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HOT WATER TREATMENTS OF PLASTIC AND STYROFOAM CONTAINERS USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

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ABSTRACT

Hot water immersion was evaluated to determine effects on persistence of potential pathogenic and saprophytic fungi on plastic pine and super cell and styroblock containers at the USDA Forest Service Nursery in Coeur d'Alene, Idaho. Most potentially-pathogenic *Fusarium* and *Cylindrocarpon* spp. were eliminated from plastic containers with immersion in water heated to 66 C for 15 seconds. Species of saprophytes *Phoma, Trichoderma, Penicillium* and *Alternaria* persisted on containers until water temperatures were 82-87 C with exposure periods of 10 seconds. Six *Fusarium* and two *Cylindrocarpon* species were routinely isolated from plastic containers. The most common *Fusarium* spp. were *F. proliferatum* and *F. oxysporum*, important pathogens of conifer seedlings in nurseries. Most potential pathogens were eliminated from styroblock containers with immersion at 79 C for 30 seconds. Operational immersion in hot water has been implemented at the nursery to sterilize reused plastic and styroblock containers.

INTRODUCTION

Production of container-grown conifer seedlings within greenhouses is important in providing reforestation stock in the Northern Region of the Forest Service. More than 4 million container seedlings are produced annually at the USDA Forest Service Nursery in Coeur d'Alene, Idaho. These seedlings are grown in either plastic or styrofoam containers of various capacities; such containers are reused several times for successive seedling crops because of the prohibitive high cost of replacement after each crop.

Root diseases caused by several groups of fungi are important limiting factors in production of container-grown seedlings at the nursery. Pathogens causing these diseases are often introduced on seed (James 1987) and reused containers (James et al. 1988). Pathogens may also reside in growing media (Coyier 1978; James 1985a) and within the interior of greenhouses on benches, floors and the roots of weeds (Chase 1993; James et al. 1987). Potentially pathogenic fungi often produce resting structures that remain viable for prolonged periods when susceptible host material is unavailable. These structures may reside on the inner surface of containers following seedling extraction (James and Gilligan 1988; James et al. 1988). Pathogens also colonize seedling roots and other organic debris that may remain in containers following

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seedling extraction (James et al. 1988; Peterson 1990; Shrimpton 1992)., When a new crop of seedlings are sown in contaminated containers, infection by resident pathogenic fungi may occur when roots penetrate into the zone where propagules occur.

Growers have traditionally washed containers after use, primarily to dislodge adhering growing media and other organic debris left behind after seedling extraction (Baker 1991). However, most methods were largely superficial and did not adequately reduce levels of potential pathogens carried within containers (James et al. 1988; Peterson 1990). Without proper sterilization, high numbers of pathogens could be introduced into new seedling crops on contaminated containers.

Several efforts to improve container sterilization have identified certain chemicals (Dumroese et al. 1993; James and Woollen 1989; Peterson 1990) and hot water (James 1992; James and Woollen 1989; Sturrock and Dennis 1988) as potential ways of treating containers. Unfortunately, chemicals that may be efficaceous are often extremely toxic and are difficult to dispose of (Dumroese et al. 1993). Probably the most promising technique for sterilizing containers is to immerse them in hot water for prescribed time periods (James et al. 1988; Peterson 1990; Shrimpton 1992; Sturrock and Dennis 1988). Immersion brings pathogen propagules into direct contact with lethal hot water and renders them non-germinative. Major problems with hot water treatments include maintenance of high enough water temperatures for prolonged time periods and deterioration of containers after exposure to hot water.

Root disease caused primarily by *Fusarium* (James 1985b, 1993) and *Cylindrocarpon* (James 1991a, 1991b) spp. have often caused problems at the Coeur d'Alene Nursery. Standard steam cleaning of containers after use has largely been ineffective in removal of most fungal inoculum. Therefore, hot water immersion treatments were evaluated to determine time/temperature exposures for adequate sterilization.

