FUNGAL COLONIZATION OF STYROBLOCK CONTAINERS -WESTERN FOREST SYSTEMS NURSERY, LEWISTON, IDAHO

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Root diseases caused by <u>Fusarium</u> spp. often limit production of containerized conifer seedlings in northern Rocky Mountain nurseries (James 1986). Recently, styroblock (James and Gilligan 1988a; James, Gilligan and Reedy 1988) and pine Leach cell (James and Gilligan 1988b) containers have been shown to harbor <u>Fusarium</u> inoculum. Standard cleaning techniques including steam treatments have usually inadequately eliminated container-borne inoculum (James and Gilligan 1988a; James, Gilligan and Reedy 1988). Residual inoculum within or on the inner walls of container cells may be available for infection of a new crop of seedlings when containers are reused.

Growers at the Western Forest Systems Nursery in Lewiston, Idaho were concerned about possible carryover of <u>Fusarium</u> inoculum within their styroblock containers. They were especially concerned about effectiveness of their standard cleaning techniques in reducing this inoculum. To help answer these questions, sampling trials were conducted using procedures developed at other nurseries (James and Gilligan 1988a; James, Gilligan and Reedy 1988).

Six styrofoam container blocks which had previously been used to grow two or more crops of seedlings were sampled. Three of the containers were uncleaned and sampled just after being used to grow a crop; the other three blocks had been cleaned using high pressure steam following seedling extraction. Twenty cells per block were randomly selected for sampling. Styroblock wall material was extraced from about 5 cm below the top and at the bottom of each selected cell. Samples were aseptically cut from the sidewall and placed, inside surface down, on a selective medium for <u>Fusarium</u> (Komada 1975). Four pieces of styrofoam (one from each of the cardinal directions) were extracted at each sample location. Plates were incubated under cool fluorescent light for 7-10 days at about 22^oC and then examined for colonization by <u>Fusarium</u> and other selected fungi. Number of styroblock pieces and sampled cells colonized by <u>Fusarium</u> and the other fungi were calculated. Extent of styroblock colonization by <u>Fusarium</u> was estimated on the basis of the proportion of surface of each piece colonized, i. e. if the entire surface was colonized, a numerical rating of 4 was given; if half of the the surface was colonized, a rating of 2 was given, and if less than half was colonized, a rating of 1 was assigned. This gave a rough approximation of the propagule density along the inner wall surface.

Comparisons of fungal colonization between cells from cleaned and uncleaned styroblock containers were made using standard "t" tests. Selected isolates of <u>Fusarium</u> were transferred to potato dextrose agar for identification using the taxonomic scheme of Nelson, Toussoun and Marasas (1983).

Results of these samples are summarized in table 1. Ninety-five percent of the cells from uncleaned styroblock containers were colonized with <u>Fusarium</u> spp. However, most of the <u>Fusarium</u> was concentrated in the bottom of cells. Cleaning reduced occurrence of <u>Fusarium</u> by approximately one-third. However, two-thirds of the cells from cleaned styroblocks were still colonized with these fungi. Walls of cells that had been cleaned still appeared "dirty" with concentrations of soil mix, organic debris, and seedling roots (Fig. 1). Cleaned styroblock containers also often had extensive epiphytic growth of <u>Thelephora terrestris</u> Ehr., a common mycorrhizal fungus (Fig.2). This fungus may produce abundant mycelial aggregations that grow over all nearby surfaces. Although this growth is difficult to remove, the fungus is not pathogenic and rarely causes damage to seedlings (James 1988b).

<u>Cylindrocarpon</u> spp., potential root pathogenic fungi (James 1988a), were also isolated from many cells (table 1). These fungi also occurred at higher concentrations at the bottom of cells, however, they were recovered in significantly (P=0.05) higher levels from cleaned cells. The other fungi assayed were mostly common saprophytes, although <u>Phoma</u> spp. may be parasitic under certain conditions (James and Hamm 1985). These fungi occurred at various concentrations and were generally reduced by cleaning.

Ninety-seven percent of the <u>Fusarium</u> isolates obtained from styroblocks were <u>F</u>. <u>oxysporum</u> Schlecht. The other 3 % were <u>F</u>. <u>acuminatum</u> Ell. & Ev. Although it is likely that some <u>F</u>. <u>oxysporum</u> isolates were saprophytes, there is also a high probability that several were pathogens. However, pathogenicity tests are required to confirm abundance of pathogens. Such tests, using isolates from Douglas-fir seed and seedlings, have previously indicated a wide range of disease-causing ability of tested <u>F</u>. <u>oxysporum</u> isolates (James et al. 1989). Unfortunately, no easy technique is available to quickly screen many isolates for their pathogenic potential.

This investigation indicates that <u>Fusarium</u> spp. and other potentially pathogenic and saprophytic fungi are common colonizers of styroblock containers at the Western Forest Systems Nursery. These organisms are found in most cells at the end of the seedling production cycle. Unfortunately, standard steam cleaning techniques employed at the nursery do not adequately reduce fungal contamination. There is sufficient viable inoculum present after cleaning to provide a threat to the next crop of seedlings. It is likely, based on investigations at another nursery (James, Gilligan and Reedy 1988), that level of contamination increases with each succeeding crop of seedlings grown in containers.

