Measuring Disease Severity of *Pythium spp.* and *Rhizoctonia solani* in Substrates Containing Pine Wood Chips

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Significance to Industry: This study demonstrates how the mini-Horhizotron can be utilized in plant pathology, and investigates the disease severity of *Pythium ultimum*, *Pythium aphanidermatum*, and *Rhizoctonia solani* on bedding plants grown in peat based substrates containing varying percentages of pine wood chips, and an industry standard potting mix. Horhizotrons, when used in addition to other disease assessment techniques, can help provide a more well-rounded and accurate assessment of root disease severity. The ability to view the rhizosphere and the accuracy with which root length can be measured suggests that the mini-Horhizotron could have broad applications in plant pathology research. The results from these experiments show that disease severity of these pathogens was equal and often less prevalent than in a traditional potting mix, which offers additional evidence of the viability of pine wood chips to be used as a replacement to perlite in substrates for greenhouse crop production.

Nature of Work: When alternative substrates are developed for container production it is important that all aspects of root development are considered, including response to soil borne pathogens. In the south eastern United States loblolly pine (*Pinus taeda* L.) has been identified as a readily available and inexpensive alternative for commonly used substrate components (3). Perlite is the most commonly used aggregate in greenhouse substrates, but it is often the most expensive, and concerns have been raised about health risks and sustainability issues (3). Recent research has shown that pine wood chips (PWC) are a suitable alternative to perlite in peat based substrates (3), but there is no information regarding disease severity of common soil borne pathogens on greenhouse plants that are grown in peat substrates amended with PWC.

Pythium spp. are some of the most common and persistent pathogens in greenhouse production, and almost all greenhouse crops are susceptible to one or more species of pythium. *Pythium aphanidermatum* and *P. ultimum* are two species that are especially common as pathogens of potted plants (1). *P. aphanidermatum* in particular is an economically important, aggressive species of pythium that causes damping off, root rot, stem rots, and blights. It has a wide host range, including many annuals and bedding plants, and favors warm temperatures and wet soils, making it an issue in greenhouse production (4). Common symptoms are yellowing foliage, stunted plant growth, and wilt (1). *Rhizoctonia solani* is an aggressive pathogen with a wide host range that causes several diseases, including damping off, stem and leaf blights, and root, stem, and crown rots. When the fungus grows over the plant foliage, it is referred to as web blight. Rhizoctonia root rot occurs on most flowering potted plants and can cause considerable economic losses (1).

Mini-Horhizotron Study. The mini-Horhizotron (developed at North Carolina State University in 2013), is a smaller version of the Horhizotron™ (5) and is a more appropriate size for smaller bedding plants and annuals. It has three concave walls (quadrants) constructed out of transparent acrylic sheets, allowing the rhizosphere to be viewed. Each quadrant has two measureable faces, giving a sum of six quadrant faces per mini-Horhizotron. Shade panels were constructed out of PVC that fit tightly against the acrylic walls to block light (2). Each mini-Horhizotron holds about the same volume of media as a 6 inch pot. The mini-Horhizotron allows for the observation and measuring of root disease and dieback over time without disturbing the root system or the container media. It allows root rot to be measured more accurately than subjective root ratings, and less severe root symptoms can be observed easily. Aggravating disease factors, such as potting media conditions and insect larvae, can also be viewed.

