Container Western White Pine Seedlings: Root Colonization by Fusarium and Cylindrocarpon Species

R.L. James

Plant Pathologist, Forest Service, Forest Health Protection, Coeur d'Alene, ID

Abstract

Healthy-appearing container seedlings of western white pine were sampled for root colonization by potentially pathogenic *Fusarium* and *Cylindrocarpon* spp. at an Idaho forest nursery. Seedlings were sampled monthly for 8 mo with the goal of better understanding epidemiological changes that might occur over time. *Fusarium* spp., especially *F. proliferatum*, were present at relatively high levels throughout the seedling production cycle. *Cylindrocarpon* (mostly *C. destructans*), however, was not detected until seedlings were 18–22 wk old. Root colonization by *Cylindrocarpon* remained much less than that by *Fusarium* spp. Although high levels of *Fusarium* contaminated seeds before sowing, potentially pathogenic species were mostly detected only at low levels. *Cylindrocarpon* spp. were detected infrequently on seeds. Very low levels of root disease occurred during the crop cycle. Good root plug condition was common on sampled seedlings; very few seedlings were culled.

Introduction

Root diseases of container-grown seedlings of western white pine (*Pinus monticola* Dougl.) periodically have damaged crops extensively, reducing the number of satisfactory seedlings produced and seedling quality (James 1985a; 1987a; 1990a; 1991a). The major fungal pathogens normally associated with such diseases include species of *Fusarium* and *Cylindrocarpon* (James 1985b; 1988a; 1990b).

In some cases, distinctive above-ground disease symptoms associated with extensive root colonization by these organisms are evident (James 1987a; 1989a; 1990a; 1991b; 2003b). All too often, however, no disease symptoms are discernable, even though root decay may be extensive (James 1988a; 1991c; 1991d; 2004a). In such cases, disease becomes evident only after seedlings are removed from containers; they may have high levels of root decay, requiring culling.

In most previous investigations, associated fungal organisms were determined at the end of the crop-growing cycle, when diseased seedlings were detected after lifting. Information on the temporal changes in fungal root colonization during a typical crop production cycle by different potentially pathogenic organisms has been lacking. This evaluation was recently conducted to provide such information, with the specific goal of determining changes in root colonization by *Fusarium* and *Cylindrocarpon* spp. during the crop production cycle.

Materials and Methods

A large container nursery in Idaho, which has traditionally produced many western white pine seedlings each year for reforestation, was selected. All seed used to produce seedlings was obtained from the same seed orchard, which produces improved seed developed for resistance to white pine blister rust (*Cronartium ribicola*). A sample of 100 seeds from bulk storage was analyzed for surface contamination by *Fusarium* and *Cylindrocarpon* spp. Seeds were placed aseptically on a selective agar medium for *Fusarium* and closely related fungal species (Komada 1975). Agar plates were incubated under diurnal cycles of cool fluorescent light at about 24 ˚C for 7–10 d. Selected emerging fungi were transferred to carnation leaf agar (Fisher and others 1982) and potato dextrose agar for identification according to the taxonomy of Nelson and others (1983) and Booth (1966). Percentages of seeds colonized by particular fungal species were determined.

Seedlings were grown in three production areas: two greenhouses (designated GH5 and GH7) and one shadehouse area (designated "Bay"). Seedlings were grown in two container sizes, 5s (120 cells block^{-1}) and 8s (91 cells block^{-1}) and sampled eight times at approximately monthly intervals, beginning about 6 wk after sowing. During each sampling period, five seedlings were randomly selected from each of the production areas and container sizes for

laboratory analysis of fungal root colonization; this resulted in four separate samples (GH7–5s; GH7–8s; GH5–8s; Bay–8s; no seedlings were grown in 5s containers in GH5 or the shadehouse) during each sampling period. Selected seedlings were carefully extracted from containers, placed into individual plastic bags, transported to the laboratory, and analyzed immediately for fungal root colonization.