MATERIALS AND METHODS

Thin wall plastic cells and polystyrene block containers were tested for effects of hot water immersion on occurrence of selected fungi on inner wall surfaces. Basically, containers were randomly selected for testing. Most tested containers had had their seedlings extracted some time (usually several months) prior to testing. They were immersed in a large vat of hot water for the prescribed time period, collected, and transported to the laboratory for analysis. Water temperatures within the vat were recorded periodically during immersion. Several untreated containers were also selected for assay to serve as checks. Tests were conducted over several months.

Generally, four pieces of container per cell were aseptically extracted from the botton of containers. Container pieces were placed on an agar medium selective for *Fusarium* and closely related species (Komada 1975). Plates were incubated under diurnal cycles of cool, fluorescent light at about 24 C for 7-10 days. Emerging fungi were identified to genus and selected *Fusarium*, *Cylindrocarpon*, and *Phoma* isolates were transferred to potato dextrose, oatmeal, and carnation leaf (Fisher et al. 1982) agar for identification. Several taxonomid guides were used to aid identification of fungal species (Booth 1966; Dorenbosch 1970; Nelson et al. 1983). Percent colonization of treated and untreated container cells by selected fungi were calculated.

RESULTS AND DISCUSSION

The major groups of potentially plant pathogenic fungi that required elimination in hot water treatments were *Fusarium* and *Cylindrocarpon* spp. Most *Fusarium* spp. were eliminated from thin wall plastic containers following treatment at 66 C for 15 seconds or more; higher temperatures at lower time periods were also fairly effective, even though occasionally some

Fusarium and *Cylindrocarpon* were detected at higher temperatures (table 1). *Fusarium* and *Cylindrocarpon* were eliminated from polystyrene block containers treated at about 79 C for 30 seconds (table 2). Although *Phoma* spp. were more persistent within containers, they are not usually important pathogens of container-grown seedlings (James and Hamm 1985), even though they may cause seedling diseases of bareroot crops (James 1979, 1984; James and Hamm 1985). Three other groups of common saprophytic fungi were monitored as well. These included *Trichoderma*, *Penicillium*, and *Alternaria* spp. *Trichoderma* spp. are commonly associated with container-grown seedlings, but are likely either saprophytic or may be antagonists of potential pathogens, particularly *Fusarium* spp. (Papavizas 1985). *Penicillium* and *Alternaria* spp. are general saprophytes that colonize dead, organic matter. These latter three groups of fungi were more persistent on containers than potential pathogens, indicating that fungi that are desirable to remove from containers are usually more susceptible to hot water treatments than other, non-pathogenic fungi.

Six *Fusarium* spp. were isolated from pine cell containers (table 1). The most common was *F. proliferatum* (Matushsima) Nirenberg, which is a common pathogen of container-grown seedlings (James et al. 1995). This species most commonly causes disease problems toward the end of the growing cycle and is especially damaging when seedlings are stressed to stop growth and set buds (James et al. 1987). Although *F. proliferatum* does not produce chlyamdospore resting structures common on other *Fusarium* spp. (Nelson et al. 1983), it apparently remains viable for long time periods on roots and organic debris left after seedling extraction. This pathogen is not commonly seedborne at the Coeur d'Alene Nursery (James 1987; James et al. 1987), nor is it isolated frequently from bareroot seedlings (James et al. 1991, 1995). *Fusarium proliferatum* routinely produces long chains of microconida that when dried are easily disseminated by air currents and water (James et al. 1995; Nelson et al. 1983). Therefore, it readily moves around and between greenhouses. The fungus often colonizes non-diseased as well as diseased seedling roots. Usually by the end of the seedling growth cycle, *F. proliferatum* is well distributed throughout greenhouse seedling crops. Selected isolates screened for pathogenicity have been extremely virulent on young Douglas-fir germinants and seedlings (James et al. 1997). Some genetic analysis work has shown that there is much less genetic variability (which may be related to virulence) on *F. proliferatum* compared with some other *Fusarium* species (Gordon and Okamoto 1992; Leslie 1991, 1995). Fortunately, most *F. proliferatum* inoculum was eliminated by immersion of thin wall plastic containers in water at 66 C for 15 seconds (table 1).

