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OCCURRENCE OF FUSARIUM ON LEACH PINE CELLS FROM THE USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

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ABSTRACT

Three hundred fifty Leach® pine cells used to grow containerized conifer seedlings at the USDA Forest Service Nursery in Coeur d'Alene, Idaho, were sampled for *Fusarium* and *Trichoderma* colonization. Cells were either sampled prior to or after cleaning. Cleaning reduced *Fusarium* colonization from 86 percent of the cells to about 50 percent. Cells stored for several months still contained high levels of *Fusarium* inoculum. Most *Fusarium* inoculum was concentrated at or near the bottom of cells. Cells with high *Fusarium* levels had corresponding low levels of *Trichoderma* and vice versa. Ability of the *Fusarium* isolates from cells to cause seedling diseases is unknown, but *Fusarium*-caused disease is common at the nursery.

INTRODUCTION

Recent investigations (James 1988; James et al. 1988b) have indicated that *Fusarium* spp., common pathogens of containerized conifer seedlings (James 1986; James and Gilligan 1985), may reside within styroblock containers and be available for infection of new crops of seedlings when these containers are reused (James 1988, James et al. 1988b). Standard cleaning techniques usually reduce levels of *Fusarium* within containers, but do not eliminate them. Generally, high percentages of sampled cells have these fungi present even after cleaning. Higher populations of *Fusarium* are detected at the bottom of cells than higher up on the cell surfaces.

Fusarium-associated diseases of containerized seedlings are commonly found at the USDA Forest Service Nursery in Coeur d'Alene, Idaho (James and Gilligan 1985; James et al. 1987), although losses from these diseases are generally low. Containerized seedlings at this nursery are produced in Leach® pine cells which are often reused for several crops following cleaning. Because of the common occurrence of *Fusarium* on styroblock containers, growers were concerned about the possible extent of these fungi on the pine cells used at Coeur d'Alene.

MATERIALS AND METHODS

Following extraction of the crop of containerized seedlings during the fall of 1987, Leach® pine cells containing dead seedlings (which had remained and not been pulled by workers) were randomly collected. A total of 50 such cells were selected, 10 each containing dead western larch (*Larix occidentalis* Nutt.), Engelmann spruce (*Picea engelmanni* Parry), and ponderosa pine (*Pinus ponderosa* Laws.), and 20 containing dead Douglas-fir (*Pseudotsuga menziesii* (Mirb.)Franco). Seedlings were carefully extracted from cells so that as much of the root system as possible remained intact. Peat-vermiculite soil mixes from each cell were stored under refrigeration for future analysis. Roots from seedlings were thoroughly washed under running tap water to remove adhering soil particles and then surface sterilized in 10 percent aqueous sodium hypochlorite and rinsed in sterile distilled water. Ten root pieces about 3-5 mm in length were aseptically cut from each root system. As many root tips as possible were included in this sample. Root pieces were placed on a selective medium for *Fusarium* spp. (Komada 1975). Plates were incubated for 7-10 days under cool fluorescent light at about 26 degrees C. Number of root pieces colonized by *Fusarium* were calculated. Representative isolates were transferred to potato dextrose agar (PDA) slants and later cultured on carnation leaf agar for identification using a standard taxonomic guide (Nelson et al. 1983).

After seedling and soil mix extraction, each cell was sampled. The bottom 3-5 mm of each cell was aseptically severed and divided into four pieces of approximately equal size. Each piece was then placed inside surface down on Komada's medium and incubated as described above. Number of cell pieces colonized with *Fusarium* and *Trichoderma* spp., common saprophytic competitors and antagonists of *Fusarium* (Papavizas 1985), were determined. Colonization of cell pieces by *Fusarium* was estimated based on the following numerical scale: 4 = the fungal colony growing around the entire circumference of the piece: 2 = the fungal colony growing around about half of the circumference of the piece: 1 = the fungal colony growing around less than half of the circumference of the piece. An average numerical colonization rating was thus derived for each group of cells sampled.

