Forest Health Protection

Numbered Report 07-05 **July 2007**

ROOT COLONIZATION OF CONTAINER WESTERN WHITE PINE SEEDLINGS BY *FUSARIUM* AND *CYLINDROCARPO*N SPECIES

R.L. James, Plant Pathologist
USDA Forest Service, Forest Health Protection
Coeur d'Alene, Idaho

ABSTRACT

Healthy-appearing container western white pine seedlings were sampled at an Idaho forest nursery eight times during the crop production cycle at monthly intervals for root colonization by potentially-pathogenic *Fusarium* and *Cylindrocarpon* spp. in an attempt to better understand epidemiological changes that might occur by these fungi over time. *Fusarium* spp., especially *F. proliferatum*, were present at relatively high levels throughout the seedling production cycle. However, *Cylindrocarpon* (mostly *C. destructans*) was not detected until seedlings were 4-5 months old. Levels of *Cylindrocarpon* root colonization remained much less than that of *Fusarium* spp. Although high levels of *Fusarium* contaminated seeds prior to sowing, those species most likely to cause disease were only detected at low levels; *Cylindrocarpon* spp. also contaminated few seeds. During the evaluation period, very low root disease occurred; few culled seedlings and good root plug condition was the standard on the sampled seedling crop.

INTRODUCTION

Root diseases of container-grown western white pine (*Pinus monticola* Dougl.) seedlings have periodically caused extensive damage to crops, resulting in reduced number of satisfactory seedlings produced and lowered seedling quality (James 1985b, 1987a, 1990a, 1991c). The major fungal pathogens routinely associated with such diseases include species of *Fusarium* and *Cylindrocarpon* (James 1985a, 1988a, 1990b). In some cases, distinctive above-ground disease symptoms associated with extensive root colonization by these organisms are evident (James 1987a, 1989a, 1990a, 1991d, 2003b). However, all too often no discernable disease

United States Department of Agriculture | Forest Service | Northern Region | 200 East Broadway P.O. Box 7669 Missoula, MT 59807

symptoms are present even though extensive root decay may have occurred (James 1988a, 1991a, 1991b, 2004a). In such cases, diseased seedlings only become evident after they are removed from containers; they often have high levels of root decay and are culled. Most previous investigations involved determining associated fungal organisms at the end of the crop growing cycle when diseased seedlings were detected following lifting. However, information on the temporal changes in fungal root colonization during a typical crop production cycle by different potentially-pathogenic organisms was lacking. An evaluation was conducted during 2006 to provide such information.

MATERIALS AND METHODS

A large container nursery in Idaho was selected for this evaluation. This nursery has traditionally produced large number of western white pine seedlings for reforestation. The goal of this evaluation was to determine changes in root colonization by *Fusarium* and *Cylindrocarpon* spp. during the crop production cycle. 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 aseptically placed 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^{o}C for 7-10 days. Selected emerging fungi were transferred to carnation leaf agar (Fisher et al. 1982) and potato dextrose agar for identification using the taxonomy of Nelson et al. 1989 and Booth 1966. Percentages of seeds colonized by particular fungal species were determined.

Seedlings were grown in three production areas: two greenhouses (designated 5 and 7) and one shade house area (designated “bay”). Seedlings grown in two container sizes (5s – 120 cells/block and 8s – 91 cells/block) were sampled eight times at approximately monthly intervals beginning in April. During each sampling period, five seedlings were randomly selected for laboratory analysis of fungal root colonization from each of the production areas and container sizes; this resulted in four separate samples (house 7 – 5s; house 7 – 8s; house 5 – 8s; bay – 8s) during each monthly sampling period. Selected seedlings were carefully extracted from containers and placed into individual plastic bags. They were transported to the laboratory and analyzed immediately for fungal root colonization.

Seedling roots were washed thoroughly to removed adhering peat growing media. Ten root pieces, each ap-proximately 5 mm, were randomly dissected from each seedling, surface sterilized in 0.5% aqueous sodium hypochlorite (10% 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.

At the end of the production cycle when seedlings were lifted from containers, a selected number of 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 seedling 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. In addition, 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.

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 apparently higher within house 7 than the two other production areas (table 2). In some cases, high levels of *Fusarium* colonization was detected early in the seedling production cycle, whereas in others, levels of colonization generally increased over time. The highest overall *Fusarium* root colonization was detected during the October sample, approximately 7 months after sowing (table 2). Eleven different *Fusarium* species were detected on seedling roots (table 3). Seven of these species were found only at extremely low levels; three others (*F. acuminatum*, *F. culmorum*, and *F. avenaceum* (Fr.) Sacc.) were isolated more frequently. However, by far the most prevalent *Fusarium* species isolated from seedling roots was *F. proliferatum*. Throughout the sampling period, *Fusarium* was isolated from nearly two-thirds of the sampled root pieces (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 four months old in one production area (house 7) or five months old in the other two areas. By the end of the production cycle, *Cylindrocarpon* spp. were only 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.

