The mini-horhizotron as a tool for assessing disease severity in container grown annuals

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Abstract

Two experiments were conducted to assess the use of the mini-horhizotron in measuring disease severity of *Pythium aphanidermatum* on the roots of bedding snapdragons and poinsettias grown in three different horticultural substrates. Plugs of *Antirrhinum majus* 'Snapshot Red' and *Euphorbia pulcherrima* 'Angelica White' were planted in mini-horhizotrons containing either a commercial potting mix or two substrates containing pine wood chips (PWC): 80% peat moss and 20% pine wood chips (80:20 PWC), or 70% peat moss and 30% PWC (70:30 PWC). Tracings of the root system were taken using clear transparencies at the time of inoculation with *Pythium aphanidermatum*, and one month later at the termination of the experiments. The tracings were uploaded into Cornell University's RootReader2D software and measured for total root length. Results showed that snapdragons grown in the 80:20 PWC substrate had a total root loss of 6%, as compared to 48% for the 70:30 PWC substrate and 81% for the commercial mix. *Pythium* infection was more severe for poinsettias, with total root losses of 71, 87 and 91% in the commercial, 80:20 PWC, and 70:30 PWC substrates, respectively. Visual observations that were noted weekly during the experiment for both snapdragon and poinsettia provided evidence of the timing and severity of root disease symptoms. Other observations included fungus gnat (*Bradysia* spp.) larva, water-soaked and necrotic roots, and loss of root hairs. The results of this study illustrate how the mini-horhizotron, when used in addition to other disease assessment techniques, can help provide a non-destructive assessment of root disease severity over time. 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.

Keywords: *Pythium*, pine wood chips, mini-horhizotron, rhizosphere

INTRODUCTION

Methods for investigating disease severity and incidence of root diseases are frequently subjective and/or destructive (Wright and Wright, 2004). Current methods that are commonly used are subjective root ratings, removal of the plant and roots from the growing medium, and assessment of above ground symptoms (which may involve subjective plant growth ratings). Methods for observation of roots in situ are not as prevalent, especially in horticultural research, and include a variety of rhizotrons (Wright and Wright, 2004). Many of these rhizotrons are expensive to construct, require specialized equipment, do not accurately represent a similar rhizosphere environment as that of greenhouse containers, and are not readily accessible for non-destructive measurements and observations of root development and substrate conditions (Campbell and Neher, 1996).

The mini-horhizotron, developed in the Horticultural Substrates Laboratory at North Carolina State University, is a device designed to observe and measure root growth for small plants during pot culture (Judd, 2013). It has three concave chambers constructed out of transparent acrylic sheets, allowing the rhizosphere to be viewed. Each chamber has two measurable faces, giving a sum of six measurable faces per mini-horhizotron. Shade panels fit tightly against the acrylic walls to block light during plant growth. Each mini-horhizotron

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holds about the same volume of substrate as a plastic greenhouse pot (16.5 cm diameter azalea pot; 11.8 cm ht; Figure 1).

Figure 1. The mini-horhizotron.

Pine wood chips (PWC) have been investigated as an alternative aggregate to perlite in peat-based greenhouse mixes (Jackson and Fonteno, 2013; Owen, 2013). 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. 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. Recent research has shown that pine wood chips (PWC) are a suitable alternative to perlite in peat-based substrates (Owen, 2013), 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.

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. *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* 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. Common symptoms are yellowing foliage, stunted plant growth, and wilt (Daughtrey, 1995).

Being a relatively new technology, there is also an absence of information about the potential of using the mini-horhizotron as a tool to assess root disease severity in horticultural crops. Therefore, the objectives of this study were to: 1) determine the disease occurrence of *Pythium aphanidermatum* and in peat-based substrates amended with either perlite or PWC aggregates; and 2) test the mini-horhizotron for possible use as a tool for root disease observation and measurement in horticultural crops.

