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FUNGAL DISTRIBUTION WITHIN PLASTIC SUPER CELL® CONTAINERS USDA FOREST SERVICE LUCKY PEAK NURSERY, BOISE, IDAHO

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ABSTRACT

An evaluation was conducted to quantify distribution of fungi, including those capable of causing seedling diseases, on the inner surfaces of plastic Super Cell® containers that had been previously used to grow seedling crops at the USDA Forest Service Lucky Peak Nursery near Boise, Idaho. *Fusarium* spp. and *Botrytis cinerea* were the most commonly-found potential pathogens on containers. Higher levels of pathogenic fungi were concentrated at or near the bottom of containers. Several different saprophytes were also common, including *Trichoderma, Phoma*, and *Penicillium* spp. Containers will require sterilization prior to being reused to grow future seedling crops at the nursery.

INTRODUCTION

The USDA Forest Service Lucky Peak Nursery near Boise, Idaho produces primarily bareroot seedlings for reforestation of Forest Service administered lands. In the past, most of the production was conifer seedlings. However, recently the nursery has begun to diversify, producing alternative crops including brush species (sagebrush, bitterbrush) , native grasses, and limited hardwood seedlings. In order to accommodate diverse plant species, growers at the nursery plan to grow selected crops within containers in a new greenhouse facility.

Plastic Super Cell® containers have been previously used at the nursery and provide several advantages for growing specific seedling stock. Ordinarily,

containers are reused to grow several crops; prior to reuse, they are cleaned to reduce potential for transmission of pathogens to new seedling crops (James et al. 1988; Peterson 1990, 1991; Sturrock and Dennis 1988). Potentially-pathogenic organisms often persist on the inner walls of container cells unless removed by adequate sanitation treatments (James et al. 1988; Sturrock and Dennis 1988). Several types of cleaning have traditionally been used, including steam treatment, chemical sterilization (Dumroese et al. 1993; James and Sears 1990), and, most recently, hot water immersion treatments (James 1992; James and Woollen 1989; Peterson 1990). Plastic containers have often been easier to sterilize than styroblock containers (James 1990; James et al. 1988).

In order to develop a viable container operation at the Lucky Peak Nursery, satisfactory cleaning/sterilization methods will be required for reused containers. Growers were initially interested in potential for colonization of containers by fungi capable of causing diseases on container-grown stock. Therefore, an evaluation was conducted to determine which potentially-pathogenic fungi were present on used containers, their populations and distribution throughout individual cells.

MATERIALS AND METHODS

The first test involved 5 randomly-selected white Super Cell® containers that had been previously used at the nursery for seedling production. In a subsequent test, 3 black Super Cell® containers returned to the nursery from clients following seedling outplanting were evaluated. Containers measured 5 cm in diameter at the top and were 18 cm high. Samples approximately 5 mm^2 were aseptically extracted from four locations within the containers: from the bottom drainage hole, and 5, 10, and 15 cm above the bottom hole. Container pieces were aseptically dissected from each of the 4 cardinal directions at each sample location (16 sample pieces per container). Sample pieces were placed directly on a selective agar medium for *Fusarium* and closely-associated fungi (Komada 1975). Plates were incubated under diurnal cycles of cool, fluorescent light at about 24°C for 7-10 days and examined for fungi colonizing container pieces. Colonizing fungi were identified to genus and selected isolates were single spored and transferred to carnation leaf agar (Fisher et al. 1982) and potato dextrose agar for identification to species. *Fusarium* spp. were identified using the taxonomy of Nelson et al. (1983), while *Cylindrocarpon* spp. were identified based on descriptions by Booth (1966).

Residual portions of the black containers which were not sampled were placed in a beaker with 500 ml of sterile, distilled water. Pieces were thoroughly mixed with water to remove adhering organic matter. One ml of the organic matter/water mixture was aseptically placed on each of 5 plates of Komada's medium and incubated as described above. Fungi colonizing organic matter were identified.

RESULTS AND DISCUSSION

The major potentially-pathogenic fungi colonizing white Super Cell® containers were *Fusarium* spp. (table 1). These fungi colonized nearly one quarter of the sampled container pieces. As in previous evaluations (James 1989b; James and Gilligan 1988a, 1988b), *Fusarium* spp. were concentrated at the bottom of containers rather than being equally distributed throughout their inner surface. The most common *Fusarium* species isolated from the white containers was *F. solani* (Mart.) Appel & Wollenw.; other isolated *Fusarium* species included *F. oxysporum* Schlecht. and *F. proliferatum* (Matsushima) Nirenberg, both of which were found at much lower levels. No *Fusarium* spp. were isolated from the inner surfaces of the black containers, although very low levels of *F. proliferatum* were isolated from organic matter obtained from these containers (table 2). All three *Fusarium* species isolated from containers or organic matter are potential pathogens of conifer seedlings (James et al. 1991). Most isolates of *F. proliferatum* are aggressive pathogens (James et al. 1995, 1997), whereas isolates of *F. solani* and *F. oxysporum* may be either pathogenic or saprophytic (James and Perez 2000; James et al. 2000).

