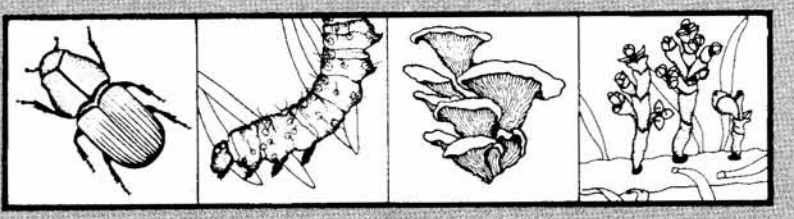
Forest Pest Management



Report 90-3 **3450**
October 1989

SPATIAL DISTRIBUTION OF FUNGI COLONIZING LEACH® PINE CELL CONTAINERS - USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

R. L. James[1]
Plant Pathologist

INTRODUCTION

Root diseases are important problems affecting production of container-grown conifer seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho and other nurseries in the northern Rocky Mountains. Losses vary among different species and season, but some level of damage usually occurs during each crop. Experience indicates that many container-grown seedlings are infected with potential root disease fungi even though they do not display disease symptoms (James and Gilligan 1988a; James and others 1987). Since most lots of conifer seed contain only limited amounts of pathogen inoculum (James 1987) and the peat-vermiculite growing media is often pathogen free (James 1985b), other possible sources of inoculum have been investigated. It was shown that Leach® pine cell containers used to grow seedlings were important sources of potential pathogenic fungi (James and Gilligan 1988b). Most containers are reused for several seedling crops. They are often discarded only after they have become brittle, cracked and no longer usable. Potential pathogens were commonly present

on containers even after they had been cleaned prior to sowing a new crop of seedlings (James and Gilligan 1988b). Sampling was concentrated at the bottom of pine cell containers where potential pathogens such as *Fusarium* spp. were often found. However, distribution of these and other fungi throughout the inner surface of containers was unknown. Therefore, an evaluation was conducted to determine spatial distribution of fungi within pine cell containers. Information from this evaluation could help growers to concentrate their efforts to improve cleaning where most of the potentially harmful fungi reside.

[1] Stationed in Coeur d'Alene, Idaho

United States Department of Agriculture | Forest Service | **Northern Region** | P.O. Box 7669 Missoula, Montana 59807

MATERIALS AND METHODS

Fifty pine cell containers were randomly selected from those that had previously been cleaned and were to be used to grow a new crop of seedlings. Brittle or broken containers were excluded from the sample. Although the number of times containers had been previously used was unknown, it appeared that most had been used for several crops. Cleaning of pine cell containers at the nursery involves removal of enclosed growing media by careful tapping followed by pressure-steam treatment with hot water (trays of seedlings are passed once under a boom with pressurized steam). Resulting "cleaned" containers often have residues of growing media on their inside surfaces (Fig. 1). Encrusted material composed mostly of media, remnants of algae, and chemical deposits are common on the outside surface at the bottom of cleaned cells (Fig. 2).

Sampled cells were aseptically bisected laterally exposing their inside surface (Fig. 1). Six pieces of cell (each approximately 3 mm^2) were aseptically excised from the extreme bottom of each cell and placed, inside surface down, on an agar medium selective for specific root pathogenic fungi (Komada 1975). Sampling was conducted at distance intervals of 33 mm from the bottom to top of each cell. At each sample interval, six pieces (3mm^2 each) of cell were excised at approximately equidistant locations and placed on the agar medium as described above. This scheme resulted in six sampling intervals, each with six pieces of container for a total of 36 locations sampled per cell. Plates with media and container pieces were incubated at about 26°C for 7-10 days under diurnal cycles of cool fluorescent light. Representative examples of colonizing fungi were transferred to potato dextrose agar (PDA) and carnation leaf agar (CLA) for identification using several taxonomic guides (Booth 1966; Dorenbosch 1970; Nelson and others 1983). Colonization percentages by particular fungi were calculated for each cell and sampling intervals. Statistical comparisons were made among the six sampling intervals using a one-way analysis of variance and Tukey's multiple range comparison test. Percentages underwent arc-sin conversions prior to analysis.