Three samples of 100 randomly selected cells each were collected for isolation of *Fusarium* and *Trichoderma*. The first sample was collected immediately after seedling extraction and included cells that had not been cleaned. The second sample was from cells that had undergone standard cleaning by the nursery (this technique consisted of high-pressure steam treatment). The third sample was from cells that had been cleaned several months before and had been stored in a warehouse. All selected cells were sampled as described above, i. e., the bottom of each cell was sampled. Number of cells, pieces, and colonization intensity were calculated as described above. In the latter two samples, those cells where *Fusarium* was detected at high levels (a high numerical colonization rating) were also sampled for colonization at three different levels throughout the length of the cell. Samples were taken from the top, and at 6 and 12 cm from the top of each selected cell. Colonization rates and intensity were calculated for these samples.

Samples of soil mix from cells containing 20 of the dead seedlings were assayed for populations of *Fusarium* and *Trichoderma* using standard soil-dilution techniques. Samples selected included soil mixes from 10 pine cells that had high levels of *Fusarium* and 10 that had high levels of *Trichoderma*. Soil mixes were air-dried for 24 hours and then sieved to remove large pieces of roots and organic matter. They were then ground into a fine powder with a mortar and pestle. Soil was mixed with 0.3 percent water agar and dispensed onto Komada's medium. After 7 days' incubation, number of *Fusarium* and *Trichoderma* colonies per plate were determined and the colony forming units per gram of soil calculated. Comparisons between populations of *Fusarium* and *Trichoderma* were made using simple linear regressions.

RESULTS AND DISCUSSION

Almost 65 percent of the Leach® pine cells sampled were colonized by *Fusarium* spp. (Table 1). Many of the pieces sampled were heavily colonized by these fungi (Fig. 1). Although standard cleaning techniques reduced amount of *Fusarium* colonization from 86 to about 50 percent of the cells sampled, there was still extensive organic debris left on the inner surfaces of cleaned cells (Fig. 2). Cells that had been stored for several months still had relatively high levels of *Fusarium* colonization.

Table 1.--Occurrence of *Fusarium* and *Trichoderma* on Leach® pine cells from the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	Dead *1/* seedlings	After lifting not cleaned	After cleaning	After cleaning and storage	All samples
Number of cells sampled	50	100	100	100	350
Number infected with *Fusarium*	38	86	51	52	227
Percent infected with *Fusarium*	76	86	51	52	64.9
Colonization intensity (*Fusarium*) 1 *2/* 2 *3/*	 75 3.35	 88 2.57	 69 2.60	 72 2.37	 76.0 2.63
Number infected with *Trichoderma*	17	21	11	15	64
Percent infected with *Trichoderma*	34	21	11	15	18.3
Colonization intensity (*Trichoderma*) *4/*	60.3	46.4	52.3	56.7	53.9

1/ Cells containing dead seedlings at the time of lifting.
2/ Percentage of pine cell pieces colonized with *Fusarium* (four sampled per cell).
3/ Numerical colonization rating based on the proportion of the circumference of the cell pieces from which *Fusarium* grew. (Highest possible rating = 4.00.)
4/ Percentage of pine cell pieces colonized with *Trichoderma* (four sampled per cell).

In two of the samples (after cleaning and after storage), it was found that *Fusarium* was most common at the bottom of containers (Table 2). An increasing amount of *Fusarium* was isolated in samples from the lower portions of cells. On the other hand, *Trichoderma* spp. were found at higher levels toward the middle of containers and seemed to decrease during storage (Table 3). In general, cells with extensive *Trichoderma* present had corresponding low levels of *Fusarium* (Fig. 3).

Table 2.--Colonization of Leach pine cells with *Fusarium* spp. after cleaning and after storage.

