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EFFECTS OF RADIO FREQUENCY WAVES ON FUNGAL COLONIZATION OF STYROBLOCK CONTAINERS

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ABSTRACT

Fungal pathogens tend to accumulate within styroblock containers, which 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 (RFs) to reduce levels of selected groups of fungi within styroblock containers was evaluated. RFs were effective only on containers that had been wetted in warm water prior to treatment. RFs were not effective on dry containers. *Fusarium proliferatum* was the most commonly encountered potentially-pathogenic fungus isolated from containers. Seven 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 RF treatment was as effective as hot water immersion, such treatments may be much more expensive due to high costs of RF equipment.

INTRODUCTION

Forest seedling nurseries growing container seedlings use a variety of containers. One of the most popular types of containers are made of styrofoam. These containers are typically reused several times to produce multiple seedling crops. However, they require sufficient cleaning prior to reuse because they can harbor potentially-pathogenic organisms that may cause important diseases on the new seedling crop (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 styroblock 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 within containers after seedlings are extracted (James et al. 1988; Peterson 1990; Sturrock and Dennis 1988).

Several approaches to cleaning styroblock 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

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et al. 1993). Because of these disadvantages, many nursery growers are looking for alternative, cost-effective techniques for container cleaning. Initially, steam treatment was often used. However, such treatments often did not adequately reduce potentially-pathogenic organisms (James 1987, 1990; James et al. 1988). Therefore, several trials were conducted to evaluate efficacy of immersion in hot water for varying lengths of time. Although time/temperature results varied among the tested nurseries (James 1992; James and Woollen 1989; Peterson 1990, 1991; Sturrock and Dennis 1988), in general exposure of styroblock containers to 60-70°C for about 120 sec. was sufficient to kill most pathogens.

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

One alternative method was to use radio frequency (RF) wave ovens to raise styroblock temperatures sufficiently to kill potential pathogens. Industrial RF ovens are used for baking, curing, and drying many different types of foods and materials. RF 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.

An evaluation was conducted to determine efficacy of RF treatment on reducing populations of selected fungi colonizing styroblock containers from a commercial forest seedling nursery. The goal was to determine if such treatment could kill potentially-pathogenic fungi and thus render containers relatively safe for reuse from a disease potential standpoint.

MATERIALS AND METHODS

Ten styroblock containers that had be used to grow several crops of conifer seedlings were tested. The containers varied in size, number of cells and manufacturer (table 1). 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 at the drainage hole because this is where the highest populations of contaminating fungi, including potential pathogens, tend to congregate (Dumroese et al. 1995; James 1987, 1989b; James and Gilligan 1988a, 1988b). 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. Styroblock "infection" was calculated as the percentage of sampled cells with a particular fungus; "colonization" was calculated as the percentage of sampled styrofoam pieces (two sampled per cell) that were colonized by a particular fungus.

After preliminary sampling, styroblock containers were treated with RF heating in a laboratory test oven (PSC, Inc., Cleveland, OH). The oven operated at 40kW at a frequency of 18MHz and was a parallel plate electrode system with variable electrode heights; the plate voltage was 12kV. The 10 styroblocks were divided into two groups of 5 containers each. Five of the containers (numbers 1-5) were "dry" treated. These containers were placed in the RF oven with electrode heights at either 19.1 or 25.4 cm (table 1) and exposed to the RF field for 2 min. Blocks were then removed and their cell surface temperatures measured with an infrared (IR) sensor. The other 5 containers (numbers 6-10) were "wet" treated. These containers were initially immersed in warm water (29.4°C) for a brief period of time, shaken to remove excess water, and placed in the RF oven with electrode heights at either 19.1 or 25.4 cm (table 1). They were exposed to the RF field for 2 min., removed, and their cell surface temperatures measured with an IR sensor.

After treatment, the styroblock containers were again sampled for fungal colonization with the same cells sampled as before treatment. Two pieces of styrofoam per cell were again sampled as described above. Statistical comparisons between pre-and post-treatment average "infection" and average "colonization" were made for the "dry" and "wet" treated containers among several different groups of fungi. Comparisons were made using the non-parametric test of Kruskal-Wallis (Ott 1984).

Table 1. Container and treatment characteristics for testing efficacy of RF heating for sterilizing styroblock containers.

