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Seedborne *Fusarium* on Douglas-fir: Pathogenicity and seed stratification method to decrease *Fusarium* contamination

PAIGE E. AXELROOD[1], MELODY NEUMANN[1], DAVID TROTTER[2], REED RADLEY[1], GWEN SHRIMPTON[3] and JOHN DENNIS[4]
[1] *B.C. Research Inc., Forest Biotechnology Centre, 3650 Wesbrook Mall, Vancouver, B.C. V6S 2L2, Canada;* [2] *B.C. Ministry of Forests, Nursery Extension Services, 14275 – 96 Ave., Surrey, B.C. V3V 7Z2, Canada;* [3] *B.C. Hydro, E16 – 6911 Southpoint Drive, Burnaby, B.C. V3N 4X8, Canada;* [4] *Forestry Canada, Pacific Forestry Centre, 506 W. Burnside Rd., Victoria, B.C. V8Z 1M5, Canada*

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Application. Diverse *Fusarium* species can be isolated from Douglas-fir seedlots, including pathogenic *Fusarium* spp., and post-stratification seedborne *Fusarium* levels were significantly less for running water imbibition compared to standing water imbibition. Douglas-fir seedling growth was not consistently different for running water and standing water seed imbibition treatments. There was no apparent relationship between the presence of seedborne *Fusarium* and subsequent disease in the nursery.

Abstract. Twelve Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlots from coastal British Columbia were assayed for seedborne *Fusarium*. All of the seedlots were contaminated with *Fusarium*. Percent of nonstratified seeds from individual seedlots harboring *Fusarium* ranged from 0.3% to 95.4%. Sixty-seven percent of the seedlots had *Fusarium* on less than 2% of the seeds. Post-stratification seedborne *Fusarium* levels were significantly less for running water imbibition compared to standing water imbibition. However, seedling growth at a container nursery was not consistently different for stratified seed imbibed initially in standing or running water. *Fusarium* disease symptoms were not observed in the nursery environment. The species of *Fusarium* isolated from seed were *F. acuminatum*, *F. avenaceum*, *F. lateritium*, *F. moniliforme*, *F. oxysporum*, *F. poae* and *F. sambucinum*. Twelve *Fusarium* isolates, comprising six species, were assessed for pathogenicity. Disease symptoms were observed after four weeks incubation and *Fusarium* isolates ranged in virulence from low to high. *Fusarium oxysporum* isolates were the most pathogenic.

Introduction

Fusarium has been associated with disease problems on Douglas-fir seedlings (*Pseudotsuga menziesii* (Mirb.) Franco) during the last 10 years in British Columbia (B.C.) container nurseries. *Fusarium* can cause pre- and post-emergence damping-off, hypocotyl rot, top blight and root rot in bare-root conifer nurseries and some of these disease syndromes are also seen in container nurseries (Bloomberg 1981; Hansen and Hamm 1988; Hamm et al. 1990; James 1986; Sutherland et al. 1989). *Fusarium* species isolated from

diseased seedlings include but are not limited to *F. oxysporum*, *F. avenaceum*, *F. sambucinum* and *F. solani*. In Canada, *Fusarium* has been isolated from seed of several conifer species including white spruce (*Picea glauca* (Moench) Voss) (Mittal and Wang 1986, 1987; Timonin 1964), eastern white pine (*Pinus strobus* L.) (Mittal and Wang 1987), Jack pine (*Pinus banksiana* Lamb.) and lodgepole pine (*Pinus contorta* Dougl var. *latifolia* Engelm.) (Timonin 1964). In the U.S. *Fusarium* has also been isolated from seedlots of Douglas-fir (Graham and Linderman 1983; James et al. 1989) and other conifers (James 1985; Huang and Kuhlman 1990; Pawuk 1978).

B.C. Ministry of Forests seed stratification procedures used through 1991 included imbibing seed in standing water for 24 h followed by incubation at 4 °C for three weeks. This method has the potential to enhance seed contamination by seedborne fungal propagules that become suspended in the water. Furthermore, seedborne *Fusarium* could play a role in the epidemiology of *Fusarium* diseases in container nurseries if pathogenic species are introduced on Douglas-fir seedlots.

The objectives of this study were (i) to evaluate the presence of seedborne *Fusarium* on B.C. Douglas-fir seedlots (ii) assess the pathogenicity of seedborne *Fusarium* isolates (iii) determine if running water seed imbibition decreased the level of seedborne *Fusarium* compared with standing water imbibition and (iv) assess seedling growth of *Fusarium* contaminated seedlots under operational conditions at a conifer nursery.

