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Hot Air Cleaning of Styrofoam Containers in Forest Nurseries

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Abstract

Fungal pathogens tend to accumulate within styrofoam containers that are reused to produce successive crops of container-grown seedlings. Most nurseries treat reused containers by immersing them in hot water for varying time periods. The efficacy of radio frequency waves (RFWs) and hot, dry air (82.2°C for 10, 20, and 60 minutes) to reduce levels of selected groups of potentially pathogenic fungi within styrofoam containers was evaluated. RFWs and hot air were effective only on prewetted containers heated under high humidity. Fungi were readily killed on container surfaces when a thin film of water was present on containers prior to treatment. Dry containers were not adequately sanitized. *Fusarium proliferatum* was the most commonly encountered potentially pathogenic fungus isolated from containers. Eight other species of *Fusarium* and two species of *Cylindrocarpon* were also isolated from containers. Common fungal saprophytes on containers included *Trichoderma* and *Penicillium* spp. Although wet RFW treatment was as effective as hot water immersion, such treatments may be much more expensive due to the high equipment costs. Wetting containers and exposure to dry, hot air is an effective alternative to hot water immersion for styroblock sanitization.

Introduction

A variety of containers are used in forest seedling nurseries. Several popular types of containers are made of styrofoam. During their effective life, these containers are typically reused several times to produce multiple seedling crops. However, they require sufficient sterilization prior to reuse because they can harbor potentially pathogenic organisms that may cause important diseases on new seedling crops (James et al. 1988; Peterson 1990, 1991; Sturrock and Dennis 1988). Potential pathogens reside on residual organic matter and within the inner cell walls of styrofoam containers (James 1987, 1989a, 1992; James and Woollen 1989; James et al. 1988). They may also colonize residual roots from the previous seedling crop that remain attached to containers after seedling extraction (James et al. 1988; Peterson 1990; Sturrock and Dennis 1988).

Several approaches to cleaning styrofoam containers have been investigated. Chemical sterilants, such as sodium hypochlorite (bleach) (James and Sears 1990) and sodium metabisulfite (Dumroese et al. 1993), have produced varying results. Problems with worker exposure to and disposal of toxic chemicals limit their desirability (Dumroese et al. 1993). Because of these disadvantages, many nursery growers have sought alternative, cost-effective techniques for container cleaning. Steam treatment has often been used. However, steam treatments may not adequately reduce potentially pathogenic organisms (James 1987, 1990; James et al. 1988). Therefore, immersion in hot water for varying lengths of time has been evaluated (James 1992; James and Woollen 1989; Peterson 1990, 1991; Sturrock and Dennis 1988). In general, exposure of styrofoam containers to 60-70°C for about 120 seconds kills most residual pathogens (Dumroese and James 2004).

Hot water immersion of large numbers of containers is time-consuming and expensive due to the high energy costs required to maintain sufficient water temperatures for efficacious treatments (Peterson 1990; Sturrock and Dennis 1988). Recently, the U.S. Department of Agriculture (USDA) Forest Service Missoula Technology and Development Center began investigating possible alternative methods for container treatment. Their goal was to evaluate other methods that might be more time and cost effective.

One alternative method was to use radio frequency wave (RFW) ovens to raise styrofoam temperatures sufficiently to kill potential pathogens. Industrial RFW wave ovens are

used for baking, curing, and drying many different types of foods and materials. RFW ovens operate at an electrical frequency of 10-100 MHz. Heating is accomplished by subjecting the material to be heated to an alternating electrical field that makes the molecules inside the material rotate and move laterally millions of times per second in an attempt to align with the changing electric field. This generates heat within the material in a manner similar to friction. The ovens can be incorporated into a conveyor system to mechanize the operation to minimize handling.

Another method was to use hot, dry air to sterilize containers within specially-fabricated ovens. This method eliminates the need to constantly maintain high water temperatures for water immersion treatments.

Evaluations were conducted to determine efficacy of RFW and hot air treatments on reducing populations of selected fungi colonizing styrofoam containers from a commercial forest seedling nursery. The goal was to determine if such treatments could kill potentially pathogenic fungi and thus render containers relatively safe for reuse from a disease potential standpoint. Some results have been previously reported (James and Trent 2001, 2002).

Materials and Methods

RFW Treatments

Ten styrofoam containers used to grow several crops of conifer seedlings were tested. The containers varied in size and manufacturer. A random-number generator was used to select cells to be sampled; 24 cells were sampled per container (the same cells–designated by row and column–were sampled in each container). Each selected cell was sampled for fungal colonization prior to treatment. Sampling for fungi was restricted to the bottom of cells adjacent to the drainage hole where the highest populations of contaminating fungi, including potential pathogens, tend to congregate (Dumroese et al. 1995; James 1987, 1989b). Two pieces of styrofoam approximately 2 x 5 mm in size were aseptically extracted from each sampled cell and placed on an agar medium selective for *Fusarium* and closely-related fungi (Komada 1975). Plates were incubated for 7-10 days at about 24°C under diurnal cycles of cool, fluorescent light. Emerging fungi were identified to genus and selected isolates were transferred to potato dextrose agar and carnation leaf agar (Fisher et al. 1982) for species identification. *Fusarium* and *Cylindrocarpon* spp. were identified using the taxonomy of Nelson et al. (1983) and Booth (1966), respectively. Container colonization was calculated as the percentage of sampled styrofoam pieces (two sampled per cell) that were colonized by a particular fungus.