Container Number	Treatment[1]	Electrode Height[2]	Container Type	Container Height[3]	Manufacturer	Initial Temperature[4]	Final Temperature[5]
1	Wet	25.4	160-7	22.9	First Choice	21.1	32.2-37.8
2	Wet	19.1	315B	15.2	First Choice	20.6	35.0-37.8
3	Wet	19.1	160/90	15.2	Beaver	21.1	43.3-48.0
4	Wet	25.4	160-7	22.9	First Choice	21.1	26.7-32.2
5	Wet	19.1	315B	15.2	First Choice	20.0	35.0-43.3
6	Dry	25.4	160-7	22.9	First Choice	20.0	26.7
7	Dry	19.1	315B	15.2	First Choice	21.1	29.4-32.2
8	Dry	19.1	160/90	15.2	Beaver	21.1	29.4-32.2
9	Dry	25.4	160-7	22.9	First Choice	20.0	29.4
10	Dry	19.1	315B	15.2	First Choice	22.2	29.4-35.0

[1] Wet treatment involved immersing container in warm (85oF) water prior to exposure to RF heat.
[2] Height (cm) of electrodes above treated container.
[3] Height (cm) of container
[4] Average temperature (°C) of container surface prior to RF heating.
[5] Average temperature (°C) of container surface just after RF heating.

RESULTS AND DISCUSSION

Effects of wet and dry RF treatments on infection of styroblock containers are summarized in tables 2 and 3, respectively. Effects of the treatments on styroblock colonization are summarized in tables 4 and 5. Basically, the wet treatment significantly reduced level of *Fusarium* and *Cylindrocarpon* infection and colonization of styroblock containers (tables 2 and 4). Levels of *Trichoderma* spp., which may be both saprophytic and potentially-antagonistic toward pathogens such as *Fusarium* and *Cylindrocarpon* (Papavizas 1985), were also significantly reduced. The number of sampled styrofoam pieces that were not colonized by any fungus were significantly greater following wet RF treatments. However, dry RF treatments did not significantly reduce level of potential pathogen (*Fusarium* and *Cylindrocarpon*) or saprophyte (*Trichoderma* and *Penicillium*) colonization (tables 3 and 5).

Table 2. Effects of wet RF treatment on infection of styroblock containers by selected fungi.

Block[1]	*Fusarium*		*Cylindrocarpon*		*Trichoderma*		*Penicillium*		Other Fungi		No Fungi	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	62.5	8.3	4.2	0	12.5	0	0	4.2	79.2	54.2	0	70.8
2	79.2	0	0	0	87.5	8.3	4.2	0	4.2	12.5	0	91.7
3	100.0	4.2	0	0	54.2	8.3	0	0	33.3	41.7	0	75.0
4	62.5	0	0	0	8.3	0	0	16.7	75.0	37.5	0	83.3
5	58.3	16.7	12.5	0	70.8	20.8	33.3	12.5	66.7	70.8	0	33.3
Average[2]	72.5a	5.8b	3.3a	0a	46.7a	7.5b	7.5a	6.7a	51.7a	43.3a	0b	70.8a

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

[2]For each fungus, average values followed by the same letter are not significantly different (P=0.05) using the Kruskal-Wallis test.

Table 3. Effects of dry RF treatment on infection of styroblock containers by selected fungi.

Block[1]	*Fusarium*		*Cylindrocarpon*		*Trichoderma*		*Penicillium*		Other Fungi		No Fungi	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
6	87.5	87.5	0	0	20.8	16.7	0	0	33.3	62.5	0	0
7	79.2	66.7	4.2	0	45.8	37.5	16.7	8.3	20.8	75.0	0	0
8	87.5	87.5	0	0	91.7	79.2	75.0	70.8	4.2	16.7	0	0
9	100.0	91.7	0	0	45.8	41.7	4.2	4.2	4.2	25.0	0	0
10	87.5	95.8	4.2	8.3	50.0	37.5	12.5	12.5	50.0	70.8	0	0
Average[2]	88.3a	85.8a	1.7a	1.7a	50.8a	42.5a	21.7a	19.2a	22.5b	50.0a	0a	0a

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

[2]For each fungus, average values followed by the same letter are not significantly different (P=0.05) using the Kruskal-Wallis test.

Table 4. Effects of wet RF treatment on colonization of styroblock containers by selected fungi.