Seedling roots were washed thoroughly to remove adhering peat growing medium. Ten root pieces, each approximately 5 mm (0.2 in.), were randomly dissected from each seedling, surface-sterilized in 0.5-percent aqueous sodium hypochlorite (10-percent bleach solution), rinsed in sterile water, placed on the selective agar medium, and incubated as described above. Associated *Fusarium* and *Cylindrocarpon* spp. were identified and percentages of sampled root pieces colonized by particular fungal species were determined.

When seedlings were lifted from containers at the end of the production cycle, a total of 63 seedlings were collected for examination of their root systems (plugs) to determine extent of noticeable root decay. Seedling root plugs were placed into one of three categories based on the extent of root decay. Poor root systems exhibited extensive root decay with few roots remaining at the bottom of the plug. Moderate root systems had an intermediate level of root decay that may have compromised the root plug integrity; i.e., some of the growing media became dislodged when seedlings were extracted from containers. Good root systems exhibited very little or no noticeable root decay, and the root plug integrity was maintained upon seedling extraction. The percentage of seedlings culled due to poor root development (indicating decay and associated effects on root plug integrity) was determined from seedlings extracted from five randomly selected containers in each of the four sampled production areas.

Table 1. Contamination of western white pine seeds with *Fusarium* and other selected fungi.

Fungal species	Percent contamination[1]
Fusarium acuminatum	73
F. culmorum	20
F. proliferatum	5
F. equiseti	3
All *Fusarium*	98
Cylindrocarpon destructans	2
Botrytis cinerea	1

[1] Sample based on 100 seeds randomly selected from bulk storage before sowing.

Results

Nearly all sampled western white pine seeds were contaminated with at least one species of *Fusarium* (table 1). Four *Fusarium* species were detected on bulk seed samples. These included, in descending order of prevalence, *F. acuminatum* Ell. & Ev., *F. culmorum* (W.G. Smith) Sacc., *F. proliferatum* (Matsushima) Nirenberg, and *F. equiseti* (Corda) Sacc.

Extent of root colonization by *Fusarium* was initially higher in GH7 than in the two other production areas (table 2). In some cases, high levels of *Fusarium* colonization were detected early in the seedling production cycle, whereas in others levels of colonization generally increased over time. (Fluctuations from month to month were the result of the small sample sizes.) The highest overall *Fusarium* root colonization was detected about 30 wk after sowing (table 2). Eleven *Fusarium* species were detected on seedling roots (table 3). By far the most prevalent *Fusarium* species isolated from seedling roots was *F. proliferatum*. Seven

Table 2. Percent colonization of container western white pine seedling roots with *Fusarium* spp.

Sample time[1]	Production area[2]				
	GH 7–5s	GH 7–8s	GH 5–8s	Bay–8s	All samples
6	97	52	11	25	48
10	66	46	22	24	37
14	96	76	18	72	67
18	62	88	68	76	74
22	74	74	36	66	68
26	94	96	50	46	72
30	80	100	66	90	84
36	59	75	62	62	63
Averages	72	79	47	60	64

[1] Week after sowing.

[2] Each seedling production area designated with greenhouse number (or open shade house area–Bay) and the container sizes used in that area (5s=120 cells block^{-1}; 8s=91 cells block^{-1}).

Table 3. *Fusarium* species colonizing roots of container western white pine seedlings.

Fusarium species	Percent of samples[1]	Percent root colonization[2]
F. proliferatum	100	48.5
F. acuminatum	88	6.0
F. culmorum	75	3.9
F. avenaceum	50	3.2
F. oxysporum	50	1.2
F. sporotrichioides	25	0.9
F. scirpi	25	0.7
F. sambucinum	12	0.4
F. equiseti	50	0.4
F. tricinctum	12	0.3
F. heterosporum	12	0.1
All species	100	64.5

[1] Percent of the 8 sampling times throughout the growing season that particular *Fusarium* species were detected.