Materials and methods

Assessment of seedborne Fusarium

Twelve Douglas-fir seedlots from the B.C. Ministry of Forests (BCMF) were assessed for the presence of seedborne *Fusarium*. Three hundred or 500 seeds per seedlot were assessed for *Fusarium* by aseptically placing dry, unstratified seeds on Komada medium (Komada 1975) supplemented with 1 mg/mL Benomyl (Later Chemicals, Richmond, B.C.). An initial trial indicated this medium was superior to Komada medium without Benomyl for reducing *Trichoderma* contaminants and enhancing the detection of *Fusarium*. Petri plates containing seeds were incubated at 23 ± 2 °C under fluorescent lights (Philips, cool white) for 14 days after which the number of seeds colonized by *Fusarium* were counted and confirmed by microscopic observation.

Five representative Douglas-fir seedlots (476, 7289, 7402, 8706 and 9983) were chosen to determine the influence of standing water or running water imbibition followed by a 21 day stratification period on seedborne *Fusarium* levels. Five hundred seeds per seedlot were assessed prior to stratification and at the end of the stratification period as previously described except Komada medium (supplemented with 1 mg/mL Benomyl) was adjusted to pH 7.0 which enhanced detection of *Fusarium* and minimized *Trichoderma* contaminants.

The water imbibition experiment was done four times. *Fusarium* colonies were purified, cultured on carnation leaf agar and identified to species (Nelson et al. 1983; Toussoun and Nelson 1976). Taxonomic identifications were confirmed by Drs. P.E. Nelson, or K. Seifert.

Seed stratification

To determine the effect of standing or running water imbibition on seedborne *Fusarium* and seedling growth the following seed stratification procedure was used. Eleven gram seed samples were placed into ethanol sterilized nylon mesh bags such that one third of the bag volume was filled with seed. Seed samples were imbibed in running or standing tap water for 24 h and each seedlot was treated separately. The running water flow rate was 1 L/minute and the containers held 4 L of water at any given time. Standing water imbibition approximated BCMF operational conditions. Four seed samples from the same seedlot were placed in a plastic bag and filled with water such that one third of the bag volume was seed and two thirds of the volume was occupied by water. Seed was surface dried for approximately 15 min at the end of both imbibition processes, placed in plastic bags and stored for 21 days in styrofoam boxes in a 2–5 °C cooler as per BCMF standards.

Laboratory and field seed germination and seedling growth

Laboratory and field germination and seedling growth assessment trials were done twice over two consecutive summers to assess the influence of standing and running water seed imbibition treatments. Stratified seeds were placed on moist seed blotter paper which was laid over moist Kimpak paper (Seedburo Equipment Chicago, IL) in seed germination plates (50 seeds/plate, 10 plates/seedlot) for laboratory germination. Plates were incubated under an eight hour photoperiod at 30 °C/20 °C, day/night temperature regime, for three weeks prior to seed germination assessment. Stratified seed was also sown (one seed/cell) in new Styroblock 312 growing containers (Beaver Plastics, Edmonton, Alberta) filled with a peat:vermiculite (3:1) seedling planting mix (50 seeds/replication, eight replications/seedlot). Seedlings were grown outdoors at the BCMF Surrey Nursery (Surrey, B.C.) using standard nursery cultural conditions which included irrigation with a liquid fertilizer solution. Field germination was recorded six weeks after sowing. Seedling height, root collar diameter, shoot dry weight and root dry weight were determined at the end of the growing season (1989: 15 seedlings per replication, eight replications per seedlot; 1990: 10 seedlings per replication, four replications per seedlot). Root collar diameter was measured with a digital caliper and seedling tissue was washed thoroughly and dried at 70 °C for 48 h prior to root and shoot dry weight determination.

Fungal isolates and cultural conditions

Fusarium isolates used in pathogenicity assays were obtained from Douglas-fir seed (Table 1), from a damped-off diseased Douglas-fir seedling (*Fusarium oxysporum* F30) and from the roots of a diseased white spruce seedling (*F. avenaceum* F8). Fungal isolates were stored as conidial suspensions in cryovials at –80 °C. Conidia were suspended in sterile distilled water containing 25% glycerol and 0.1% agar, incubated at room temperature for four hours, then placed at –80 °C for long-term storage.

A medium containing carnation leaves has been used successfully to promote sporulation of *Fusarium* (Snyder and Hansen 1947; Toussoun and Nelson 1976). A medium containing either conifer needles or carnation leaves, was used to grow inoculum for pathogenicity assays. This medium was used to promote sporulation, to more closely approximate the growth of the fungus under more natural conditions and to minimize changes in cultural morphology which may occur on a medium such as potato dextrose agar. Conifer needles were harvested from pesticide-free two to three month-old Douglas-fir or white spruce seedlings, rinsed with distilled water and blotted dry. Carnation leaves were harvested from the stems of pesticide-free cut flowers and processed in a similar fashion. The needles and leaves were cut into 2–4 mm pieces, dried at 70 °C for 12–14 h, placed in glass vials and sterilized by gamma-irradiation (2.5 megarads). Conifer needle agar (CNA) or carnation leaf agar (CLA) was prepared in Petri plates by sprinkling the gamma-irradiated needles or

Table 1. *Fusarium* seed contamination prior to stratification and laboratory germination of coastal Douglas-fir seedlots.

