Assessing the severity of damping-off caused by *Pythium ultimum* and *Rhizoctonia solani* in peat-based greenhouse substrates amended with pine wood chip aggregates

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Abstract

Processed pine wood has potential as a greenhouse substrate component to replace perlite. However, there is limited information regarding processed pine wood's suppressiveness to soilborne diseases. A series of experiments were conducted to evaluate pine wood chips (PWC) suppressiveness to *Pythium ultimum* and *Rhizoctonia solani* on cucumber (*Cucumis sativus* L. 'Straight Eight') seedling growth. In Experiment 1, cucumber seeds were sown in substrates formulated to contain either 10, 20, or 30% perlite or PWC aggregates and were inoculated with no pathogen (control), 0.1 g L^{-1} *Pythium ultimum*, or 0.05 g L^{-1} *Rhizoctonia solani* isolates. Fourteen-day-old cucumber seedlings were evaluated to determine disease severity. *P. ultimum* inoculum concentration of 0.1 g L^{-1} did not cause damping-off in substrates amended with perlite or PWC aggregates. Regardless of aggregate amendment rate, *R. solani* severity of damping-off was generally similar among substrates amended with perlite. In general, less disease was observed with PWC aggregates than with perlite across all amendment volumes. In Experiment 2, cucumber seedlings were sown in substrates formulated to contain either 20, 30, or 40% perlite or PWC aggregates and were inoculated with 0 (control), 1.2, 2.4, or 3.6 g L^{-1} *P. ultimum* isolates. Disease severity of cucumber seedlings were similar among all substrates amended with 20 to 40% PWC aggregates. In Experiment 3, cucumber seedlings were sown in substrates formulated to contain either 20, 30, or 40% perlite or PWC aggregates and were inoculated with 0 (control), 1.2 g L^{-1} of *P. ultimum*, or 0.05 g L^{-1} *R. solani* inoculum isolates. Results found potential suppressiveness of *P. ultimum* and *R. solani* when peat-based substrates were amended with PWC.

Keywords: aggregate, bioassay, disease prediction, horticultural substrate, loblolly pine

INTRODUCTION

Containerized plants grown in composted bark substrates are considered to be free of root diseases. Hoitink et al. (1975, 1976) suggests the absence of root rots might be due to the eradication of pathogens during composting due to the suppressive nature of the composted bark. Demonstration by Hoitink (1982) found nursery, floriculture, and foliage crops to be free of root rot diseases when grown in substrates formulated with 4 bark:1 peat (v/v). The tree species in which bark is removed and processed can affect the spectrum of pathogens suppressed. For example, Hoitink (1982) observed composted pine bark (PB) to suppress *Phytophthora* and *Pythium* root rots, but not *Rhizoctonia* damping-off. Additionally, Hoitink (1982) also found hardwood bark (HB) contaminated with 60% wood chips did not suppress *Phytophthora* root rot of *Rhododendron* sp. in nurseries. Nelson et al. (1983) evaluated the suppression of *Rhizoctonia* damping-off in container substrates amended with composted HB and reported increased aging of composted HB was more suppressive compared to green

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(non-composted) HB. In comparison to a Canadian peat substrate, Nelson et al. (1983) also found green HB amended substrates to be slightly suppressive to *Rhizoctonia* damping-off and suggest the involvement of chemical inhibitors were the source of suppression. However, a study evaluating substrate components by Waller et al. (2008) demonstrated *R. solani* to be absent in various sources of peat moss, wood fiber, bark, and coir.

Substrate pathogens can be assessed in greenhouse and nursery substrates by conducting suppressiveness bioassays. Chen et al. (1988) determined the suppressiveness of peat, composted PB and composted HB to *Pythium* damping-off by utilizing a cucumber bioassay. In this study, cucumber seeds were direct sown into substrates inoculated with *Pythium ultimum* to determine the severity of damping-off of seedlings. They reported peat to be conducive whereas the composted PB and HB were suppressive to the disease.

Pine wood chips (PWC) have been investigated as an alternative aggregate to perlite in peat-based greenhouse mixes (Jackson and Fonteno, 2013; Owen, 2013). No information is available regarding the disease incidence, susceptibility, or suppressiveness of *P. ultimum* or *R. solani* in peat-based substrates amended with PWC aggregates. Therefore, the objective of this study was to determine the disease occurrence or suppressiveness of *P. ultimum* and *R. solani* damping-off in peat-based substrates amended with either perlite or PWC aggregates.

