RESEARCH REPORT 10-12

PYTHIUM POPULATIONS IN BAREROOT NURSERY SOILS

by
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INTRODUCTION

Bareroot seedlings lifted and held in long-term cold storage (> 1 wk) from October to mid-December can result in poor seedling survival compared to when seedlings are lifted and stored (> 1 wk) in January (Kahler and Gilmore 1961; Dierauf 1976; Hebb 1982; Venator 1984). During lifting, bareroot seedling roots can be damaged, causing wounds that could serve as infection sites for soil-borne fungi. *Pythium* is a common pathogen found in nursery soils and is associated with "damping-off disease" before and after germination and "fine feeder root disease" several weeks after germination (Kelley and Oak 1989). *Pythium* is considered a "water mold" and can become active after heavy rainfall or irrigation, but it also has the ability to live in soil for years feeding on dead organic material. If *Pythium* is being transferred from nursery soils and into cold storage on infected seedling roots, once seedlings are placed in storage, the moist, cool (1-5°C) conditions may provide an environment conducive for *Pythium* growth that results in seedling mortality after outplanting.

In 1996, Sun reported steady decreases in bareroot longleaf pine survival after inoculating seedlings with increasing levels of *Pythium dimorphum* and storing them for 4 weeks. Thus, his report indicates that *Pythium* can kill seedlings after long-term storage and may be one reason for the poor outplanting survival observed. If bareroot seedlings are being infected with *Pythium*, the fungus must be present in nursery soils at the time of lifting. Therefore, this soil survey was conducted in nurseries within the Nursery Cooperative to determine the relative levels of *Pythium* populations in soils in November and January.

METHODOLOGY

In November and January 2008/2009 (Year 1) and 2009/2010 (Year 2), soil collecting kits, containing a soil sampling box, collecting instructions, and return postage, were mailed to 28 bareroot seedling nurseries in the southern U. S. Soils were sampled from an operational nursery bed prior to lifting seedlings. First, the top 5 cm was removed and samples collected from the next 15-20 cm at every 20-30 m within the nursery bed. Samples were labeled with the tree species grown in the sample area, the date of sampling, and the date of the last soil fumigation (Year 2). Upon receipt at Auburn University, soils were placed in cold storage (4-5°C) until processed.

Soil samples collected from the nurseries were assayed in May 2009 (Year 1) and March 2010 (Year 2). Soils were transferred from the sampling boxes into plastic bags and mixed thoroughly. From the soil sample, a 1 g sub-sample was added to a 250 mL flask containing 100 mL distilled water/agar solution (2 g Difco® agar/1000 mL distilled water). Two flasks per soil sample were placed on a shaker for 20 min. From each flask, 13 drops (1 mL) of the soil solution was plated onto *Pythium* selective media (Feng and Dernoeden 1999). The dropper was sterilized in 20% bleach/water solution and rinsed in distilled water before plating the next sample. A total of ten plates (repetitions) and 10 mL of soil solution were assayed per soil sample. *Pythium* recovery was the number of colony forming units (CFUs) identified on the selective media. In cases where soils were assayed from the same nursery in both November and January, a two sample paired t-test was performed using SAS statistical software (9th ed., SAS Institute, Cary, NC). Data from Year 1 and Year 2 were analyzed separately.

RESULTS

Year 1

Of the 16 nurseries with *Pythium* recovered from the soil, eight were collected in the fall (Table 1). Two nurseries (U and V) accounted for 74% of the total *Pythium* recovered in the fall (Table 2). Of the 9 nurseries that had no *Pythium* in November, five were positive and four remained negative for *Pythium* in January (Table 2). *Pythium* numbers were similar from the 13 nurseries that sent soil samples in both November and January ($P = 0.5774$).

Year 2

Similar to the Year 1 soil sample surveys, two nurseries (A and X) accounted for 64% of the total *Pythium* in the fall (Table 3). During the 2009/2010 soil sample survey, sixteen nurseries provided soil samples in both the fall and winter collections; six were negative for *Pythium* in each season and two had no change (Table 3). Of the other eight nurseries that submitted samples, one had lower levels of *Pythium* in the winter, while *Pythium* levels increased in the winter soil sample by 14,000 CFUs/mg soil in seven nurseries (Table 3). Overall, *Pythium* numbers were similar from the 16 nurseries that sent soil samples in both November and January ($P = 0.3492$).

Regardless of season, *Pythium* numbers were numerically higher in soils that were fumigated in the last year. On average, soils sampled at ≤ 1 year since the last fumigation had 50% more *Pythium* (11,071 CFUs) than samples taken from nursery beds at > 1 year since the last fumigation (5,429 CFUs) (Table 3).