After preliminary sampling, containers were treated with RFW heating in a laboratory test oven (PSC, Inc., Cleveland, OH). The oven operated at 40kW at a frequency of 18MHz and contained a parallel plate electrode system with variable electrode heights; the plate voltage was 12kV. The 10 containers were divided into 2 groups of 5 containers each. Five of the containers were "dry" treated; the other 5 containers were "wet" treated. These latter containers were initially immersed in cold water for a brief period of time, shaken to remove excess water, and placed in the RFW oven. Electrode heights were either 19.1 or 25.4 cm above containers that were exposed to the RFW field for 2 minutes. Blocks were then removed and their cell surface temperatures measured with an infrared (IR) sensor. Final temperatures varied somewhat among containers, but averaged 33.5°C (range 26.7-48.0°C).

After treatment, containers were again sampled for fungal colonization using the same presampling cells. Two pieces of styrofoam per cell were again sampled as described above. Statistical comparisons of fungal colonization (number of sampled styrofoam pieces colonized by particular fungi) between pre- and post-treatment were made for the "dry" and "wet" treated containers. Comparisons were made using the nonparametric test of Kruskal-Wallis (Ott 1984).

Hot Air Treatments

Six additional styrofoam containers used to grow several crops of seedlings at an Idaho nursery were tested. Each container was cut into thirds; within each third, 12 cells were randomly sampled for selected fungi. The same cells were sampled before and after treatment. Cell sampling and associated fungal identification was conducted as outlined above.

Five of the containers were exposed to hot, dry air in a small oven. Each third was exposed to 82.2°C for 10, 20, and 60 minutes, respectively. The sixth container was first wetted with tap water, shaken to remove excess water, and then exposed to dry heat; each third was exposed to the same temperature–time regime as the other five containers. After treatment, containers were again sampled for

presence of fungi. Results for nonwetted containers were collated and average colonization means for particular fungal groups before and after treatment were compared statistically using the Kruskel-Wallis Test (Ott 1984). The same statistical tests were used to evaluate treatment effects on fungal colonization for the wetted styrofoam container.

Results and Discussion

Effects of wet and dry RFW treatments on colonization of styrofoam containers are summarized in table 1. Basically, styrofoam containers had to be wetted prior to treatment for RFWs to significantly reduce level of *Fusarium* and *Cylindrocarpon* (the only potentially pathogenic fungi assayed) colonization. Levels of *Trichoderma* spp., which are saprophytic and sometimes potentially antagonistic toward pathogens, such as *Fusarium* and *Cylindrocarpon* (Papavizas 1985), were also significantly reduced when containers were wetted prior to treatment. Wetting blocks prior to treatment also resulted in essentially sterilizing major portions of sampled containers. In contrast, if blocks were not wetted prior to treatment, RFWs did not significantly reduce level of potential pathogen (*Fusarium* and *Cylindrocarpon*) or saprophyte (*Trichoderma* and *Penicillium*) colonization (table 1).

Effects of treating styrofoam containers with hot, dry air for 10, 20, and 60 minutes on colonization by selected fungi are summarized in table 2. Although statistical differences varied, prewetting containers greatly improved efficacy of hot air treatments. This was particularly evident by the much larger number of sampled cells without any detectable fungal colonization after being wetted and exposed to hot air. The temperature to which containers were exposed (82.2°C) was at the upper limit possible because containers became disfigured and unusable at higher temperatures.

We found that air heated with either RFWs or in a standard oven did not adequately penetrate containers to kill resident microorganisms unless containers are wet prior to treatment. Since immersion of blocks into hot water is also usually efficacious (James 1992; James and Woollen 1989; Peterson 1990; Sturrock and Dennis 1988), apparently the water conducts heat to surfaces of cells where microorganisms reside.

Table 1. *Effects of RFW treatments on colonization of styrofoam containers by selected fungi.*

Fungus[1]	Percent colonization[2]			
	Wet containers[3]		Dry containers	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Fusarium	54a	3b	72a	71a
Cylindrocarpon	2a	0b	1a	1a
Trichoderma	35a	5a	34a	30a
Penicillium	4a	3a	15a	13a
Other fungi	37a	29a	14b	39a
No fungi	0b	60a	0a	0a

[1] For each fungus, average values comparing pre- and post-treatments followed by the same letter (across columns) are not significantly different (P=0.05) using the Kruskal-Wallis test.

[2] Twenty-four cells sampled per container; the same cells were sampled before (pre) and after (post) treatment.

[3] Container surfaces wetted prior to RFW treatments.

[4] Unidentified fungi isolated from styrofoam pieces.

