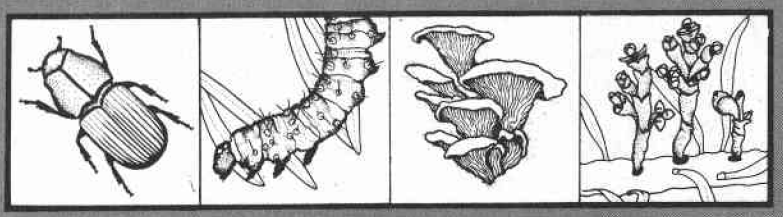
Forest Pest Management



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AN EVALUATION OF THE EFFICACY OF HOT WATER-CHEMICAL TREATMENTS TO CLEAN STYROBLOCK CONTAINERS CHAMPION TIMBERLANDS NURSERY PLAINS, MONTANA

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ABSTRACT

Treating styroblock containers in hot water (68°C) with a very dilute bleach and detergent solution effectively reduced populations of *Fusarium* and *Cylindrocarpon* spp., two potential root pathogens of containerized conifer seedlings. However, the treatment did not significantly reduce levels of *Phoma* (another potential pathogen) nor saprophytic *Penicillium* and *Alternaria* spp. The treatment also eliminated *Fusarium* and *Cylindrocarpon* spp. from pieces of seedling roots which had penetrated walls of container cells. This cleaning technique will be used in the future at the Champion Timberlands Nursery, Plains, Montana.

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INTRODUCTION

Containerized seedling production is increasing in importance in the northern Rocky Mountains to meet demands for reforestation seedling stock. Unfortunately, diseases may contribute significantly to the problems of growing containerized tree seedlings in greenhouses (Jarvis 1989). Environmental conditions necessary to produce seedlings are also often conducive to buildup and spread of important seedling pathogens (James 1984; Jarvis 1989).

Recent investigations (James and Gilligan 1988a, 1988b; James, Dumroese and Wenny 1988; James, Gilligan and Reedy 1988; Sturrock and Dennis 1988) showed that one of the major sources of pathogen inoculum in container operations is contaminated styroblock and pine cell containers. Because of their expense, containers are usually used for several crops of seedlings before being discarded. All nurseries try to clean containers after each crop. Unfortunately, steam cleaning or normal washing techniques are often unsatisfactory in significantly reducing potential pathogenic fungi from containers (James and Gilligan 1988b; James, Gilligan and Reedy 1988). To improve cleanliness of containers that must be reused for several crops, several nurseries have tried to improve their standard cleaning techniques. Use of such chemical sterilants as standard bleach (sodium hypochlorite), methyl bromide, and sodium metabisulfite have been or are being tested for operational use by growers (James 1989; James, Dumroese and Wenny 1988; Sturrock and Dennis 1988).

The Champion Timberlands Nursery produced about 560,000 container conifer seedlings each year for the past several years at their greenhouses in Plains, Montana. However, recently they began growing two crops each year, almost doubling their production, to meet increasing demand for seedling stock for their forest lands. Fusarium root disease, caused by a complex of *Fusarium* species (mostly *F. oxysporum* Schlect.), has been a recurring problem at this nursery (James 1986a, 1986b, 1986c; James, Gilligan and Reedy 1988). The disease has been especially damaging to Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) and western larch (*Larix occidentalis* Nutt.). Attempts to reduce impact of this disease by applying fungicide drenches once seedlings display disease symptoms have proven unsuccessful.

Previous investigations (James, Gilligan and Reedy 1988) showed that much of the *Fusarium* inoculum was introduced into new crops of seedlings on contaminated styroblock containers. Also, standard high pressure steam-cleaning techniques employed at the nursery were not significantly reducing pathogen levels on containers. Therefore, growers at the Champion Nursery decided the most practical approach to controlling Fusarium root disease was prevention of seedling infection by greatly reducing amounts of fungal inoculum carried within containers. A new cleaning technique using hot water was instituted to try to improve cleanliness of containers and reduce pathogen levels. This report summarizes this technique and describes effects on fungal colonization of containers.

MATERIALS AND METHODS

Two groups of styroblock containers were tested in this evaluation. An older group 5 or more years old (had been used to grow 5 or more crops of seedlings) and a newer group 3 years old. Selected styroblocks were cut in half; one half was cleaned using the treatment described below and the other half served as an untreated check.

Treated blocks were first washed with steam under pressure. Following steam treatment, the blocks were soaked for 10 min. in hot water (about 68°C) which also contained a very dilute solution of standard household bleach (0.04 percent Sani-kleen® detergent. After soaking, blocks were quickly immersed in a colder water

(about 24°C) solution (0.23 ml/gal.) of Consan® (a multi-purpose algicide, fungicide, and bactericide - Del Tek, Inc., Pearland, TX). Blocks were then air dried and evaluated for fungal populations.