Percent of culled seedlings at lifting was generally quite low (table 5). Seedlings were culled for various reasons, but primarily because they failed to reach size and quality specifications. 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 root decay that was evident (table 5). In some cases (house 7 – 8s; house 5 – 8s), none of the examined seedlings had root systems considered to be in poor condition.

DISCUSSION

Excessive root decay of container western white pine seedlings, resulting in high cull levels, and poor outplanting performance, has normally be ascribed to high levels of root colonization by *Cylindrocarpon*, spp., especially *C. destructans* (James 1988a, 2003a, 2004a; James et al. 1994). These fungi are routinely isolated from seedling roots exhibiting decay symptoms, especially loss of cortical cell tissues (James 1988b, 1995, 2000; James et al. 1994). 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, usually being replaced with other, less innocuous, fungi (Dumroese et al. 2000).

Although *Cylindrocarpon* has been associated with important conifer seedling diseases in nurseries (Beyer-Ericson et al. 1991; Bloomberg and Sutherland 1971; Evans 1967; James 1988a, 2004b; Unestam and Beyer-Ericson 1990), the aggressiveness of this species has been questioned, especially when seedlings are grown under non-stressful conditions (Dahm and

Strzelcayk 1987a, 1987b). In fact, many white pine seedlings with extensive root decay attributed to *Cylindrocarpon* do not exhibit any disease symptoms during the production cycle; they are only detected once seedlings have been removed from their containers (James 1988a; James et al. 1994).

In this evaluation, *Cylindrocarpon* spp., primarily *C. destructans*, were isolated at fairly low levels, especially when compared with 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 (house 7) by the end of the growth cycle.

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 often been previously implicated in container seedling root diseases (James and Dumroese 2006; James et al. 1995); some isolates have been shown to be highly virulent on young conifer seedlings, at least under controlled greenhouse growing conditions or within *in vitro* laboratory experiments (James et al. 1997). Although previous evaluations indicated that *F. proliferatum* increases in intensity of root colonization as the seedling crop ages (James 1991c, 1991d; James and Gilligan 1990), in the current evaluation relatively high levels of root colonization by this fungus were found on very young seedlings.

Fusarium and *Cylindrocarpon* inoculum has often been detected on sown seeds (James 1987b, 1987c, 1988a, 1989b), containers used to grown previous seedling crops (Dumroese et al. 2002), and on various types of organic matter within and adjacent to greenhouse environments (James 2003a; James and Dumroese 2006). In the current evaluation, *Cylindrocarpon* was detected on only 2% of the sampled seeds. Although *Fusarium* spp. were detected at high levels on seeds, *F. proliferatum*, the species with the highest disease potential (James et al. 1995, 1997) was detected on only 5% of the seeds. Therefore, it appears that contaminated seeds were not an important source of either *Fusarium* or *Cylindrocarpon* inoculum.

Styrofoam containers used to produce seedlings were not sampled in this evaluation. However, growers use standard hot water sterilization treatments 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 et al. 2002). Therefore, it is unlikely that high levels of either *Cylindrocarpon* or *Fusarium* were introduced into the white pine seedling crop via contaminated containers.

Organic debris within or surrounding seedling production greenhouses or shade houses may have contributed *Cylindrocarpon* and *Fusarium* inoculum. Likewise, weeds can also harbor these fungi. However, neither organic debris or weeds were assayed for potential pathogens, so the extent of these two sources as a source of *Cylindrocarpon* or *Fusarium* inoculum is unknown.

Root diseases caused by *Cylindrocarpon* and/or *Fusarium* spp. 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 of growers will be necessary to make sure seedling crops are not stressed to the point where these potential pathogens can cause important losses.

LITERATURE CITED

Beyer-Ericson L, Dahm E, Unestam T. 1991. An overview of root dieback and its causes in Swedish nurseries. European Journal of Forest Pathology 21:439-443.

Bloomberg WJ, Sutherland JR. 1971. Phenology and fungus-nematode relations of corky root disease of Douglas-fir. Annals of Applied Biology 69:265-276.

Booth C. 1966. The genus *Cylindrocarpon*. The Commonwealth Mycological Institute. Kew, Surrey, England. Mycological Papers No.104. 56p.

