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Prevalence and incidence of the root-inhabiting fungi, *Fusarium, Cylindrocarpon* and *Pythium*, on container-grown Douglas-fir and spruce seedlings in British Columbia

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Application. Species of *Fusarium, Cylindrocarpon* or *Pythium* were readily isolated from the roots and growth medium of container-grown Douglas-fir and spruce seedlings. Differences in their occurrence varied with nursery, but not consistently with conifer species, indicating that cultural practices which alter conditions such as growth medium moisture and temperature can influence their prevalence.

Abstract. Surveys were made at the end of the 1990 and 1991 growing seasons for root-inhabiting fungi in the genera *Fusarium, Cylindrocarpon* and *Pythium* from the roots of one year-old container-grown Douglas-fir and spruce seedlings grown under greenhouse conditions. In the 1990 survey of four nurseries, it was found that 61–97% of both Douglas-fir and spruce roots were colonized with *Fusarium, Cylindrocarpon* or *Pythium*. There were significantly ($p \leq 0.05$) more Douglas-fir roots than spruce roots colonized by *Fusarium* at all nurseries, however, there were significantly ($p \leq 0.05$) more spruce roots than Douglas-fir roots colonized by *Cylindrocarpon* and *Pythium*. Root colonization of Douglas-fir and spruce by the three fungal genera during 1991 varied from 0–82% at three nurseries, however, only at a south coastal nursery was there significantly ($p \leq 0.05$) more spruce than Douglas-fir roots colonized by *Cylindrocarpon*. Significantly more seedlings were infected in 1990 than in 1991. In 1991, there were few significant differences between Douglas-fir and spruce, in the percentage of seedlings with colonized roots and in the percentage of growth medium colonized by the fungi. However, there were significant differences between nurseries.

Introduction

Fungi in the genera *Fusarium, Cylindrocarpon* and *Pythium* have often been isolated from roots of container-grown Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, or spruce, *Picea* spp., in North America (Landis 1989; Sutherland et al. 1989; Hamm et al. 1990) and Europe (Galaaen and Venn

1979; Perrin and Gomez 1991). These fungi are common inhabitants of container nurseries, having been isolated from seedling growth containers, media, greenhouse benches, irrigation water and air (James et al. 1991; Landis 1989; Sutherland et al. 1989) and from conifer seeds (Sutherland et al. 1987; James and Genz 1982). Although, there is a substantial amount of information available on *Fusarium, Cylindrocarpon* and *Pythium* incidence in container-grown seedlings (Sutherland et al. 1989, Unestam et al. 1989; Lilja et al. 1992; Dumroese et al. 1993), the prevalence and potential importance of these fungi on asymptomatic container-grown seedlings (Landis 1989) remains largely unknown.

Fusarium, Cylindrocarpon and *Pythium* have been isolated from roots of container-grown Douglas-fir and spruce seedlings in British Columbia (B.C.), Canada, however, little is known about how their incidence relates to specific nursery, cultural practices, or hosts (Sutherland et al. 1989). The objective of this study was to determine prevalence and incidence of *Fusarium, Cylindrocarpon* and *Pythium* late in the 1990 and 1991 growth seasons on the roots and in the 1991 growing season in the growth media of Douglas-fir and spruce seedlings in four container nurseries in B.C.

Materials and methods

1990 and 1991 surveys for root inhabiting fungi

Two surveys were made, one in November 1990 and the other in November 1991, to determine presence and incidence of fungi in the genera *Fusarium, Cylindrocarpon* and *Pythium* on roots of asymptomatic 1-year-old container-grown Douglas-fir, and various spruce species, (*Picea* spp.) grown under greenhouse conditions.

In the 1990 survey, 100 randomly-selected one-year-old container-grown seedlings of each tree species were collected in each of four nurseries throughout B.C., i.e., one each on Vancouver Island, in the southern interior, in the northern interior and a south coastal nursery (Table 1). Interior Douglas-fir was sampled at a southern and northern interior nursery, whereas, coastal Douglas-fir was sampled at a Vancouver Island and a south coastal nursery. Engelmann spruce (*P. engelmannii* Parry ex Engelm.) was sampled at the southern interior and south coastal nurseries, while Sitka spruce (*P. sitchensis* (Bong.) Carr.) and white spruce (*P. glauca* Moench) were sampled at the Vancouver Island and the northern interior nurseries, respectively.

In 1991, three B.C. nurseries, a northern and a southern interior nursery, and a south coastal nursery, were surveyed. From each nursery, one year old, container-grown seedlings were randomly selected from 13 STYROBLOCK®

Table 1. Number of seedlings sampled, the respective seedlot number, and the nurseries and conifer species sampled at the end of the 1990 or 1991 growing season.

