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HOT WATER STERILIZATION OF STYROBLOCK CONTAINERS PLUM CREEK NURSERY, PABLO, MONTANA

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INTRODUCTION

Growers of container conifer seedlings have had recurring problems with root disease for many years (James and others 1988a). Investigations of the epidemiology of such diseases have revealed that containers which are re-used for several successive crops may contain fungal pathogen inoculum which infects new crops of seedlings (James and others 1988b). Most growers have previously used some sort of steam cleaning to dislodge growing media and other debris that may remain within containers after a seedling crop is extracted. Previous work has indicated that this type of cleaning is usually not effective in removing all residual organic debris (figure 1) nor eliminating propagules of pathogenic fungi that may be present (James 1990; James and others 1988b, 1988c). Most of these propagules are concentrated in the bottom of containers (James 1989); these fungi often colonize organic material including seedling roots which have egressed into the side walls of styrofoam containers (figure 1) (James and others 1988b, 1988c).

Several alternatives have been evaluated for improving container cleaning. Chemicals such as bleach (aqueous sodium hypochlorite), sodium metabisulfite, and synthetic surface sterilants (i.e., Physan®) have been evaluated with mixed results (James and others, unpublished). The most efficaceous, non-toxic treatment investigated so far has been immersion in hot water (James and Woollen 1989). Hot water treatment has proven effective for both styrofoam and plastic (Ray Leach® pine cell) containers (James, unpublished). However, dose response (time-temperature interactions) for maximum efficacy must still be determined.

Growers at the Plum Creek Nursery in Pablo, Montana have previously cleaned their styroblock containers with an initial immersion in R-11 (a surfactant) followed by spraying with cold water to dislodge organic materials and with high-pressure steam and a final immersion in a Physan®-water solution. Because hot water immersion had proven effective at other nurseries (James and Woollen 1989; James, unpublished), growers at Plum Creek were anxious to evaluate this type of treatment at their nursery. Although high levels of root diseases are not normally high at the Plum Creek Nursery, disease problems that have occurred may be related to carry-over of pathogen inoculum on containers (James and Gilligan 1990).

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MATERIALS AND METHODS

Styroblock containers were evaluated for presence of fungi, especially those capable of causing root diseases of conifer seedlings, following hot water immersion treatment. Three treatments were evaluated: (A) standard cleaning procedures previously used at the nursery, (B) spraying with cold water, immersion in R-11, followed by immersion in hot water (75°C for 1 min.), and (C) the same as (B) except hot water immersion was for 3 min. Following treatment, two containers from each of the three treatments were randomly selected for sampling. Within each selected container, ten cells were randomly sampled. Four small pieces of styrofoam (one from each cardinal direction) were aseptically removed from the bottom of each sampled cell and placed on an agar medium selective for *Fusarium* spp. and closely-related fungi (Komada 1975). Within each sampled container, 7-10 pieces of root which had grown into the walls of styrofoam and remained embedded after extraction of seedlings were also sampled for colonization by fungi. Root pieces about 2 cm in length were aseptically cut and placed on Komada's medium. Plates with styrofoam and root pieces were incubated at about 24°C under diurnal cycles of cool, fluorescent light for 7-10 days, after which they were examined for fungal colonization.

Figure 1. Cross-section of styroblock container with organic debris and embedded roots remaining after seedling extraction and "normal" cleaning at the Plum Creek Nursery. Propagules of potentially pathogenic fungi often reside on the inner styrofoam surface of cells or within seedling roots that penetrate the styrofoam.

Gross morphological characteristics were used to identify genera of fungi. However, selected isolates had to be tranferred to potato dextrose agar or carnation leaf agar for identification (Fisher and others 1982; Nelson and others 1987). Taxonomic treatises by Nelson and others (1987) and Booth (1966) were used for identification of *Fusarium* and *Cylindrocarpon* spp., respectively.

RESULTS AND DISCUSSION

Results of this evaluation (Table 1) indicated that hot water treatment effectively eliminated carry-over inoculum of potential root-disease organisms. The 75°C treatment for only 1 min. was as effective as the treatment for 3 mins. *Fusarium oxysporum* Schlecht. and *F. acuminatum* Ell. & Ev., both potential root-pathogenic fungi (James and others 1989), were found at relatively low levels on normally-treated containers (treatment A). However, *Cylindrocarpon destructans* (Zins.)Scholten, a major cause of root decay of white pine seedlings at the nursery (James and Gilligan 1990), was very common. A related species of *Cylindrocarpon*, *C. tenue* Bugn., was also encountered, although at much lower levels than *C. destructans*. Fortunately, all *Fusarium* and *Cylindrocarpon* spp. were completely eliminated by hot water immersion treatments.