MATERIALS AND METHODS

Experiment 1

On December 19, 2011, eight-year-old loblolly pine trees (*Pinus taeda* L.) were harvested (Chatham County, NC) at ground level, de-limbed, and subsequently stored under shelter for protection from the weather. On January 3, 2012, pine logs were chipped in a DR Chipper (18 HP DR Power Equipment, model 356447; Vergennes, VT) resulting in small wood chips (1 L × 0.2 W × 0.9 H – cm). Wood chips were then spread out (2.5-cm deep) on a concrete pad under shelter, turn periodically and allowed to air dry for 2 d. Wood chips were air-dried to reduce the initial moisture content (MC), which has been shown in unpublished studies to aid in the processing of the wood chips through the hammer mill. Moisture content for fresh wood chips was 43% initially and 35% after air-drying for 2 d. Wood chips were then hammer-milled through a 6.35-mm screen (Meadows Mills, North Wilkesboro, NC) to produce pine wood chips (PWC; 0.11 L × 0.4 W × 0.2 H – cm). Pine wood chips were subsequently stored in closed bulk bags under shelter.

On March 8, 2011 moistened (50%) sphagnum peat (Pro-Moss Sphagnum Peat, Quakertown, PA) was amended with either 10, 20, or 30% perlite (Carolina Perlite Company, Gold Hill, NC) or PWC (by vol.), to produce a total of six substrate treatments. After formulation of the substrates, initial substrate pH was determined by the 2:1 saturated media extract method (SME method; 2 parts deionized water:1 part substrate) using a Hanna HI 9813-6 pH instrument (Hanna Instruments, Woonsocket, RI). Dolomitic limestone (Mississippi Lime Company, Vicksburg, MS) was incorporated in all substrate treatments at the rate of 4.5 kg m^{-3}. Substrates were allowed to incubate for 45 d in sealed plastic bags for lime equilibration before potting. Using the SME method previously described, all substrates were found to have a pH of 5.4±0.2 units.

On April 23, 2012, 25 g of white long grain rice was weighed, placed into a beaker containing 18 mL of deionized water, sealed with aluminum foil, and autoclaved for 12 h. On April 25, autoclaved rice grains were inoculated with colonized agar disc samples of *P. ultimum* (isolate 1110) or *R. solani* (isolate RS3, anastomosis group 4 (AG-4), deposited in the US Department of Agriculture-Agriculture Research Service culture collection at Peoria, IL, as NRRL 22805). Beakers were sealed to allow 6 d of fungal colonization. On May 1, colonized rice grains were pulverized in a Waring blender (Stamford, CT) and the resulting particles were screened through a 2-mm diameter sieve to produce inoculum of uniform size.

Substrates formulated on March 8 were measured and bagged separately to contain 12 L of each substrate to produce six control substrates, six substrates inoculated with 0.1 g L^{-1} of *P. ultimum*, and six substrates inoculated with 0.05 g L^{-1} *R. solani*, for a total of 18 treatments. Inoculation of substrates was conducted by incorporating the inoculum into each individual

bag of substrate and gently rotating the sealed bags for 2 min to allow for even distribution. Ten replications of 15.4-cm (1.3-L) diameter plastic containers (ITML Horticultural Products, Middlefield, OH) were filled with each of the 18 substrates treatments. Ten seeds of *Cucumis sativus* L. 'Straight Eight' (cucumber) were evenly placed on the substrate surface using a circular plastic stencil and direct sown to a depth of 1 cm. Pots were placed on bench level in a glass-glazed greenhouse at North Carolina State University, Raleigh, NC (lat. 36°N) and irrigated with clear water as needed. The experimental design was a complete randomized block (by treatment) design with three treatments (control, *P. ultimum*, *R. solani*) × six substrates × 10 pot replications.