[2] Overall percent of sampled root pieces colonized by particular *Fusarium* species—total number of root pieces sampled=1,953.

were found only at extremely low levels; three others [*F. acuminatum*, *F. culmorum*, and *F. avenaceum* (Fr.) Sacc.] were isolated more frequently. *Fusarium* was isolated from an average of nearly two-thirds of the sampled root pieces throughout the sampling period (table 3).

The other assayed group of root-colonizing organisms was *Cylindrocarpon*. These fungi were detected at much lower levels than *Fusarium* spp. (table 4). *Cylindrocarpon* spp. were not detected until seedlings were 18 wk old in one production area (GH7) or 22 wk old in the other two areas. By the end of the production cycle, *Cylindrocarpon* spp. were detected on a little more than a third of the sampled roots (table 4). By far the most common *Cylindrocarpon* species isolated from roots was *C. destructans* (Zins.) Scholten. These pathogens probably get into the crop via contaminated seeds, containers, and debris within and adjacent to greenhouses (James and Dumroese 2007). They are not commonly found in the irrigation supply or the peat-based media. Some species, such as *F. proliferatum*, likely can be spread by air movements (James and others 1997).

Table 4. Percent colonization of container western white pine seedling roots with *Cylindrocarpon* spp.

Sample time[1]	Production area[2]				
	GH 7–5s	GH 7–8s	GH 5–8s	Bay–8s	All samples (mean)
6	0	0	0	0	0
10	0	0	0	0	0
14	0	0	0	0	0
18	40	4	0	0	14
22	70	8	42	22	36
26	12	2	44	34	23
30	38	0	20	20	20
36	61	31	26	17	35
Averages	37.5	10	19	13.5	20

[1] Week after sowing.

[2] Each seedling production area designated with greenhouse number (or open shade house area–Bay) and the container sizes used in that area (5s=120 cells block^{-1}; 8s=91 cells block^{-1}).

[3] *Cylindrocarpon* isolates comprised 99-percent *C. destructans* and 1-percent *C. gracile*.

Table 5. Percent of sampled seedlings within root plug condition categories and percent culls of container western white pine seedlings at the time of lifting (36 wk after sowing).

Production area	Root plug condition[1]			Percent seedling culls[2]
	Poor	Moderate	Good	
GH7–5s	26	21	53	2.0
GH7–8s	0	8	92	2.0
GH5–8s	0	15	85	2.5
Bay–8s	21	21	58	7.3
Averages	14.3	17.5	68.2	3.5

[1] Visible condition of plugs at the time of lifting, based on extent of noticeable root decay (poor=extensive root decay and/or few roots remaining at the bottom of the plug; moderate=moderate root decay with compromised root plug integrity; good=little or no root decay evident; root plug integrity maintained). Number of seedlings sampled: GH7–5s =19; GH7–8s=12; GH5–8s=13; Bay–8s=19; total=63.

[2] Five randomly selected styrofoam blocks with seedlings sampled per production area at the time of lifting. Number of cells sampled: GH7–5s=600; GH7–8s=455; GH5–8s=728; Bay–8s=455; total=2,238.

Percent of seedlings culled due to poor root condition was quite low (table 5). More than two-thirds of the examined root systems at the time of lifting were considered to be in good condition, based primarily on the extent of noticeable root decay (table 5). In some cases (GH7–8s; GH5–8s), no seedlings examined had poor root systems.

Discussion

Excessive root decay of container western white pine seedlings, resulting in high cull levels and poor outplanting performance, is normally ascribed to high levels of root colonization by *Cylindrocarpon* spp., especially *C. destructans* (James 1988a; James and others 1994; James 2003a, 2004a). These fungi are routinely isolated from seedling roots exhibiting decay symptoms (James 1988b; James and others 1994; James 1995, 2000). High seedling losses in nurseries have often been associated with excessive moisture being maintained for prolonged periods within root plugs. Fortunately, *Cylindrocarpon* levels on colonized roots tend to decrease over time following outplanting onto forest sites and usually do not adversely affect seedling survival (Dumroese and others 2000).

