FUNGI ASSOCIATED WITH LONGLEAF PINE CONTAINERS BEFORE AND AFTER CLEANING

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Abstract

Soil was collected from used containers before and after they were cleaned at four nurseries that produce longleaf pine seedlings. The nurseries were located in Florida (FL), Georgia (GA), North Carolina (NC), and Mississippi (MS). The GA and MS nurseries used 5% and 10% bleach (sodium hypochlorite), respectively to clean containers, while the NC nursery used chlorine (5 g/L). The FL nursery did not clean their containers. *Fusarium* spp. were routinely isolated from the residual container soil; no *Pythium* spp. or *Phytophthora spp.* were isolated. The most common *Fusarium* spp. isolated were F. *oxysporum*, *F. proliferatum*, and F. *solani*. The average number of *Fusarium* colony forming units (cfu) per container cavity was much higher for FL (2,752 cfu) than for the other nurseries (49 to 309 cfu). No cleaning method was effective in eliminating *Fusarium* inoculum. Pathogenicity tests with *F. prol feratum* and F. *oxysporum* from MS resulted in a significant amount of cankers and some mortality of longleaf pine seedlings. *Fusarium prol feratum* from FL, GA, and NC caused a mild cankering response. Isolates of F. *proliferatum* from MS caused damping-off of germinating seedlings. Low levels of *F. proliferatum* were isolated from unused potting soil at the GA and NC nurseries, but none of these isolates were pathogenic.

Key Words

Container sanitation, soil testing, pathogenicity, container nursery, longleaf pine

The practice of cleaning seedling containers before reuse is standard at most nurseries. Growers sanitize containers to reduce the potential for transfer of pathogens to the next seedling crop (Dumroese and others 1990). Andrew's Nursery in the state of Florida is one nursery that has not practiced cleaning containers as a standard treatment. The nursery is interested in maintaining beneficial fungi in their containers (for example, mycorrhizae), and tries to maintain as much soil as possible in the containers after seedling removal. The incidence of disease development has not differed between used and new containers in the FL operation. Containers at the other three nurseries are washed with bleach or chlorine.

Information is lacking on the fungi associated with used containers producing longleaf pine seedlings, and on the effectiveness of cleaning containers. In the northwestern United States and British Columbia, researchers found treatments with a bleach solution did not eliminate fungi from the used containers Games and others 1988, Sturrock and Dennis 1988). Information has not been available on the efficacy of chlorine as a container treatment. Some Southern nursery managers have expressed concern about the effectiveness of their cleaning technique on containers and about the potential for pathogenic fungi being introduced into containers from fresh potting soil. Because of these concerns, the objectives of this study were to: 1) evaluate the fungi associated with soil collected from used containers; 2) evaluate the *Fusarium* spp. associated with unused potting soil; and 3) test the fungi for pathogenicity.

METHODS

Samples were collected from used containers from Claridge Nursery in Goldsboro, North Carolina (NC), T.W. Earle Nursery in Statesboro, Georgia (GA), Andrew's Nursery in Chiefland, Florida (FL), and W.W. Ashe Nursery in Brooklyn, Mississippi (MS). The used containers were sampled twice before they were cleaned. The first sampling occurred within a day of seedling removal. The second sampling occurred 2 to 3 weeks after seedling removal. A final sample of residue soil was collected after the nurseries operationally cleaned their containers. Six containers (12 for the MS nursery) were randomly selected for each of the precleaning samples, and 10 containers were randomly selected following cleaning. Soil particles retained on the surface of the containers were removed with a bristle brush. Samples were placed in plastic bags and stored at 3 °C for up to 1 week.

A 1-ml soil dilution of 0.1 grams of soil in 200 ml of cooled sterile water with 0.25% agar was placed on 10 plates of 2 selective medias. The selective medias were an Oomycetes media (Kannwischer and Mitchell 1981) and a *Fusarium* media made with PCNB (Nelson and others 1983). Only the *Fusarium* media was used with the postcleaning soil samples. The population of fungi in soil samples was based on the colony forming units (cfu) per gram of soil (wet weight). The dry weight of soil was not determined due to the small amount of soil collected. Only *Fusarium spp.* were recovered from soil collected before the containers were cleaned. Four colonies of *Fusarium* spp. were selected for identification by rotating the plate clockwise and sampling the first colony in the lower half of each quarter. All *Fusarium spp.* were single-spored and placed on carnation and PDA agar for identification.