RESULTS AND DISCUSSION

Colonization of pine cell containers by *Fusarium* spp. was common and concentrated near the bottom of containers (Table 1). Significantly higher levels ($P=0.05$) of both *Fusarium acuminatum* Ell. & Ev. and *Fusarium oxysporum* Schlect. were isolated from the bottom of containers. Samples toward the top of containers yielded progressively less amounts of *Fusarium*. None of these potentially pathogenic fungi were detected at the top of containers. Levels of colonization were higher than previously detected on pine cell containers from the Coeur d'Alene Nursery (James and Gilligan 1988b), i.e., about 50 percent of the cleaned cells previously sampled were colonized with *Fusarium* spp. whereas the current evaluation indicated that 90 percent of the cells were colonized.

Cylindrocarpon spp., another group of potential pathogens (James 1988), were also detected at higher numbers near the bottom of cells (Table 1). However, levels of colonization were generally less than *Fusarium* spp.

Several common saprophytic fungi were also assayed on containers. *Trichoderma* spp. were distributed fairly equally throughout the inner surface of containers. Likewise, *Penicillium* and *Alternaria* species were also well distributed. Levels of *Trichoderma* were often higher than most other fungi. These potentially important competitors/antagonists of pathogens (Papavizas 1985) are common inhabitants of growing media (James 1985b, 1989). Their presence within containers may help explain relatively low levels of disease despite high levels of infection (James and Gilligan 1988a).

Figure 1.--Cross-section of a Leach® pine cell container that has been steam cleaned at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Figure 2.--Outside surface of the bottom of a steam cleaned Leach® pine cell.

One of the more common groups of fungi colonizing pine cell containers was *Phoma* spp. Two different species were detected: *P. eupyrena* Sacc. and *P. herbarum* Westend. Both species occurred at relatively high levels and were equally distributed throughout the inner surface of most containers. *Phoma eupyrena* is a common soil-borne fungus, especially in nursery soils (James and Hamm 1985). It can be a pathogen (James 1983; Kliejunas and others 1985), although it is usually not very aggressive. *Phoma herbarum* has been associated with conifer seedling diseases (James 1985a). However, it is also a common saprophyte (Dorenbosch 1970). Neither of these *Phoma* species have often been associated with root diseases of container-grown seedlings at the Coeur d'Alene Nursery.

In conclusion, this evaluation has shown that *Fusarium* spp. and other possible pathogenic fungi are concentrated toward the bottom of containers. However, fungi which may be either saprophytic or antagonistic toward pathogens are well distributed throughout the container profile. If growers concentrate their cleaning efforts on the bottom of containers, levels of pathogens might be reduced. Either immersing containers (at least the bottoms) in a sterilizing solution (biocide or hot water) or inverting them prior to treatment with an overhead steam boom might be helpful.

Table 1.--Spatial distribution of fungi within Leach® pine cell containers - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Cells[1]	Arrange Fungal Colonization (Percent)[3]										
Sample Location[2]	FACU	FOXY	ALL FUS	CYL	TRI	PEN	A	PE	PH	ALL P	NONE
Bottom	40 B[4]	60 B	90 C	20 AB	40 A	0 A	10 A	30 A	40 A	70 A	0 A
33 mm	30 B	60 B	60 B	50 B	60 AB	0 A	50 AB	30 A	50 A	80 A	0 A
66 mm	0 A	40 B	40 B	20 AB	80 B	20 A	20 A	50 A	50 A	100 A	0 A
99 mm	0 A	10 A	10 A	10 A	90 B	10 A	60 B	50 A	40 A	90 A	30 A
132 mm	30 B	20 AB	40 B	10 A	60 AB	10 A	30 AB	40 A	60 A	100 A	30 A
Top	0 A	0 A	9 A	0 A	50 AB	0 A	30 AB	40 A	60 A	100 A	10 A
Totals[5]	60	70	90	60	100	30	90	50	60	100	50
Pieces[1]											
Bottom	23B	45 C	68 C	12 AB	17 AB	0 A	2 A	18 A	40 A	58 A	0 A
33 mm	8 A	28 B	37 B	27 B	22 B	0 A	20 B	18 A	40 A	58 A	0 A
66 mm	0 A	8 A	8 A	7 A	25 B	5 A	13 AB	37 A	45 A	82 AB	0 A
99 mm	0 A	3 A	3 A	2 A	27 B	5 A	15 AB	35 A	38 A	73 AB	5 A
132 mm	5 A	5 A	10 AB	2 A	12 A	2 A	10 A	28 A	50 A	78 AB	7 A
Top	0 A	0 A	0 A	0 A	10 A	09 A	10 A	35 A	58 A	93 B	3 A
Totals[5]	6	15	20	9	19	2	12	29	45	74	3