Seedlot[1]	Year Collected	% Germination[2]	% *Fusarium* Contamination[3]
476[3]	1959	92	60.0
7289	1982	66	0.3
7402[3]	1982	78	1.8
9983[3]	1982	90	0.5
8706[3]	1982	95	1.2
9556	1983	99	0.7
6282	1987	92	0.7
6789	1989	93	1.3
6791	1989	94	1.3
6759	1989	95	0.3
6758	1989	96	0.7
6513	1989	97	3.7

[1] British Columbia (B.C.) Ministry of Forests seedlot numbers.

[2] Data for laboratory germination of Douglas-fir seed was provided by the B.C. Ministry of Forests (1991 seed registry).

[3] *Fusarium* species were isolated and identified from the following seedlots: 476 – *F. moniliforme* and *F. oxysporum*; 7402 – *F. avenaceum* and *F. sambucinum*; 9983 – *F. avenaceum* and *F. lateritium*; 8706 – *F. oxysporum*, *F. poae* and *F. acuminatum*.

leaves onto the surface of molten 2% water agar. Working cultures of *Fusarium* were prepared from –80 °C storage cultures by scraping the frozen conidial culture with a sterile wooden stick and streaking the surface of CNA or CLA. Cultures were incubated at 23 ± 2 °C under fluorescent lights until sporulation was abundant on carnation leaf or conifer needle tissue (two to three weeks). Cultures were stored for up to four weeks at 4 °C prior to use.

Pathogenicity assays

Pathogenicity assays were developed previously using *F. avenaceum* (isolate F8) and *F. oxysporum* (isolate F30). These two isolates and 10 seedborne *Fusarium* isolates, comprising six species, were assessed in pathogenicity assays. Douglas-fir seedlot 16501 was used in pathogenicity assays since preliminary evaluation indicated it was free of *Fusarium*. Seed was stratified prior to use as previously described except imbibition was under running tap water for 24 h, seed was placed on sterile paper toweling for approximately 15 min in a laminar flow hood to remove surface water and placed in plastic screw-cap containers at 4 °C for three to four weeks.

Two assays were developed to assess the pathogenicity of *Fusarium* isolates. Glass vials (25 × 95 mm) or Magenta GA-7 jars (Magenta Corp., Chicago, IL) were filled to a depth of 5.0 cm with growing medium [6 L peat; 2 L vermiculite; 19 g dolomite; 3.8 g micronutrients (Plant Products Nutritrace)] moistened to a moisture content of approximately 79% (dry weight basis) with Plant Products soluble fertilizer 20–8–20 (0.65 g/L). Conifer seedlings (uninoculated) did not grow well when the growing medium was autoclaved and therefore unsterilized medium was used in the assays. The growing medium bulk density was 132 kg/m^3 and the pH was 5.5. *Fusarium* inoculum, in sterile distilled water, was prepared by harvesting conidia from colonized conifer needles (CNA) or carnation leaves (CLA). The spore concentration was adjusted to 70,000 conidia per mL (± 0.4 log unit) with a hemacytometer and checked by dilution plating.

The first assay used conifer seeds which were rinsed 10 times with sterile distilled water and blotted dry. Two mL of conidial inoculum (fungal isolate treatment) or sterile distilled water (control treatment) were pipetted onto the seedling growing medium surface in each vial (20 vials/treatment). Three seeds were evenly spaced on the surface, seed was covered with 2 cc granite grit (mesh size no. 1) moistened with 1.0 mL distilled water and vials were capped with clear Magenta double-rimmed polypropylene caps to allow air exchange. Alternatively, eight 1.0 mL spots of inoculum or sterile distilled water were pipetted onto the planting mix surface in eight evenly spaced spots in Magenta GA-7 jars (eight jars/treatment). One seed was then placed on top of each of the eight inoculum spots and the eight seeds were covered with granite grit. The grit surface was moistened with distilled water and jars were then covered with double-rimmed polypropylene lids. Jars and vials were placed in a plant growth room under an 18 h photoperiod (photosynthetically

active radiation (PAR) 360 m^{-2} s^{-1}) at 28°/22 °C, day/night regime. Disease was assessed after four weeks of incubation.