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	Number of seedlings sampled (seedlot number)						
	Vancouver Island	Southern Interior		Northern Interior		South Coastal	
Conifer species	*1990*	1990	1991	1990	1991	1990	1991
Picea engelmannii	–	100 (5681)	52, 52 (8311), (4019)	–	52 (4019)	–	34 (4019)
Picea glauca	–	–	–	100 (3984)	–	100 (4162)	–
Picea glauca x engelmannii	–	–	–	–	52 (29157)	–	43 (6701)
Picea sitchensis	100 (4753)	–	–	–	–	–	–
Pseudotsuga menziesii interior	–	100 (5653)	52, 52 (0269), (8192)	100 (8612)	48, 52 (25783), (8192)	–	–
Pseudotsuga menziesii coastal	100 (8703)	–	–	–	–	100 (6399)	33 (6542)

(Beaver Plastics, Edmonton, AB) seedling growth containers from each of six different seedlots (three Douglas-fir and three spruce). Due to variations in availability of seedlings, 33 coastal Douglas-fir and 43 hybrid Engelmann spruce (*P. glauca* × *engelmannii* Engelm.)were sampled from the south coastal nursery, where as, 52 seedlings of both interior Douglas-fir and Engelmann spruce were sampled from both the northern and southern interior nurseries (Table 1).

Also, at each of the three nurseries a common, interior Douglas-fir and Engelmann spruce, seedlot, of one year-old seedlings, were sampled. However, variations were present in the number of seedlings sampled, i.e. no Douglas-fir and 34 spruce were sampled from the south coastal nursery, 48 Douglas-fir and 52 spruce from the northern interior nursery, and 52 seedlings each of Douglas-fir and spruce from the southern interior nursery (Table 1).

During the 1991 survey only, both roots and the growth medium were assayed for the above fungi.

Isolation of fungi from seedling roots

Whole root systems of each seedling were thoroughly washed free of adhering growth medium. A 3-cm long section including all roots of the same physiological age, was cut out from the middle of the root system and surface sterilized with a 10% bleach solution (0.525% aqueous sodium hypochlorite), for 5 minutes and rinsed three times in sterile, distilled water. Twelve, 3-cm long root segments were chosen at random and placed onto Komada's medium (Komada 1975) acidified with 0.1% HCl to pH 4.5, plus the antibiotic aueromycin (100 mg/L), to slow the growth of bacteria. Six root pieces each were placed on the media in the 2 Petri plates, and incubated at 18–24 °C with a 14–10 h (day–night) photoperiod and examined for *Fusarium* and *Cylindrocarpon* over the next 20 days.

Pythium spp. were isolated from roots using a medium composed of a 17% (v/v) of V8 juice (Campbell Soup Co., Toronto, ONT), 1.5% water agar, plus, per liter, the antibiotics Pimaricin (10 mg), Rose Bengal (20 mg), Rifampin (12 mg), Ampicillin (250 mg), and Pentachloronitrobenzene (120 mg). Again, 12 root pieces from each seedling (six in each of two Petri plates) were incubated at room temperature (22–25 °C) in the dark for 2–4 days.

The number of root segments colonized, divided by the number of root segments assessed (from infected plants only) was used to determine the percent root colonization.

The percentage of colonized seedlings was calculated by dividing the number of seedlings infected by the number of seedlings sampled.

Isolation of fungi from the seedling growth medium

The peat : vermiculite (3 : 1) growth medium, from around the roots of four randomly selected seedlings per STYROBLOCK was removed and thoroughly mixed. Two 1.25 g subsamples of the growth medium, plus 200 mL of 0.1% water agar, were placed into each of two Erlenmeyer flasks and vigorously shaken. One mL from each flask was plated onto each of two Petri plates containing Komada's medium and incubated as for seedling roots. The moisture content of the growth medium was determined (40 g sample dried for 72 h, allowed to cool and then weighed) to obtain the number of Colony Forming Units per gram (CFU/g) of dry growth medium. *Pythium* spp. were isolated by adding 50 mL of 0.1% water agar to each of two flasks containing 1.25 g of growth medium, and shaking vigorously. From each flask, a 1 mL sample was plated onto each of two V8 juice based agar medium plates and incubated at 22–25 °C in the dark. After 2 days the plates were washed with sterile, distilled water to remove the particles of growth medium. The number of CFUs per gram of dry growth medium was then determined as above.