Pathogenicity tests were conducted on *Fusarium* isolates from soil sampled after cleaning and from the fresh potting soil. No more than four isolates were tested for each *Fusarium sp.* Five 2-month-old longleaf pine seedlings were inoculated for each isolate. The resulting canker lengths were measured after 6 months. The cause of seedling mortality or cankers was determined from diseased tissue by isolation on PCNB media and transferring the fungi to carnation agar for identification.

Fusarium isolates from FL, GA, and MS were evaluated for their ability to cause damping-off. The wheat berry method used by Pawuk (1981) was modified to infest soil. Two randomly selected isolates of each *Fusarium sp.* from each nursery were grown on 10 grams of sterilized wheat berries soaked in 12 ml water. The two isolates were combined, ground-up and added to sterilized potting soil at a rate of 1:10 by weight. Ten germinating seedlings were placed in the soil. The cause of seedling mortality was determined by using PCNB media and transferring the resulting fungi to carnation agar for identification.

RESULTS

Fusarium spp. were routinely isolated from the residual soil of used containers prior to cleaning. *Pythium* and *Phytophthora* were not isolated from used containers. No attempt was made to isolate *Pythium* and *Phytophthora* from postcleaning and potting-soil samples. There was no clear trend in the numbers of *Fusarium* cfu/g soil of the precleaning samples (Tablel). At the MS and NC nurseries, the number of *Fusarium* cfu/g was much less in the postcleaning than the precleaning evaluations. At the GA nursery, the number of *Fusarium* cfu/g in the postcleaning was greater than the 3-week storage and less than the no storage precleaning evaluations. The FL *Fusarium* cfu/g soil was greater in the last sample before sowing than the first two samplings following seedling removal. *Fusarium spp.* were isolated from the unused potting soil at the GA and NC nurseries.

Table 1. *Fusarium* spp. colony forming units per gram of residual-container soil and potting soil before and after cleaning.

Nursery	Precleaning		Post-cleaning	Potting soil
	No storage	2-3 weeks storage		
FL	36,400	42,800	88,000*	0
GA	116,000	29,600	48,200	2,600
MS	43,800	72,800	14,200	0
NC	13,200	40,600	7,800	6,200

The nurseries that used a cleaning method had much lower *Fusarium* cfu/cavity than the FL nursery (Table 2). Among nurseries, there was no clear trend between the percentage of *Fusarium* spp. isolated from used container soil and the sampling dates (Table 3 through Table 6). The

most common *Fusarium spp.* isolated from container soils at the nurseries was *F. oxysporum,* followed by *F. proliferatum* and *F. solani. A Fusarium* spp., originally identified as *F. subglutinans* (Nelson and others 1983), was isolated from the residual soil of used containers from the FL, *GA,* and NC nurseries. This *Fusarium spp.* does not fit a more recent taxonomic key for the *Gibberella fujikuroi* species complex (Nirenberg and O'Donnell 1998) and remains unnamed. The only *Fusarium spp.* isolated from fresh potting soil was *F. prolferatum.*

In pathogenicity tests on 2-month-old longleaf pine seedlings, *F. oxysporum* and *F. proliferatum* from MS caused 20% and 10% mortality, respectively. No other isolates killed seedlings. The *F. prolferatum* isolates from the container soil at all four nurseries caused a larger canker response than the control (Table 7). The *F. proliferatum* isolates from MS caused a significantly greater cankering response in seedlings than those isolates of *F. proliferatum* from the *GA* and NC nurseries. The only other *Fusarium sp.* that caused a significant cankering response in

Table 2. Mean amount of soil collected and *Fusarium* colony forming units (cfu) per cavity following the cleaning method used on containers

Nursery	Precleaning		Postcleaning	Potting soil
	No storage	2-3 weeks storage		
FL	36,400	42,800	88,000*	0
GA	116,000	29,600	48,200	2,600
MS	43,800	72,800	14,200	0
NC	13,200	40,600	7,800	6,200

*Average cavity size from 9 trays at 98.34 cm^2 and one tray at 147.51 cm^2.