Block[1]	*Fusarium*		*Cylindrocarpon*		*Trichoderma*		*Penicillium*		Other Fungi		No Fungi	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	41.7	4.2	2.1	0	6.3	0	0	2.1	58.3	39.6	0	54.2
2	56.3	0	0	0	75.0	4.2	2.1	0	2.1	6.3	0	87.5
3	91.7	2.1	0	0	35.4	4.2	0	0	20.8	29.2	0	64.6
4	43.8	0	0	0	4.2	0	0	8.3	58.3	27.1	0	64.6
5	37.5	8.3	6.3	0	56.3	14.6	18.8	6.3	45.8	41.7	0	27.1
Average[2]	54.2a	2.9b	1.7a	0b	35.4a	4.6b	4.2a	3.3a	37.1a	28.8a	0b	59.6a

[1]Twenty-four cells sampled per block; the same cells were sampled before (pre) and after (post) treatment.
[2]For each fungus, average values followed by the same letter are not significantly different (P=0.05) using the Kruskal-Wallis test.

Table 5. Effects of dry RF treatment on colonization of styroblock containers by selected fungi.

Block[1]	*Fusarium*		*Cylindrocarpon*		*Trichoderma*		*Penicillium*		Other Fungi		No Fungi	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
6	77.1	70.8	0	0	12.5	10.4	0	0	25.0	50.0	0	0
7	58.3	54.2	2.1	0	31.3	22.9	12.5	6.3	12.5	66.7	0	0
8	58.3	68.8	0	0	72.9	66.7	52.1	52.1	2.1	8.3	0	0
9	95.8	85.4	0	0	22.9	25.0	2.1	2.1	2.1	18.8	0	0
10	72.9	75.0	2.1	4.2	31.3	22.9	8.3	6.3	58.3	52.1	0	0
Average[2]	72.5a	70.8a	0.8a	0.8a	34.2a	29.6a	15.0a	13.3a	14.2b	39.2a	0a	0a

[1]Twenty-four cells sampled per block; the same cells were sampled before (pre) and after (post) treatment.
[2]For each fungus, average values followed by the same letter are not significantly different (P=0.05) using the Kruskal-Wallis test.

Table 6. Effects of wet RF treatment on colonization of residual seedling roots within styroblock containers by selected fungi.

Block[1]	*Fusarium*		*Cylindrocarpon*		*Trichoderma*		*Penicillium*		Other Fungi		No Fungi	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	20	50	0	0	10	0	10	0	70	40	0	10
2	50	0	30	0	40	0	20	0	0	0	0	100
3	40	0	0	0	10	0	0	0	70	30	0	70
4	20	0	0	0	30	0	0	0	60	30	0	70
5	30	10	20	0	0	0	0	0	50	80	0	10
Average[2]	32a	12b	10a	0b	18a	0b	6a	0b	50a	36a	0b	52a

[1]Ten randomly-selected pieces of residual roots sampled per block both before (pre) and after (post) treatment.
[2]For each fungus, average values followed by the same letter are not significantly different (P=0.05) using the Kruskal-Wallis test.

Table 7. Effects of dry RF treatment on colonization of residual seedling roots within styroblock containers by selected fungi.

Block[1]	*Fusarium*		*Cylindrocarpon*		*Trichoderma*		*Penicillium*		Other Fungi		No Fungi	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
6	20	10	0	0	0	0	0	0	80	70	0	20
7	80	40	0	0	0	10	0	0	20	80	0	0
8	50	60	10	0	0	30	10	30	50	40	0	0
9	70	60	0	0	40	10	0	0	0	40	0	0
10	40	50	30	0	20	30	0	20	30	60	0	0
Average[2]	52a	44a	8a	0b	12a	16a	2b	10a	36b	58a	0a	4a

[1]Ten randomly-selected pieces of residual roots sampled per block both before (pre) and after (post) treatment.

[2]For each fungus, average values followed by the same letter are not significantly different (P=0.05) using the Kruskal-Wallis test.

The wet RF treatment also significantly reduced the level of residual root colonization by both *Fusarium* and *Cylindrocarpon* spp. (table 6); wet treatment also reduced root colonization by the common saprophytes *Trichoderma* and *Penicillium* and resulted in significant increases in the number of sampled roots that were not colonized by any fungus (table 6). However, the dry treatment did not significantly reduce levels of *Fusarium* root infection (table 7). Likewise, root colonization by other fungi (most notably *Trichoderma* spp.) were also not affected by this treatment. Several *Fusarium* spp. colonized both the styroblock containers and residual roots within the containers (tables 8 and 9, respectively). By far the most common *Fusarium* species encountered was *F. proliferatum* (Matsushima) Nirenberg. This species is commonly associated with root diseases of container-grown seedlings (James et al. 1995) and can be an aggressive pathogen under the right environmental conditions (James et al. 1995, 1997). The next most common species was *F. sporotrichioides* Sherb., isolates of which may or may not be pathogenic on conifer seedlings (James and Perez 1999). Other *Fusarium* species isolated from styroblock containers included *F. oxysporum* Schlecht., *F. avenaceum* (Fr.) Sacc., *F. acuminatum* Ell & Ev., *F. sambucinum* Fuckel, *F. culmorum* (W.G. Smith) Sacc. and *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas. All these species except the latter two were also isolated from sampled residual roots. Some of these other *Fusarium* species are potential pathogens on conifer seedlings, whereas others are probably saprophytic (James et al. 1991).