At 10, 15, and 17 days after sowing (DAS), disease severity was assessed with the following scale: 1 = healthy, vigorous seedling; 2 = seedling emerged but stunted; 3 = emergence but seedling diseased; and 4 = dead, no emergence. The mean disease severity rating from 10 seedlings represented one replication per pot (10 pots per treatment). Data were subjected to analysis of variance (ANOVA) by the general linear model procedures (PROC GLM) and means were subjected to Duncan's means separation and separated significant differences at $P \leq 0.05$ (SAS Institute, Cary, NC).

To verify the seedlings in the bioassay were infected with either *P. ultimum* or *R. solani*, selective agar plates were prepared to determine the occurrence of fungal infection. On May 10, glycerol asparagine agar selective for *P. ultimum*, was produced by autoclaving 15 g of agar and 1 L of water. Once cooled, the addition of 500 mL CMA, 63 mg PCNB, 125 mg Ampic, 1 mL Pimaricin, and 1 mL Rifamycin was added to the solution. Alkaline water agar, selective for *R. solani* was produced by autoclaving 15 g of agar and 1 L of water. Once cooled, 100 mg streptomycin sulfate, penicillin G potassium, and 700 µL sodium hydroxide 1 N was added to the solution for a pH of 8.5. The agars were then poured into petri dishes, allowed to cool, and sealed with parafilm.

On May 15, 10 seedlings randomly sampled from each treatment was collected and bagged. Seedling were washed with cold tap water to remove any remaining substrate components from cotyledons or roots and allowed to air dry. Following protocols established by Olson and Benson (2007), root systems of three representative samples of each of the 18 treatments were excised with sterilized scissors, placed on formulated agar plates, sealed, and placed in a climate controlled dark location for colonization. On May 22, agar plates were removed to determine colonization of *P. ultimum* or *R. solani* or any other substrate pathogen that may have been present in the substrates formulated.

Experiment 2

The objective of this experiment was to determine the inoculum threshold for *P. ultimum*. The previous inoculation rate used in Experiment 1 was lower than anticipated. Except where indicated, procedures for Experiment 2 were as described in Experiment 1. Freshly harvested nine-year-old loblolly pine trees were chipped on January 8, 2013, allowed to air dry for 1 d, and on January 10, wood chips were then hammer-milled through a 6.35-mm screen. On January 15 moistened (50%) sphagnum peat was amended with 20, 30, or 40% (v/v) PWC, to produce a total of three substrate treatments. The 10% PWC amended substrate was not included in this experiment because results from Experiment 1 indicated similar disease severity ratings between 10 and 20% PWC amended substrates. The methodology of increasing the percentage of PWC to 40% was to determine the disease severity and potential suppressiveness for a substrate formulated with an aggregate percentage higher than previously tested. Substrates formulated to contain perlite were not included in this experiment as it was designed to determine the inoculum rate for *P. ultimum* in PWC amended substrates and as a threshold for future studies. After formulation of the substrates, initial substrate pH was determined and dolomitic limestone was incorporated to adjust substrate pH to 5.4. Substrates were incubated for 1 d in sealed plastic bags to allow for lime activation and pH equilibration before potting.

Using the similar procedure for Experiment 1, rice grains were autoclaved and inoculated with *P. ultimum* (isolate 1110) to produce inoculum for the bioassay. Substrates were measured and bagged separately to contain 3.6 L of each substrate to produce 12 total

substrate treatments. One substrate of each ratio was inoculated with 0.0, 1.2, 2.4, or 3.6 g L^{-1} of *P. ultimum*. Five replications of 15.4-cm (1330 mL) diameter plastic containers were filled with each of the 12 substrates treatments and 10 cucumber seeds were evenly placed on the substrate surface and direct sown to a depth of 1 cm. The pots were placed on bench level in a glass-glazed greenhouse at North Carolina State University and thoroughly irrigated as needed upon weather conditions. The experimental design was a completely randomized design with five replications × four rates of *P. ultimum* inoculum × three substrates. Disease severity was assessed 16 d after sowing.

