Occurrence and Persistence *of Fusarium* within Styroblock and Ray Leach Containers [1,2]

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Abstract.--Fusarium spp. are common pathogens of containerized conifer seedlings. They often colonize inner walls of styroblock and Ray Leach° pine cell containers. Highest amounts of Fusarium were detected at the bottom of cells. As many as 95 percent of the cells at some nurseries, sampled prior to cleaning, were colonized with Fusarium. Hot water cleaning and dipping in bleach solutions reduced, but did not eliminate these fungi within containers. Fumigation with methyl bromide was no more effective than standard hot water treatments for styroblock containers; however, it was more effective in reducing levels of Fusarium within pine cells. Contaminated containers may be an important inoculum source of these pathogens for subsequent seedling crops.

INTRODUCTION

Fusarium spp. cause important diseases of containerized conifer seedlings in northern Rocky Mountain nurseries (James 1984a, 1986). Most conifer species are susceptible to these fungi, but losses are often greatest on Douglas-fir (Pseudotsuga menziesii (Beissn.) Franco), western larch (Larix occidentalis Nutt.), and Engelmann spruce (Picea_ engelmannii Parry)(James 1984a, 1985b; James and Gilligan 1985). Several investigations were previously conducted to help understand the disease cycle on containerized seedling stock to improve efficacy of control techniques. One important aspect of these investigations involves determining possible sources of Fusarium inoculum for seedling infection. It has been shown that seed (James 1984b, 1986, 1987), soil mixes (James 1985a), and greenhouse debris such as weeds (James and others 1987) may all act as important inoculum sources. However, experience in some nurseries has shown these potential sources do not provide enough inoculum to account for the high disease levels encountered. Therefore, investigations were conducted to ascertain the relative abundance of Fusarium inoculum on containers reused several times to grow successive crops of seedlings. Evaluations were also made on the relative efficacy of standard cleaning techniques to reduce inoculum.

MATERIALS AND METHODS

Styroblocks and Ray Leach pine cells were analyzed from several different container nurseries in the northern Rocky Mountains (table 1). Sampling intensity varied among the nurseries, but analysis techniques were similar. Styroblock cells were selected for sampling using a random number generator. Pieces of styroblock adjacent to the inner wall were aseptically cut from selected cells and placed, inside surface down, on an agar medium selective for Fusarium (Komada 1975). Usually 2-4 pieces of styroblock were collected from each cell. Although most samples were collected from the bottom of cells, some samples were taken higher up the cell. Pine cells were sampled similarly with pieces cut from the bottom of cells. Sampling was designed to determine: 1) percentage of cells colonized with Fusarium and 2) a measure of colonization intensity which roughly indicated density of fungal propagules within cells available for infection of seedlings. Plates with container pieces were incubated at about 22-24^0 C under cool

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fluorescent light for 5-7 days. The number of pieces from which Fusarium grew, as well as an approximation of the percentage of the piece colonized, were determined.

Table 1. Northern Rocky Mountain nurseries sampled for occurrence of Fusarium spp. on containers.

Container Type	Nursery and Location
Styroblock	Plum Creek, Pablo, MT
	Champion Timberlands, Plains, MT
	Potlatch Corporation, Lewiston, ID
	Western Forest Systems, Lewiston, ID
	University of Idaho, Moscow, ID
Ray Leach® Pine Cell	USDA Forest Service, Coeur d'Alene, ID

Cells were sampled both before and after cleaning. Cleaning techniques varied somewhat among the different nurseries. In most cases, cleaning consisted of washing containers with hot water under pressure. Some nurseries added commercial cleansers such as Saniclean° and followed the water treatment with immersions in a bleach solution. In one case, styroblocks and pine cells were fumigated with methyl bromide (under polyethylene tarps) following standard hot water cleaning. Some containers were also sampled several months after being cleaned. These containers were either stored outside or within greenhouses or warehouses.

Comparisons between "cleaned" and "uncleaned" container cells were made using standard "t" tests. Percentages underwent arc-sin transformation prior to analysis.

RESULTS AND DISCUSSION

Extent of styroblock container colonization by Fusarium spp. varied widely among the nurseries sampled (table 2). For example, levels at the Plum Creek and University of Idaho nurseries were generally lower than those at the Champion Timberlands, Potlatch, and Western Forest Systems nurseries. At Plum Creek and Potlatch nurseries there were also some differences in Fusarium colonization of containers sown with different seedlots. In most cases, standard cleaning significantly (P=0.05) reduced amount of Fusarium within containers, although high residual populations often remained. Fusarium spp. were isolated from the bottom of cells more frequently than from near the top. These fungi were also often isolated from root pieces that penetrated the side walls of cells and were not removed during cleaning.