For each half of each block, 20 cells were randomly selected for sampling using a random number generator. At the bottom of each selected cell, four small pieces of styroblock (2-3 mm diam.) were aseptically cut (one from each cardinal direction). Pieces were placed inside surface down on an agar medium selective for *Fusarium* and closely related fungi (Komada 1975). In addition, seedling root pieces that were present in sampled blocks were aseptically cut (3-5mm in length) and placed on the selective medium. Plates were incubated at about 22°C under diurnal cycles of cool fluorescent light for 7-10 days. After incubation, plates were examined for fungal growth from styroblock and root pieces. Colonization percentages were determined for the following groups of fungi readily identified on the selective medium: *Fusarium*, *Cylindrocarpon*, *Trichoderma*, *Penicillium*, *Phoma*, and *Alternaria*. Selected isolates of *Fusarium* were transferred to potato dextrose (PDA) and carnation leaf agar and identified to species using the taxonomic guide of Nelson et al. (1983).

Colonization percentages for treated and untreated styroblocks were compared using paired "t" (Sokal and Rohlf 1973) tests. Percentages underwent arc-sin transformations prior to analysis.

RESULTS AND DISCUSSION

Effects of the hot water-chemical treatment on styroblock colonization by selected fungi are summarized in Tables 1 and 2. Table 1 provides data on percentage of sampled cells colonized with different types of fungi, whereas Table 2 compares colonization intensity (based on percent of styrofoam pieces colonized) by selected fungi. The treatment significantly reduced occurrence of *Fusarium* and *Cylindrocarpon*, the two most important genera of potential root pathogens. These results are especially impressive since *Fusarium* spp. were colonizing untreated blocks at very high levels. *Trichoderma* spp., potential antagonists toward pathogens and therefore desirable in container operations (Papavizas 1985), were also significantly reduced by the treatment. Three other groups of fungi (*Penicillium*, *Phoma*, and *Alternaria*) were not significantly reduced.

An overall summary of the treatment's ability to kill fungi colonizing styroblock containers is provided by analysis of "clean" cells (sampled styrofoam pieces not colonized by any fungi). More than half of the pieces sampled in treated blocks were without any fungal colonization, whereas all pieces from untreated blocks were colonized by at least some fungi (Table 2). As expected, older styroblocks (used for five or more seedling crops) were colonized to a greater extent with *Fusarium* and other potential pathogens, such as *Cylindrocarpon* and *Phoma* spp., than newer blocks.

During seedling production, roots may grow into the side walls of styroblock container cells. When seedlings are extracted, some of these roots invariably break and remain attached to walls. These roots are usually not completely removed when containers are cleaned, even if high-pressure steam is used. Fungi often colonize these roots, particularly *Fusarium* spp. which are well adapted to infecting seedling roots (James, Gilligan and Reedy 1988). the hot water-chemical treatment evaluated at the Champion Nursery completely eliminated *Fusarium*, *Cylindrocarpon*, and *Trichoderma* on seedling roots (Table 3). On the other hand, almost half of the sampled roots were colonized with *Fusarium* spp. in untreated blocks.

Table 1.--Effects of hot water-chemical treatment on colonization of styroblock container cells with *Fusarium* and other selected fungi at the Champion Timberlands Nursery, Plains, Montana.

	Percent of Cells Colonized					
	5 year-old Blocks		3 year-old Blocks		All Blocks Combined	
Fungi	Treated	Untreated	Treated	Untreated	Treated	Untreated
F. oxysporum	5.0	72.5	0	50.0	3.3[1]	65.0[1]
Other *Fusarium*	0	45.0	0	45.0	0	45.0
All *Fusarium*	5.0	85.0	0	60.0	3.3	76.7
Cylindrocarpon	2.5	35.0	5.0	25.0	3.3	31.7
Trichoderma	5.0	65.0	10.0	85.0	6.7	71.7
Penicillium	90.0	72.5	60.0	100.0	80.0*	81.7*
Phoma	17.5	42.5	25.0	30.0	20.0*	38.3*
Alternaria	5.0	7.5	0	0	3.3*	5.0*
Clean (no fungi)	10.0	0	30.0	0	16.7	0

[1]Within each row, treated and untreated means followed by an asterisk are not statistically different (P=0.05) using a paired "t" test. All other pairs of means are significantly different (P=0.05). All percentages underwent arc-sin conversions prior to analyses.

Table 2.--Effects of hot water-chemical treatment on colonization intensity of styroblock containers with *Fusarium* and other selected fungi at the Champion Timberlands Nursery, Plains, Montana.

