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FUSARIUM POPULATIONS WITHIN PEAT-BASED GROWING MEDIA USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

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ABSTRACT

Peat-based container growing media used in 2004 for tree improvement and production stock at the USDA Forest Service Coeur d'Alene Nursery contained high populations of *Fusarium*, particularly *F. oxysporum*. Using contaminated media resulted in disease of western white pine tree improvement trees, but did not cause abnormally high levels of disease in conifer production stock. This may have been due to relatively large populations of *Trichoderma* spp., also detected in the media. Since peat-based growing media has not normally contained high populations of potentially-pathogenic fungi in the past, it is important to determine if further shipments of media from the applicable manufactures are contaminated. If so, steps should be taken to reduce future contamination.

INTRODUCTION

Growing media used for the production of container forest seedlings in nurseries is usually composed of sphagnum peat moss plus other organic or inorganic materials such as vermiculite, perlite, sawdust, or tree bark (James 1985; Sutherland et al. 1989). Most nurseries obtain this media commercially from companies that mix components and package the mixed media for direct use. Commercially-prepared growing media is not necessarily treated to eliminate potential pathogenic fungi that may have contaminated one or more of its components. Some companies routinely treat media with aerated steam to reduce potential for harboring pathogens; such treatments often kill pathogenic fungi while allowing other microorganisms, particularly spore-forming bacteria, to

survive (James 1985). Steam-treated growing media may be more pathogen suppressive because surviving bacteria tend to be antagonistic toward common nursery pathogens.

Most nursery growers have not encountered problems with pathogen contamination of peat-based growing media. Previous direct assays of media have indicated high levels of common saprophytes, such as *Trichoderma* and *Penicillium* spp., but usually no, or very low levels of pathogens (James 2005).

During 2004 at the USDA Forest Service Nursery, Coeur d'Alene, Idaho, unusually high levels of disease in container-grown western white pine (*Pinus monticola* Dougl.) stock being grown for tree improvement purposes was noticed. Although some level of disease usually occurred in the past, disease severity was unusually high on some stock. Growers wanted to know which potentially-pathogenic fungi were associated with decline and mortality of this container stock. Because disease levels were much higher than normal, growers were also interested if the peat-based growing media being used was contaminated with potentially-pathogenic fungi. This pre-packaged media was from a new source and used throughout the nursery on most container stock. Therefore, an evaluation was conducted to help answer these grower concerns.

MATERIALS AND METHODS

Isolations were made from declining tree improvement white pine stock and from container growing media being used to grow tree improvement and production stock. In addition, isolations were made from grit used to cover seed following sowing in container operations.

Three young white pine trees grown in one gallon plastic pots displaying decline symptoms (chlorotic foliage and dieback of branch terminals) were selected for analysis. Trees were extracted carefully from containers and their roots washed thoroughly to remove adhering growing media. Several lateral roots per tree were randomly selected and dissected into pieces approximately 5 mm in length. Sampled root pieces were from throughout the attached root system. Root pieces were surface sterilized in 0.525% aqueous sodium hypochlorite (10% household bleach solution), rinsed in sterile water and placed on selective agar media for *Fusarium* and related organisms (Komada 1975) and V-8 juice agar amended with several antibiotics (James et al. 1990b; Stone et al. 1995) for detection of Oomycete pathogens (*Pythium* and *Phytophthora*). Plates with Komada's medium were incubated under diurnal cycles of cool, fluorescent light at about 24°C for 7 days. Selected emerging fungi were transferred to carnation leaf agar (Fisher et al. 1982) and potato dextrose agar for identification using the taxonomy of Nelson et al. 1983. Plates with V-8 juice agar were incubated in the dark at about 24°C for three days. Selected isolates growing within the medium were transferred to water agar, identified to genus using agar/water slants, and identified to species using the taxonomy of Waterhouse 1968. Percent of sampled root pieces colonized by selected fungi were calculated.

Analyses of potential pathogen populations within peat-based growing media were conducted using a modified dilution technique. The growing media was composed of standard sphagnum peat mixed with non-composted sawdust material from wood-processing mills. The media had not been steam treated. Four samples were analyzed from media being used for tree improvement plantings; two of these samples were collected directly from bagged media and two were screened to remove larger pieces of wood that were present. Five samples were randomly collected from bagged media being used to grow production container stock. Each sample was collected from a different bag. For each media sample, 1.25g were mixed with 200 ml of 0.1% water agar in a blender. One ml of the mixture was placed on each of three plates of selective agar media (Komada and V-8 juice). Plates were incubated and selected fungal isolates identified as described above. Approximately 5 g of each sample were dried in an oven at about 100°C for 24 hrs. to determine oven-dry weight for standardization of population calculations. Populations of *Fusarium* and Oomycetes were calculated as number of colony-forming units (cfu) per gram of oven-dried media.

Five collections of fine rock grit used to cover seeds following sowing of production container seedlings were made. Samples were selected that displayed superficial fungal sporulation (orange to pink sporodochia) and collected directly from containers. Pieces of grit with fungal growth were placed directly on Komada's medium. Plates were incubated and selected fungal isolates identified as described above.

RESULTS

Declining container-grown western white pine trees used for tree improvement had roots extensively colonized with either *Fusarium oxysporum* Schlecht. or *Cylindrocarpon destructans* (Zins.) Scholten (table 1). Low levels of another *Cylindrocarpon* species (*C. tenue* Bugn.) and *Pythium irregulare* Buisman were also isolated from some trees.