DISCUSSION

Pythium populations in bareroot nursery soils were variable between the fall and winter collection periods for both years. Because *Pythium* was present during the fall and winter, it is possible that seedling roots can become infected with *Pythium* during the fall lifting season. While *Pythium* was detected in the winter soil sample survey, long-term cold storage is possible for bareroot loblolly pine (*Pinus taeda*) during this time (Barnett & Brissette 1987). Root exudates such as sugars, amino acids, and other ions can stimulate *Pythium* spore germination (Hendrix and Campbell 1973), and root exudation is common following root undercutting in the fall before lifting. Seedlings roots are not undercut in the winter, and a favorable environment for *Pythium* activity is not present. Other studies from soils sampled on two agricultural sites in two consecutive years have shown a steady increase in *Pythium* populations from September to December followed by a decrease from December to May with the highest levels reaching > 2,500 CFUs/g dry soil (Ali-Shtayeh et. al.1986). The authors speculated that higher levels of *Pythium* in the fall may be caused by increases in soil organic material, lower levels of antagonistic fungi, or spore activation in favorable conditions.

One possibility for the variation in *Pythium* populations observed among the nurseries sampled could be the timing since the last soil fumigation. Numerically, more *Pythium* colony forming units was recovered from nursery soils sampled less than one year since soil fumigation. Fumigation time, type, and rates can differ between nurseries, which may result in variable control of *Pythium*. For example, spring fumigation using a standard 67% methyl bromide/33% chloropicrin combination at 393 kg/ha, chloropicrin at 336 kg/ha, and a combination of metam sodium at 90 kg/ha and chloropicrin at 163 kg/ha reduced *Pythium* spp. in April, but by November of the same year, *Pythium* populations were similar to those in non-fumigated soils (Cram et al. 2007). Similarly, a spring fumigation with MC33 at 360 kg/ha controlled *Pythium*, but populations rebounded by spring of the next year resulting in disease (Tanaka et al. 1986). These results suggest that *Pythium* populations can rebound within the same year of fumigation.

Another factor that contributes to *Pythium* survival in the soil is a suitable environment that favors fungal growth. Cultural practices used to produce forest tree seedlings are well established and similar between nurseries. However, the various methods used to implement these cultural practices may differ based on the idiosyncrasies of the nursery manager. For example, *Pythium* requires high soil moisture conditions that can develop in areas with high soil compaction or improper field drainage. For example, *Pythium* CFUs increased in nursery bed depths of 12-24 cm when tilled with a moldboard plow but decreased at 0-15 cm depths when a disc was used (Juzwik et al. 1999). Thus, a simple change in soil cultivation method can affect *Pythium* populations in nursery soils.

MANAGEMENT IMPLICATIONS

The variability of *Pythium* spp. in forest tree nursery soils between seasons may be attributed to either the time since soil fumigation or nursery practices that promote an environment for fungal development. Controlling irrigation frequencies and establishing good soil drainage are two ways to minimize soil conditions that favor fungal growth. Other control methods include maintaining a low soil pH and avoiding the use of contaminated equipment (Kelley and Oak 1989). Due to the ability of *Pythium* to survive on seedling roots and grow during storage (Sun 1996; Jackson

2010), the infection of even one seedling during lifting could cause *Pythium* to infect other seedlings in storage and result in seedling mortality after outplanting.

LITERATURE CITED

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Table 1. Number of soil survey responses and nurseries that had a positive or negative *Pythium* sample.

	Year 1		Year 2	
	Nov 2008	Jan 2009	Nov 2009	Jan 2010
Responding Nurseries	19	16	20	19
Nurseries with *Pythium*	8	8	9	13
Nurseries without *Pythium*	11	8	11	6

Table 2. Number of colony forming units (CFUs) per mg of soil in November 2008 and January 2009 (Year 1).

	CFU/mg soil	
Nursery	November 2008	January 2009
A	7,000	——
B	——	2,000
C*	0	5,000
D*	0	9,000
E*	0	3,000
F	0	——
H	0	——
I	13,000	——
J	1,000	——
K*	0	0
L*	0	1,000
M*	1,000	0
N*	0	0
O*	1,000	1,000
R*	0	0
U*	57,000	9,000
V	33,000	——
X*	0	0
Y*	0	10,000
Z*	9,000	0
BB	——	0
Total	122,000	41,000
Mean of (+) Nurseries	15,000	5,000

* = paired t-test results (T = 0.57; P > T = 0.5774; Error DF= 12)

Table 3. Number of colony forming units (CFUs) per mg of soil in November 2009 and January 2010 (Year 2).

Nursery	CFU/mg soil Nov 2009	Years Last Fum[z]	CFU/mg soil Jan 2010	Years Last Fum
A	37,000	1	——	——
B	——	——	1,000	2
C*	0	1	0	1
D*	0	——	0	2
E*	0	1	4,000	2
F	0	1	——	——
G	9,000	0.5	——	——
H*	0	1	4,000	0.5
I*	0	1.5	0	——
K*	0	1	4,000	2
L*	3,000	1	12,000	1
M*	4,000	1.5	33,000	——
N*	0	3	11,000	2
O*	2,000	3	2,000	——
Q*	0	2	0	——
R*	7,000	1	8,000	——
T	——	——	11,000	1
U	——	——	11,000	2
W	4,000	1	——	——
X*	30,000	1	4,000	1
Y*	0	1	5,000	1
Z*	0	1	0	3
AA	8,000	1	——	——
BB*	0	1.5	0	——
Total	104,000		110,000	
Mean of (+) Nurseries	12,000		8,000	

[z] = # of years since last soil fumigation
* = paired t-test results (T = -0.97; P > T = 0.3492; Error DF = 15)