	Colonization Intensity[1]					
	5 year-old Blocks		3 year-old Blocks		All Blocks Combined	
Fungi	Treated	Untreated	Treated	Untreated	Treated	Untreated
F. oxysporum	1.2	56.9	0	15.0	0.8[2]	42.9[2]
Other *Fusarium*	0	20.6	0	25.0	0	22.1
All *Fusarium*	1.2	77.5	0	40.0	0.8	65.0
Cylindrocarpon	0.6	17.5	2.5	7.5	1.2	14.2
Trichoderma	1.9	31.9	2.5	51.2	2.1	38.3
Penicillium	43.8	45.0	22.5	93.8	36.7	61.2
Phoma	4.4	12.5	6.2	10.0	5.0*	11.7*
Alternaria	1.2	2.5	0	0	0.8*	1.7*
Clean (no fungi)	50.6	0	68.8	0	56.7	0

[1]Percent of styrofoam pieces sampled (four per cell) colonized with appropriate fungi.

[2]Within each row, treated and untreated means followed by an asterisk are not statistically different (P=0.05) using a paired "t" test. All other pairs of means are significantly different (P=0.05). All percentages underwent arc-sin conversions prior to analyses.

Table 3.--Effects of hot water-chemical treatment on colonization of root pieces from styroblock containers at the Champion Timberlands Nursery, Plains, Montana.

	Percent Root Piece Colonization	
Fungi	Treated Blocks	Untreated Blocks
F. oxysporum	0[1]	14.3[1]
Other *Fusarium*	0	33.3
All *Fusarium*	0	42.8
Cylindrocarpon	0	0
Trichoderma	0	76.2
Penicillium	28.2*	47.6*
Phoma	0*	4.8*
Clean (no fungi)	53.8	0

[1]Within each row, treated and untreated means followed by an asterisk are not statistically different (P=0.05) using a paired "t" test. All other pairs of means are significantly different (P=0.05). All percentages underwent arc-sin conversions prior to analyses.

General cleanliness, based on external appearance, was also very different for treated and untreated blocks. Before cleaning, tops of blocks had extensive algal growth over their entire surface (Figure 1). In addition, the interior of untreated cells usually had extensive amounts of residual soil and organic debris, which may harbor potentially pathogenic fungi (James, Gilligan and Reedy 1988). However, following treatment, most external algal growth was removed (Figure 2) as well as the mix and debris within cells. It should be noted that seedling roots attached to the walls of styroblock cells were not removed by the treatment, even though they were not colonized by potentially pathogenic fungi.

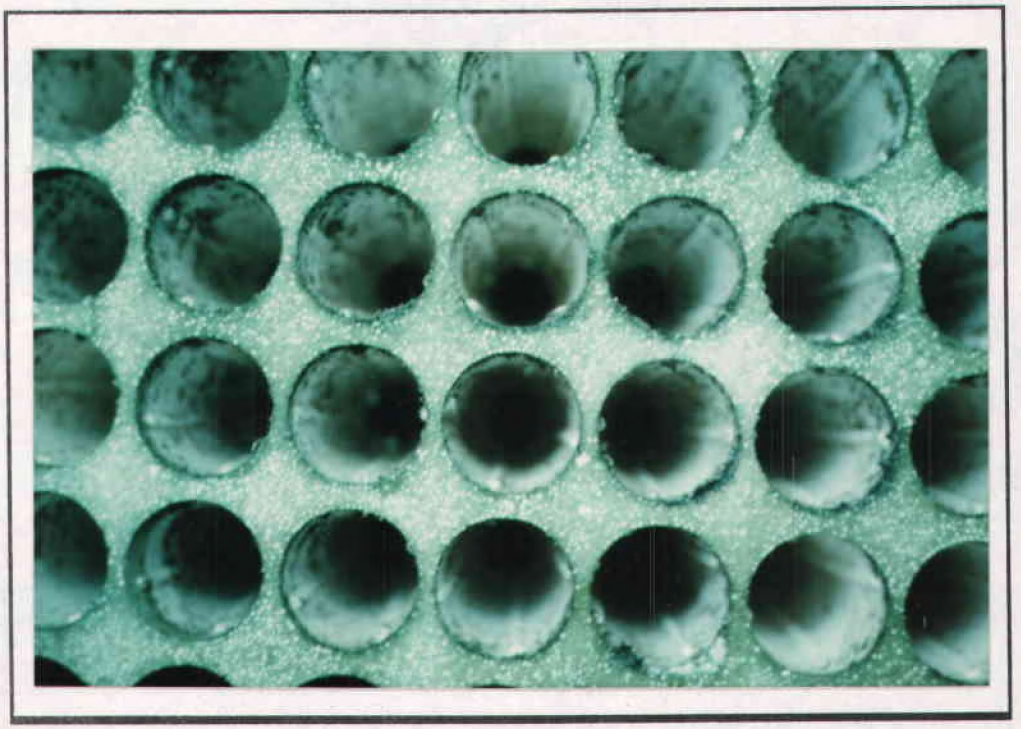

Figure 1.–Styroblock container prior to hot water treatment at the Champion Timberlands Nursery. Extensive algal growth occurred on the tops of untreated containers.

