Mini-Horhizotron: An Apparatus for Observing and Measuring Root Growth of Container-grown Plant Material In Situ

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Abstract. An apparatus was developed that allows for a range of non-destructive measurements on root growth in containers (pot culture). The mini-Horhizotron was designed to measure root growth of small plant material such as seedlings, herbaceous plugs, or woody plant liners normally grown in containers less than 3.8 L. The mini-Horhizotron design has three chambers extending away from the center that could be filled with the same substrate or filled separately with different substrates/treatments to observe root growth response from a single plant. The objectives were: 1) to test the suitability of the mini-Horhizotron's design and its effects on plant growth with several different species; 2) to test two different experimental designs on the mini-Horhizotrons for research purposes; and 3) to test the effect of wood-amended substrates on root length of a single species. Measurement included quantification of the longest roots growing away from the center (where the plug was transplanted). Herbaceous and woody plants grown in the mini-Horhizotrons included: *Echinacea purpurea* (L.) Moench 'Prairie Splendor', *Chrysanthemum* L. 'Garden Alcala Red', *Rudbeckia hirta* L. 'Becky Yellow', and *Ilex crenata* Thunb. 'Steeds'. These plants produced root and shoot growth similar to plants grown in traditional greenhouse containers with approximately equal heights and volumes, allowing for root observations in the mini-Horhizotrons to be considered simulations of traditional container-grown crop production. Results from the initial root growth measurements provide evidence that the mini-Horhizotron may be used with a different substrate in each chamber, effectively altering a portion of the rhizosphere of one plant and reducing the number of mini-Horhizotrons needed for replications during scientific studies. Root growth was measured in three substrates containing by volume 70:30 peat:perlite (control), peat:pine-wood chips, or peat:shredded pine wood. For the species grown in pine-wood chips or shredded pine wood-amended substrates, root growth equaled or exceeded that observed in the control substrate at all time periods. The mini-Horhizotron was used to non-destructively measure treatment/substrate effects on root growth while providing full visual access to the root zone and developing root system.

A large portion of the U.S. green industry is involved with growing plants in containers, including bedding plants, vegetable plants, foliage plants, potted flowering plants, potted nursery stock, and other assorted floriculture crops. Root growth of crops grown in containers is a central element in overall plant performance, whether it is during propagation, production, or post-production (e.g., transplant

success) as a result of the combined functions of roots being anchorage, support, and water and nutrient uptake (Wraith and Wright, 1998). Considering the large portion of the industry involved with growing plants in containers and the importance of understanding the physiology and morphology of roots, the factors that influence root growth in container production need to be continually investigated. However, root growth and root architecture are frequently excluded in horticultural research (Wright and Wright, 2004), and the study of natural root development is a challenge as a result of the difficulty of root observations in containers during crop production (Silva and Beeson, 2011).

Strategies and techniques for observing, studying, and quantifying root growth have been reported over the past nine decades. Observing and measuring root growth of crops began in the field, and methods such as hand-drawing were time-consuming and difficult (McDougall, 1916; Weaver et al.,

1922). Several of the field techniques, like photography, have since been modified to also measure root growth of plants grown in containers. Fortunately, many advances have been made over the decades in the study of root measurements, including techniques that can be easier, faster, and more descriptive of root growth, like the rhizotron or mini-rhizotron for field-grown plants (Taylor et al., 1990).

Currently, the most common root system evaluations of plants grown in containers are 1) subjective root ratings; and 2) root dry weight determination. Subjective root ratings can be a simple and easy way to qualitatively describe rootballs, washed roots, and propagated rooted cuttings. Ratings can evaluate root density, appearance, branching, and distribution. However, the person rating the root system must first understand how to accurately rate the quality of the root system (Walters and Wehner, 1994), and because the root rating is subjective, it often varies with each examiner. Root dry weight is a destructive method, which involves extracting and drying the plant root system (Aung, 1974). For dry weight measurements, and possibly for root ratings, substrate must be washed from the roots and many of the fine roots and root hairs are lost in this process as are the natural positions and arrangements of the roots. In standard methods of washing and storing root samples, losses of dry weight from 20% to 40% may occur (Oliveira et al., 2000; van Noordwijk and Floris, 1979).

The HorhizotronTM was developed at Auburn University and Virginia Tech as a non-destructive technique to measure horizontal root growth from rootballs of plants grown in nursery containers, allowing for post-transplant assessment (Wright and Wright, 2004). The HorhizotronTM is constructed of eight panels of glass attached to an aluminum base to form four wedge-shaped quadrants and is suitable for greenhouse or field use and fits a range of nursery stock rootballs. Previous studies with the HorhizotronTM have shown the design allows for each quadrant to be modified in different ways to examine the effects of different rhizosphere conditions (Jackson et al., 2005; Price et al., 2009; Wright and Wright, 2004). However, the HorhizotronTM works best with large-sized rootballs (3.8 to 11.4 L); the glass panels are not permanent and can move and crack; and the shade box does not restrict all light from the root system. Silva and Beeson (2011) developed a large-volume rhizotron to observe root growth in an environment closer to natural soil conditions and still have the apparatus above ground and therefore relatively easier to collect measurements. However, this design is even larger than the HorhizotronTM and intended for woody plants with large rootballs to imitate post-transplant/or field growing conditions.