The mini-Horhizotron study was implemented at the Marye Ann Fox Teaching Laboratories Greenhouse at North Carolina State University. Three different substrates were used: Fafard 4P (containing 45% sphagnum peat moss, processed pine bark, perlite, vermiculite, wetting agent, starter nutrients and dolomitic limestone), a substrate containing 80% peat moss and 20% PWC, and a third substrate containing 70% peat moss and 30% PWC. Substrates containing PWC had the following amendments added per cubic yard at mixing: 7.5 pounds 200 mesh dolomitic limestone. There were six pathogen treatments compared in this study: Trt. 1) 4P-U (uninoculated), Trt. 2) 30PWC-U, Trt. 3) 20PWC-U, Trt. 4) 4P-I (inoculated), Trt. 5) 30PWC-I, Trt. 6) 20PWC-I. Mini-Horhizotrons were filled with each individual substrate and one plug of *Antirrhinum majus* 'Snapshot Red' (bedding snapdragon) was planted in each. The mini-Horhizotrons were arranged in a complete randomized block design with three substrate replicates per treatment, yielding a total of 18 mini-Horhizotrons. The plants were watered as needed with 200 ppm nitrogen derived from a 20-10-20 water soluble fertilizer and allowed to grow until root systems were well developed and had reached the end of the quadrants in the mini-Horhizotrons. On 23 April, 2013 treatments 4, 5, and 6 were inoculated with *Pythium aphanidermatum* from colonized rice grains. The inoculum was created by placing 25 g of long grain white rice in a beaker with 25 mL of water and autoclaving twice over the course of two days, which took place on 14 April 2013 and 15 April, 2013. The autoclaved rice grains were then inoculated on 16 April, 2013 with four colonized agar discs of *P. aphanidermatum*. Six grains of inoculum were inserted two inches below the substrate surface of each mini-Horhizotron. The root systems of all plants were traced by hand using a blue marker onto transparencies at the time of inoculation, and again one month later at the termination of the study.

Transparencies were cut to match the size of each quadrant, and were held in place by binder clips while roots were being traced. Only healthy, white roots were traced; roots showing any sign of disease (off color, water soaked, no root hairs, cortex sloughing off, etc.) were not included for measurement. The traced roots were photographed and the images were converted to black and white using a high contrast red filter in Adobe Photoshop CS5, and were then uploaded to Cornell University's RootReader 2D software where they were measured for total root length. The digital images were set to a resolution of 182.9 pixels/cm., with an error criterion of 9.1 pixels. At termination shoots were visually inspected for disease symptoms and rated on a scale of 1 to 5 with 5 = healthy, 4 = slightly stunted, 3 = chlorosis/moderate stunting/delayed flowering, 2 = wilting/severe stunting, and 1 = dead. Shoots were removed at the substrate surface and weighed to determine fresh weight, and a bloom count was taken. Data were analyzed using Tukeys Studentized Range Test ($p \leq 0.05$) (SAS Institute version 9.1, Cary, NC). On 24 May, 2013 root samples from each plant were plated onto PARP media (a pythium selective media) to confirm the presence of *P. aphanidermatum* in the inoculated treatments. On 25 May, 2013 *P. aphanidermatum* was observed growing from all of the substrate treatments, including the uninoculated controls. As a result, the uninoculated treatments for each substrate were not included in the means, therefore disease occurrence and severity was just compared among substrates.

Disease survey. The disease survey was implemented at the Marye Ann Fox Teaching Laboratories Greenhouse at North Carolina State University on 9 May 2013, using the same substrates as the mini-Horhizotron study, with the same amendments added to the substrates containing PWC. There were nine pathogen treatments compared in this study: Trt. 1) 4P-U (uninoculated), Trt. 2) 30PWC-U, Trt. 3) 20PWC-U, Trt. 4) 4P-P(Pythium), Trt. 5) 30PWC-P, Trt. 6) 20PWC-P , Trt. 7) 4P-R (Rhizoctonia), Trt. 8) 30PWC-R, Trt. 9) 20PWC-R. Plastic pots (5 inch diameter) were filled with the substrates and one plug of vinca (*Catharanthus roseus)*, marigold (*Tagetes patula)*, wax begonia (*Begonia semperflorens-cultorum)*, or impatiens (*Impatiens walleriana)* was planted. Containers were arranged in a randomized split block with six replicates per species, and four species per treatment. Plants were fertilized the same as in the mini-Horhizotron study, and on 22 May 2013 treatments 4 – 6 were inoculated with *Pythium ultimum* and treatments 7 – 9 were inoculated with *Rhizoctonia solani.* The inoculum for each species was created on 19 May 2013 using the same techniques as outlined in the mini-Horhizotron study, and seven rice grains were inserted one inch below the substrate surface in each pot. Data collected for this study included the date when symptoms were first observed, and visual ratings of the shoots and roots. Shoots were visually inspected for disease symptoms and rated on a scale of 1 to 5 with 5 = healthy, 4 = slightly stunted/few cankers or leaf blight, 3 = chlorosis/moderate stunting/delayed flowering/moderate stem and crown rot or leaf blight, 2 = wilting/severe stunting/severe stem and crown rot or leaf blight, and 1 = dead. Roots were visually inspected for root rot and rated on a scale of 1 to 5 with 5 = healthy white roots / no disease recovered, 4 = 25% root rot or seemingly healthy roots + disease recovered, 3 = 50% root rot, 2 = 75% root rot, and 1 = brown/dead roots. Data were analyzed using Tukeys Studentized Range Test ($p \leq 0.05$) (SAS Institute version 9.1, Cary, NC).