[1] Cells = 50 cells were sampled; each cell was sampled at six locations.
Pieces = at each sample location, six pieces about 3 x 3 mm were sampled.

[2] Samples were taken at 33 mm intervals from the bottom to the top of each container. At each sampling interval, six pieces of container were sampled.

[3] FACU = *Fusarium acuminatum*; FOXY = *Fusarium oxysporum*; ALL FUS = All *Fusarium*; CYL = *Cylindrocarpon* spp.; TRI = *Trichoderma* spp.; PEN = *Penicillium* spp.; A = *Alternaria* spp.; PE = *Phoma eupyrena*; PH = *Phoma herbarum*; ALL P = *Phoma*; NONE = no fungi growing on sample.

[4] Within each column for cells and pieces, means followed by the same capital letter are not significantly different (P = 0.05) using Tukey's multiple range comparison test.

[5] Total percent of sampled cells (50 total) and pieces (1,800 total) colonized with appropriate fungi or root colonized with any fungi.

LITERATURE CITED

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

Dorenbosch, M. M. J. 1970. Key to nine ubiquitous soil-borne *Phoma*-like fungi. Persoonia 6(1):1-14.

James, R. L. 1983. Phoma blight of conifer seedlings. USDA Forest Service, Northern Region. Nursery Disease Notes #3. 8p.

James, R. L. 1984. Characteristics of *Phoma* isolates from nurseries of the Pacific Northwest Region. USDA Forest Service, Northern Region. Nursery Disease Notes #14. 26p.

James, R. L. 1985a. Characteristics of *Phoma herbarum* isolates from diseased forest tree seedlings. USDA Forest Service, Northern Region. Nursery Disease Notes #22. 6p.

James, R. L. 1985b. Diseases associated with containerized seedling soil mixes. Tree Planters' Notes 36(2):3-5.

James, R. L. 1987. Occurrence of *Fusarium* on conifer tree seed from northern Rocky Mountain nurseries. *In*: Landis, T. D. (tech. coord.). Proceedings: Combined Western Forest Nursery Council and Intermountain Nursery Association Meeting. USDA Forest Service, Gen. Tech. Rept. RM-137. pp. 109-114.

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

James, R. L. 1989. Fungi colonizing tree improvement peat-vermiculite media, USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region. Nursery Disease Notes #85. 4p.

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. Seed and seedling infection, symptom production, and disease progression. USDA Forest Service, Northern Region. Rept. 87-13. 22p.

James, R. L. and C. J. Gilligan. 1988a. Association of *Fusarium* with non-diseased containerized ponderosa pine seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region. Rept. 88-5. 10p.

James, R. L. and C. J. Gilligan. 1988b. Occurrence of *Fusarium* on Leach pine cells from the USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region. Rept. 88-8. 10p.

James, R. L. and P. B. Hamm. 1985. Chlamydospore-producing species of *Phoma* from conifer seedlings in Pacific Northwest forest tree nurseries. Proc. Mont. Acad. Sci. 45:26-36.

Kliejunas, J. T., J. R. Allison, A. H. McCain and R. S. Smith, Jr. 1985. Phoma blight of fir and Douglas-fir seedlings in a California nursery. Plant Disease 69:773-775.

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.

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