MATERIALS AND METHODS

The mini-horhizotron study was implemented at the Marye Ann Fox Teaching Laboratories Greenhouse at North Carolina State University on March 7, 2013. Three different substrates were used: a commercial mix (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 (80:20 PWC), and a substrate containing 70% peat moss and 30% PWC (70:30 PWC). The PWC substrates were amended with 3.86 $kg \cdot m^3$ 200 mesh dolomitic limestone. Six mini-horhizotrons were filled with each individual substrate. To account for substrate settling which occurs after initial irrigation events, the

mini-horhizotrons were tapped three times by lifting the unit approximately 10 cm from a hard surface and gently dropping to settle the substrate, and then filled to the top with substrate again. One plug of *Antirrhinum majus* 'Snapshot Red' (bedding snapdragon) was planted in each box. Plants were fertilized at each watering with 200 mg L^{-1} nitrogen derived from 20N-4.4P-16.6K (Peatlite Special, Peters Professional; The Scotts Co., Marysville, OH) and injected at 1:100 ratio by Dosatron injector (D14MZ2; Dosatron International, Inc., Clearwater, FL). Plants were allowed to grow until at least 5 roots reached the end of each measurable face of the mini-horhizotrons.

On April 23, 2013, the mini-horhizotrons 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 April 14 and 15, 2013. The autoclaved rice grains were then inoculated on April 16, 2013 with four colonized agar discs of *P. aphanidermatum*. Six grains of inoculum were inserted five cm below the substrate surface of each mini-horhizotron using tweezers.

To assess root system growth and development, roots of all plants were traced by hand on May 24 to determine total root length. Cumulative root length was measured by tracing the roots on a transparency sheet (27.9×7.6 cm transparency film; 3M Visual Systems Division, Austin, TX) with a thin-tip, wet-erase transparency marker. Only root growth against the clear chamber walls could be traced. Tracings were made on the date of inoculation, and again one month later at the termination of the study. Transparencies were cut to match the size of each measurable face, and were held in place by binder clips while roots were being traced. The root tracings were digitally photographed, and the images were calibrated and converted to black and white using a high contrast red filter in Adobe Photoshop CS5 and uploaded to RootReader 2D software (RootReader 2D version 4.3.1; Cornell University, USDA-ARS, Ithaca, NY). The RootReader 2D software selected the traced roots and measured total root length of the traced roots. Weekly visual observations were made regarding the condition of the plant roots and progression of visual root disease symptomology. On May 24, 2013 root samples from each plant were plated onto PARP media (a *Pythium* selective media) to confirm the presence of *P. aphanidermatum.* Data were analyzed using LSD ($P \leq 0.05$) (SAS Institute version 9.2, Cary, NC).

This experiment was repeated with poinsettia. On March 1, 2013 rooting hormone was applied to cuttings of *Euphorbia pulcherrima* 'Angelica White' that were stuck in Oasis Strips and placed in a misting system to root. On May 27, 2013 mini-horhizotrons were filled with each substrate (same as snapdragon experiment) and one rooted cutting was planted in each. On June 28, 2013, the mini-horhizotrons were inoculated with *Pythium aphanidermatum* from colonized rice grains, following the procedures outlined in the snapdragon study. On July 26 roots of all plants were traced, and the experiment was concluded. For further observations of the potential usefulness of using the mini-horhizotron for root growth assessment four other species were grown in the mini-horhizotron, at the same time as the poinsettia trial (May 27, 2013). Additional species included wax begonia (*Begonia semperflorens-cultorum*), vinca (*Catharanthus roseus* 'Cooler Deep Orchid'), impatiens (*Impatiens walleriana*), and marigold (*Tagetes patula* 'Janie Deep Orange'). These species were not inoculated and measured like the snapdragon and poinsettia, but instead were grown in the mini-horhizotron for visual observation of their root systems, and to determine if this apparatus can be used to successfully grow and test multiple species for future work.