The second assay used six day-old conifer germinants. Seed was rinsed under running tap water for two hours and then rinsed three times with sterile distilled water. Seed was placed on sterile, moist Kimpak paper and incubated for six days in a germination box in a plant growth room under conditions previously described. The radicle was approximately 1.0–1.5 cm at the time of planting. A depression was made in the seedling growing medium (6 mm diam × 15 mm deep) and the interior of the depression (planting hole) was inoculated with 2.0 mL sterile distilled water (control) or with 2.0 mL of conidial inoculum at the concentration previously described. One germinant was placed in each planting hole, the planting mix was pressed around the radical, the planting mix surface was covered with granite grit (2 cc, mesh size no. 1), moistened and capped as described above (20 vials/treatment). Vials or Magenta jars were arranged in a completely randomized design and incubated in a plant growth room under an 18 hr photoperiod for four weeks prior to assessing disease. A heat stress (11 h of 28–30 °C) was imposed each day during the illumination period to enhance disease development (Bloomberg 1981). Pathogenicity assays using seed and germinants were each repeated once. Two additional seed assays were done in Magenta jars.

The number of nonemerged, healthy (symptomless) and diseased seedlings for each treatment (*Fusarium* isolates and control) were recorded in all experiments. Diseased seedlings exhibited one or more of the following symptoms: damping-off, shoot necrosis, and/or root rot. *Fusarium* was often sporulating on diseased stem and needle surfaces. Diseased and healthy seedlings were sampled from representative isolates from each *Fusarium* species from some of the experiments planted with conifer seed. Diseased tissue was usually very desiccated and was rinsed gently with sterile distilled water, placed in 10% bleach for 30 s, rinsed four times with sterile distilled water and placed on Komada medium supplemented with 1 mg/mL Benomyl and 100 mg/mL aeuromycin sulfate. Plates containing plant tissue were incubated under lights as previously described and *Fusarium* colonies recovered from diseased plant tissues were identified and compared with the treatment isolate. Healthy seedlings were also selected to determine if *Fusarium* root infection occurred in the absence of symptom expression. Healthy seedlings were assessed for *Fusarium* infection as described above except a three minute 10% bleach soak treatment was used for the root tissue.

Statistical analysis

The Wilcoxon signed-rank test (one-tailed) was used to determine if running water imbibition significantly reduced seedborne *Fusarium* levels compared to standing water imbibition (Dixon and Massey 1983). The Bonferroni multiple comparison procedure was used to compare the proportion of healthy seedlings for each treatment in pathogenicity assays (Brown and Hollander

1977; Devore and Peck 1986; Mendenhall et al. 1986). A two-tailed t-test was used to detect significant differences in seedling growth (height, root collar diameter, shoot and root dry weight) between the standing and running water imbibition treatments for each individual seedlot in the nursery experiment.

Results

Seedborne Fusarium

Fusarium was detected in all 12 Douglas-fir seedlots that were assessed prior to stratification (Table 1). *Fusarium* was isolated from seedlots which ranged in germination from 66%–99% and had been cold stored at –20 °C for two to 33 years post harvest. Percent of nonstratified seeds from individual seedlots harboring *Fusarium* ranged from 0.3% to 95.4% (Tables 1 and 2). Sixty-seven percent of the seedlots had less than 2% *Fusarium* contamination of the seed (Tables 1 and 2). The number of seeds contaminated with *Fusarium* was higher for some seedlots when seed was assessed on Komada medium supplemented with 1 mg/mL Benomyl and adjusted to pH 7.0 (Table 2) compared to pH 4.0 (Table 1). For example, *Fusarium* was detected on 95.4% and 60% of the seed from seedlot 476 when seed was plated on Komada medium at pH 7.0 and pH 4.0, respectively. Fewer fungal contaminants were present on the medium adjusted to pH 7.0 which appeared to improve the detection of seedborne *Fusarium*.

Five Douglas-fir seedlots (476, 7289, 7402, 8706 and 9983) were selected for further study. Seven species of *Fusarium* were isolated from Douglas-fir seed from four of these seedlots and included *Fusarium acuminatum* Ell. & Ev., *F. avenaceum* (Fr.) Sacc., *F. lateritium* Nees, *F. moniliforme* Sheldon, *F. oxysporum* Schlecht, *F. poae* (Peck) Wollen and *F. sambucinum* Fuckel (Table 1). Each *Fusarium* spp. was not isolated from more than two seedlots. Seedlot 476 had the most *Fusarium* contamination and the majority of the isolates were *F. moniliforme*.

Fusarium moniliforme and *F. proliferatum* are closely related *Fusarium* species, both producing microconidia in chains, differentiated primarily by the production of polyphialides by the latter species (Nelson et al., 1983). In practice, this distinction has caused problems because some isolates produce a very low percentage of polyphialides (<1%). In this study, these isolates are considered *F. moniliforme*. Isolates with 10 % or more polyphialides are considered *F. proliferatum*. No intermediate isolates were found. (K. Seifert, personal communications.)

Seed stratification

The percent of seeds contaminated with *Fusarium* increased during stratification for some seedlots (Table 2). This was most pronounced in standing

Table 2. The percent of Douglas-fir seeds contaminated with *Fusarium* prior to stratification and after standing or running water imbibition and a 21 day stratification period.