Experiment 3

The objective of this experiment was to determine the disease severity of *R. solani* and an increased inoculum rate of *P. ultimum* determined in Experiment 2 in greenhouse substrates formulated to contain either 20, 30 or 40% perlite or PWC aggregates. Except where indicated, procedures for Experiment 3 were as described in Experiment 1. On February 12, 2013, nine-year-old loblolly pine trees were harvested at ground level and de-limbed. On February 13, 2013, pine logs were chipped, allowed to air dry for 1 d, and on February 14, wood chips were then hammer-milled through a 6.35-mm screen. Moistened (50%) sphagnum peat moss was amended with 20, 30, or 40% (v/v) perlite or PWC, to produce a total of six substrate treatments. Based on the results of Experiment 1, utilizing aged PWC (three mo.), fresh PWC aggregates were utilized to determine if aggregate age affects the potential to suppressive *P. ultimum* and *R. solani* in greenhouse substrates formulated to contain PWC aggregates. After formulation of the substrates, initial substrate pH was determined and dolomitic limestone was incorporated to adjust substrate (pH 5.4). Substrates were allowed to incubate for 1 d in sealed plastic bags for lime equilibration before potting. On February 15, 2013, inoculated rice grains of *P. ultimum* (isolate 1110) and *R. solani* (isolate RS3) were pulverized and sieved. Substrates were measured, bagged separately to contain 6 L of each substrate ratio, inoculated with 0.0 g or 1.2 g L^{-1} of *P. ultimum* or 0.05 g L^{-1} *R. solani* inoculum. Disease severity was assessed 14 d after sowing.

RESULTS AND DISCUSSION

In Experiment 1, significant differences in disease severity occurred in uninoculated control substrates amended with 10% perlite (Table 1). Increased disease severity in substrates amended with 10% perlite may be likely caused by poor cucumber seed germination, unfavorable environmental conditions, or loss of cucumber seeds from splashing water caused by overhead irrigation. It is also unknown if potential differences in physical properties of the different substrates (which were not measured in this experiment) had any effect of the seedling growth. Disease severity ratings for *Pythium* were similar in substrates amended with 10 and 30% perlite or PWC aggregates. In general, the *R. solani* isolate caused more symptoms of what appeared to be consistent with damping-off or rat tailing on cucumber seedlings than the *P. ultimum* isolate in substrates amended with 10-30% perlite. These data indicate peat-based substrates amended with 10, 20, or 30% perlite were conducive to damping-off with mean disease severity values of 3.1, 2.9, and 3.1, respectively. Substrates amended with PWC aggregate had some potential of suppressiveness to *R. solani*, with the greatest suppression occurring in substrates amended with 10 or 20% PWC aggregates.

In Experiment 2, disease severity of cucumber seedlings were similar among all substrates amended with 20 to 40% PWC aggregates (Table 2). Compared to the uninoculated controls, substrates amended with increasing PWC aggregates increased in conduciveness. Therefore, data indicates that substrates amended with <20% PWC aggregates may likely suppress *P. ultimum.*

In Experiment 3, disease severity ratings of uninoculated controls were similar among substrates amended with 20 and 40% perlite or PWC aggregates. However, substrates amended with 30% PWC were observed to have increased severity rate of 1.4, compared to the severity rate 1.0 of 30% perlite amended substrate. In general, substrates formulated with 20 and 30% perlite were more conducive to *P. ultimum* with disease severity ratings of 4.0 and

4.0, respectively, compared to similar ratios amended with PWC aggregates. However, substrates amended with 40% PWC aggregates were more conducive with a disease severity rating of 4.0, compared to the similar ratio of perlite. *Rhizoctonia solani* disease severity differed significantly between substrates amended with 40% perlite and PWC aggregates (Table 3). The data suggest 40% PWC-amended substrates to be more suppressive with a severity rate of 2.1, compared to the 40% perlite substrate with a rate of 2.7.

Table 1. *Cucumis sativus* L. 'Straight Eight' bioassay conducted to determine disease severity of *Pythium ultimum* and *Rhizoctonia solani* inoculated substrates amended with 10, 20, or 30% (v/v) perlite or pine wood chip (PWC) aggregates.