Root growth in transparent containers/root boxes may be quantified using different methods. Digital imaging can be used by computer programs to evaluate root systems, as Silva and Beeson (2011) reported with a rhizotron. Digital imaging includes photographs or videos, scanned images of exposed roots, or scanned

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root tracings. Advantages to using image analysis include the advancing technology that allows computers to determine structure and segmentation of root growth, which will aid in describing rate of change in roots and their growth pattern (Spalding and Miller, 2013). However, images can often be unfocused or blurry, and when taking images of glass or reflective surfaces, the glare from the sun or artificial lighting is a problem. In some cases, the soil/substrate might not contrast with the plant roots enough to be completely visible. This causes the program user to have to manually pick out each root and trace the length of it themselves and, depending on the number of roots per image, could be time-consuming. Root growth rates can also be quantified by measuring the length of the longest roots against the transparent walls. Measuring the length of the five longest roots on each side of a quadrant is commonly used with the Horhizotron™ (Jackson et al., 2005; Price et al., 2009; Wright and Wright, 2004) with the roots of two sides of one quadrant averaged to obtain the experimental value for that quadrant (Wright and Wright, 2004).

The use of different wood products as substrate components have been widely researched in the past decade and have been proven to be an acceptable and sustainable alternative to greenhouse and nursery substrates as well as suitable substrate components when amended to peat-moss and pine bark. Gruda and Schnitzler (2004b) examined spruce (*Picea* sp.) wood fiber substrates (WFS) and noted particularly well-developed root systems of plants grown in the WFS compared with plants grown in peat and rockwool substrate. The physical properties of both a coarser and finer WFS resembled peat substrates with the amount of total pore space; the finer WFS had a high air volume compared with peat, and mixing both WFS and peat substantially improved the air volume (Gruda and Schnitzler, 2004a). Wright and Browder (2005) also noted an increase in root growth with plants grown in 75% pine bark:25% loblolly (*Pinus taeda* L.) pine chips compared with 100% pine bark. Jackson et al. (2010) observed the highest root rating for plants grown in loblolly pine tree substrates compared with plants grown in peat-lite or pine bark. However, these observations were quantified with root dry weights or subjective ratings, so the extent of the apparent root growth enhancement remains to be accurately quantified and explained.

Further investigations of plant root growth in containers and additional understanding of the factors that affect it are critical for improving overall growth and quality of container-grown plants. Developing new techniques to study, observe, and measure root growth of seedlings and small-sized plants during production (vegetable transplants, plugs for floricultural crops, and nursery liners) will be beneficial in future research of root development. The premise of this work was to develop a new apparatus to measure root growth in a greenhouse production setting using a system/technique similar to the Horhizotron™ but with a different purpose, design, and construction components

(discussed below). The name of this apparatus is the mini-Horhizotron, based off of the name Horhizotron™ (horizontal root growth measurement instrument), which is used with permission of the Horhizotron™ designers (Wright and Wright, 2004). The objectives of this work were: 1) to design the mini-Horhizotron and test its suitability for observing and measuring container-grown plant roots non-destructively; 2) to compare the effect of two experimental designs (number of substrates/treatments per mini-Horhizotron) on plant root growth; and 3) use the mini-Horhizotrons to compare root growth of plants in different container substrates.

Materials and Methods

Design and construction of the mini-Horhizotron. The mini-Horhizotron was designed to maximize visible substrate surface area for root measurements. The final design included six flat surfaces producing three radiating chambers forming a geometric deltoid (Fig. 1). The mini-Horhizotron (10.2 cm ht) was designed with a total volume of 2.1 L compared with a common greenhouse container (16.5 cm o.d. azalea pot; 11.8 cm ht), which holds 1.7 L, respectively. This provided similar air and water profiles in the pot and mini-Horhizotron. The surface area of the pots used (measured) in these studies was 480 cm², whereas the six flat surfaces of the mini-Horhizotron were 1260 cm², almost a 3-fold increase.

Thirty mini-Horhizotrons were made, each having a triangular base with an area of 907.2 cm² (44.3 cm base length with corners removed) and was cut from a polyvinyl chloride (PVC) board (Plasticlad®, Franklin, VA). Three end caps (PVC trimboard; Plasticlad®) were placed at each corner of the triangle base and permanently fastened with 4.45-cm wood screws (Power Pro #8; Hillman Group, Inc., Cincinnati, OH; Fig. 2A). Each end cap had a trapezoid shape 12.7 cm tall with two 6.35-mm notches (2.54 cm apart) to hold the ends of two chamber walls (Fig. 1). Transparent acrylic sheets (6.35 mm diameter; Lucite International, U.K.) were cut to form the three chambers, 10.2 cm tall and 41.9 cm in length (Fig. 1). The concave curves of the acrylic sheets were created by heating the acrylic sheets in an oven at 150 °C for 3 min and then placing the sheet on a form-fitting, curved mold, which was constructed to have the desired angle for each chamber. On the mini-Horhizotron, the acrylic sheets are held in place by screws (#5 × 1.3 cm; Hillman Group, Inc.), which were drilled into the end cap notches and through the acrylic sheets to hold them in place. To minimize leaks, the acrylic sheets were attached to the base with adhesive caulk (Loctite® Polyseamseal®, Westlake, OH). All components of the mini-Horhizotron base, chamber walls, and end caps are permanently bonded together with screws or caulking. End caps are 2.5 cm taller than the walls so when stacked during storage, weight is not transferred to the acrylic walls. Mini-Horhizotrons were constructed

with lightweight components; average weight of the mini-Horhizotron is 2.25 kg (n = 6). To facilitate drainage, three 0.07-cm holes were drilled into the base of each chamber 5.5 cm apart starting 3 cm from the end caps (Fig. 2B). Shade panels (6.35 mm diameter, PVC panel; Plasticlad®) were constructed to fit flush against the concave clear acrylic walls to block sunlight from the rhizosphere, and a flange strip was attached with a staple gun to create a “lip” over the acrylic sides to aid in the ability to remove the shade panel and to block sunlight from entering at any angle from the top (40 cm length, 11.4 cm ht, 1 cm width; Fig. 2B–D). Shade panels were constructed in the same procedure as the acrylic sides, and flange strips (1.3 cm ht, 1 cm width; Fig. 2B–D) were cut from the same material as the shade panels (Fig. 3A–C).