Sample location	Time of Sample - After cleaning	Time of Sample - After cleaning and storage	Time of Sample - Both samples
Top			
Percent colonization	6.7	20.0	11.1
Colonization intensity *1/*	2.5	6.7	3.9
Colonization intensity *2/*	2.67	3.25	3.00
Down 6 cm			
Percent colonization	20.0	53.3	31.1
Colonization intensity *1/*	10.0	25.0	15.0
Colonization intensity *2/*	3.83	2.73	3.22
Down 12 cm			
Percent colonization	63.3	86.7	71.1
Colonization intensity *1/*	35.0	61.7	43.9
Colonization intensity *2/*	3.62	3.73	3.67
Bottom			
Percent colonization	100.0	100.0	100.0
Colonization intensity *1/*	92.5	100.0	95.0
Colonization intensity *2/*	2.95	3.33	3.09
Sample size	30	15	45

1/ Percentage of pine cell pieces colonized with *Fusarium* (four sampled per location).

2/ Numerical colonization rating based on the proportion of the circumference of the pine cell pieces from which *Fusarium* grew. (Highest possible rating = 4.00.)

Table 3.--Colonization of Leach® pine cells with *Trichoderma* spp. after cleaning and after storage.

Sample location	Time of Sample: After cleaning	Time of Sample: After cleaning and storage	Time of Sample: Both samples
Top			
Percent colonization	36.7	0	24.4
Colonization intensity *1/*	15.8	0	10.6
Down 6 cm			
Percent colonization	63.3	20.0	48.9
Colonization intensity *1/*	21.7	5.0	16.1
Down 12 cm			
Percent colonization	40.0	20.0	33.3
Colonization intensity *1/*	20.8	10.0	17.2
Bottom			
Percent colonization	3.3	13.3	6.7
Colonization intensity *1/*	3.3	5.0	3.9
Sample size	30	15	45

1/ Percentage of pine cell pieces colonized with *Fusarium* (four sampled per location).

Seedlings that were dead (culled) at the time of lifting were usually colonized by *Fusarium* (Table 4). Also, pine cells in which these seedlings were growing were also heavily colonized, but not more than cells picked at random from lifted seedlings (Table 1). Intensity of colonization of pine cells by either *Fusarium* or *Trichoderma* were good indicators of the amounts of these respective fungi detected in the soil mix within these cells (Table 5). In other words, those cells heavily colonized with *Fusarium* had large populations of these fungi within the adjacent soil mix.

Table 4.--Colonization of roots from dead containerized seedlings with *Fusarium* at the USDA Forest Service Nursery.

Species	Number seedlings sampled	Percent colonized	Colonization intensity 1/
Douglas-fir	20	75.0	32.7
Western larch	10	90.0	40.0
Engelmann spruce	10	40.0	92.5
Ponderosa pine	10	80.0	77.1
Totals	50	72.0	50.3

1/ Based on the number of root pieces colonized for infected root systems only.

Table 5.--Colony-forming units of *Fusarium* and *Trichoderma* within soil mixes of containerized seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	- - - - - Set 1 1/ - - - - -		- - - - - Set 2 2/ - - - - -	
Number	*Fusarium*	*Trichoderma*	*Fusarium*	*Trichoderma*
1	267	3,660	8,200	660
2	533	10,000	2,067	0
3	67	1,000	4,060	0
4	0	1,467	6,134	0
5	0	2,000	5,134	0
6	200	10,000	267	133
7	0	3,600	6,400	0
8	0	1,667	8,600	0
9	0	4,400	4,460	0
10	0	4,860	10,000	0
Average	106.7	4,085.4	5,532.2	79.3

1/ From Leach® pine cells colonized with low amounts of *Fusarium* and high amounts of *Trichoderma*.

2/ From Leach® pine cells colonized with high amounts of *Fusarium* and low amounts of *Trichoderma*.