Two *Cylindrocarpon* species were isolated from either styroblock containers or residual seedling roots: *C. destructans* (Zins.) Scholten and *C. tenue* Bugn. (tables 8 and 9). Both species were encountered at much lower frequencies than *Fusarium* species. *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).

Effective removal of potentially-pathogenic fungi from reused styroblock containers was achieved at lower temperatures than was required for hot water immersion by treating with RF waves following wetting of containers. Although not all potentially-pathogenic *Fusarium* propagules were killed

by the wet RF treatment, sufficient inoculum was eliminated to greatly reduce the potential for disease in future seedling crops utilizing treated containers (James et al. 1988). Apparently, the RF waves heated the water remaining after wetting containers to sufficient temperatures to kill fungal propagules. It is possible that exposure to RF for a shorter period of time might be just as effective as the two minute exposure evaluated in this test. There was no indication in our tests that the RF waves themselves were toxic to pathogen propagules because the dry treatments were totally ineffective.

Table 8. Effects of wet and dry RF treatment on colonization of styroblock containers by selected *Fusarium* and *Cylindrocarpon* species[1].

Fusarium[2]	Dry Treatment		Wet Treatment	
	Pre	Post	Pre	Post
FPRO	59.2	66.3	50.8	2.9
FSPO	8.3	6.7	1.2	0
FOXY	4.1	0	0	0
FAVE	0.8	0	0	0
FACU	0	0	0.4	0
FSAM	0	0.8	0	0
FCUL	0	0.4	0.8	0
FSUB	0	0	0.8	0
Cylindrocarpon[3]				
CYDE	0.8	0.8	2.1	0

[1]. Values in table are percent of sampled pieces of styrofoam colonized by appropriate fungus.
[2]. FPRO = *F. proliferatum*; FSPO = *F. sporotrichioides*; FOXY = *F. oxysporum*; FAVE = *F. avenaceum*; FACU = *F. acuminatum*; FSAM = *F. sambucinum*; FCUL = *F. culmorum;* FSUB = *F. subglutinans*.
[3] CYDE = *C. destructans*.

Table 9. Effects of wet and dry RF treatment on colonization of residual roots by selected *Fusarium* and *Cylindrocarpon* species[1].

Fusarium[2]	Dry Treatment		Wet Treatment	
	Pre	Post	Pre	Post
FPRO	30	36	26	10
FSPO	2	6	4	0
FOXY	4	0	2	0
FAVE	4	0	0	0
FACU	10	0	0	0
FSAM	2	0	0	2
Cylindrocarpon[3]				
CYDE	8	2	0	0
CYTE	0	0	2	0

[1]. Values in table are percent of sampled root pieces colonized by appropriate fungus.
[2]. FPRO = *F. proliferatum*; FSPO = *F. sporotrichioides*; FOXY = *F. oxysporum*; FAVE = *F. avenaceum*; FACU = *F. acuminatum*; FSAM = *F. sambucinum.*
[3] CYDE = *C. destructans*; CYTE = *C. tenue*.

Effective removal of potentially-pathogenic fungi from reused styroblock containers was achieved at lower temperatures than was required for hot water immersion by treating with RF waves following wetting of containers. Although not all potentially-pathogenic *Fusarium* propagules were killed by the wet RF treatment, sufficient inoculum was eliminated to greatly reduce the potential for disease in future seedling crops utilizing treated containers (James et al. 1988). Apparently, the RF waves heated the water remaining after wetting containers to sufficient temperatures to kill fungal propagules. It is possible that exposure to RF for a shorter period of time might be just as effective as the two minute exposure evaluated in this test. There was no indication in our tests that the RF waves themselves were toxic to pathogen propagules because the dry treatments were totally ineffective.

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

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