Results and Discussion:

Mini-Horhizotron study: Results from data taken at termination indicated that the root systems and shoots of snapdragons grown in the substrates containing 20% and 30% PWC were less effected by *Pythium aphanidermatum* infection than those grown in Fafard 4P (Table 1 and Figure 1). Plants grown in the 80:20 peat/PWC substrate had the highest total root length at the end of the experiment (Figure 1), and plant health ratings were significantly higher than Fafard 4P (Table 1). The peat/PWC plants displayed no wilting. Snapdragons grown in 80:20 peat/PWC displayed minimal stunting as a result of infection, and the mean fresh weight of those plants was 32g heavier than those grown in Fafard 4P (Table 1). The mean bloom count for the 80:20 peat/PWC substrate was 20 blooms, 70:30 peat/PWC was 5 blooms, and Fafard 4P had zero blooms (Table 1).

Disease survey: Results from the disease survey indicated that disease severity in substrates containing pine wood chips was equal to Fafard 4P, except in the case of vinca root ratings for both *P. ultimum* and *R. solani* treatments, impatiens shoot ratings for *R. solani* treatments, and impatiens root ratings for *P. ultimum* treatments, where disease severity was less than Fafard 4P (Tables 2 and 3). The plants grown in peat/PWC substrates exhibited symptoms at later dates than Fafard 4P (Figure 2). The Rhizoctonia treatments for all of the substrates exhibited web blight, leaf blight, crown rot, stem rot and/or root rot. Impatiens seemed particularly susceptible to Rhizoctonia infection and the impatiens and begonias had stem and crown rot more so than root rot, with numerous cankers observed and some loss of shoots from stem rot. Web and leaf blights seemed to be most prevalent on the marigolds, and root rot was most prevalent on the vinca, with some leaf blight also observed. Ratings were significantly higher for vinca root health in the 70:30 peat/PWC substrate than the Fafard 4P, and shoot health was significantly higher on the impatiens grown in the 80:20 peat/PWC substrate. (Table 2) The impatiens grown in the 80:20 peat/PWC displayed the least amount of wilting caused by disease infection.

Pythium symptoms were not as obvious as the Rhizoctonia. Shoot symptoms observed were mild to moderate stunting, and stem cankers near the substrate level. Substrates containing peat/PWC were shown to have less disease severity on the root systems of vinca and impatiens than Fafard 4P, with the 80:20 peat/PWC having the highest mean rating for those species (Table 3).

Literature Cited:

1. Daughtrey, M.L. 1995. Compendium of flowering potted plant diseases. APS Press, St. Paul, MN.
2. Judd, L.A. 2013. Rhizometrics: Novel techniques to observe and measure root growth of container-grown crops. North Carolina State University, North Carolina. Master's Thesis.
3. Owen, W.G. 2013. Pine wood chips as an alternative to perlite in greenhouse substrates: Cultural parameters to consider. North Carolina State University, North Carolina. Master's Thesis.

4. Parker, K.C. Pythium aphanidermatum. http://www.cals.ncsu.edu/course/pp728/Pythium/Pythium_aphanidermatum.html
5. Wright, A.N. and R.D. Wright. 2004. The Horhizotron™: A new instrument for measuring root growth. HortTechnology 14:560-563

Table 1. Comparison of fresh weights, plant health ratings, and bloom counts of bedding snapdragons grown in three different substrates that were infected with *Pythium aphanidermatum*.

Substrates	Fresh Weight (g)	Plant Health Rating[v]	Bloom Count
80:20 P/PWC[z]	50.7a[w]	4.2a	20.0a
70:30 P/PWC[y]	39.0a	3.8ab	5.0a
4P[x]	18.7b	2.8b	0.0a

[z] Substrate containing 80% peat moss and 20% pine wood chips.
[y] Substrate containing 70% peat moss and 30% pine wood chips.
[x] Fafard 4P.
[w] Tukeys Studentized Range Test ($p \leq 0.05$). Means with same letter in column = no significant difference.
[v] Plant Health Rating on a scale of 1 to 5 with 5 = healthy, 4 = slightly stunted, 3 = chlorosis/moderate stunting/delayed flowering, 2 = wilting/severe stunting, and 1 = dead.