Table 2. Occurrence of Fusarium spp. on styroblock containers from five nurseries in the northern Rocky Mountains.

			Percent of Cells Colonized with Fusarium					
			Prior to Cleaning			After Cleaning		
Nursery[1]	Seedlot	No. Cells Sampled	Top	Bottom	Both	Top	Bottom	Both
Plum Creek	631	50	6	16	22*	0	6	6*
	632	50	12	20	28*	2	14	14*
	17045	50	6	34	36*	2	16	16*
	Totals	150	8	23	29	1	12	12
Champion	-	100	44	-	44NS	12	65	65NS
Potlatch	Spur 10	50	20	86	88*	0	18	18*
	Camp 55	50	22	92	96*	2	36	36*
	Robinson	50	18	86	86*	2	20	20*
	Blackwell	50	12	76	76*	2	18	18*
	Totals	200	18	85	86*	2	24	24*
Western Forest Systems	-	60	13	93	95*	15	65	67*
University of Idaho	FN	80	-	44	44NS	-	33	33NS

[1] See table 1 for nursery locations.

* Denotes significant differences (P=0.05) in Fusarium colonization of cells prior to and after cleaning.

NS Denotes no significant differences (P=0.05) in Fusarium colonization of cells prior to and after cleaning.

Table 3. Occurrence of Fusarium spp. on Ray Leach® pine cells from the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	Prior to Cleaning	After Cleaning	After Cleaning and Storage	All Samples
Percent Cells Infected	86*	51*	52*	65
Percent Colonization Intensity	88*	69*	72*	76

[1] **Percentage of pine cell pieces colonized with Fusarium.**

*** Denotes significant differences (P=0.05) in Fusarium colonization of cells prior to cleaning and after cleaning or after storage.**

At the USDA Forest Service Nursery, Coeur d'Alene, ID, 86 percent of pine cells sampled prior to cleaning were infected with Fusarium spp. (table 3). This was reduced to about 50 percent by standard steam cleaning. However, storage in a warehouse for several months failed to significantly reduce Fusarium populations within pine cell containers.

Treatment of styroblock containers with methyl bromide had no effect on occurrence of Fusarium (table 4). Percent of cells colonized with these fungi were similar before and after methyl bromide treatment. On the other hand, the fumigant significantly reduced percentage of pine cells which were colonized with Fusarium. Differences in effectiveness of methyl bromide between the different types of containers may be due to the ability of the fumigant to penetrate the container side walls or problems with methodology.

Table 4. Effects of methyl bromide fumigation on occurrence of Fusarium within styroblock and Ray Leach® pine cell containers.

Container Type	Cells Sampled	Percent Cells Colonized with Fusarium - After Cleaning	Percent Cells Colonized with Fusarium - After Methyl Bromide
Styroblock	100	22.5NS	26.3NS
Pine Cells	120	55.5*	29.2*

* Denotes significant differences (P=0.05) in Fusarium colonization of cells before and after methyl bromide treatment using a standard "t" test.

NS Denotes non-significant differences (P-0.05) in Fusarium colonization of cells before and after methyl bromide treatment using a standard "t" test.

The most common species of Fusarium isolated from containers was F. oxysnorum Schlect. Other major species included F. sambucinum Fuckel, F. tricinctum (Corda) Sacc. and F. acuminatum Ell. & Ev. Some of these isolates were probably pathogens whereas others were likely saprophytic. Pathogenicity tests will be required to evaluate how extensive the occurrence of pathogenic strains of Fusarium are in isolates from containers.

Our investigations indicated styroblock and pine cell containers commonly harbor Fusarium inoculum after being used to grow a crop of container seedlings. Standard techniques for cleaning containers after use are ineffective in reducing amounts of Fusarium to very low levels. Experience indicates that the more containers are used to grow several crops of seedlings, the more they become contaminated with these fungi. Several managers use older containers to grow species like ponderosa pine (Pinus ponderosa Laws.), which are not damaged by Fusarium as much as other conifer species (James and Gilligan 1988).

These investigations also showed that standard techniques of hot water, steam, and bleach treatment are relatively ineffective in reducing Fusarium to acceptable levels. Methyl bromide was also not very effective, especially in styroblocks. However, some growers in Canada have begun to use sodium metabisulfite, a chemical used in fermentation, to clean containers and report good success (Dennis and Sturrock 1988).

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