Initial tests with the mini-Horhizotron. Three substrates were used in the initial testing of the mini-Horhizotron, 70% peat amended with 30% perlite (PL), pine-wood chips (PWC), or shredded pine wood (SPW; v/v). On 19 Dec. 2011, 8-year-old loblolly pine trees (*Pinus taeda* L.) were harvested in Chatham County, NC, at ground level, delimbed, and stored under shelter from weather. On 2 Jan. 2012 the delimbed pine logs were chipped in a DR Chipper (Model 356447; 18 HP DR Power Equipment, Vergennes, VT) to produce coarse wood chips. The pine logs destined for shredding were processed in a Wood Hog shredder (Morbark® Model 3800, Winn, MI). Both the chipped and shredded pine wood was then processed in a hammermill through a 6.35-mm screen (Meadows Mills, North Wilkesboro, NC) to produce two products, PWC and SPW. The SPW particles have been reported to have physical properties similar to peat and the PWC component properties similar to perlite (Fields, 2013). Both wood materials have been investigated as perspective new horticultural substrate components (Jackson and Fonteno, 2013). Both the PWC and SPW were air-dried to a moisture content of 35% and stored under shelter in unsealed 1.5-m³ bags for 23 weeks before the initiation of this study.

Substrates were mixed on 1 June 2012, tested for initial pH, and then amended with dolomitic limestone (#200; Mississippi Lime Company, Vicksburg, MS) at 3.86 kg-m³ to achieve a desired pH of 5.8. On 2 June 2012, three mini-Horhizotrons were filled with each individual substrate. To account for substrate settling that occurs after initial irrigation events, the mini-Horhizotrons were tapped three times by lifting the unit 10 cm from a hard surface and dropping to settle the substrate and then filled to the top with substrate again. Three species were used in this experiment: coneflower (*Echinacea purpurea* ‘Prairie Splendor’; 162-tray; C. Raker & Sons, Inc., Litchfield, MI), mum (*Chrysanthemum* ‘Garden Alcala Red’; 51-tray; C. Raker & Sons, Inc.), and holly (*Ilex crenata* ‘Steeds’; 10 cm, 16-liners; Casey Nursery, Inc., Goldsboro, NC). One plug or liner (holly) was planted into the center of each mini-Horhizotron. One mini-Horhizotron was considered a replication because all three chambers contained

Mini Horhizotron Section

Fig. 1. Schematic drawing of the mini-Horhizotron. Top view of the mini-Horhizotron with the end cap, the acrylic sides and chambers, and the base, before the corners of the end caps and base were trimmed. Shade panel section that fits directly against the clear acrylic side shows the flange strip to assist in the removal of the shade panel to view rhizosphere.

the same substrate. Three substrates $\times$ three replications of each substrate $\times$ three species made a total of 27 mini-Horhizotrons. Plants were also grown in the same substrates in traditional greenhouse containers to compare root dry weights at the end of the study to discern any effects the mini-Horhizotron's shape and increased surface area may have contributed to root growth compared with the plastic container of relatively similar substrate volume. Six greenhouse containers (16.5-cm-diameter pots; Dillen Products, Middlefield, OH) were filled with each substrate on 2 June 2012 and filled to the top of the container and lightly tapped three times to settle the substrate. One plug/liner of each species was planted into the center of the containers. Three substrates $\times$ six replications of each substrate $\times$ three species made a total of 54 containers. Mini-Horhizotrons and containers were randomized by species on a greenhouse bench in Raleigh, NC. As a result of preliminary physical property results showing all substrate physical properties were similar,

plants in the mini-Horhizotrons and containers could then be similarly hand-watered as needed depending on weather conditions and never showed symptoms of water stress. As a result of the design of the mini-Horhizotron and its shade panels, the shade panels can remain in place when irrigating plants because the substrate surface is exposed, similar to plants grown in a traditional plastic container. If shade panels are removed during irrigations, the clear-sided chambers allow for visual observations of water percolation and substrate hydration, which are events seldom viewed (Fig. 3D). 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 a Dosatron injector (D14MZZ; Dosatron International, Inc., Clearwater, FL).