Table 3. *Fusarium* species associated with soil remaining on containers after seedling removal at three different times and in fresh potting soil at Andrew's Nursery in Chiefland, FL

Species	No storage		Stored 2 weeks		Before sowing		Potting soil	
	%	number	%	number	%	number	%	number
F. oxysporum	60	24	73	29	77	31	0	0
F. solani	35	14	15	6	0	0	0	0
F. proliferatum	3	1	5	2	13	5	0	0
F. chlamydosporum	0	0	5	2	0	0	0	0
Fusarium species*	0	0	0	0	10	4	0	0

*Identified and confirmed as *Fusarium subglutinans* in 1997; does not fit a recent key for *Gibberella fujikuroi* species complex (Nirenberg and O'Donnell 1998).

Table 4. *Fusarium* species associated with soil remaining on containers before and after cleaning and in fresh potting soil at the T. W. Earle Nursery in Statesboro, GA

Species	Precleaning				Postcleaning		Potting soil	
	No storage		Stored 3 weeks					
	%	number	%	number	%	number	%	numb
F. oxysporum	55	22	73	29	65	26	0	0
F. proliferatum	8	3	20	8	18	7	100	0
Fusarium species*	15	6	0	0	10	4	0	0
F. solani	0	0	0	0	2	1	0	0
F. equiseti	5	2	0	0	0	0	0	0
F. avenaceum	5	2	0	0	0	0	0	0
F. chlamydosporum	3	1	3	1	0	0	0	0

*Identified and confirmed as *Fusarium subglutinans* in 1997; does not fit a recent key for *Gibberella fujikuroi* species complex (Nirenberg and O'Donnell 1998).

Table 5. *Fusarium* species associated with soil remaining on containers before and after cleaning and in fresh potting soil at the W. W. Ashe Nursery near Brooklyn, MS

Species	Precleaning				Postcleaning		Potting soil	
	No storage		Stored 3 weeks					
	%	number	%	number	%	number	%	number
F. oxysporum	40	16	83	33	60	24	0	0
F. proliferatum	45	18	13	5	10	4	0	0
F. solani	8	3	2	1	30	12	0	0
F. avenaceum	5	2	0	0	0	0	0	0
F. lateritium	2	1	0	0	0	0	0	0

Table 6. *Fusarium* species associated with soil remaining on containers before and after cleaning and in potting soil at the Claridge Nursery, Goldsboro, NC

Species	Precleaning				Post-cleaning		Potting soil	
	No storage		Stored 3 weeks					
	%	number	%	number	%	number	%	number
F. oxysporum	34	13	73	29	18	5	0	0
F. proliferatum	47	18	3	1	74	20	100	19
F. solani	11	4	20	8	4	1	0	0
Fusarium species*	3	1	0	0	0	0	0	0

*Identified and confirmed as *Fusarium subglutinans* in 1997; does not fit arecent key for *Gibberella fujikuroi* species complex (Nirenberg and O'Donnell 1998).

Table 7. Average length of cankers on 8-month-old longleaf pine seedlings 6 months after inoculation with *Fusarium* spp. from container soil (C) following cleaning and fresh potting soil (P)

Nursery	Soil type	Inoculation	Mean canker length (mm) +		Confirmed re-isolation (%)
-	-	*Control*	0.1	a	-
FL	C	*F. oxysporum*	1.3	ab	20‡
FL	C	*F. proliferatum*	4.5	cde	100
FL	C	*Fusarium* species*	0.9	a	100
GA	C	*F. oxysporum*	1.9	a	86‡
GA	C	*F. proliferatum*	3.5	bcd	100
GA	P	*F. proliferatum*	0.6	a	100
GA	C	*Fusarium* species*	0.9	a	100
GA	C	*F. solani*	2.5	abcd	100
MS	C	*F. oxysporum*	5.7	de	86§
MS	C	*F .proliferatum*	7.1	e	100
MS	C	*F. solani*	0.9	a	60¶
NC	C	*F. oxysporum*	1.1	ab	100
NC	C	*F. proliferatum*	4.4	cd	100
NC	P	*F. proliferatum*	1.4	ab	100
NC	C	*F. solani*	0.9	abc	50‡