Dahm H, Strzelczyk E. 1987a. Cellulolytic and pectolytic activity of *Cylindrocarpon destructans* isolates pathogenic and non-pathogenic to fir (*Abies alba*) and pine (*Pinus sylvestris*). Journal of Phytopathology 118:76-83.

Dahm H, Strzelczyk E. 1987b. Effect of pH, temperature and light on the pathogenicity of *Cylindrocarpon destructans* to pine seedlings in associative cultures with bacteria and actinomycetes. European Journal of Forest Pathology 17:141-148.

Dumroese RK, James RL, Wenny DL. 2000. An assessment of *Cylindrocarpon* on container western white pine seedlings after outplanting. Western Journal of Applied Forestry 15(1):5-7.

Dumroese RK, James, RL, Wenny DL. 2002. Hot water and copper coatings in reused containers decrease inoculum of *Fusarium* and *Cylindrocarpon* and increase Douglas fir seedling growth. HortScience 37:943-947.

Evans G. 1967. The production of a phytotoxin, nectrolide, by some root-surface isolates of *Cylindrocarpon radicicola*. Plant and Soil 26:253-260.

Fisher NL, Burgess LW, Toussoun TA, Nelson PE. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. Phytopathology 72:151-153.

James RL. 1985a. Containerized western white pine seedling mortality at the Bonners Ferry Ranger District, Idaho Panhandle National Forests. USDA Forest Service, Northern Region, Forest Pest Management. Report 85-18. 7p.

James RL. 1985b. Root diseases of transplanted western white pine seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes #21. 4p.

James RL. 1987a. Containerized western white pine seedling root disease - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes #66. 4p.

James RL. 1987b. Fungi on bleach-treated western white pine seed, Raintree Nursery, Libby, Montana. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes #48. 1p.

James RL. 1987c. Occurrence of fungi on western white pine seed - Plum Creek Nursery, Pablo, Montana. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes #45. 3p.

James RL. 1988a. Diseases of conifer seedlings associated with *Cylindrocarpon* species: a review. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes #76. 14p.

James RL. 1988b. Field mortality of western white pine transplants - Kootenai National

Forest. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes #71. 3p.

James RL. 1989a. Chlorosis of container-grown western white pine seedlings - Fisher River Ranger District, Kootenai National Forest, Montana. USDA Forest Service, Northern Region, Forest Pest Mangement. Nursery Disease Notes #87. 4p.

James RL. 1989b. Occurrence of fungi on western white pine seed - Idaho Department of Lands. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes #84. 6p.

James RL. 1990a. Needle tip dieback of container-grown western white seedlings - Champion Timberlands Nursery, Plains, Montana. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes #103. 4p.

James RL. 1990b. Needle-tip necrosis of container-grown western white pine seedlings - University of Idaho Research Nursery. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes #106. 4p.

James RL. 1991a. Cylindrocarpon root disease of container-grown whitebark pine seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Report 91-8. 10p.

James RL. 1991b. Fungal colonization of roots from western white pine transplant seedlings outplanted on the Wallace Ranger District, Idaho Panhandle National Forests, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes #120. 10p.

James RL. 1991c. Root decay of container-grown lodgepole pine and western white pine seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes #116. 4p.

James RL. 1991d. Wilting of container-grown western white pine seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes #123. 5p.

James RL. 1995. Root diseases of western white pine transplants - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Insect and Disease Manage-ment. Report 95-8. 10p.

James RL. 2000. Diseases associated with whitebark pine seedling production – USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 00-8. 11p.

James RL. 2003a. Diseases in forest nurseries: implications for forest managers. Western Forester 48(5):8-9.

James RL. 2003b. Fungal associates of corky root syndrome of bare root 2-0 western white pine seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Nursery Disease Notes No. 150. 7p.

James RL. 2004a. *Cylindrocarpon destructans* associated with root disease of container-grown western white pine tree improvement stock - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Nursery Disease Notes No. 155. 9p.

James RL. 2004b. Pathogen infection and colonization of container-grown whitebark pine seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service,

Northern Region, Forest Health Protection. Nursery Disease Notes No. 154. 10p.

James RL, Dumroese RK. 2006. Investigations of *Fusarium* diseases within inland Pacific Northwest Forest Nurseries. In: Guyon, J.C. [compiler]. Proceedings of the 53rd Western International Forest Disease Work Conference: 2005 August 26-29; Jackson, WY; USDA Forest Service Intermountain Region. pp. 3-11.

James RL., Dumroese RK, Wenny DL. 1994. Observations on the association of *Cylindrocarpon* spp. with diseases of container grown conifer seedlings in the inland Pacific Northwest of the United States. *In:* Perrin, R. and J. R. Sutherland (eds.). Diseases and Insects in Forest Nurseries. Dijon, France, October 3-10, 1993. Institut National De La Recherche Agronominque. Les Colloques No. 68. pp. 237-246.