RESULTS

Snapdragon plants grown in the commercial substrate had an average of 41 cm of total root length at the time of *Pythium* inoculation compared to 52 and 48 cm for plants grown in the 80:20 and 70:30 PWC substrates, respectively (Figure 2). The total root length decreased to 8 cm in the commercial substrate, 49 cm in the 80:20 PWC substrate, and 25 cm in the 70:30 PWC substrate four weeks after inoculation (Figure 2). This equates to a total root loss of 6% for the 80:20 PWC substrate and 48% for the 70:30 PWC substrate, as compared to 81% for the commercial mix.

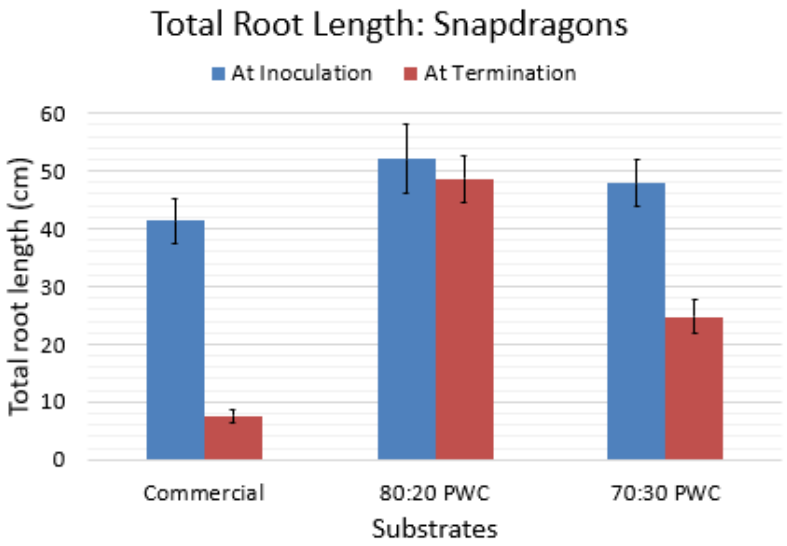

Figure 2. Comparison of mean total root lengths of snapdragons (*Antirrhinum majus* 'Snapshot Red') grown in mini-horhizotrons containing a commercial mix, a substrate containing 80% peat moss and 20% pine wood chips (80:20 PWC), and a substrate containing 70% peat moss and 30% pine wood chips (70:30 PWC). Measurements were taken at the time of inoculation with *Pythium aphanidermatum*, and four weeks later at the termination of the experiment.

Pythium infection was more severe for poinsettias, with total root losses of 71, 87 and 91% in the commercial, 80:20 PWC, and 70:30 PWC substrates, respectively (Figure 3). Poinsettia plants grown in the commercial substrate had higher average root length at the time of inoculation than both the PWC substrates (Figure 3).

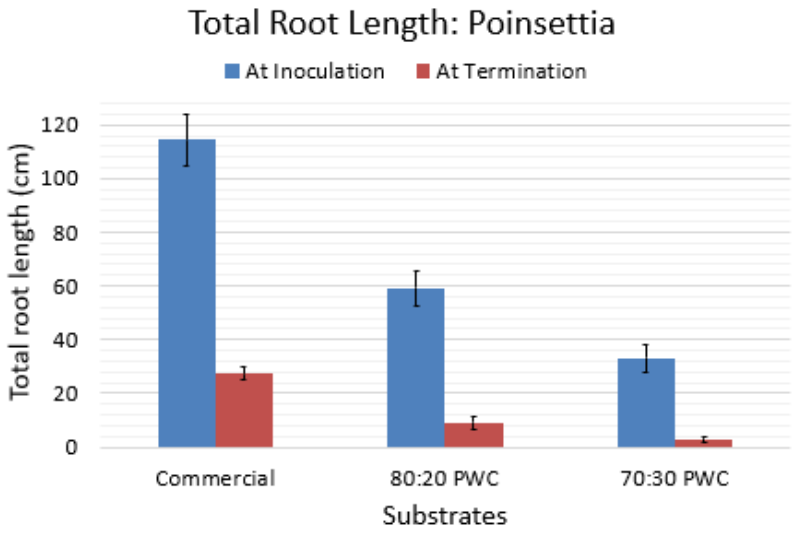

Figure 3. Comparison of mean total root lengths of poinsettias (*Euphorbia pulcherrima* 'Angelica White') grown in mini-horhizotrons containing a commercial mix, a substrate containing 80% peat moss and 20% pine wood chips (80:20 PWC) and a substrate containing 70% peat moss and 30% pine wood chips (70:30 PWC). Measurements were taken at the time of inoculation with *Pythium aphanidermatum*, and four weeks later at the termination of the experiment.