Once per week root length measurements (cm) were taken on the three longest roots appearing on the clear sides of each chamber starting at 11 d after planting (DAP) and

finishing at 46 DAP. Only roots growing against the clear chamber walls were measured; any root growth occurring in the substrate not against the walls was therefore not visible and could not be measured. However, the width of each chamber was only 2.54 cm, so the center of each chamber was only 1 cm away from the chamber walls. This greatly increased the surface-to-volume ratio compared with traditional containers. The main/primary roots were not always the ones measured; roots grew from the plugs or liners similar to growing in a container, meaning the roots grew downward until an obstruction and either changed direction or sent out lateral roots. As a result of the roots growing down and sending out lateral roots, the longest root measured was the root tip furthest out from the plug/liner (center of the mini-Horhizotron). When the three longest roots of a species reached the end of each chamber, the experiment was ended. Each chamber had two measureable sides (Fig. 2A–D) giving a sum of six viewing/measurable sides per mini-Horhizotron. Measurements were

Fig. 2. (A) Design of the mini-Horhizotron depicting the three chamber configuration, (B) the removable shade panels, which fit directly against the acrylic chambers to restrict all light from the rhizosphere, (C) mini-Horhizotrons planted with herbaceous plugs and woody nursery liners (coneflower, mum and holly) and randomized on a greenhouse bench, and (D) mini-Horhizotrons with and without shade panels depicting seeding growth.

taken by attaching a transparent sheet (20.5 cm length $\times$ 10.5-cm ht transparency film; 3M Visual Systems Division, Austin, TX) with a printed cm^2 grid on each side, and roots were measured from the start of the gridlines, which was at the center of the mini-Horhizotron (where the plug/liner was planted) to the end of the gridlines, which reached the end of the chambers (at the end cap; 20.5 cm). Measuring three roots per side $\times$ six sides per mini-Horhizotron $\times$ three replications per substrate equals data taken on 54 roots $\times$ six measurement dates equaled 324 data points per plant species tested.

Both the mini-Horhizotrons and the container-grown plants were harvested on 54 DAP; shoots were removed at the substrate surface and the rootballs were carefully washed to remove substrate in preparation for dry weight determination. Both the shoots and washed root systems were oven-dried at 70 °C for 48 h.

Physical properties including air space (AS), container capacity (CC), and total porosity (TP) were determined for each substrate blend at experiment initiation using the North Carolina State University porometer method (Fonteno et al., 1995). Properties were determined using three representative samples of each substrate.

Single versus multiple treatments. Three substrates were used in different experimental

designs with the mini-Horhizotrons, 70% (v/v) peatmoss amended with 30% PL, PWC, SPW. On 4 July 2012, 9-year-old loblolly pine trees were harvested in Chatham County, NC, at ground level, delimbed, and subsequently stored under shelter for protection from the weather. The delimbed pine logs were then either chipped or shredded and hammermilled in the same process as previously described. Substrates were mixed on 28 July 2012, and all were tested for initial pH and then amended with dolomitic limestone (#200; Mississippi Lime Company) at $4.45 \text{ kg}\cdot\text{m}^{-3}$ to achieve a desired pH of 5.8. On 30 July 2012, three mini-Horhizotrons were filled with each individual substrate and tapped in the same manner as previously described for a total of nine mini-Horhizotrons. Six additional mini-Horhizotrons were separated in the center with a three-sided divider (3 cm length; 12 cm ht) positioned at 120° angles from one another so that the three chambers were separated and the substrate filling each chamber met in the middle of the mini-Horhizotron. Each chamber was filled with one of the three substrates in random order and the same tapping and refilling procedure occurred. Once filled, the divider was gently removed allowing the interface of the three substrates to be united. One plug of rudbeckia (*Rudbeckia hirta* 'Becky Yellow'; 288-tray; C. Raker & Sons,

Inc) was planted into the center of each mini-Horhizotron. Each of the three mini-Horhizotrons that contained the same substrate in all chambers was considered a single replication. The six mini-Horhizotrons with a different substrate in each chamber was considered a block design with six replications. A total of 15 mini-Horhizotrons were used in this experiment. Mini-Horhizotrons were completely randomized on a greenhouse bench grown in Raleigh, NC. Plants were watered and fertilizer in the same manner as previously described. Root length measurements (cm) were taken on the three longest roots appearing on the face of each chamber every 4 d starting at 15 DAP and ending at 67 DAP. Measurements of root length and substrate physical properties were taken as previously described.

Comparing rates of PWC substrates. Three substrates were tested in the mini-Horhizotron; peatmoss was amended with 20%, 30%, or 40% (v/v) PWC. Loblolly pine trees used to make PWC in this experiment were harvested and processed as previously explained. Substrates were mixed on 28 July 2012, initial pH of the substrates was tested, and all the substrates were amended with dolomitic limestone (#200; Mississippi Lime Company) at $4.45 \text{ kg}\cdot\text{m}^{-3}$ to achieve the desired pH of 5.8. On 30 July 2012, three

mini-Horhizotrons were filled and tapped with each individual substrate in the same manner previously described. One plug of rudbeckia (*Rudbeckia hirta* 'Becky Yellow'; 288-tray; C. Raker & Sons, Inc.) was planted into the center of each mini-Horhizotron. One mini-Horhizotron was considered a replication because all three chambers contained the same substrate. Three substrates $\times$ three replications of each substrate $\times$ one species made a total of nine mini-Horhizotrons. Mini-Horhizotrons were completely randomized on a greenhouse bench in Raleigh, NC. Plants were watered and fertilizer in the same manner as previously described. Root length measurements (cm) were taken on the three longest roots appearing on the face of each chamber every 4 d starting at 15 DAP and ended at 67 DAP. Measurements of root lengths and substrate physical properties were taken as previously described. Based on similar physical properties of the substrates, the nine mini-Horhizotrons were randomized on one greenhouse bench with a similar watering regime.