The high levels of *Botrytis cinerea* (Pers.) ex. Fr. on the black containers (table 2) was interesting since these pathogens of container-grown crops were not isolated from the white containers. *Botrytis cinerea* is mostly pathogenic on foliage and other above-ground portions of crops (James 1984). The fungus produces resting structures (sclerotia) and readily colonizes organic matter (Coley-Smith 1980). This allows the pathogen to remain viable for extended periods between susceptible crops (Coley-Smith 1980; James 1984). Apparently, *B. cinerea* remained viable on the inner surfaces of the black containers by colonizing organic matter that remained after seedlings were extracted.

Table 1. Colonization of white Super Cell® containers by selected fungi – USDA Forest Service Lucky Peak Nursery, Boise, Idaho[1].

Sample Location[2]	*Fusarium*[3]					Other Fungi[4]
	FSOL	FOXY	FPRO	All Fus	*Trichoderma*	
Bottom	55	0	0	55	65	25
5 cm	10	0	0	10	65	40
10 cm	0	5	5	10	55	35
15 cm	5	5	0	10	60	40
All Pieces	17.5	2.5	1.2	21.2	61.2	35.0

[1] Values in table are percent of sampled pieces (four at each location per container; 16 total pieces per container) colonized with appropriate fungus.
[2] Sample locations in reference to the bottom (drainage hole) of containers.
[3] *Fusarium* spp.: FSOL = *F. solani*; FOXY = *F. oxysporum*; FPRO = *F. proliferatum*.
[4] Includes *Phoma* spp., *Cylindrocarpon destructans*, *C. tenue*, *Penicillium* spp., and various species of Mucorales.

Table 2. Colonization of black Super Cell® containers by selected fungi – USDA Forest Service Lucky Peak Nursery, Boise, Idaho[1].

Sample Location[2]	*Fusarium proliferatum*	*Trichoderma* spp.	Other Fungi[3]	*Botrytis cinerea*	No Fungi
Bottom	0	0	25.0	91.7	0
5 cm	0	16.7	33.3	41.7	16.7
10 cm	0	0	16.7	66.7	16.7
15 cm	0	25.0	33.3	50.0	8.3
All Pieces	0	10.4	27.1	62.5	10.4
Org. Matter[4]	0.4	14.0	0	3.8	-

[1] Values in table (except organic matter) are percent of sampled pieces (four at each location per container; 16 total pieces per container) colonized with appropriate fungus.
[2] Sample locations in reference to the bottom (drainage hole) of containers.
[3] Includes *Phoma* spp., *Cylindrocarpon tenue*, *Penicillium* and various species of Mucorales.
[4] Organic matter from water suspensions; values are colonies of appropriate fungi/ml of solution.

Other fungi isolated from both white and black containers included *Cylindrocarpon destructans* (Zins.) Scholten, *C. tenue* Bugn., *Trichoderma* spp., *Phoma* spp., *Penicillium* spp. and several unidentified species in the order Mucorales. Most of these are common saprophytic organisms living on dead organic matter. The possible exception is *Cylindrocarpon* spp. which may be slightly-moderately pathogenic under certain nursery conditions (Beyer-Ericson et al. 1991; Dahm and Strezelczyk 1987; James et al. 1994). In addition, some species of *Trichoderma* may be antagonistic toward nursery pathogens such as *Fusarium* and *Cylindrocarpon*, and are therefore desirable colonizers of containers (Papavizas 1985).

This work indicated that steps are required to adequately clean used Super Cell® containers before being used to grow future crops of nursery seedlings in order to prevent introducing potential pathogens on the new crop. Although pathogens may persist on the containers, they usually can be satisfactorily killed by immersing containers in hot water (82°C for 120 sec.) after loose organic matter is removed (James 1992; James and Woollen 1989; Peterson 1990; Sturrock and Dennis 1988). Chemical sterilization may also be satisfactory, but is usually not as desirable as hot water treatment because of potential problems with worker exposure to and disposal of toxic chemicals (Dumroese et al. 1993). Pressurized steam treatment sprays are usually not adequate, primarily because much pathogen inoculum resides well inside container cavities, often at the bottom of cavities (James et al. 1988).

It is recommended that adequate sterilization of reused containers be implemented at the Lucky Peak Nursery. Hot water immersion seems to be the best current method available for most nurseries and should adequately remove potential pathogens and protect future seedling crops. New techniques including heating containers with radio frequency waves (James and Trent 2001b) and dry heat (James and Trent 2001a) may be as effective as hot water

immersion and could be available for operational use in the near future.

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