Seedlot	Pre-stratification % *Fusarium* contamination	Experiment	Post-stratification *Fusarium* contamination of seeds[1]			
			Standing Water		Running Water	
		#	#	%	#	%
476	95.4	1	483	96.6	477	95.4
		2	483	96.6	486	97.2
		3	185	37.0	180	36.0
		4	427	85.4	495	99.0
7289	0.6	1	47	9.4	3	0.6
		2	14	2.8	11	2.2
		3	0	0.0	1	0.2
		4	2	0.4	30	6.0
7402	2.6	1	201	40.2	80	16.0
		2	309	61.8	150	30.0
		3	111	22.2	39	7.8
		4	491	98.2	492	98.4
8706	8.0	1	103	20.6	63	12.6
		2	220	44.0	59	11.8
		3	107	21.4	7	1.4
		4	230	46.0	134	26.8
9983	1.0	1	5	1.0	26	5.2
		2	1	0.2	42	8.4
		3	22	4.4	1	0.2
		4	x[2]	x[2]	x[2]	x[2]

[1] Five hundred seeds were assayed for each treatment for every experiment. Seeds were imbibed in standing or running water for 24 h and incubated for 21 days at 4 °C prior to determining the percent *Fusarium* contamination after stratification. Running water imbibition significantly decreased the post-stratification number (#) of seeds contaminated with *Fusarium* compared with standing water imbibition (p = 0.027, one-tailed Wicoxon signed-rank test).

[2] Missing value.

water imbibition treatments for seedlots 7402 and 8706 which had 2.6% and 8.0% of the seeds contaminated with *Fusarium* prior to stratification, respectively. Following stratification, the percent of seeds contaminated with *Fusarium* increased approximately 21-fold in seedlot 7402 and five-fold for seedlot 8706 (mean of post stratification *Fusarium* values, Table 2). Seedlot 476 had a very high level of seedborne *Fusarium* (95%) and this high level did not change after stratification.

Running water imbibition significantly decreased the level of post-stratification seedborne *Fusarium* compared to standing water imbibition when results from the five seedlots were assessed from four different experiments (Table 2, $p = 0.027$, one-tailed, Wilcoxon signed-rank test). This decrease was most dramatic in seedlots 7402 and 8706 which had a low level of *Fusarium* on dry seed (2.6% and 8.0%, respectively) but increased dramatically after standing water imbibition and stratification. Running water imbibition did not decrease seedborne *Fusarium* levels in seedlot 476 which was heavily contaminated with *Fusarium* (95% pre-stratification *Fusarium* contamination).

Pathogenicity assays

Fusarium isolates ranged in virulence from low to high (Figs. 1 and 2) and disease symptoms included pre- and post-emergence damping-off as well as fungal colonization of root, stem and needle tissues. *Fusarium oxysporum* isolates produced significantly more disease than other species in the seed assay and caused emergence failure or disease ranging from 64–88% of inoculated seed for individual isolates (Fig. 1). *Fusarium moniliforme* isolates caused emergence failure or disease ranging from 49–69% of inoculated seed for individual isolates. *Fusarium avenaceum*, *F. sambucinum*, *F. lateritum* and *F. acuminatum* usually resulted in fewer diseased and non-emerged seedlings that were not significantly different from the control treatment in the seed experiment. Differences in disease levels among the *Fusarium* species were not as great in the germinant assay and slightly higher levels of disease were observed for *F. avenaceum*, *F. lateritium* and *F. acuminatum* isolates (Fig. 2). However, trends in the levels of disease caused by *Fusarium* spp. in seed and germinant experiments were similar to each other and when both experiments were repeated. Similar levels of disease within a species were also observed after inoculation with *F. oxysporum* and *F. avenaceum* isolates obtained from seed and diseased seedlings.

Fusarium oxysporum, *F. moniliforme*, *F. acuminatum*, and *F. sambucinum* were isolated from diseased seedlings from their respective treatments in seed pathogenicity assays. These fungi were also isolated from bleach treated roots of seedlings which appeared healthy indicating cortical infection most likely occurred in the absence of symptom expression. The utilization of an isolate specific stain and microscopic observation of root sections would be required to confirm this observation. *Fusarium avenaceum* F8 was isolated from diseased seedlings in assays done prior to this study. Disease levels were low