The majority of isolates obtained from pine cells were *F. oxysporum* Schlect. (Table 6). Other isolated species included *F. sambucinum* Fuckel, *F. lateritium* Nees, *F. equiseti* (Corda)Sacc., *F. tricinctum* (Corda)Sacc., and *F. acuminatum* Ell. & Ev. This is the first time that either *F. lateritium* or *F. equiseti* have been found by the authors as associates of containerized conifer seedlings. Some of these organisms are undoubtedly saprophytes and not able to elicit diseases of seedlings. However, based on previous pathogenicity investigations (James et al. 1988a), it is likely that some of the isolates are pathogens. Unfortunately, it is not easy to recognize which organisms are pathogens without conducting extensive tests. Tests are planned to evaluate ability of some of the isolates obtained from pine cells to cause disease.

Table 6.--Occurrence of *Fusarium* spp. on Leach® pine cells from the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	Percent of Isolates *1/*					
Isolate Source	FOXY	FLAT	FACU	FTRI	FSAM	FEQU
Uncleaned cells	37.5	50.0	0	0	12.5	0
Recently cleaned cells	64.5	25.8	0	3.2	3.2	3.2
Cells cleaned & stored	66.7	16.7	5.5	5.5	0	5.5
All sources	61.4	26.3	1.7	3.5	3.5	3.5

1/ FOXY = *F. oxysporum*; FLAT = *F. lateritium*; FACU = *F. acuminatum*; FTRI = *F. tricinctum*; FSAM = *F. sambucinum*; FEQU = *F. equiseti*.

These investigations indicated that *Fusarium* spp. are common inhabitants of Leach® pine cells used to grow containerized conifer seedlings at the USDA Forest Service Nursery in Coeur d'Alene, Idaho. Cleaning containers reduced levels of colonization, but even after cleaning more than half of the cells sampled were still contaminated. Standard cleaning techniques have also been shown to be largely ineffective in removing *Fusarium* from styroblock containers (James et al. 1988b). The common occurrence of *Fusarium* on the roots of nondiseased container seedlings at the Nursery (James et al. 1987; James and Gilligan 1988) may be due to carryover of inoculum on pine cells from one crop to another. In any event, large amounts of inoculum may or may not result in significant disease losses. Factors of seedling stress may be related to production of disease symptoms. There may also be other factors which affect disease production.

The problem of determining whether or not most of the *Fusarium* isolates on Leach® pine cells are pathogens may not be easily solved. Many individual strains of these fungi can be classified rather easily using vegetative compatibility tests (Pohalla 1985). However, organisms within identified strains may vary in virulence, although they usually exhibit host specificity. Other techniques, such as isozyme analyses (Otrasina and Cobb 1987), may also be useful. On the other hand, it is possible that presence of pathogenic strains of *Fusarium* may not necessarily mean that much disease will occur.

These research questions should be addressed in order to evaluate importance of the findings of this investigation.

Figure 1. Colonization of pieces of Leach® pine cells with *Fusarium* spp. from the USDA Forest Service Nursery, Coeur d'Alene, Idaho. The four colonized pieces on the left are from a different cell than the four on the right. All pieces have been incubated on Komada's medium for 7 days.

Figure 2. Bisected Leach® pine cell that had undergone standard cleaning at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. Cleaning reduced extent of *Fusarium* colonization, but many propagules remained.