Statistical analysis. For all experiments, data were subjected to regression analysis of root growth over time using repeated measures, and data from measuring substrate physical properties were analyzed using GLM procedures (SAS Institute Version 9.2, Cary, NC). Means were separated by the least significant difference (LSD) at $P \leq 0.05$, except when a Tukey-Kramer significant difference ($P \leq 0.05$) was used to compare unbalanced data sets. Additionally, the comparison of root dry weights between plants in the mini-Horhizotrons and plants in the greenhouse containers were subjected to general linear model procedures and means were separated by LSD at $P \leq 0.05$.

Results and Discussion

Initial tests with the mini-Horhizotron. Shoot and root dry weights of coneflower and mum plants were the same in both the mini-Horhizotron and traditional containers (Table 1). The plants were also similar in all three substrates. Holly was the same in the two systems using the PL substrate. However, holly had both more shoot and root growth in the mini-Horhizotron in the PWC substrate and more shoot growth in the SPW substrate in the mini-Horhizotrons (Table 1).

All species exhibited nearly linear root growth over the course of the experiment in all three substrates (Fig. 4). At 11 DAP, all three substrates produced similar root growth in each species. From 18 DAP to 25 DAP, mums grown in PWC had longer root lengths than plants grown in PL and SPW. At 39 DAP, mum plants growing in PL had more root growth than plants growing PWC or SPW. However, at the end of the study (46 DAP), root growth of mums in all three substrates were not different. At 18 and 25 DAP, coneflower had more root growth in the PWC and SPW substrates compared with plants grown in PL substrate. The fourth (32 DAP) and fifth measurement (39 DAP)

dates for coneflower had greater root growth in SPW substrate compared with PL substrate, but plants in PWC substrate were not different from either. The final measurement date (46 DAP) for coneflower had more root growth in PWC substrate compared with plants grown in PL substrate, and plants grown in SPW substrate were not different from either. Holly had greater root growth in

PWC at 25 DAP through 32 DAP; at 39 DAP through 46 DAP, holly plants grown in all three substrates had no significant difference in root growth. Observed for all species, the PWC and SPW components either met or exceeded root growth for all time periods compared with the PL substrate. Physical properties of the three substrates used in this first experiment showed no differences in AS,

Fig. 3. (A) Seven-week-old *Rudbeckia* plants grown in a mini-Horhizotron during a growth trial, (B) shade panels removed during plant trial for accessibility to view the rhizosphere and measure root growth, (C) view of a root system (after shoot portion was removed) in the mini-Horhizotron with root length, branching/architecture, and root hairs easily visible, and (D) frontal view of the mini-Horhizotron showing water percolation/movement and substrate hydration at the initial irrigation event after planting.

Table 1. Comparison of shoot and root dry weights of *Echinacea*, *Ilex*, and *Chrysanthemum* species grown in greenhouse containers or in the mini-Horhizotrons.^z

Plant	Substrate	Shoot ^y (g)		Root ^x (g)	
		CT ^w	MH ^v	CT	MH
<i>Echinacea</i>	PL ^u	5.7 a ^t	6.3 a	3.0 a	2.5 a
	PWC ^s	6.1 a	6.5 a	4.4 a	3.4 a
	SPW ^r	6.8 a	8.4 a	3.3 a	3.5 a
<i>Ilex</i>	PL	4.4 a	5.1 a	1.0 a	1.1 a
	PWC	4.1 b	7.1 a	1.1 b	1.8 a
	SPW	4.0 b	5.6 a	1.0 a	1.3 a
<i>Chrysanthemum</i>	PL	7.1 a	7.0 a	4.4 a	4.6 a
	PWC	5.6 a	6.5 a	4.3 a	4.5 a
	SPW	6.4 a	5.6 a	5.0 a	6.9 a

^z*Echinacea purpurea* 'Prairie Splendor'; *Ilex crenata* 'Steeds'; *Chrysanthemum* 'Garden Alcala Red'.

^yShoot dry weight, severed plant at substrate surface, and oven-dried.

^xRoot dry weight, washed root system to remove all substrate, and oven-dried.

^wCT = container-grown plants (n = 6).

^vMH = mini-Horhizotron-grown plants (n = 3).

^uPL = 70:30 peat:perlite (v/v).

^tMeans separated within row separated for shoot and root by Tukey-Kramer significant difference, $P \leq 0.05$. Means followed by the same letter are not significantly different.

^sPWC = 70:30 peat:pine-wood chips (v/v), PWC produced by chipping and hammermilling loblolly pine logs (*Pinus taeda* L.) through a 6.35-mm screen.

^rSPW = 70:30 peat:shredded pine wood (v/v), SPW produced by shredding and hammermilling loblolly pine logs through a 6.35-mm screen.

CC, or TP (Table 2), yet, as just mentioned, root response was not identical.

When comparing plants grown in the mini-Horhizotrons or containers, all three species (coneflower, mum, holly) showed no root or shoot growth differences when grown in the traditional PL substrate (Table 1). With holly plants, shoot and root growth was greater in the mini-Horhizotron with the PWC substrate, whereas only shoot growth was greater in the SPW substrate. There seemed to be a greater effect from the wood in the substrates than the system the plants were grown in. It appears that the design of the mini-Horhizotron did not significantly influence overall root growth for the herbaceous plants evaluated from that measured in traditional containers.