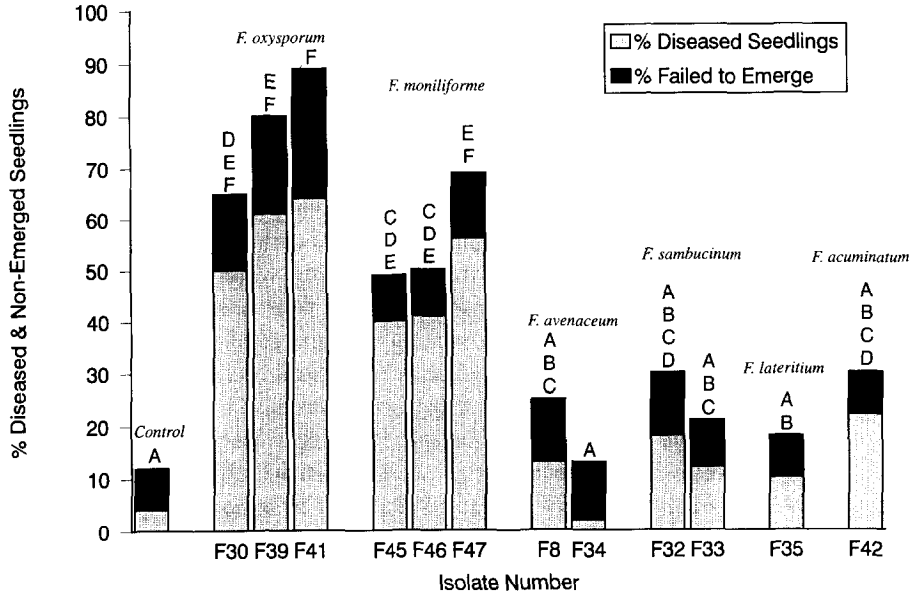

Fig. 1. Pathogenicity assessment of *Fusarium* isolates after inoculation of Douglas-fir seed and incubation for four weeks. Isolates were obtained from Douglas-fir seed except isolates F30 and F8 which were from diseased Douglas-fir and spruce seedlings, respectively. Reading across the graph, percentages which share at least one common letter are not significantly different ($p = 0.05$).

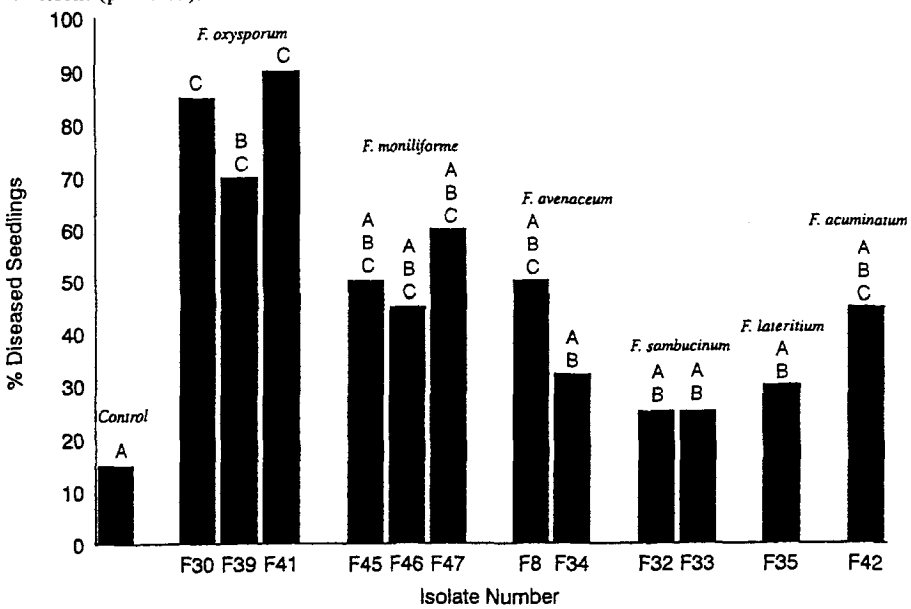

Fig. 2. Pathogenicity assessment of *Fusarium* isolates after inoculation of six day-old Douglas-fir germinants and incubation for four weeks. Isolates were obtained from Douglas-fir seed except isolates F30 and F8 which were from diseased Douglas-fir and spruce seedlings respectively. Reading across the graph, percentages which share at least one common letter are not significantly different ($p = 0.05$).

for *F. avenaceum F34* and *F. lateritium F35* and these *Fusarium* species were not recovered from representative seedlings which were recorded as diseased or healthy from two seed assays. *Fusarium lateritium F35* was recovered from a few healthy seedlings from a third assay.

The level of emergence failure was similar for the control treatment compared with most of the *Fusarium* treatments and may have been due to nonviable seed (Fig. 1). A few diseased seedlings were observed in control treatments. This was likely the result of an undetected pathogen or a low level of *Fusarium* inoculum introduced on seed which was not detected in seed isolation experiments. *Fusarium oxysporum* and *F. moniliforme* were isolated from a few seedlings which were not inoculated with these fungi indicating *Fusarium* may have been introduced at a very low frequency on the seed used in some trials. *Fusarium* was not recovered from growing medium which was assessed four times over the last several years. However, the growing medium was not assessed for all assays.