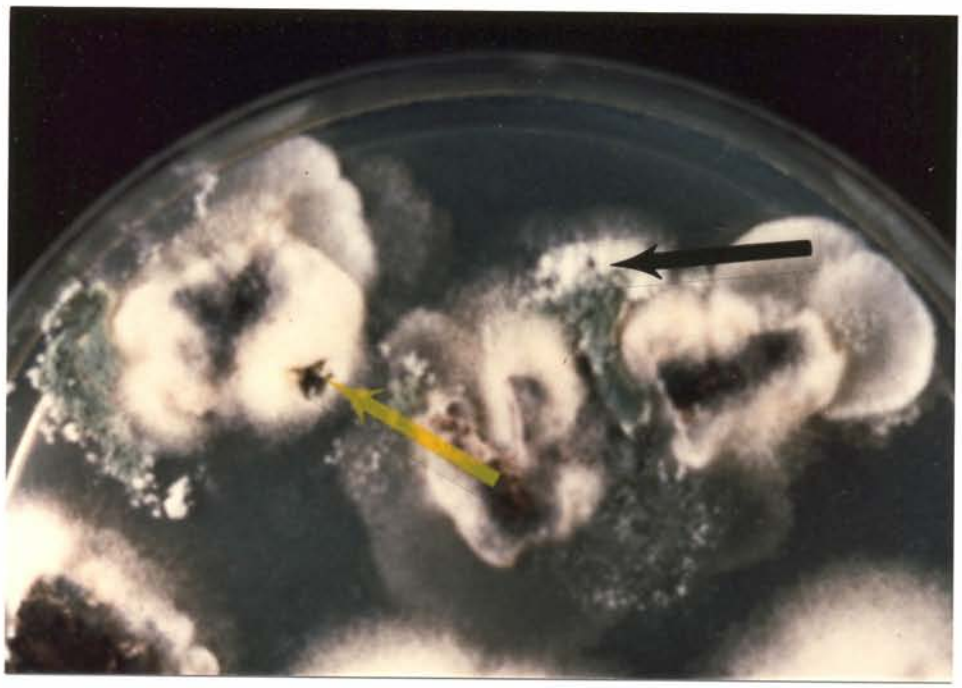

Figure 3. Colonization of pieces of Leach® pine cells with *Fusarium* (yellow arrow) and *Trichoderma* (black arrow). High levels of *Fusarium* were usually detected in cells that had low levels of *Trichoderma* and vice versa.

LITERATURE CITED

James, R. L. 1986. Diseases of conifer seedlings caused by seed-borne *Fusarium* species. *In*: Shearer, R. C. (compiler). Proceedings: Conifer Tree Seed in the Inland Mountain West Symposium. USDA Forest Service, Intermountain Res. Sta., Gen. Tech. Rept. INT-203. pp. 267-271.

James, R. L. 1988. Root disease of containerized conifer seedlings-Western Forest Systems Nursery, Lewiston, Idaho. USDA Forest Service, Northern Region. Rept. 88-3. 5p.

James, R. L. and C. J. Gilligan. 1985. Containerized Engelmann spruce seedling diseases at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region. Rept. 85-17. 15p.

James, R. L. and C. J. Gilligan. 1988. Association of *Fusarium* with nondiseased containerized ponderosa pine seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region (In preparation).

James, R. L., R. K. Dumroese, D. L. Wenny, J. F. Myers and C. J. Gilligan. 1987. Epidemiology of *Fusarium* on containerized Douglas-fir seedlings. 1. Seed and seedling infection, symptom production, and disease progression. USDA Forest Service, Northern Region. Rept. 87-13. 22 pp.

James, R. L., R. K. Dumroese, C. J. Gilligan and D. L. Wenny. 1988a. Epidemiology of *Fusarium* on containerized Douglas-fir seedlings. 3. Pathogenicity of selected *Fusarium* isolates from Douglas-fir seed and seedlings. USDA Forest Service, Northern Region (In preparation).

James, R. L., C. J. Gilligan and V. Reedy. 1988b. Evaluation of root diseases of containerized conifer seedlings at the Champion Timberlands Nursery, Plains, Montana. USDA Forest Service, Northern Region (In preparation).

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Prot. Res. 8:114-125.

Nelson, P. E., T. A. Toussoun and W. F. O. Marasas. 1983. *Fusarium* species: an illustrated manual for identification. The Pennsylvania State University Press, University Park. 193p.

Otrasina, W. J. and F. W. Cobb, Jr. 1987. Analysis of allozyma of three distinct variants of *Verticicladiella wageneri* isolated from conifers in western North America. Phytopathology 77: 1360-1363.

Papavizas, G. C. 1985. *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. Ann. Rev. Phytopathol. 23:23-54.

Pohalla, J. E. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Can. J. Bot. 63: 179-183.