Table 1.	Colonization of cleaned and uncleaned styroblock containers with
	Fusarium and other selected fungi - Western Forest Systems Nursery,
	Lewiston, Idaho ¹ .

	Location of Sample Within Top Bottom Uncleaned Cleaned Uncleaned Cleaned			Aggregate ²			
Pieces,	3.8	5.0	70.0*	65.0* 40.8* 3.18	36.9*	22.9*	
<u>Cylindroca</u> Cells Pieces	46.7				51.7 * 15.0		
Trichoderm Cells Pieces	25.0*	5.0 * 2.5*		38.3* 19.6*	66.7 * 24.4*		
Penicilliu Cells Pieces		11.7 * 3.8 *	45.0 * 28.8*	11.7 * 5.4*		16.7 * 4.6 *	
Phoma Cells Pieces	16.7 6.3		35.0 * 18.8 *	16.7 * 9.6*		33·3* 7·3*	
Alternaria Cells Pieces		18.3 5.0	23.3 9.2	21.7 7.1	33.3 6.7	40.0 12.1*	

¹ Figures in the table are percentage of cells and/or styrofoam pieces sampled which were colonized by appropriate fungi. Percentages followed by an asterisk (*) indicate significant differences (P=0.05) between samples from cleaned and uncleaned containers using a standard "t" test.

- ² An aggregate value which includes samples from both the bottom and top of cells.
- ³ An average numerical rating based on surface of styrofoam pieces colonized by <u>Fusarium</u>: 4 = entire surface colonized, 2 = half the surface colonized, 1 = less than half the surface colonized.



Figure 1. Cross section of a "cleaned" styroblock cell from the Western Forest Systems Nursery. Note abundance of soil mix and other organic material along the inner surface of the cell.



Figure 2. Epiphytic growth of <u>Thelephora</u> <u>terrestris</u> (dark brown crust near center of photograph) on the bottom of a styroblock container from the Western Forest Systems Nursery.

It is important that alternatives to existing cleaning techniques be tested. Recent trials in British Columbia (Sturrock and Dennis 1989) have indicated that sodium metabisulfite, a chemical used to inhibit fermentation in the brewing process, and hot water baths (80°C or higher) were effective in greatly reducing occurrence of <u>Fusarium</u> and other fungi within styroblock containers. Evaluations of these promising techniques should be done at nurseries in the northern Rocky Mountains to provide alternatives to existing cleaning procedures which are unsatisfactory.

LITERATURE CITED

- James, R. L. 1986. Diseases of conifer seedlings caused by seed-borne <u>Fusarium</u> species. <u>In</u>: Shearer, R. C. (compiler). Proceedings: Conifer Tree Seed in the Inland Mountain West Symposium. USDA Forest Service, Intermountain Research Sta., Gen. Tech. Rept. INT-203. pp. 267-271.
- James, R. L. 1988a. Diseases of conifer seedlings associated with <u>Cylindrocarpon</u> species: a review. USDA Forest Service, Northern Region. Nursery Dis. Notes #76. 14p.
- James, R. L. 1988b. <u>Thelephora</u> <u>terrestris</u> on Douglas-fir seedlings -Champion Timberland Nursery, Plains, Montana. USDA Forest Service, Northern Region. Nursery Dis. Notes #70. 2p.
- James, R. L. and C. J. Gilligan. 1988a. Fungal colonization of styroblock containers - Plum Creek Nursery, Pablo, Montana. USDA Forest Service, Northern Region. Rept. 88-10. 9p.
- James, R. L. and C. J. Gilligan. 1988b. Occurrence of <u>Fusarium</u> 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 <u>Phoma</u> from conifer seedlings in Pacific Northwest forest tree nurseries. Proc. Montana Academy of Sciences 45:26-36.
- James, R. L., C. J. Gilligan and V. Reedy. 1988. Evaluation of root diseases of containerized conifer seedlings at the Champion Timberlands Nursery, Plains, Montana. USDA Forest Service, Northern Region. Rept. 88-11. 21p.
- James, R. L., R. K. Dumroese, C. J. Gilligan and D. L. Wenny. 1989. Epidemiology of <u>Fusarium</u> on containerized Douglas-fir seedlings. 3. Pathogenicity of selected <u>Fusarium</u> isolates from Douglas-fir seed and seedlings. Idaho Experiment Station Bulletin (In press).
- Komada, H. 1975. Development of a selective medium for quantitative isolation of <u>Fusarium</u> oxysporum from natural soil. Rev. Plant Protec. Res. 8:114-125.

- Nelson, P. E., T. A. Toussoun and W. F. O. Marasas. 1983. <u>Fusarium</u> species: an illustrated manual for identification. The Pennsylvania State University Press, University Park. 193p.
- Sturrock, R. N. and J. J. Dennis. 1989. Styroblock sanitization: results of laboratory assays from trials at several British Columbia forest nurseries. In: Landis, T. D. (compiler). Proceedings of the combined meeting of the Western Forest Nursery Council, Intermountain Nursery Association and Forest Nursery Association of British Columbia, Vernon, B. C. August, 1988. (In press).