Although *Cylindrocarpon* has been associated with important conifer seedling diseases in nurseries (Evans 1967; Bloomberg and Sutherland 1971; James 1988a; Unestam and Beyer-Ericson 1991; Beyer-Ericson and others 1991; James 2004b), the aggressiveness of this species has been questioned, especially when seedlings are grown under nonstressful conditions (Dahm and Strzelcayk 1987a, b). In fact, many western white pine seedlings with extensive root decay attributed to *Cylindrocarpon* exhibit no disease symptoms during the production cycle; they are detected only once seedlings have been removed from their containers (James 1988a; James and others 1994).

In this evaluation, *Cylindrocarpon* spp., primarily *C. destructans*, were isolated at fairly low levels, especially when compared to root colonization by *Fusarium* spp. *Cylindrocarpon* was not detected early in the crop production cycle, and relatively high colonization frequency was found only in one production area (GH7) at the time of lifting.

On the other hand, *Fusarium* root colonization was generally much higher during all sampling periods. Although a wide range of species were isolated from seedling roots, *F. proliferatum* was by far the most common. This species has been implicated often in container seedling root diseases (James and others 1995; James and Dumroese 2006); some isolates can be highly virulent on young conifer seedlings, at least under controlled greenhouse growing conditions or during *in vitro* laboratory experiments (James and others 1997). Although previous evaluations indicated that *F. proliferatum* increases root colonization as the seedling crop ages (James and Gilligan 1990; James 1991a, 1991b), relatively high levels of root colonization by this fungus were found on very young seedlings in this evaluation.

Fusarium and *Cylindrocarpon* inocula have often been detected on sown white pine seeds (James 1987b; 1987c; 1988a; 1989b), on containers used to grow previous seedling crops (Dumroese and others 2002), and on various types of organic matter within and adjacent to greenhouses (James 2003a; James and Dumroese 2006). In this evaluation, *Cylindrocarpon* was detected on only 2 percent of the sampled seeds. Although *Fusarium* spp. were detected at high levels on seeds, *F. proliferatum*, the species with the highest disease potential (James and others 1995, 1997), was found on only 5 percent. Therefore, it appears that contaminated seeds were not an important source of potentially pathogenic *Fusarium* or *Cylindrocarpon* spp.

Styrofoam containers used to produce seedlings were not sampled in this evaluation. However, growers use standard hot water sterilization to clean containers that have been used to produce previous seedling crops. These treatments have usually been quite effective in eliminating inoculum of potentially pathogenic fungi (Dumroese and others 2002). Therefore, it is unlikely that high levels of either *Cylindrocarpon* or *Fusarium* were introduced into the white pine seedling crop by contaminated containers.

Organic debris within or surrounding seedling production greenhouses or shade houses may have contributed *Cylindrocarpon* and *Fusarium* inoculum. Weeds can also harbor these fungi. Neither organic debris nor weeds were assayed for potential pathogens, however, so the extent of these two sources as a source of *Cylindrocarpon* or *Fusariu*m inoculum is unknown.

Root diseases caused by *Cylindrocarpon*, *Fusarium* spp., or both will continue to be of concern to container seedling growers. Both groups of fungi can cause devastating losses when virulent fungal isolates and conducive environmental conditions are present. Although losses during the current evaluation were very low, continued low disease levels cannot be guaranteed for the future. Careful vigilance by growers will be necessary to make sure seedling crops are not stressed to the point where these potential pathogens can cause important losses.

Address correspondence to: R.L. James, Forest Service, Forest Health Protection, 3815 Schreiber Way, Coeur d'Alene, ID 83815; e-mail: rjames@fs.fed.us; (208) 765-7421.

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