Seed germination and seedling growth

Germination of seeds sown in styroblocks at Surrey Nursery and on germination plates in the laboratory was not significantly different for standing or running water imbibed seed (results not shown). The high level of seedborne

Table 3. Douglas-fir seedling growth as influenced by standing or running water seed imbibition.[1]

Seedlot	Height (cm)		Root Collar Diameter (mm)		Shoot Dry Wt. (g)		Root Dry Wt. (g)	
	Stand	Run	Stand	Run	Stand	Run	Stand	Run[1]
Trial 1 (1989)								
476	11.7*	12.4	1.7	1.7	2.20	2.30	0.79	0.82
7289	10.2*	10.7	1.7*	1.8	2.04	2.20	0.75*	0.87
7402	11.7*	10.7	1.7	1.6	2.03	2.05	0.69*	0.79
8706	11.4	12.2	1.6	1.6	2.03*	2.25	0.74	0.78
9983	12.0*	13.3	1.7*	1.8	2.26	2.55	0.75	0.83
Trial 2 (1990)								
476	7.0	9.9	1.7	1.7	1.40*	1.68	1.10	1.19
7289	7.0	6.6	1.8*	1.7	1.60	1.41	1.22	1.07
7402	6.9	6.5	1.7	1.7	1.41	1.28	1.08	1.00
8706	6.6	6.2	1.6	1.6	1.25	1.20	0.98	0.96
9983	7.9	7.7	1.8	1.8	1.58	1.55	1.09	1.13

[1] Seed was imbibed with standing (stand) or running (run) water and incubated for 21 days at 4 °C prior to sowing seed in stryroblocks and growing seedlings operationally at a conifer nursery.

* Indicates a significant difference between standing and running water imbibition for each seedlot using a two-tailed *t*-test ($p < 0.05$).

Fusarium on seedlot 476 did not appear to influence seed germination (Table 1).

Some significant differences in seedling growth were observed during the 1989 trial when comparing standing and running water imbibition treatments for the five seedlots (Table 3). Seedling height was significantly greater for the running water imbibition treatment compared to the standing water imbibition treatment for four of the five seedlots and significantly less for one seedlot (7402). A few other seedlots also exhibited significant increases in root collar diameter, shoot and root dry weight for the running water imbibition treatment in 1989. However, none of these growth differences were observed in the 1990 trial. Seed was sown several weeks late during the 1990 trial and seedling size was therefore smaller than seedlings harvested at the end of the 1989 trial.

Discussion

Fusarium was isolated from all of the coastal Douglas-fir seedlots which were assessed in this study. Furthermore, seven *Fusarium* species were isolated from four representative seedlots indicating Douglas-fir seed harbors a diversity of *Fusarium* contaminants. In a recent evaluation by Forestry Canada, 82% of 138 B.C. Douglas-fir seedlots were found to be contaminated with *Fusarium* (J. Dennis, personal communications). These combined results indicate *Fusarium* is a common seedborne inhabitant.

Seedborne *Fusarium* levels increased during the stratification process for some seedlots and running water imbibition significantly decreased the post-stratification level of seedborne *Fusarium* compared to the standing water imbibition treatment. However, this decrease was not observed for all seedlots. For example, seedborne *Fusarium* levels on seedlot 476 remained high (90–95%) after the running water imbibition treatment possibly because the contamination was not due to seed surface *Fusarium* propagules. *Fusarium* seed contamination of seedlot 476 was reduced significantly using a running water imbibition in combination with a post stratification eight hour treatment of 3% hydrogen peroxide indicating additional seed sanitation methods can further reduce seedborne *Fusarium* (Neumann and Axelrood, unpublished). James (1987) found running water seed imbibition significantly reduced seedborne *Fusarium* on spruce compared to standing water imbibition. Treatments with hydrogen peroxide, ethanol, bleach or hot water also reduced *Fusarium* seed contamination (Dumroese et al. 1988). Recommendations for conifer seed sanitation treatments have been summarized by Campbell and Landis (1990). As an outcome of the present study and other publications, the B.C. Ministry of Forests is in the process of implementing a running water stratification treatment for Douglas-fir seed as a change to their operational procedures.

Evaluation of the percent of seeds contaminated with seedborne *Fusarium*

can be influenced by the selective medium used for isolation. Although Komada medium was developed to isolate *F. oxysporum* (Komada 1975) this medium supplemented with 1 mg/mL Benomyl proved useful in decreasing *Trichoderma* contaminants and detecting a variety of *Fusarium* species in this study. For some seedlots, higher levels of seedborne *Fusarium* were detected if Komada medium supplemented with 1 mg/mL Benomyl was adjusted to pH 7.0 which appeared to further reduce *Trichoderma* and other fungal contaminants. P. Hamm (personal communications) found Komada medium supplemented with 1 mg/mL Benomyl successfully inhibited the growth of many *Trichoderma* contaminants and enhanced recovery of *Fusarium* species (*F. oxysporum, F. avenaceum, F. acuminatum, F. sambucinum, F. moniliforme, F. proliferatum* and *F. poae*) from conifer seed, seedling roots and soil compared with Nash and Snyder medium (Nash and Snyder 1962) or PCNB medium (Papavizas 1967). Komada medium supplemented with 1 mg/mL Benomyl is also routinely used by other scientists to isolate *Fusarium* from conifer seed (R. James and J. Dennis, personal communications).