Visual observations that were noted weekly during the experiment for both snapdragon and poinsettia provided evidence of the timing and severity of root disease symptoms (Table 1). The ability to observe the root system in situ allowed for mycelium growth/presence to be documented as it occurred (time after inoculation), which is difficult to do when plants are grown in traditional containers.

Table 1. Weekly observations of disease symptoms shown by bedding snapdragons and poinsettias grown in mini-horhizotrons containing either a commercial mix or substrates containing peat moss and pine wood chips, inoculated with *Pythium aphanidermatum.*

Snapdragon[a]	
Week 1	Mycelium seen growing from rice grains one day after inoculation[b]
Week 2	Water-soaked roots were visible. Roots with the cortex sloughed off were visible on some plants
Week 3	Numerous roots had brown tips. Many necrotic roots were visible, and noticeable root dieback had occurred on some plants. Fungus gnat[c] larvae were visible in some of the mini-horhizotrons
Week 4	Many water-soaked and necrotic roots were visible. Extensive root dieback was observed in the commercial mix. Many of the roots in the 80:20 PWC[d] substrate showed lesser symptoms such as tan roots, and roots lacking root hairs. New root growth was visible in both substrates, but more so in the 80:20 peat/PWC
Poinsettia[e]	
Week 1	Mycelium seen growing from rice grains one day after inoculation[f]
Week 2	Brown root tips were observed and some water-soaked roots were visible
Week 3	Fungus gnat larvae[c] were observed in some mini-horhizotrons. Root dieback was visible
Week 4	Necrosis of multiple large roots was observed on plants in both treatments
Week 5	Continued root dieback was observed, at a much more dramatic rate than that of the snapdragons. Extensive algae growth was seen in some of the mini-horhizotrons. Many black, rotten roots were observed in both substrates
Week 6	Extensive root dieback was observed on the plants grown in the 80:20 PWC[d] substrate, and several of the mini-horhizotrons had no healthy roots visible at all along the measurable face. Other mini-horhizotrons showed new root growth alongside the diseased roots, especially those in the commercial mix

[a]*Antirrhinum majus* 'Snapshot Red.' Plugs were planted into mini-horhizotrons on March 7, 2013.
[b]Inoculation took place on April 23, 2013. Six colonized rice grains were used per mini-horhizotron.
[c]*Bradysia* spp.
[d]Substrate containing 80% peat moss and 20% pine wood chips.
[e]*Euphorbia pulcherrima* 'Angelica White.' Cuttings were planted in Oasis strips on March 1, 2013 and planted in mini-horhizotrons on May 27, 2013.
[f]Mini-horhizotrons were inoculated on June 28, 2013. Six colonized rice grains were used per mini-horhizotron.

Other observations included fungus gnat (*Bradysia* spp.) larva, water-soaked and necrotic roots, root hairs, and root necrosis/die-bark (Table 1; Figure 4).

The annual species (begonia, vinca, impatiens, and marigold) grown in mini-horhizotrons developed healthy root systems (visually assessed) and provided additional understanding of how plants (and their roots) grow in this apparatus and how disease treatments/inoculations may be conducted in the future (Figure 5).