Other *Fusarium* spp. isolated from containers included *F. oxysporum* Schlecht., *F. acuminatum* Ell. & Ev., *F. sambucinum* Fuckel, *F. sporotrichioides* Sherb., and *F. tricinctum* (Corda) Sacc. *Fusarium oxysporum* is a well known pathogen of conifer seedlings, both container-grown (James et al 1987; 1991) and bareroot (Bloomberg 1971, 1973; James et al. 1991). It is most commonly associated with damping-off and root disease of container seedlings early in the growth cycle. The fungus is routinely seed-borne and producing resting structures (chlamydospores and sclerotia) that may remain viable for long time periods (James 1987; James et al. 1991). *Fusarium oxysporum* is genetically very diverse (Gordon and Okamoto 1992) with common pathogenic and saprophytic strains. Morphologically, the different strains may appear similar, being difficult to separate (James et al. 1991) . The species is extremely diverse in controlled pathogenicity assays (James et al. 1989). The other *Fusarium* species are likely more saprophytic than pathogenic, although some aggressive strains of *F. acuminatum* and *F. sporotrichioides* may affect conifer seedlings (James and Gilligan 1984; Rathbun 1922). Therefore, it is important not to label all *Fusarium* as "bad", but to more clearly evaluate which species are most commonly

represented to determine potential hazard of these fungi.

Cylindrocarpon spp. have often been associated with different conifer seedling diseases, including those affecting container-grown seedlings (James 1991a, 1991b). In some cases, diseases have been severe, causing extensive damage (James 1991b). However, *Cylindrocarpon* spp. are often not very aggressive pathogens (Booth 1966; James et al. 1994), but rather are common colonizers of root rhizospheres (Booth 1966; James et al. 1994) and may cause disease when hosts are stressed (James et al. 1994). The two species isolated from plastic containers were *C. destructans* (Zins.) Scholten and *C. tenue* Bugn.; both species have been implicated in conifer seedling diseases, but are not considered as important as *Fusarium* spp. as potential pathogens (James et al. 1994).

Two species of *Phoma* were likewise isolated from plastic containers: *P. eupyrena* Sacc. and *P. herbarum* Westend. The former species has most often been associated with conifer seedling diseases (James and Hamm 1985), especially with tip dieback of bareroot seedlings (James 1979, 1984; James and Hamm 1985). *Phoma herbarum* is usually not considered an important conifer seedling pathogen, although little work has been done to confirm this conclusion.

CONCLUSIONS

This evaluation showed that hot water immersion is the best may to ensure that potentially-pathogenic fungi are eliminated from both plastic and styroblock containers before reuse. This procedure has been adapted throughout the container seedling industry (Peterson 1990; Shrimpton 1992; Sturrock and Dennis 1988). In most cases, growers use hot water alone or in combination with soap, which tends to help remove surface tension and ensure penetration of water into styroblock walls (Peterson 1990). Some growers may still use some chemical sterilizants (Peterson 1990; Sturrock and Dennis 1988), but in most cases, water alone is sufficient if kept at high enough temperatures (James and Woolen 1989; Peterson 1990). Problems have developed designing the proper apparatus to clean many containers quickly while keeping water temperatures high enough (Peterson 1990; Sturrock and Dennis 1988). However, commercial machinery has become recently available that works quite well. At the Coeur d'Alene Nursery, a large vat is filled with water heated with a boiler. A forklift is used to lift containers in and out of the water. This cost-effective procedure has worked well for several years.