Single versus multiple treatments. In comparing the two experimental designs, mini-Horhizotron chambers were filled with the same substrate (replication) vs. each chamber filled with a different substrate (block). Rudbeckia root growth in the PL substrate was similar from 15 DAP through 55 DAP, after which root growth was greater in the replication experimental design to the end (67 DAP; Table 3). Root growth in the PWC substrate was greater in the replication experimental design from 15 to 27 DAP and again from 55 to 67 DAP. From 31 to 51 DAP, plants grown in the PWC substrate were the same. Root growth in the SPW substrate was the same from 15 to 51 DAP, except at 43 DAP when root growth in the replication design was greater (Table 3). At and beyond 55 DAP, root growth in the SPW substrate was greater in the replication design. Differences between root growth in the two experimental designs for PL and SPW substrates started at 51 to 55 DAP, whereas differences in the PWC substrates used in the two designs started at the beginning of the study through 27 DAP, and then differences were observed again at 55 DAP.

Both the experimental design and the substrate had a significant effect on root growth (Table 3), showing that there was a substrate effect on root growth of rudbeckia. The differences noticed between the design of either replication or block at/after 55 DAP could be an effect of substrate type; at 67 DAP, the difference in rudbeckia root length between block and replication mini-Horhizotrons was 1.8 cm for all three substrates. Container capacity of the PL substrate (66.6%) was significantly lower compared with the SPW substrate (74.1%), and the PL substrate (84.7%) was lower in TP compared with both PWC and SPW substrates (88.4% and 89.4%, respectively; Table 2). Dividing the mini-Horhizotron into the block can reduce the number of mini-Horhizotrons used for replications in the design of the experiment; however, the species used in this experiment indicated no effect until after 4 weeks from transplant. These results indicate that using the mini-Horhizotron to observe three different substrates would potentially provide similar results as if using only one of the substrates in the mini-Horhizotron for crops grown 3 to 4 weeks; however, the effect of

Fig. 4. Root length measurements of *Chrysanthemum* 'Garden Alcala Red', *Echinacea purpurea* 'Prairie Splendor', and *Ilex crenata* 'Steeds' planted in mini-Horhizotrons from 11 to 46 d after planting (DAP) for peatmoss amended with 30% perlite (PL), pine-wood chips (PWC), or shredded pine wood (SPW). SE bars are shown to indicate sample variation.

different species would need to be further explored.

Comparing rates of PWC substrates. Plants grown in all three rates of PWC substrates had similar root growth from the beginning through 35 DAP (Fig. 5). Beginning at 39 DAP, plants grown in 40% PWC substrate had greater root growth compared with plants grown in the other substrates (Fig. 5). LSD analysis indicates that at 51 DAP, root growth was similar between the 40% PWC and 30% PWC substrates, but plants grown in 40% PWC substrate were significantly longer than plant roots in 20% PWC substrate. At 55 DAP through the end of the study, plants grown in 40% PWC and 30% PWC substrates had significantly longer plant roots compared with plants grown in 20% PWC substrate. These data provide evidence of a general increase in rudbeckia root growth in 40% PWC substrate starting at 39 DAP through 51 DAP (Fig. 5). The increase shown in the higher percentage of PWC could have been

affected by the physical properties; 40% PWC substrate had a greater percentage of AS (3% to 6% more) compared with the other substrates (Table 2), and therefore this substrate could potentially have higher humidity/air pores that may promote root growth.

Conclusion

Using the mini-Horhizotron, plant roots can be easily observed and data easily collected on root growth of small herbaceous and woody plants in a greenhouse production setting. Based on the ease and quality of the data collected in a non-destructive method on actively growing plant roots, the mini-Horhizotron could have potential for use in scientific study and quantification of root growth that would be indicative of plant growth in traditional containers.

During the initial trial of the mini-Horhizotron, root dry mass of the herbaceous species tested and root length measurements provide evidence that there was little difference

in growth between the substrate treatments or between the mini-Horhizotron and containers for the species tested at the end of the experiment. However, measuring root growth during the experiment revealed that the three plant species (coneflower, mum, holly) varied in their rates of root growth in the different substrates. These differences over time, not detectable with traditional root washings at the end of an experiment, would have been lost. The ability to repeatedly and non-destructively measure root systems in the mini-Horhizotron can provide valuable insight into the process of root growth and development and the factors that influence it. The first experiment used PWC that was older (stored longer after harvest and processing) than the

PWC in the other experiments; however, the effect of PWC age on plant/root growth was not an objective of these experiments and any possible effects it may have had were not tested.

For all species tested in these trials, PWC and SPW substrate components equaled or exceeded root growth at all time periods compared with the PL substrate. The third experiment yielded data of coneflower root growth increasing with increasing amounts of PWC in the peat substrate. Root growth differences between plants grown in PWC substrates compared with the traditional PL substrate may not have been large; however, plants grown in the PWC substrate appear to develop faster (have longer roots) in the early

portions of the growth period. This may have occurred as a result of potentially higher humidity/air pores of the PWC component compared with perlite.

Another possibility is that roots may grow more easily along the straight edges of the PWC particles compared with the rounded shape of the perlite particles. During several of the experiments highlighted in this work as well as other unpublished and unrelated experiments conducted in the past 3 years, plant roots have been observed growing along the flat sides and contour of wood particles in a manner not seen with plant roots growing on/near perlite particles. Plant roots after wood particles have been observed in substrates amended with both PWC and SPW. Roots grow best in substrates (and soils) where there is adequate air and water and a suitable chemical environment. Even in a substrate with those optimal conditions, roots have been observed and measured (unpublished data) growing faster, likely a result of taking the path of least resistance and growing where there is less obstruction or along a linear and flat surface as has been seen on wood or bark particles.