James RL, Dumroese RK, Wenny DL. 1995. *Fusarium proliferatum* is a common, aggressive pathogen of container-grown conifer seedlings. Phytopathology 85(10): 1129.

James RL., Dumroese RK, Wenny DL. 1997. Pathogenicity of *Fusarium proliferatum* in container-grown Douglas-fir seedlings. *In:* James, R. L. (editor). Proceedings of the third meeting of IUFRO Working Party S7.03-04 (Diseases and Insects in Forest Nurseries). USDA Forest Service, Northern Region, Forest Health Protection. Report 97-4. pp. 26-33.

James RL, Gilligan CJ. 1990. Root decay of container-grown western white pine seedlings - Plum Creek Nursery, Pablo, Montana. USDA Forest Service, Northern Region, Forest Pest Management. Report 90-10. 18p.

Komada H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Review Plant Protection Research (Japan) 8:114-125.

Nelson PE, Toussoun TA, Marasas WFO. 1983. *Fusarium* species: an illustrated manual for identification. The Pen-nsylvania State University Press, University Park. 193p.

Unestam T, Beyer-Ericson L. 1990. Diseases of containerized conifer seedlings in Swedish Nurseries. *In*: Sutherland, J. R. and S. G. Glover (eds.). Proceedings of the first meeting of IUFRO Working Party S2.07-09 (Diseases and Insects in Forest Nurseries). Forestry Canada. Pacific and Yukon Region. Information Report BC-X-331. pp. 105-108.

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

Fungal Species	Percent Contamination[1]
Fusarium acuminatum	73
Fusarium culmorum	20
Fusarium proliferatum	5
Fusarium equiseti	3
All *Fusarium*	98
Cylindrocarpon destructans	2
Botrytis cinerea	1

[1]Sample based on 100 randomly-selected seeds from bulk storage prior to sowing.

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

Sample Date	Production Area[1]				All Samples
	A – House 7; 5s	B – House 7; 8s	C – House 5; 8s	D – Bay; 8s	
4/06	97	52	11	25	48
5/06	66	46	22	24	37
6/06	96	76	18	72	67
7/06	62	88	68	76	73.5
8/06	74	74	36	66	67.5
9/06	94	96	50	46	71.5
10/06	80	100	66	90	84
12/06	59	75	62	62	63
Averages	72	79	47	60	64

[1]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; 8s = 91 cells/block).

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

Fusarium Species	Percent of Samples[1]	Percent Root Colonization[2]
Fusarium proliferatum	100	48.5
Fusarium acuminatum	87.5	6.0
Fusarium culmorum	75	3.9
Fusarium avenaceum	50	3.2
Fusarium oxysporum	50	1.2
Fusarium sporotrichioides	25	0.9
Fusarium scirpi	25	0.7
Fusarium sambucinum	12.5	0.4
Fusarium equiseti	50	0.4
Fusarium tricinctum	12.5	0.3
Fusarium heterosporum	12.5	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.

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

Sample Date	Production Area[1]				All Samples
	A – House 7; 5s	B – House 7; 8s	C – House 5; 8s	D – Bay; 8s	
4/06	0	0	0	0	0
5/06	0	0	0	0	0
6/06	0	0	0	0	0
7/06	40	4	0	0	13.5
8/06	70	8	42	22	35.5
9/06	12	2	44	34	23
10/06	38	0	20	20	19.5
12/06	61	31	26	17	34.9
Averages	37.5	10	19	13.5	20.5[2]

[1]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; 8s = 91 cells/block).
[2]*Cylindrocarpon* isolates comprised 99% *C. destructans* and 1% *C. gracile*.

Table 5. Root plug condition and percent culls of container western white pine seedlings at the time of lifting [12/06].

Production Area	Root Plug Condition[1]			Percent Seedling Culls[2]
	Poor	Moderate	Good	
A-House 7 – 5s	26	21	53	2.0
B-House 7 – 8s	0	8	92	2.0
C-House 5 – 8s	0	15	85	2.5
D-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: A = 19; B = 12; C = 13; D = 19; total = 63.
[2]Five randomly-selected styrofoam blocks with seedlings sampled per production area at the time of lifting. Number of cells sampled: A = 600; B = 455; C = 728; D = 455; total = 2238.

R.L. James is Plant Pathologist, USDA Forest Service, Northern Region, Forest Health Protection.
Address: USDA Forest Service, 3815 Schreiber Way, Coeur d'Alene, ID 83815; email: rjames@fs.fed.us.