Table 2. Root and shoot health ratings of annuals grown in three different substrates that were infected with *Rhizoctonia solani*.

	Vinca		Impatiens		Begonia		Marigold	
Substrate	Root[w]	Shoot[v]	Root	Shoot	Root	Shoot	Root	Shoot
80:20 P/PWC[z]	2.9ab[u]	3.3a	2.1a	3.3a	2.7a	3.4a	3.3a	3.1a
70:30 P/PWC[y]	3.7a	4.1a	1.8a	2.3b	2.3a	3.1a	3.3a	3.3a
4P[x]	2.7b	3.3a	1.8a	2.2b	2.6a	2.6a	3.5a	2.9a

[z] Substrate containing 80% peat moss and 20% pine wood chips.
[y] Substrate containing 70% peat moss and 30% pine wood chips.
[x] Fafard 4P.
[w] Root health rated on a scale of 1 to 5 with 5 = healthy white roots / no disease recovered, 4 = 25% root rot or seemingly healthy roots + disease recovered, 3 = 50% root rot, 2 = 75% root rot, and 1 = brown/dead roots.
[v] Shoot health rated on a scale of 1 to 5 with 5 = healthy, 4 = slightly stunted/few cankers or leaf blight, 3 = chlorosis/moderate stunting/delayed flowering/moderate stem and crown rot or leaf blight, 2 = wilting/severe stunting/severe stem and crown rot or leaf blight, and 1 = dead.
[u] Tukeys Studentized Range Test ($p \leq 0.05$). Means with same letter in column = no significant difference.

Table 3. Root and shoot health ratings of annuals grown in three different substrates that were infected with *Pythium ultimum.*

	Vinca		Impatiens		Begonia		Marigold	
Substrate	**Root**[w]	**Shoot**[v]	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**
80:20 P/PWC[z]	3.4a[u]	4.3a	3.0a	4.0a	2.9a	3.5a	3.4a	3.8a
70:30 P/PWC[y]	3.3a	4.0a	2.7ab	3.6a	3.2a	3.9a	3.1a	3.8a
4P[x]	2.5b	3.6a	2.3b	3.3a	2.9a	3.5a	2.9a	3.5a

[z] Substrate containing 80% peat moss and 20% pine wood chips.
[y] Substrate containing 70% peat moss and 30% pine wood chips.
[x]Fafard 4P.
[w]Root health rated on a scale of 1 to 5 with 5 = healthy white roots / no disease recovered, 4 = 25% root rot or seemingly healthy roots + disease recovered, 3 = 50% root rot, 2 = 75% root rot, and 1 = brown/dead roots.
[v]Shoot health rated on a scale of 1 to 5 with 5 = healthy, 4 = slightly stunted, 3 = chlorosis/moderate stunting/delayed flowering, 2 = wilting/severe stunting, and 1 = dead.
[u]Tukeys Studentized Range Test ($p \leq 0.05$). Means with same letter in column = no significant difference.