Substrates[e]	Disease severity[a]		
	Control[b] 0.0 g	*Pythium*[c] 0.10 g	*Rhizoctonia*[d] 0.05 g
		(g L^{-1})	
Perlite (%)			
10	1.4±0.30 a[f]	1.3±0.22 b	3.1±0.20 a
20	1.1±0.23 b	3.1±0.10 a	2.9±0.32 ab
30	1.1±0.13 b	1.2±0.28 b	3.1±0.20 a
PWC[g] (%)			
10	1.1±0.17 b	1.2±0.16 b	2.1±0.74 d
20	1.1±0.12 b	1.2±0.28 b	2.6±0.43 bc
30	1.1±0.18 b	1.1±0.24 b	2.3±0.57 cd

[a] Disease severity was assessed with the following scale: 1 = healthy, vigorous seedling; 2 = seedling emerged but stunted; 3 = emergence but seedling diseased; and 4 = dead, no emergence.
[b] Control, uninoculated.
[c] *Pythium ultimum.*
[d] *Rhizoctonia solani.*
[e] Substrates were formulated on a by volume basis to contain 10, 20, or 30% perlite or PWC aggregates.
[f] Mean separated within column by Duncan's multiple range test ($P \leq 0.05$).
[g] PWC were made from loblolly pine trees that were harvested, delimbed, chipped, and hammer milled through a 6.35-mm screen.

Table 2. Cucumber 'Straight Eight' bioassay used to determine the disease severity of *Pythium ultimum* inoculated substrates amended with 20, 30, or 40% (v/v) pine wood chip (PWC) aggregates.

Substrates[d]	Disease severity[a]			
	Control[b] 0.0 g	*Pythium*[c] 1.2 g	2.4 g	3.6 g
		(g 6 L^{-1})		
PWC[e] (%)				
20	1.3±0.44 a[f]	1.5±0.52 a	2.0±0.29 a	2.6±0.49 a
30	1.3±0.31 a	2.3±0.46 a	2.5±0.57 a	3.2±0.25 a
40	1.6±0.31 a	2.7±0.15 a	2.5±0.12 a	2.8±0.20 a

[a] Disease severity was assessed with the following scale: 1 = healthy, vigorous seedling; 2 = seedling emerged but stunted; 3 = emergence but seedling diseased; and 4 = dead, no emergence.
[b] Control, uninoculated.
[c] *Pythium ultimum*
[d] Substrates were formulated on a volume basis to contain 20, 30, or 40% PWC aggregates.
[e] PWC were made from loblolly pine trees (*Pinus taeda*) that were harvested, delimbed, chipped, and hammer milled through a 6.35-mm screen.
[f] Mean separated within column by Duncan's multiple range test ($P \leq 0.05$).

Table 3. Cucumber 'Straight Eight' bioassay used to determine the disease severity of *Pythium ultimum* and *Rhizoctonia solani* inoculated substrates amended with 10, 20, or 30% (v/v) perlite or pine wood chip (PWC) aggregates.

Substrates[e]	Disease severity[a]		
	Control[b] 0.0 g	*Pythium*[c] 1.2 g	*Rhizoctonia*[d] 0.05 g
		(g L^{-1})	
Perlite (%)			
20	1.1±0.10 a[f]	4.0±0.08 a	2.5±0.99 a
30	1.0±0.00 b	4.0±0.00 a	1.8±0.58 a
40	1.3±0.35 a	3.7±0.30 b	2.7±0.56 a
PWC[g]			
20	1.8±1.53 a	3.1±0.48 b	1.6±0.48 a
30	1.4±0.20 a	3.5±0.39 b	1.9±0.34 a
40	1.2±0.25 a	4.0±0.00 a	2.1±0.33 b

[a] Disease severity was assessed with the following scale: 1 = healthy, vigorous seedling; 2 = seedling emerged but stunted; 3 = emergence but seedling diseased; and 4 = dead, no emergence.
[b] Control, uninoculated.
[c] *Pythium ultimum*
[d] *Rhizoctonia solani*
[e] Substrates were formulated on a by volume bases to contain 20, 30, or 40% perlite or PWC aggregates.
[f] Mean separated within column by Duncan's multiple range test ($P \leq 0.05$).
[g] PWC were made from loblolly pine trees (*Pinus taeda*) that were harvested, delimbed, chipped, and hammer milled through a 6.35-mm screen.

CONCLUSIONS

Results from these root rot studies with PWC were equal or similar compared to perlite. Further work is needed to investigate but in general, there is no increased threat of these diseases due to the use of PWC in place of perlite. This information assessing the biological and chemical characteristics of PWC aggregates will help growers and investigators alike to make PWC a suitable replacement for perlite in greenhouse substrates.

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