An important part of any integrated pest management system in conifer seedling greenhouses will be maintaining a growing environment where pathogen levels are kept very low. Sterilizing growing media and containers has recently become important in maintaining such an environment (Baker 1991; Chase 1993). Likewise, ensuring that greenhouse interiors are clean and that seedlots are properly treated before sowing will help reduce introduction of pathogen inoculum into seedling crops. Most success in disease control in greenhouses is achieved by prevention rather than trying to control disease once it occurs (Jarvis 1989).

Table 1. Effects of hot water immersion on persistence of selected fungi on plastic pine and super cell containers - USDA Forest Service Nursery, Coeur d'Alene, Idaho.*

		Temperature (degrees C) Time (seconds)									
Fungus	Check	37.8 10	43.3 10	48.9 10	54.4 10	60.0 5	60.0 10	60.0 15	65.6 5	65.6 10	65.6 15
All *Fusarium*	44.0	64.6	41.7	52.1	52.1	43.8	17.7	10.4	8.4	50.0	0
F. proliferatum	24.5	52.1	20.8	16.7	39.6	37.5	10.4	1.0	0	37.5	0
F. oxysporum	10.2	8.3	14.6	8.3	12.5	6.3	5.2	4.2	0	10.4	0
F. acuminatum	7.9	0	0	16.7	0	0	1.0	2.1	0	0	0
F. sambucinum	1.4	0	0	6.3	0	0	1.0	0	0	0	0
F. sporotrichioides	0	4.2	8.3	8.3	0	0	0	1.0	2.1	0	0
F. tricinctum	0	0	0	0	0	0	0	0	6.3	0	0
All *Cylindrocarpon*	5.5	0	4.2	0	0	0	0	4.2	12.5	0	0
C. destructans	4.2	0	4.2	0	0	0	0	4.2	4.2	0	0
C. tenue	2.8	0	0	0	0	0	0	0	8.3	0	0
All *Phoma*	26.4	10.4	6.3	16.7	2.1	31.3	13.5	11.5	41.7	27.1	43.8
P. eupyrena	4.2	4.2	2.1	0	0	12.5	1.0	0	0	4.2	6.3
P. herbarum	22.2	6.3	4.2	16.7	2.1	18.8	12.5	11.5	41.7	22.9	37.5
Trichoderma	47.2	50.0	83.3	68.8	83.3	33.3	27.1	54.2	16.7	12.5	18.8
Penicillium	41.2	6.3	16.7	27.1	27.1	54.2	34.4	21.9	2.1	16.7	25.0
Alternaria	1.4	0	0	0	0	0	2.1	2.1	14.6	35.4	12.5
No Fungi	0.9	0	0	0	0	0	35.4	16.7	16.7	0	12.5
Number of Samples	216	48	48	48	48	48	96	48	48	48	16

*Percent of sampled container pieces colonized with particular fungus (four pieces sampled per cell).

Table 1. (continued). Effects of hot water immersion on persistence of selected fungi on plastic pine and super cell containers - USDA Forest Service Nursery, Coeur d'Alene, Idaho.*

