[3] percent root colonization (A)	(B)
F. oxysporum			
23C	2.0	52	20
26C	2.0	100	20
27B	2.4	99	0
29G	3.0	85	14
43B	2.1	97	10
46H	2.1	52	0
49S	2.0	77	39
56M	3.5	99	0
75B	1.6	87	32
75C	2.2	41	41
Average	2.3	78.9	17.6
F. "roseum"			
5E	2.2	98	0
23G	2.3	98	0
26E	2.0	77	0
28G	2.2	96	11
30F	2.1	95	0
39F	1.9	100	0
46E	2.0	100	0
50A	2.6	98	1
52F	2.3	70	0
69B	2.4	90	5
Average	2.2	92.2	1.7
Controls	2.0	—	25

[1] See table 1 for isolate descriptions.
[2] See table 2 for description of disease severity rating system. Only one seedling (isolate 23G) died during the experiment.
[3] Seedlings not killed by *Fusarium* within 68 days of inoculation. Root colonization (A) is the percent of sampled root system colonized by inoculated isolates; root colonization (B) is the percent of sampled root system colonized by other *Fusarium* spp.

Older Seedling Test

Only one of the inoculated older seedlings died during this test. Disease severity ratings (extent of root disease symptoms) were highest for seedlings inoculated with the same *F. oxysporum* isolates (29G and 56M) that were most virulent on young seedlings (table 7 and plate 3). This was not always the case for the *F. "roseum"* isolates.

Several older seedlings inoculated with *F. oxysporum* had roots colonized with fusaria other than the inoculated isolates (table 7, column b). Most of these other fusaria were isolates of *F. oxysporum;* they may have colonized seedling roots prior to inoculation with the test isolates. These other fusaria were recovered from seedling roots more frequently and inoculated isolates found less frequently in the *F. oxysporum* group than in the *F. "roseum"* group.

Plate 3. Older seedlings 68 days after inoculation. Note needle tip dieback on seedlings inoculated with isolate 56M.

Combination of All Tests

Numerical rankings were used to compare isolate virulence in each of four tests: seed germination, post-emergence damping-off, young seedling disease, and older seedling disease (table 8). Rankings were assigned on the basis of ability to cause disease. The more virulent isolates had lower numerical rankings, i.e., the most virulent isolate was ranked first. Rankings were equally weighted for each test to calculate a composite ranking for all tests. Comparisons were made within each group of isolates (*F. oxysporum and F. "roseum"*) and for all isolates combined.

With few exceptions, isolate virulence was consistent across the four tests. For example, of the *F. oxysporum* isolates, 29G and 56M were consistently the most virulent. Likewise, *F. "roseum"* isolates 23G and 46E were consistently virulent. Many of the *F. oxysporum* isolates were more virulent than the *F. "roseum"* iso-

Table 8. Comparative virulence of selected *Fusarium* isolates on Douglas-fir seed and seedlings[1].

Isolate[2]	Seed germination[3]		Post emergence damping-off[3]		Young seedling		Older seedling		Composite[6]	
	FOXY[4]	ALL	FOXY	ALL	FOXY	ALL	FOXY	ALL	FOXY	ALL
23C	8	13	8	13	10	20	7	14	10	18
26C	7	12	5	9	5	7	7	14	5	10
27B	9	15	10	16	8	12	3	4	8	11
29G	2	2	1	2	2	2	2	2	1	1
43B	10	18	9	14	6	10	5	11	8	14
46H	4	7	6	10	9	19	5	11	5	11
49S	5	8	2	6	4	6	7	14	3	6
56M	1	1	4	8	1	1	1	1	1	2
75B	3	5	3	7	3	5	10	20	4	8
75C	6	11	7	11	7	11	4	8	5	9
	FROSE[5]		FROSE		FROSE		FROSE		FROSE	
5E	3	6	3	4	3	8	5	8	2	5
23G	2	4	1	1	2	4	3	6	1	3
26E	6	14	6	15	9	17	8	14	9	18
28G	5	10	2	3	6	14	5	8	4	7
30F	8	17	8	18	6	14	7	11	9	18
39F	4	9	5	12	6	14	10	19	6	16
46E	1	3	4	5	1	3	8	14	2	4
50A	9	19	7	17	9	17	1	3	8	17
52F	7	16	9	19	5	12	3	6	5	14
69B	10	20	10	20	3	8	2	4	6	13

[1] Values in table are numerical rankings of virulence based on isolate effects on (1) seed germination (percent of control), (2) post-emergence damping-off (percent of control) and (3) numerical disease ratings for both young and older seedlings. Rankings are given for each group of isolates (FOXY and FROSE) and for all isolates combined.