Cylindrocarpon spp. also commonly colonized pieces of root remaining embedded in styrofoam containers after seedling extraction (Table 2). However, treatment with hot water immersion effectively eliminated these fungi on roots.

Some isolates of *Trichoderma* and *Penicillium* and several unidentified bacterial species persisted on styroblock containers and seedling roots after hot water immersion (tables 1 and 2). However, these organisms are most likely saprophytes that commonly inhabit growing media and the rhizospheres of container-grown seedlings. Some may even be antagonistic to or competitive with root-pathogenic fungi (Papavizas 1985); if so, these organisms would be desirable to keep within containers.

Although this evaluation indicated that immersion of styroblock containers in water heated to 75°F for 1 min. was effective in eliminating potentially pathogenic fungi, exact dose-response criteria were not determined. A lower temperature (68°C) was effective at another nursery with a longer exposure time (10 min.)(James and Woollen 1989). In another evaluation with plastic (Ray Leach®) containers (James, unpublished), exposure for 15-30 sec. was usually sufficient to effectively eliminate pathogenic fungi when water temperatures were above 65°C. Apparently, hot water "shocks" are lethal to propagules of most fungi. Immersion for longer time periods may not greatly improve efficacy of treatments, especially if a surfactant is added to the water to ensure uniform contact of hot water with container surfaces. Future evaluations should pinpoint time-temperature requirements for sterilizing styroblock containers; because of their porosity, styrofoam containers are probably more difficult to sterilize than those made of plastic.

Table 1. Effects of hot water treatments on colonization of styroblock containers by selected fungi - Plum Creek Nursery, Pablo, Montana.

Percent Cells Colonized[1]

	Fusarium[3]			*Cylindrocarpon*[4]			Other Organisms[5]				
Treatment[2]	FOXY	FACU	All	CYDE	CYTE	All	TRI	PEN	PHO	BAC	CLEAN
A	10	5	15	75	15	80	70	0	20	10	0
B	0	0	0	0	0	0	5	0	0	25	75
C	0	0	0	0	0	0	10	40	0	0	60

Colonization Percentage[6]

	Fusarium[3]			*Cylindrocarpon*[4]			Other Organisms[5]				
Treatment[2]	FOXY	FACU	All	CYDE	CYTE	All	TRI	PEN	PHO	BAC	CLEAN
A	5.0	1.2	6.2	63.8	8.8	72.5	38.8	0	7.5	3.8	0
B	0	0	0	0	0	0	1.3	0	0	7.5	92.5
C	0	0	0	0	0	0	5.0	12.5	0	0	83.8

[1]Ten cells sampled within each of two styroblock containers per treatment.
[2]A = normal container treatment (immersion in R-11, cold water spraying, high-pressure steam spraying, immersion in Physan®-water solution).
B = immersion in hot water (75°C) for 1 min. following normal treatment.
C = immersion in hot water (75°C) for 3 min. following normal treatment.
[3]FOXY = *Fusarium oxysporum*; FACU = *Fusarium acuminatum*
[4]CYDE = *Cylindrocarpon destructans*; CYTE = *Cylindrocarpon tenue*
[5]TRI = *Trichoderma* spp.; PEN = *Penicillium* spp.; PHO = *Phoma* spp. BAC = Bacteria; CLEAN = No fungal or bacterial organisms colonizing styrofoam
[6]Based on percentage of styrofoam pieces colonized by appropriate fungi (four sampled per cell).

Table 2. Effects of hot water treatment on colonization of detached seedling roots by selected fungi within styroblock containers - Plum Creek Nursery, Pablo, Montana.

Percent Roots Colonized[1]

Treatment[2]	*Cylindrocarpon*	*Trichoderma*	*Phoma*	Bacteria	Clean[3]
A	70.6	29.4	11.8	29.4	0
B	0	0	0	52.9	47.1
C	0	0	0	15.0	85.0

[1]7-10 root pieces sampled from each of two styroblock containers per treatment.
[2]A = normal container treatment (immersion in R-11, cold water spraying, high-pressure steam spraying, immersion in Physan®-water solution).
B = immersion in hot water (75°C) for 1 min. following normal treatment.
C = immersion in hot water (75°C) for 3 min. following normal treatment.
[3]Percentage of sampled root pieces not colonized with fungi or bacteria.

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