Other observations and potential data collection methods were also noted and conceived while using the mini-Horhizotron in these and other trials since 2011. Although not included as a tested method of data collection or growth assessment in this work, pictures were taken of roots growing in the mini-Horhizotron. The clear, curved sides of the mini-Horhizotron chambers often caused picture-taking to be a challenge; it was difficult to overcome the glare and focus cameras on the rhizosphere/chambers. Glare on the clear-sided walls of the mini-Horhizotron could be lessened/minimized by covering the white PVC base/edges with dark cloth and using a large dark cloth or umbrella over the mini-Horhizotrons to block light from above. It may also be advantageous to photograph the mini-Horhizotrons indoors, away from sunlight, where the lighting can be adjusted or minimized to reduce the glare.

Table 2. Physical properties of all substrates used in mini-Horhizotron experiments in 2012.^z

Ratio ^v	Substrate	Container capacity ^y (%)	Air space ^x (%)	Total porosity ^w (%)
			Expt. 1 ^u	
70:30	PL ⁱ	73.3 a ^s	15.0 a	88.3 a
	PWC ^r	71.9 a	17.5 a	89.3 a
	SPW ^q	76.1 a	14.2 a	90.2 a
			Expt. 2 ^u	
70:30	PL	66.6 b	18.1 a	84.7 b
	PWC	70.3 ab	18.1 a	88.4 a
	SPW	74.1 a	15.3 a	89.4 a
			Expt. 3 ^u	
80:20	PWC	73.7 a	15.2 b	89.0 a
70:30	PWC	70.3 a	18.1 ab	88.4 a
60:40	PWC	69.8 a	21.2 a	90.9 a

^zPhysical properties data were collected from three samples per substrate for the three experiments and represented as means. Analysis performed using the North Carolina State University porometer method (Fonteno et al., 1995).

^yContainer capacity = (wet weight – oven dry weight) ÷ volume of the sample.

^xAir space = volume of water drained from the sample ÷ volume of the sample.

^wTotal porosity = container capacity + air space.

^vRatio = peat substrate amended with PL, PWC, or SPW as specified percent ratios (v/v).

^uExpt. 1: testing the design of the mini-Horhizotron.

ⁱPL = peat amended with perlite.

^sMeans separated by experiment and within columns for physical properties by least significant difference, $P \leq 0.05$. Means followed by the same letter are not significantly different.

^rPWC = peat amended with pine-wood chips, PWC is produced by chipping and hammermilling (6.35-mm screen) loblolly (*Pinus taeda* L.) pine logs.

^qSPW = peat amended with shredded pine wood, SPW is produced by shredding and hammermilling (6.35-mm screen) loblolly pine logs.

^uExpt. 2: Comparison of experimental design of the mini-Horhizotron using replication vs. block.

^uExpt. 3: Comparing rates of PWC substrates in the mini-Horhizotron.

Table 3. Comparison of *Rudbeckia hirta* 'Becky Yellow' root growth in different experimental designs using the mini-Horhizotrons with three different substrates.

Substrate	Expt. design	Root length measurements (cm) ^z													
		Days after planting													
		15	19	23	27	31	35	39	43	47	51	55	59	63	67
PL ^y	Block ^x	1.7 a ^w	2.9 a	5.2 a	6.7 a	8.5 a	10.9 a	14.3 a	16.7 a	17.6 a	18.0 a	18.7 a	18.7 b	18.7 b	18.7 b
	Rep ^v	1.4 a	2.7 a	5.7 a	7.4 a	8.9 a	10.0 a	14.0 a	16.1 a	17.1 a	18.3 a	19.6 a	20.1 a	20.4 a	20.5 a
PWC ^u	Block	1.4 b	2.6 b	5.3 b	6.9 b	9.7 a	11.2 a	14.6 a	16.6 a	17.2 a	17.8 a	18.4 b	18.5 b	18.6 b	18.6 b
	Rep	2.9 a	4.1 a	6.7 a	8.3 a	10.3 a	12.3 a	14.9 a	17.2 a	18.4 a	19.0 a	19.8 a	20.1 a	20.3 a	20.4 a
SPW ⁱ	Block	2.6 a	3.6 a	7.0 a	8.6 a	11.9 a	13.0 a	15.4 a	16.3 b	17.6 a	18.3 a	18.5 b	18.5 b	18.5 b	18.5 b
	Rep	2.8 a	4.4 a	7.5 a	9.4 a	12.6 a	14.1 a	17.1 a	18.3 a	18.9 a	19.6 a	20.1 a	20.2 a	20.3 a	20.3 a
Significance ^s		Experimental design				Substrate				Interaction				Regression ^r	
		***				***				NS				L***, Q***	

^zRoot length (cm) averaged over three longest roots per chamber wall; block design n = 54, replication (Rep) design n = 36.

^yPL = 70:30 peat:perlite (v/v).

^xBlock was the experimental design with each chamber of six mini-Horhizotrons filled with an individual substrate.

^wMeans separated by substrate within column by Tukey-Kramer significant difference, $P \leq 0.05$. Means followed by the same letter are not significantly different.

^vRep was the experimental design with each chamber of three mini-Horhizotrons filled with the same substrate.

^uPWC = 70:30 peat:pine-wood chips (v/v), PWC produced by chipping and hammermilling loblolly pine logs (*Pinus taeda* L.) through a 6.35 mm screen.

ⁱSPW = 70:30 peat:shredded pine wood (v/v), SPW produced by shredding and hammermilling loblolly pine logs through a 6.35-mm screen.