*Identified and confirmed as *Fusarium subglutinans* in 1997; does not fit new key for *Gibberella fujikuroi* species complex (Nirenberg and O'Donnell 1998).
+Data followed by the same letter do not differ significantly (alpha = 0.05) according to Tukey's Studentized Range (HSD) test.
‡*F. proliferatum* isolated.
§*F. solani* isolated.
¶*Penicillium* sp. contamination.

seedlings was the F. *oxysporum* isolates from the MS nursery. In pathogenicity tests using germinating seedlings, *F. proliferatum* from MS caused damping-off of all seedlings. No other *Fusarium* sp. caused damping-off from the three nurseries that were tested.

DISCUSSION

Fusarium spp. were found to be regularly associated with the residual soil of used longleaf pine containers before and after cleaning. These results are similar to studies conducted in the West that found treating containers with bleach did not eliminate Fusarium from the containers games and others 1988, Sturrock and Dennis 1988). Other studies that tested methyl bromide, hot water games and others 1988) and steam games and Gilligan 1988) for cleaning containers also failed to eliminate *Fusarium* spp. The only treatment documented as eliminating *Fusarium* spp. from containers was a 3 minute dip in 80 °C to 100 °C water (Sturrock and Dennis 1988). Currently, many container operations in the Northwest use a hot water treatment to sanitize used containers (per comm. Robert James, Forest Service, Coeur d'Alene, ID).

All cleaning methods tested resulted in less *Fusarium* cfu/cavity than the untreated containers from FL. Physically removing soil appeared to be as important as chemical sterilization for reducing *Fusarium* cfu/cavity. The 10% concentration of bleach did seem to reduce the number of Fusarium cfu/g of soil, whereas the 5% concentration did not. However, the 5% bleach treatment had much less residual soil than the 10% bleach treatment resulting in lower *Fusarium* cfu/cavity.

Fusarium proliferatum was the only species isolated from the unused potting soil at the GA and NC nurseries. In general, the species most isolated from the container soil of all four nurseries was F. *oxysporum,* which is consistent with other surveys of soil fungi from used containers games and Gilligan, 1988, James and others 1988). Unlike these surveys, *F. proliferatum* and F. *solani* were the second and third most common species overall, except at the GA nursery, where an unnamed *Fusarium* spp. was more common.

The variability of the *Fusarium* isolates in causing seeding disease and mortality in the pathogenicity tests is similar to the findings of other scientists (Huang and Kuhlman 1990, James and others 1989, Tint 1945). *Fusariumproliferatum* from the MS nursery was the only isolate that may have been a threat to the next-year's container crop. The other *F. proliferatum* isolates from used containers produced only a weak-canker forming response, while the isolates from unused potting soil were nonpathogenic. Although the F. *oxysporum* isolate from MS did cause 20% mortality in the 2-month-old seedlings, it was not considered a significant threat to seedling production because the mortality was associated with wounding and there was no damping-off response in germinating seedlings.

Sanitation is a cornerstone of any integrated pest management program (Tinus and McDonald 1979), and most nurseries attempt to sanitize their containers to reduce all microorganisms (Dumroese and others 1990, James and Gilligan 1988, Sturrock and Dennis 1988). The ability of Andrew's Nursery to operate without cleaning used containers may be due to the possibility that pathogenic fungi are not present. Another possibility is that beneficial microorganisms have

been maintained in the residual soil of containers, which displaces or are antagonistic to potential pathogenic fungi. Regardless, the results of the pathogenicity testing of the FL isolates suggest that Andrew's Nursery apparently has no need at this time to clean used containers. This evaluation was intended as an initial
investigation of fungi associated with used containers before and after cleaning. Further studies would be required to determine if cleaning treatments are significantly different in reducing fungi and whether the reduction in fungi decreases disease development in container seedlings.

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