[5] No fungal growth from sampled styrofoam pieces.

Table 2. *Effects of hot air treatments for varying time periods on colonization of styrofoam containers by selected fungi.*

Fungus[1]	Percent colonization[2]											
	10 minutes				20 minutes				60 minutes			
	Dry		Wet[3]		Dry		Wet[3]		Dry		Wet[3]	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Fusarium	11a	19a	17a	0b	7a	8a	4a	4a	7a	7a	12a	0b
Cylindrocarpon	2a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a
Trichoderma	28a	36a	8a	4a	42a	46a	4a	0a	37a	33a	4a	0a
Penicillium	22a	19a	33a	4b	28a	13a	37a	0b	27a	17a	29a	0b
Other fungi	66a	65a	75a	0b	55a	52a	83a	12b	62a	51a	79a	8b
No fungi	0a	0a	0a	87b	0a	0a	0a	87b	0a	136	0a	92b

[1] For each fungus, average values comparing pre- and post-treatments followed by the same letter (across columns) are not significantly different (P=0.05) using the Kruskal-Wallis test.

[2] Twenty-four cells sampled per container; the same cells were sampled before (pre) and after (post) treatment.

[3] Container surfaces wetted prior to hot air treatments.

[4] Unidentified fungi isolated from styrofoam pieces.

[5] No fungal growth from sampled styrofoam pieces.

Several *Fusarium* spp. colonized styrofoam containers (table 3). One of the most common *Fusarium* species encountered was *F. proliferatum* (Matsushima) Nirenberg. This species is commonly associated with root diseases of container-grown conifer seedlings (James et al. 1995) and can be an aggressive pathogen (James et al. 1995, 1997). Another common species was *F. sporotrichioides* Sherb., which may or may not be pathogenic on conifer seedlings (James and Perez 1999). Other *Fusarium* species isolated from styrofoam containers included *F. oxysporum* Schlecht., *F. avenaceum* (Fr.) Sacc., *F. acuminatum* Ell & Ev., *F. sambucinum* Fuckel, *F. equiseti* (Corda) Sacc., *F. culmorum* (W.G. Smith) Sacc., and *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas. Several of these *Fusarium* species are potential pathogens on conifer seedlings, whereas others are probably saprophytic (James et al. 1991).

Two *Cylindrocarpon* species were isolated from either styrofoam containers: *C. destructans* (Zins.) Scholten and *C. tenue* Bugn. Both species were encountered at much lower frequencies than *Fusarium* species (tables 1 and 2). *Cylindrocarpon destructans* may be an important pathogen of conifers (Beyer-Ericson et al. 1991; Dahm and Strezelczyk 1987; James et al. 1994), whereas *C. tenue* is usually saprophytic (Booth 1966; James et al. 1994).

Table 3. Fusarium *species colonizing styroblock containers tested with hot air treatments.*

***Fusarium* Species**	**Percent of *Fusarium* species**	
	RFW treatment	**Hot air treatment**
F. proliferatum	87	25
F. sporotrichioides	8	14
F. acuminatum	1	31
F. oxysporum	3	10
F. solani	0	4
F. avenaceum	1	6
F. equiseti	0	8
F. culmorum	1	0
F. subglutinans	1	0

Use of RFWs on wet containers effectively removed potentially pathogenic fungi from reused styrofoam containers at lower temperatures than required for hot water immersion. Although not all potentially pathogenic *Fusarium* propagules were killed by the wet RFW treatment, sufficient inoculum was reduced from used containers to greatly limit disease potential in future seedling crops (James et al. 1988). Apparently, RFWs heated the thin water film on containers to sufficient temperatures to kill fungal propagules. It is possible that exposure to RFWs for a shorter time period might be just as effective as the 2 minute exposure evaluated in this test. Because the dry treatments were ineffective, there was no indication in our tests that the RFWs themselves were toxic to pathogen propagules

The major disadvantage of wet RFW treatments is the cost of equipment. The oven and conveyor system required is much more expensive than existing hot water immersion tank systems. However, lower energy costs required for the RFW system as compared to hot water immersion may help offset the high initial equipment costs. In any event, our results indicated that wet RFW treatments may provide a suitable alternative to standard hot water immersion for cleaning reused styrofoam containers.

Only a thin film of water was necessary to conduct hot, dry air to where undesirable organisms reside. Heating large amounts of water is unnecessary and more costly than heating equal volumes of air. Therefore, heating air can replace heating large volumes of water to obtain similar efficacy in sanitizing containers, resulting in lower energy costs. However, systems must be designed to reduce heat loss when replacing containers within ovens and container surfaces must be wetted prior to heat exposure.

One question not adequately addressed is the effects of either hot air or hot water immersion treatments on longevity and useful life span of styrofoam containers. One treatment may be more damaging to containers than the other, resulting in another important "cost" of treatment.

Our work indicated that hot air may be as effective as hot water immersion treatments for sanitizing styroblock containers if the containers are wetted prior to treatment and heated under humid conditions.

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