Some samples of peat-based container media used for growing of both tree improvement and production container stock were contaminated with high populations of *Fusarium* (tables 2 and 3). Both *F. oxysporum* and *F. solani* (Mart.) Appel & Wollenw. were frequently isolated from growing media. *Trichoderma* spp., which may ameliorate potential pathogens by exhibiting antagonism and/or mycoparasitism (Papavizas 1985), were also found at high levels is most media samples. The only other group of fungi that was commonly isolated from media was *Penicillium* spp.

Table 1. Colonization of roots from tree improvement container-grown western white pine trees with potentially-pathogenic fungi - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Tree Number	Percent Root Colonization[1]			
	FOXY[2]	CYDE[3]	CYTE[4]	*Pythium*[5]
1	82.2	11.1	31.1	0
2	11.1	95.5	0	38.1
3	11.1	97.7	0	4.8
Averages	34.8	68.1	10.4	14.3

[1]Based on a random sample of 45 (*Fusarium, Cylindrocarpon*) and 21 (*Pythium*) root pieces per tree.
[2]*Fusarium oxysporum*
[3]*Cylindrocarpon destructans*
[4]*Cylindrocarpon tenue*
[5]*Pythium irregulare*

Table 2. Colonization of peat-based container media used to grow tree improvement stock by selected fungi - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Sample		Average Fungal Population (cfu/g)		
Number	Description	*Fusarium*[1]	*Trichoderma*	T/F Ratio[2]
1	Super Cell Mix #1	2945	4426	1.50
2	Super Cell Mix #2	1967	3222	1.64
3	Screen Mix #1	0	5280	0
4	Screen Mix #2	30	5684	189.47

[1]From a sample of 292 isolates, 99.3% were *F. oxysporum* and 0.7% were *F. solani*.
[2]Ratio of *Trichoderma* to *Fusarium* populations; higher numbers indicate greater potential for disease suppressiveness by *Trichoderma* spp.

Table 3. Colonization of peat-based container media used to grow production container stock by selected fungi - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Sample Number	Average Fungal Population (cfu.g)[1]			
	Fusarium	*Trichoderma*	T/F Ratio[2]	*Penicillium*
1	1867	3627	1.94	0
2	2880	4053	1.41	0
3	0	1280	0	853
4	267	3840	14.38	1067
5	0	0	0	1867

[1]From a sample of 94 isolates, 44.7% were *F. oxysporum* and 55.3% were *F. solani*.
[2].Ratio of *Trichoderma* to *Fusarium* populations; higher numbers indicate greater potential for disease suppressiveness by *Trichoderma* spp.

DISCUSSION

Peat-based growing media are commonly used to grow a wide variety of container plants. Media contains sphagnum peat moss, often mixed with either other organic matter (sawdust, bark residues) or inorganic materials (vermiculite, perlite). Media is often premixed by manufacturers so it is ready for immediate filling of containers. Pathogen contamination of peat-based media is unusual (James 1985) because either the media is steam-treated prior to shipment or organic and inorganic sources of media are not contaminated with pathogens.

This is the first report of high populations of potential pathogenic *Fusarium* spp. contaminating container growing media used at the Coeur d'Alene Nursery. Contaminated media probably contributed to the decline of western white pine trees being grown in containers for tree improvement. Similar organisms were isolated from both declining trees and the container medium. *Fusarium* levels were also high in portions of the container media used for production stock at the nursery, even though resulting disease levels were not unusually high. Severe disease levels of production stock may have been prevented by presence of relatively high *Trichoderma* populations in the media, i.e., high *Trichoderma* to *Fusarium* ratios. *Trichoderma* spp. are commonly used as biocontrol agents against fungal plant pathogens, including *Fusarium* spp. (Papavizas 1985). It is also possible that the majority of the *Fusarium* population was comprised of non-pathogenic isolates. Populations of *Fusarium oxysporum* are often made up of genetically diverse individuals, many of which are non-pathogenic on plants (Gordon and Martyn 1997). Although isolates of *Fusarium solani* are frequently encountered in forest nurseries (James et al. 1991; James and Perez 2000), most isolates exhibit low virulence or are non-pathogenic on young conifer germinants (James and Perez 2000).

Although abnormally severe disease did not result from using *Fusarium*-contaminated growing media on the 2004 seedling crop, the presence of such high populations of these potentially-pathogenic fungi within the media is of concern. Using *Fusarium*-contaminated media in the future may result in excessive disease (James 1985; James and Gilligan 1984; James et al. 1990), particularly if pathogenic isolates are present and environmental conditions conducive for pathogen buildup (James and Gilligan 1984). Therefore, it is advisable to continue to monitor media obtained from the same manufacturer, and if high populations of *Fusarium* persist, it may be necessary to steam treat the media prior to use. Steam treatment is not sterilization. Rather, it tends to kill propagules of potential pathogens while not adversely affecting some desirable organisms, such as spore-forming bacteria which may act as potential biocontrol agents (James 1985).

It is important to reduce introduction of inoculum of potential pathogens into container seedling production operations (James et al. 1990a). Peat-based growing media is usually not an important source of pathogen inoculum. When it becomes

so, steps should be taken to limit contamination to reduce potential disease risks.

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