Statistical analysis

All data were analyzed using the Chi-square test (Zar 1984) to detect significant differences between conifer species and nurseries for the occurrence of *Fusarium, Cylindrocarpon* and *Pythium* in seedling roots and in the growth medium.

Results

The 1990 survey

At the end of the growing season, 61–97% of seedlings of both Douglas-fir and spruce, at all four nurseries, were colonized by members of one, two or all three fungal genera (Table 2).

The incidence of *Fusarium* on seedling roots (percent root colonization) from Douglas-fir (5–36%) was more than from spruce seedling roots (1–11%). There were significantly ($p \leq 0.05$) more *Fusarium* infected roots of Douglas-fir than spruce, at all four nurseries. *Cylindrocarpon* on seedling roots (percent root colonization was comparable in prevalence, and was consistently high, in both Douglas-fir (25–57%) and spruce (26–73%) roots (Table 2). *Cylindrocarpon* was equally prevalent on both conifers at the northern interior and south coastal nurseries, but there were significant ($p \leq 0.05$) differences in its occurrence at the southern interior and at the Vancouver Island nurseries. *Pythium* was more often isolated from roots of spruce (1–27%) than

Table 2. Percent of Douglas-fir and spruce seedlings yielding *Fusarium*, *Cylindrocarpon* or *Pythium* from the roots at four nurseries at the end of the 1990 growing season.

Nursery	Northern Interior		Southern Coastal		Southern Interior		Vancouver Island	
Seedling species	Interior Douglas-fir	White Spruce	Coastal Douglas-fir	White Spruce	Interior Douglas-fir	Engleman Spruce	Coastal Douglas-fir	Sitka Spruce
Seedlot number	8612	3984	6399	4162	5653	5681	8703	4753
Percent seedlings colonized								
Total[1]	97	86	89	90	61	83	89	64
Percent root colonization[2]								
Fusarium	36.0a[3]	11.0b	10.0a	2.0b	5.0a	1.0b	12.0a	3.0b
Cylindrocarpon	41.0a	30.0a	57.0a	73.0a	25.0a	54.0b	41.0a	26.0b
Pythium	14.0a	27.0b	3.0a	19.0b	0.0a	19.0b	0.0a	1.0a

[1] Based on 100 seedlings, roots yielded one or more, or any combination of the three fungi.
[2] Frequently more than one fungus was obtained from a seedling, therefore, columns do not total 100%.
[3] Within nurseries, comparing conifer species, adjacent means followed by the same letter are not significantly different (χ^2, $p = 0.05$).

from Douglas-fir (0–14%). *Pythium* was equally prevalent ($p \leq 0.05$) on Douglas-fir and spruce at the Vancouver Island nursery, but its occurrence was significantly ($p \leq 0.05$) lower on Douglas-fir than on spruce, at the other three nurseries.

The 1991 survey

The following data, for percent seedlings colonized and percent root colonization are the combined results for all seedlots at the three sampled nurseries.

At the end of the growing season, for all Douglas-fir and spruce seedlots, 0–82% of the roots yielded *Fusarium, Cylindrocarpon* or *Pythium* (Table 3).

Fusarium was particularly abundant on seedling roots (percent root colonization) of both Douglas-fir, (62.0%) and spruce, (70.1%) at the northern interior nursery, and on Douglas-fir, (41.7%) and spruce, (93.3%) at the southern coastal nursery. There were significant ($p \leq 0.05$) differences for *Fusarium* (86.3 and 0.0%) between Douglas-fir and spruce, respectively, at the southern interior nursery. *Cylindrocarpon* occurred on seedling roots of Douglas-fir (83.0%) and spruce (100%) at the northern interior nursery and on Douglas-fir, (69.1%) and spruce, (96.9%) at the southern coastal nursery. The lowest incidence of *Cylindrocarpon* (41.2%) from Douglas-fir roots occurred at the southern interior nursery. There were significant ($p \leq 0.05$) differences for *Cylindrocarpon* (41.2 and 0.0%) between Douglas-fir and spruce, respectively, at the southern interior nursery. At the south coastal nursery there were significant differences for *Cylindrocarpon* (61.9 and 96.9%) on roots of Douglas-fir and spruce, respectively. *Fusarium* and *Cylindrocarpon* levels were high and equally prevalent on Douglas-fir and spruce at the northern interior nursery (Table 3). None of the three fungi were isolated from spruce roots at the southern interior nursery.