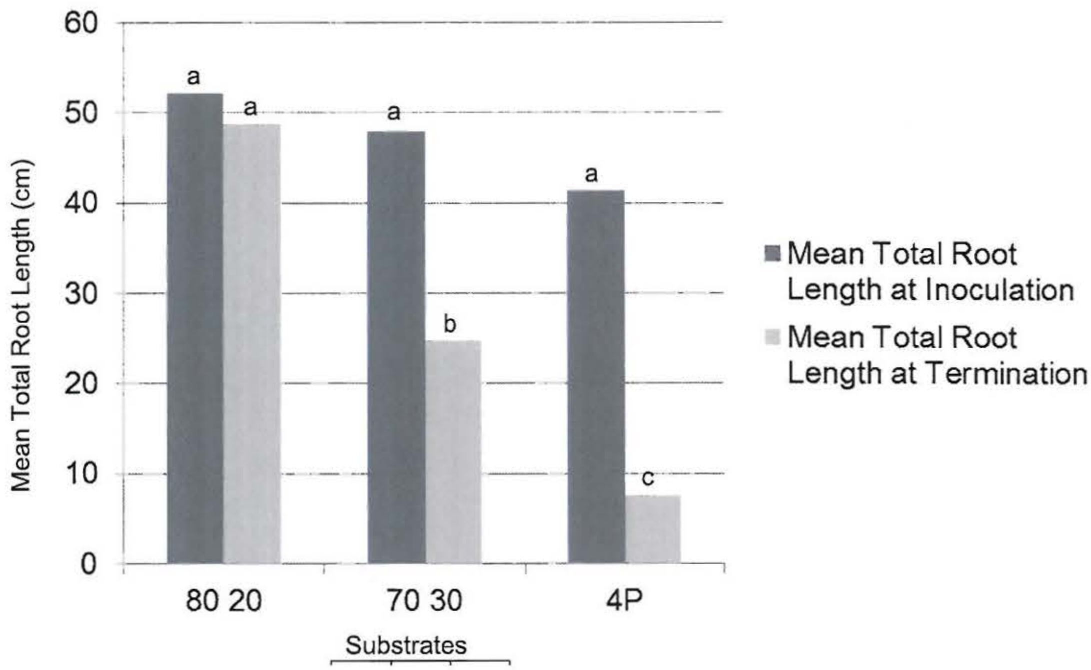

Figure 1. Comparison of mean total root lengths of snapdragons grown in mini-Horhizotrons containing 80% peat moss and 20% pine wood chips, 70% peat moss and 30% pine wood chips, and Fafard 4P at the time of inoculation with *Pythium aphanidermatum*, and at the termination of the experiment.

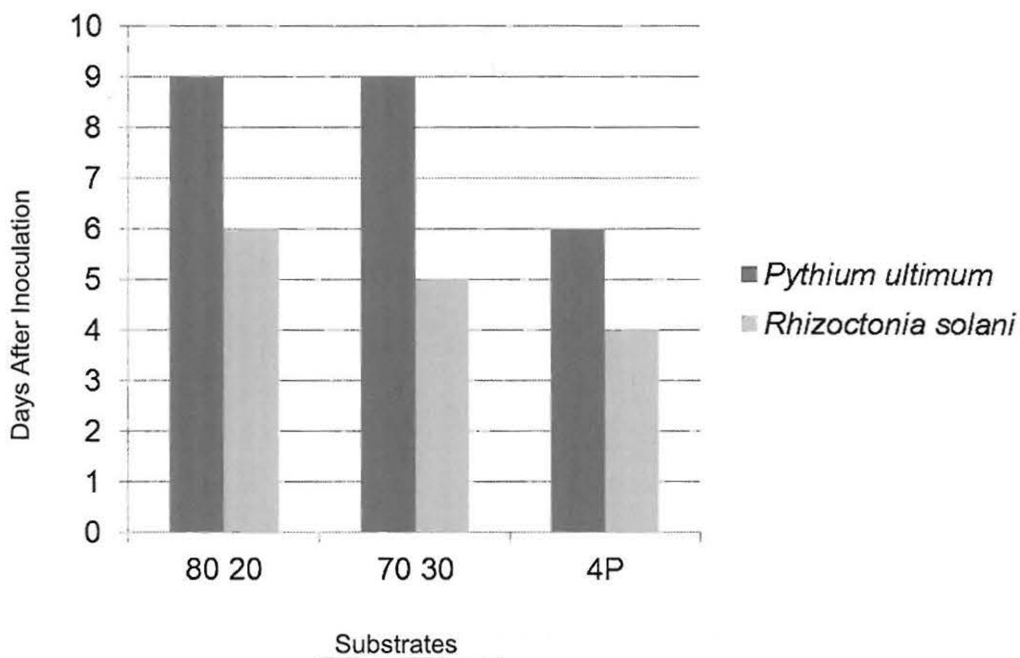

Figure 2. The number of days after inoculation with *Rhizoctonia solani* and *Pythium ultimum* when symptoms were visible on bedding annuals grown in substrates containing 80% peat moss and 20% pine wood chips, 70% peat moss and 30% pine wood chips, and Fafard 4P.