	Temperature (degrees C) Time (seconds)										
Fungus	65.6 30	66.1 30	68.3 15	68.3 30	70.0 15	71.1 5	71.1 10	71.1 15	71.1 30	71.1 45	71.1 60
All *Fusarium*	0	12.5	12.5	0	0	0	17.2	0	0	0	2.5
F. proliferatum	0	0	0	0	0	0	13.3	0	0	0	0
F. oxysporum	0	0	0	0	0	0	0	0	0	0	0
F. acuminatum	0	25.0	12.5	0	0	0	0	0	0	0	0
F. sambucinum	0	0	0	0	0	0	6.2	0	0	0	2.5
F. sporotrichioides	0	0	0	0	0	0	0	0	0	0	0
F. tricinctum	0	0	0	0	0	0	0	0	0	0	0
All *Cylindrocarpon*	0	0	0	0	0	2.1	0	0	0	0	0
C. destructans	0	0	0	0	0	0	0	0	0	0	0
C. tenue	0	0	0	0	0	2.1	0	0	0	0	0
All *Phoma*	16.7	0	25.0	25.0	0	8.3	11.0	20.0	8.3	12.5	2.5
P. eupyrena	0	0	0	0	0	0	0.8	0	0	0	0
P. herbarun	16.7	0	25.0	25.0	0	8.3	10.2	20.0	8.3	12.5	2.5
Trichoderma	58.3	25.0	0	58.3	0	8.3	36.7	30.0	0	0	7.5
Penicillium	20.8	83.3	75.0	29.2	50.0	2.1	21.1	22.5	10.4	5.0	0
Alternaria	8.3	0	25.0	16.7	25.0	14.6	16.4	27.5	27.1	2.5	0
No fungi	8.3	8.3	12.5	8.3	25.0	70.8	26.6	20.0	72.9	82.5	80.0
Number of Samples	24	12	8	24	4	48	128	40	48	40	

*Percent of sampled container pieces colonized with particular fungus (four pieces sampled per cell).

Table 1 (continued). Effects of hot water immersion on persistence of selected fungi on plastic pine and supper cell containers - USDA Forest Service Nursery, Coeur d'Alene, Idaho.*

	Temperature (degrees C) Time Seconds										
Fungus	71.1 180	71.1 300	73.3 10	73.9 10	73.9 15	75.6 10	76.7 10	76.5 15	79.4 15	80.0 10	87.8 10
All *Fusarium*	2.5	0	0	0	0	0	0	0	0	4.2	0
F. proliferatum	2.5	0	0	0	0	0	0	0	0	4.2	0
F. oxysporum	0	0	0	0	0	0	0	0	0	0	0
F. acuminatum	0	0	0	0	0	0	0	0	0	0	0
F. sambucinum	0	0	0	0	0	0	0	0	0	0	0
F. sporotrichioides	0	0	0	0	0	0	0	0	0	0	0
F. tricinctum	0	0	0	0	0	0	0	0	0	0	0
All *Cylindrocarpon*	0	0	0	0	0	0	0	0	0	0	0
C. destructans	0	0	0	0	0	0	0	0	0	0	0
C. tenue	0	0	0	0	0	0	0	0	0	0	0
All *Phoma*	0	2.5	25.0	31.3	6.3	25.0	35.0	25.0	31.3	41.7	0
P. eupyrena	0	0	0	0	0	0	0	0	0	4.2	0
P. herbarum	0	2.5	25.0	31.3	6.3	25.0	35.0	25.0	31.3	37.5	0
Trichoderma	0	0	87.5	51.3	50.0	25.0	20.0	0	62.5	4.2	25.0
Penicillium	0	2.5	25.0	37.5	31.3	25.0	20.0	20.0	18.8	29.2	50.0
Alternaria	0	0	0	37.5	12.5	0	30.0	40.0	12.5	0	25.0
No Fungi	97.5	92.5	0	0	18.8	25.0	22.5	20.0	0	25.0	0
Number of Samples	40	40	8	16	16	4	40	20	16	24	8

*Percent of sampled container pieces colonized with particular fungus (four pieces sampled per cell).

Table 2. Effects of hot water immersion on persistence of selected fungi on styroblock containers - USDA Forest Service Nursery, Coeur d'Alene, Idaho.*

	Temperature (degrees C)/Time (seconds)			
Fungus	Check	79.4/30	80.0/60	80.0/90
Fusarium	0	0	3	0
Cylindrocarpon	3	0	0	0
Phoma	93	10	3	0
Trichoderma	35	5	5	0
Penicillium	5	0	5	5
No Fungi	0	85	85	95
No. Samples	40	40	40	40

* Percent of sampled container pieces colonized with particular fungus (10 cells per styroblock; 4 pieces per cell sampled).

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