A comparison of *Fusarium, Cylindrocarpon* and *Pythium* prevalence in the growth media at the three nurseries showed that *Fusarium* was significantly ($p \leq 0.05$) more prevalent in the growth medium of Douglas-fir than in that of the spruce growth medium (Table 3). The same comparison for *Cylindrocarpon* and *Pythium* showed that they were equally prevalent at two of the nurseries, while at the northern interior nursery *Pythium* was more prevalent on spruce (Table 3).

The 1991 survey – common seedlots

A comparison of the nurseries was made by using a common interior Douglas-fir (seedlot # 8192) and Engelmann spruce (seedlot # 4109) seedlot grown at each of the three nurseries. Isolations from the roots of the common Douglas-fir and spruce seedlots showed that there were significant ($p \leq 0.05$) dif-

Table 3. Percent of Douglas-fir and spruce seedlings yielding *Fusarium, Cylindrocarpon* or *Pythium*, from the roots, and the colony forming units in the growing medium, at three nurseries at the end of the 1991 growing season.

Nursery	Northern Interior		Southern Coastal		Southern Interior	
Seedling species[1]	Interior Douglas-fir	Hybrid + Engelmann Spruce	Coastal Douglas-fir	Hybrid + Engelmann Spruce	Interior Douglas-fir	Engelmann Spruce
Seedlot number	25783, 8192	29157, 4019	6542	6701, 4019	0269, 8192	8311, 4019
	$n = 100$	$n = 104$	$n = 33$	$n = 77$	$n = 104$	$n = 104$
Percent seedlings colonized	42	42	82	65	22	0
Percent root colonization[2]						
Fusarium	62.0a[3]	70.1a	41.7a	93.3a	86.3	0.0
Cylindrocarpon	83.0a	100.0a	69.1a	96.9b	41.2	0.0
Pythium	83.3a	90.0a	0.0a	50.3b	0.0	0.0
CFU[4]						
Fusarium	16.9a	6.4b	95.9a	25.0b	0.0	8.3
Cylindrocarpon	1.9a	1.4a	0.0a	19.6b	0.0a	2.1a
Pythium	1.9a	61.7b	0.0a	15.1a	0.0a	0.0a

[1] The data were combined for the two seedlots of each species sampled at each nursery.
[2] More than one fungus was obtained from each seedling, therefore, columns do not total 100%.
[3] Within nurseries, and between conifer species, adjacent means followed by the same letter are not significantly different (χ^2, $p = 0.05$).
[4] Colony forming units x 10^3/gram oven dry growing medium.

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ferences between all nurseries for Douglas-fir and spruce seedlings roots (percent root colonization) yielding *Fusarium* (Table 4). *Cylindrocarpon* was only obtained from roots of spruce at the northern interior and southern coastal nurseries. Differences between numbers of seedlings from which root fungi were isolated were related to nursery with there being no significant ($p \leq 0.05$) differences among nurseries for *Pythium* on Douglas-fir or spruce. However, *Pythium* numbers were consistently low at all nurseries on both Douglas-fir and spruce.

Isolations from the growth medium of the common Douglas-fir and Engelmann spruce seedlots at the three nurseries revealed that, regardless of nursery, there were no significant ($p \leq 0.05$) differences in *Fusarium* or *Cylindrocarpon* CFU levels for either tree species (Table 4). However, there was a significant ($p \leq 0.05$) difference for spruce at the northern interior nursery for *Pythium* prevalence than at the other two nurseries (Table 4).

Discussion

Our results show that fungi in the genera *Fusarium, Cylindrocarpon* and *Pythium* can be readily isolated from the roots and growth medium of healthy looking, container-grown Douglas-fir and spruce seedlings. All three genera are known to have species which are either minor pathogens or facultative parasites (Garrett 1970) which colonize roots without adversely affecting shoot growth. We found that the differences in the percentage of seedlings whose roots contained such fungi were related to specific nurseries, and by implication to cultural practices, more so than to the seedling species. However, colony forming unit levels from Douglas-fir and spruce were significantly different for numerous sample comparisons. The CFUs of *Fusarium, Cylindrocarpon* and *Pythium* in the seedling growth medium was seen not to be correlated with fungal counts on the roots.