[2] See table 1 for isolate descriptions.

[3] The average values for seed germination and post-emergence damping-off at two inoculum concentrations (1:25 and 1:50) were used to rank the isolates.

[4] FOXY = *F. oxysporum.*

[5] FROSE = *F. "roseum."*

[6] The composite rank is based on equal weights for each disease category.

lates. However, some *F. oxysporum* were non-pathogenic and some *F. "roseum"* were among the most virulent.

Discussion

Our results confirm previous studies (Bloomberg 1981, James and Gilligan 1984, James et al. 1986, Tint 1945) showing *Fusarium* isolates obtained from conifer seed and seedling roots often display a wide range of pathogenicity. Some isolates readily cause disease in infected plants; others do not (Bloomberg 1971). Bloomberg and Lock (1972) concluded from their work in bareroot nurseries that the several different diseases caused by *F. oxysporum* are probably caused by different strains of the fungus.

However, even though they may not cause disease symptoms, most isolates readily colonize cortical cells of seedling roots (Bloomberg 1966, James and Gilligan 1988b). These organisms may remain "dormant" indefinitely or may become active pathogens when the host is stressed (Bloomberg 1971, 1973). In any event, it is not unusual to obtain several different *Fusarium* isolates from the roots of non-diseased seedlings (Bloomberg 1966, 1971; James and Gilligan 1988a, 1988b; James et al. 1987).

Results of our pathogenicity tests indicate some of the most virulent isolates came from roots of non-diseased seedlings or from apparently healthy seed (*F. oxysporum* isolates 29G and 56M and *F. "roseum"* isolates 5E and 46E). Conversely, some of the least pathogenic isolates came from roots of diseased seedlings. Therefore, the relative virulence or aggressiveness of isolates cannot be accurately predicted from their isolation source. Experience has also shown cultural characteristics are unreliable indicators of the virulence of isolates from conifer seedlings (James and Gilligan 1984, James et al. 1986).

Recent work indicates pathogenic *Fusarium* isolates may be located using heterokaryosis analysis of auxotrophic mutants (Puhalla 1985) and isozyme pattern analysis (Reddy and Stahman 1972). These procedures stress

the importance of genetic control of pathogenicity and have proven fairly accurate in identifying pathogenic strains of *Fusarium* from agricultural crops. However, such work on conifer seedling isolates has not been reported.

The most virulent *F. "roseum"* isolates were classified as either *F. acuminatum* or *F. avenaceum*. Actual differences between these two species are minimal and based only on production of chlamydospores in culture (Nelson et al. 1983). They may be the same species.

Isolates classified as *F. sambucinum* (30F and 69B) were morphologically distinct from the other *F. "roseum"* isolates (Nelson et al. 1983). Both *F. sambucinum* isolates were less pathogenic than isolates of other species in our tests. Even though this species has been isolated frequently from conifer seedlings (Hansen and Hamm 1988, James et al. 1988), it may not be as important a pathogen as other *Fusarium* species (Hansen and Hamm 1988, James and Gilligan 1984, Tint 1945).

Our investigations point out the complexity of the *Fusarium* disease situation in containerized Douglas-fir seedlings. The different species of *Fusarium* may elicit symptoms at different stages of the crop cycle. Some isolates are capable of causing rapid disease, whereas others are mainly saprophytic. There is also an unclear relationship between species and source of isolate and relative virulence. Some *Fusarium* isolates may even be beneficial if they protect seedling roots from infection by pathogens (Baker and Cook 1974).

More work is needed to clarify the role of environmental conditions (moisture, temperature, etc.) on pathogenicity of *Fusarium* in Douglas-fir and other conifer species. We also need to understand the role of inherent genetic susceptibility. For example, it is possible that seedlots with poor germination capacity or low germination energy are more prone to *Fusarium* damage. Also, under the right conditions of environmentally induced stress and host susceptibility, it is possible most *Fusarium* strains will cause disease in conifer seedlings. It is important to identify the important factors contributing to disease expression in conifer seedlings so more effective control measures can be developed.

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