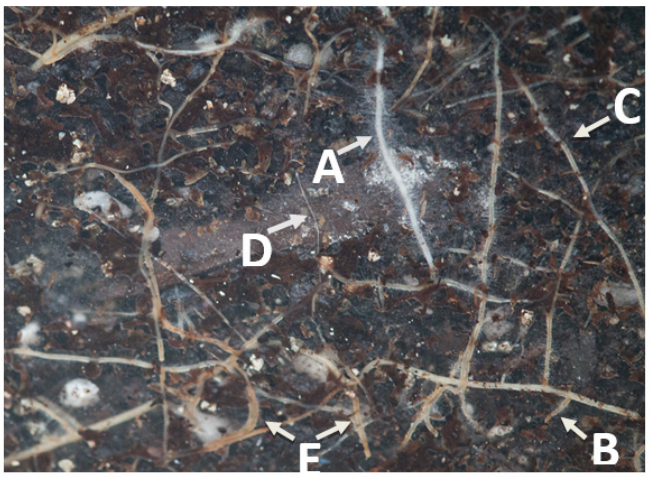

Figure 4. Different stages of root rot/disease on snapdragon roots that were visible in a mini-horhizotron, (A) healthy white root with visible root hairs, (B) discolored root lacking root hairs, (C) water-soaked root, (D) root with cortex sloughed off, and (E) necrotic roots.

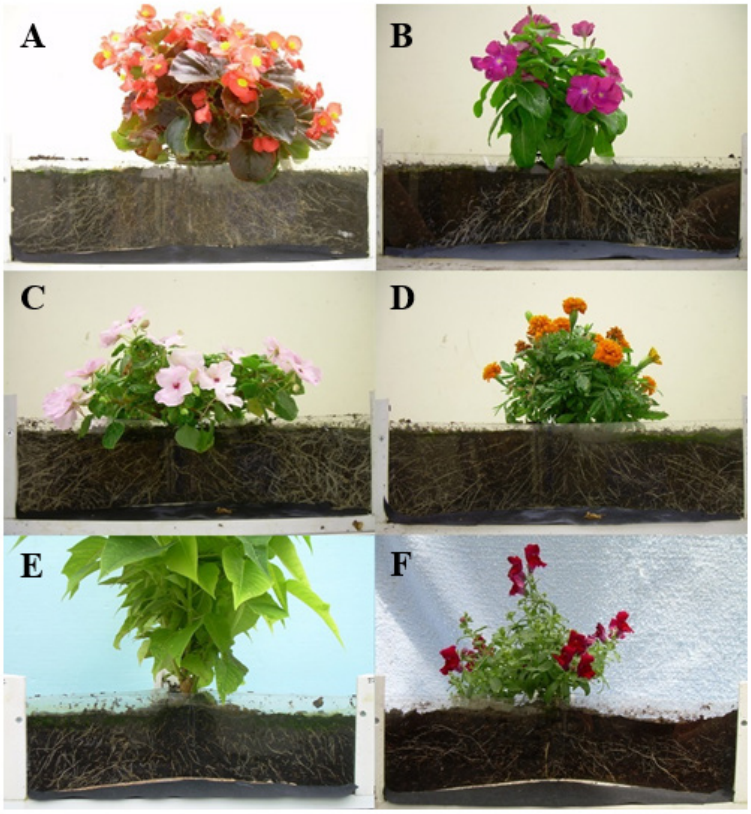

Figure 5. Root systems of floriculture crops grown in mini-horhizotrons for root observation and measure: A) wax begonia (*Begonia semperflorens-cultorum*), B) vinca (*Catharanthus roseus* 'Cooler Deep Orchid'), C) impatiens (*Impatiens walleriana*), D) marigold (*Tagetes patula* 'Janie Deep Orange'), E) poinsettia (*Euphorbia pulcherrima* 'Angelica White'), and F) snapdragon (*Antirrhinum major* 'Snapshot Red').

DISCUSSION

The results of these experiments demonstrate how the mini-horhizotron can be utilized to investigate the disease severity of *Pythium aphanidermatum* on bedding plants grown in peat-based substrates containing varying percentages of pine wood chips, and an industry standard potting mix. The results from these experiments indicate that disease severity was equal and often less prevalent in snapdragons grown in substrates containing PWC than in a traditional potting mix; however, poinsettias had larger root systems when grown in a commercial mix, and less dieback after *Pythium* dieback, although root loss was severe in all of the substrates. In addition, mini-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 and root disease research.

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