In both 1990 and 1991, Douglas-fir and spruce roots contained a high percentage of *Cylindrocarpon* at all nurseries, except for spruce at the southern interior nursery in 1991. *Cylindrocarpon* was always the most common fungus isolated from seedling roots, suggesting that the conditions late in the growing season favor *Cylindrocarpon* over *Fusarium*. Unestam et al. (1989) showed that *C. destructans* (Zins.) Scholt. is typically limited to the rhizoplane of healthy Scots pine (*Pinus sylvestris* L.) seedling roots. Salt (1979) found that *C. destructans* survives indefinitely in young rootlets, root hairs and superficial cortical cells without adversely affecting seedling health, similarly, *Cylindrocarpon* encountered in our studies was not adversely affecting seedling health. However, Unestam et al. (1989) showed that *C. destructans*

Table 4. Percentage[1] of root colonization of Interior Douglas-fir and Engelmann spruce seedlings, yielding *Fusarium, Cylindrocarpon* or *Pythium* from the roots and the growing medium, at three nurseries at the end of the growing season in 1991.

	Roots					Growing medium				
	Interior Douglas-fir[2]		Engelmann Spruce[3]			Interior Douglas-fir		Engelmann Spruce		
Fungi	Northern Interior $n = 48$[4]	Southern Interior $n = 52$	Northern Interior $n = 52$	Southern Interior $n = 52$	Southern Coastal $n = 34$	Northern Interior $n = 12$[5]	Southern Interior $n = 13$	Northern Interior $n = 13$	Southern Interior $n = 13$	Southern Coastal $n = 13$
Fusarium	32.0a[6]	62.5b	32.6x	0.0y	60.0z	15.8a	25.3a	6.1x	8.3x	7.6x
Cylindrocarpon	40.0a	17.0b	67.9x	0.0y	41.6z	0.0a	0.0a	1.4x	5.4x	2.1x
Pythium	33.3a	0.0b	90.0x	0.0y	17.0z	0.0a	0.0a	61.7x	0.0y	0.0y

[1] Frequently more than one fungus was recovered from each root or growing medium, therefore column totals do not equal 100%.
[2] B.C. Forest Service seedlot, 8192.
[3] B.C. Forest Service seedlot, 4019.
[4] n = seedlings sampled.
[5] n = styroblocks sampled.
[6] Reading across, adjacent means within a tree species and fungus genus, that are followed by the same letter are not significantly different (χ^2, $p = 0.05$).

could kill roots of Scots pine when the seedlings were stressed by factors such as low light intensity, waterlogged growth medium, and fungicide treatments.

Pythium spp. flourish in waterlogged growth media and may cause root rot of containerized seedlings (Sutherland et al. 1989). Their presence indicates poor drainage of the growth medium (Venn et al. 1986) which predisposes roots to infection by these fungi (Landis 1989). The fact that *Pythium* spp. were isolated, indicates that there have been relatively high moisture conditions in the growing containers. An area of waterlogged medium often occurs in the lower half of seedling growing containers due to the compaction of peat-vermiculite growth media (Landis 1989). At the northern interior nursery in 1990, a high percentage of spruce roots yielded *Pythium* spp., suggesting that cultural conditions e.g., moisture levels in the growth medium, could be implicated in the growth and establishment of *Pythium* spp.

Environmental factors can significantly affect *Fusarium* colonization of roots of conifer seedlings (James et al. 1991). Several *Fusarium* species colonize conifer roots at temperatures above 24 °C (Bloomberg 1971), where soil moisture levels are high (Stoner 1981) and they can colonize peat growth media, provided the media are suitably enriched (Stoner 1981), or otherwise altered by cultivation. Bloomberg (1979) suggested for the practical control of *Fusarium*, nurseries should maintain cooler growth medium (soil) temperatures during the first part of the growing season. Warm temperatures early in the growing season initiated the establishment of *Fusarium* within the roots and once established there was an increased rate of spread from root to root (Bloomberg 1979). Differences between the three nurseries in seedling infection by *Fusarium* suggests differences in nursery cultural conditions conducive to *Fusarium* presence, e.g., conditions affecting the growth medium, early on in the growing season, that allowed the establishment of *Fusarium*, and its resulting spread throughout the root system.

Interactions of many interrelated factors probably influence root colonization and although present, *Cylindrocarpon, Fusarium* and *Pythium*, may not necessarily initiate above or below-ground disease symptoms. Their presence might even be beneficial if they can protect seedling roots from infection by pathogens (James et al. 1991). Their presence suggests that they are common inhabitants of roots of container-grown seedlings, however, root colonization is an inadequate predictor of disease levels (James et al. 1987).

We view this study as exploratory, recognizing the fact that our sample size was small considering the diversity of nurseries upon which we drew our conclusions and that information was gathered for two growing season only. Certainly, an extensive exploration of the relationship between nursery environmental conditions, and nursery practices could yield significant insight into the distribution patterns exhibited by root-inhabiting fungi in nurseries.

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