Differences in virulence were observed among the six seedborne *Fusarium* species with *F. oxysporum* producing the highest levels of disease, *F. moniliforme* producing moderate levels of disease and *F. sambucinum* and *F. lateritum* producing less disease. Virulent isolates of *F. oxysporum, F. acuminatum* and *F. avenaceum* were isolated from diseased and/or healthy Douglas-fir seed or seedlings grown in western U. S. conifer nurseries and these isolates caused more disease than *F. sambucinum* (James et al. 1989). Seedling disease levels caused by *F. acuminatum, F. avenaceum, F. lateritium,* and *F. sambucinum* were not significantly different than the control non-inoculated treatment in seed pathogenicity assays in this study. However, *F. avenaceum* and *F. acuminatum* caused a significant increase in disease in one germinant assay indicating the inoculation method can cause variability in disease development. Few isolates of these *Fusarium spp.* were obtained from the four seedlots which were assessed in this study to evaluate differences in virulence. *F. proliferatum* has been isolated from Norway spruce seed (Urosevic 1961) and damped-off southern pine seedlings (Pawuk and Barnett 1974). Furthermore, Huang and Kuhlman (1990) have identified seedborne isolates of *F. moniliforme var. moniliforme, F. moniliforme var. intermedium* and *F. moniliforme var. subglutinans* as pathogenic on slash pine germinants. Pawuk (1978) found no correlation between species of seedborne *Fusarium* and seedling mortality of longleaf pine. This may have been because the germination of all treatments was extremely low resulting in insignificant differences in pathogenicity.

The presence of seedborne *Fusarium* does not necessarily indicate a high probability of subsequent disease as indicated by the low levels of disease produced by several of the seedborne *Fusarium* species. In addition, *F. oxysporum, F. moniliforme, F. acuminatum,* and *F. sambucinum* were isolated from bleach treated roots of healthy looking seedlings in this study. Bloomberg (1966) and Vaartaja and Cram (1956) saw a similar situation in seedlings

grown in bareroot nurseries as did James et al. (1989) in containers. This would indicate pathogen presence does not necessarily relate to symptom development. Furthermore, disease symptoms were not observed in nursery-grown seedlings from the *Fusarium* contaminated seedlots used in this study.

The environmental conditions which are present and the stresses placed on seedlings are influential in the expression of disease (Bloomberg 1981; James 1985). A heat stress treatment was needed to produce symptom development in pathogenicity assays in this study. Furthermore, nonpathogenic *Fusarium* isolates have been identified in pathogenicity assays on Douglas-fir (James 1986) and slash pine (Huang and Kuhlman 1990).

Douglas-fir seedling growth was not consistently different for the running water and standing water imbibition treatments over two field seasons. Some significant differences were observed during one field season and seedling growth was greater for several of the running water treatments compared to the standing water treatments. James and Genz (1981) found fewer germinant disease problems occurred on ponderosa pine when seed was imbibed in running water compared to standing water. Furthermore, running water imbibition in combination with a hydrogen peroxide post-stratification seed treatment significantly increased Douglas-fir seedling growth (Neumann et al. 1992).

Pathogenic Fusaria are present on conifer seed (Pawuk 1978; Timonin 1964), including Douglas-fir (Graham and Linderman 1983; James 1986; James et al. 1989) and this study provides evidence that pathogenic *Fusarium* species are also present on B.C. seedlots of Douglas-fir. However, the results reported herein indicated seedling growth was not consistently less on seedlots with high levels of *Fusarium* and disease symptoms were not apparent on nursery-grown seedling. Seedborne *Fusarium* may have potential to cause pre- and post-emergence damping-off and root rot of conifer seedlings (James et al. 1986). Specific environmental and cultural conditions appear to be required for disease expression. Although epidemiological information and a *Fusarium* root disease model has been developed for bareroot-grown Douglas-fir seedlings (Bloomberg 1979), this information is not easily applied to container nurseries due to differences in cultural practices and environmental conditions. For instance, seedling containers are routinely sown with two to three seeds per cavity and thinned at a later date. If seedborne inoculum incites pre- or post emergence damping-off, disease may be compounded at a later date if a healthy seedling shares the same cavity with a damped-off seedling. In addition, *Fusarium* can be introduced on used styroblocks (Sturrock and Dennis 1988) and nursery debris. At present, the relative importance of these different *Fusarium* inoculum sources is unknown. The development of disease models for container nurseries, including an evaluation of the impact of different inoculum sources and specific cultural and environmental parameters which influence disease progression would increase the understanding of *Fusarium* related diseases in container nurseries.

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