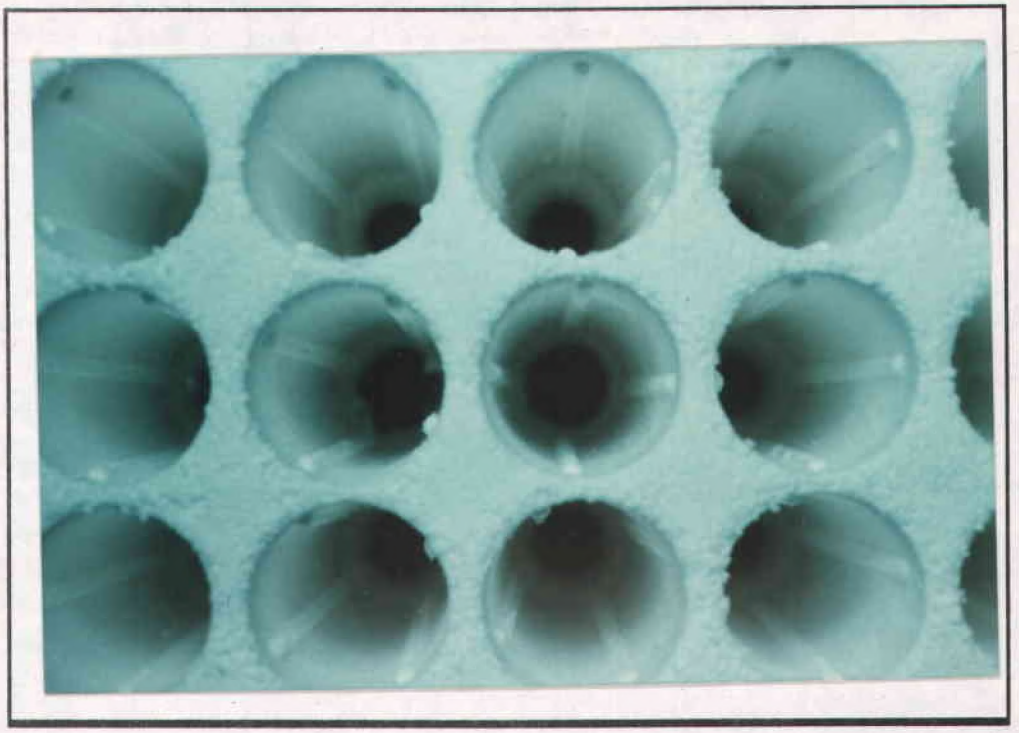

Figure 2.–Styroblock container after hot water treatment at the Champion Timberlands Nursery. Note absence of algal growth on the tops of treated containers.

For this evaluation, *Fusarium* spp. isolated from pieces of styroblock were divided into two species. The most common species was classified as *F. oxysporum* and two morphologically different groups were commonly isolated. One group produced profuse white aerial mycelium with a deep violet pigment under the colony when grown on PDA while the other group lacked aerial mycelium and was mostly orange to peach colored with no violet pigmentation. The other species of *Fusarium* spp. was *F. acuminatum* Ell. & Ev. Both these species have previously been isolated from root diseased seedlings at the Champion Nursery (James 1986a; James, Gilligan and Reedy 1988).

Our results confirm the ability of hot water and sterilant chemicals to remove most potentially pathogenic fungi from styroblock containers. Sturrock and Dennis (1988) achieved complete elimination of *Fusarium*, *Cylindrocarpon*, and *Pythium* after immersion of blocks for 3 min. in water at 80°C. However, they were unable to reduce levels of *Phoma* with their treatment. We were also not able to significantly reduce levels of *Phoma* spp. with our treatment. Sturrock and Dennis (1988) indicated that water temperatures much above 80°C caused distortion to the structural integrity of styroblocks.

Duration of exposure to hot water is probably very important in the ability to kill fungi. Sturrock and Dennis (1988) showed that exposure to 80°C for 1 min. was not very effective in reducing fungal levels, whereas extending the exposure period to 3 min. greatly improved treatment efficacy. In our evaluation, blocks were immersed in a cooler water solution (68°C) but for a longer duration. This added exposure time probably compensated for the reduced water temperature. Of course, one problem with such long exposure periods is being able to treat hundreds of containers operationally this way. Likewise, being able to maintain high enough water temperatures for so long may be difficult.

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