^sSignificance = *** and ns represent significant effects when $P \leq 0.001$ and nonsignificant response, respectively.

^rRegression for root growth over time in all substrates, L and Q represent linear or quadratic response, respectively, and *** represent significant effects when $P \leq 0.001$.

Fig. 5. Root length measurements of *Rudbeckia hirta* 'Becky Yellow' from 15 to 67 d after planting (DAP) in mini-Horhizotrons individually filled with peat-based substrates amended with 20%, 30%, or 40% pine-wood chips (PWC). SE bars are shown to indicate sample variation.

In addition to the possibility of taking photographs of roots growing in the mini-Horhizotrons, another possible procedure for collecting root growth data may be root tracings. Roots (or entire root systems) could be traced on a transparency sheet placed on the outside of the mini-Horhizotron sides in a similar method as was described in this article to measure root length over time. These tracings could provide data on root system development, which is more of a qualitative assessment compared with root growth, which is a more quantitative assessment. Root tracings may then be analyzed by scanning or uploading an image of the tracing to a computer program like WinRHIZO (Regents Instruments, Quebec City, Canada) or RootReader 2D (Cornell University, USDA-ARS, Ithaca, NY), which analyzes the tracings and calculates total root length. There are numerous computer programs both commercially and freely available that can be used to measure roots digitally (Lobet, 2011). Preliminary use of this technique has shown potential.

The clear-sided chambered design of the mini-Horhizotron also allowed for clear observations of root hairs against the chamber

walls, root dieback and regeneration, and root branching/architecture patterns. Other biological observations in the rhizosphere observed on several occasions included fungus gnat larvae (*Bradysia* species) and fungal and mycelium growth. The ability to observe plant roots, other biological inhabitants or occurrences, water movement (penetration, infiltration, percolation, saturation), etc., within the substrate/rhizosphere could potentially improve the use of the mini-Horhizotron beyond a research scope as a tool for teaching purposes.

Literature Cited

Aung, L.H. 1974. Root-shoot relationships, p. 29–61. In: Carson, E.W. (ed.). *Plant root and its environment*. University Press of Virginia, Charlottesville, VA.

Fields, J.S. 2013. Hydrophysical properties and hydration efficiency of traditional and alternative greenhouse substrate components. MS thesis, NC State Univ., Raleigh, NC.

Fonteno, W.C., C.T. Hardin, and J.P. Brewster. 1995. Procedures for determining physical properties of horticultural substrates using the NCSU porometer. Horticultural Substrates Laboratory, North Carolina State University, Raleigh, NC.

Gruda, N. and W.H. Schnitzler. 2004a. Suitability of wood fiber substrate for production of vegetable transplants. I. Physical properties of wood fiber substrates. *Sci. Hort.* 100:309–322.

Gruda, N. and W.H. Schnitzler. 2004b. Suitability of wood fiber substrates for production of vegetable transplants. II. The effect of wood fiber substrates and their volume weights on the growth of tomato transplants. *Sci. Hort.* 100:333–340.

Jackson, B.E. and W.C. Fonteno. 2013. New media components—Are they worth their weight in dirt? *OFA Bul.* 938:14–18.

Jackson, B.E., A.N. Wright, J.L. Sibley, and J.M. Kemble. 2005. Root growth of three horticultural crops grown in pine bark amended cotton gin compost. *J. Environ. Hort.* 23:133–137.

Jackson, B.E., R.D. Wright, and M.C. Barnes. 2010. Methods of constructing a pine tree substrate from various wood particle sizes, organic amendments, and sand for desired physical properties and plant growth. *HortScience* 45:103–112.

Lobet, G. 2011. Root image analysis software chooser. 5 Mar. 2013. <<http://www.root-image-analysis.org/root-image-analysis-software-chooser/>>.

McDougall, W.B. 1916. The growth of forest tree roots. *Amer. J. Bot.* 3:384–392.

Oliveira, M.R.G., M. van Noordwijk, S.R. Gaze, G. Brouwer, S. Bona, G. Mosca, and K. Hairiah. 2000. Auger sampling, ingrowth cores and pinboard methods, p. 175–210. In: *Root methods: A handbook*. Springer-Verlag, Berlin, Germany.

Price, J.G., A.N. Wright, and K.M. Tilt. 2009. Organic matter application improves posttransplant root growth of three native woody shrubs. *HortScience* 44:377–383.

Silva, D.D. and R.C. Beeson, Jr. 2011. A large-volume rhizotron for evaluating root growth under natural-like soil moisture conditions. *HortScience* 46:1677–1682.

Spalding, E.P. and N.D. Miller. 2013. Image analysis is driving a renaissance in growth measurement. *Curr. Opin. Plant Biol.* 16:100–104.

Taylor, H.M., D.P. Upchurch, and B.L. McMichael. 1990. Applications and limitations of rhizotrons and minirhizotrons for root studies. *Plant Soil* 129:29–35.

van Noordwijk, M. and J. Floris. 1979. Loss of dry weight during washing and storage of root samples. *Plant Soil* 53:239–243.

Walters, S.A. and T.C. Wehner. 1994. Evaluation of the U.S. cucumber germplasm collection for root size using subjective rating technique. *Euphytica* 79:39–43.

Weaver, J.E., F.C. Jean, and J.W. Crist. 1922. *Development and activities of roots and crop plants*. Carnegie Institution of Washington, Washington, DC. Publ. 316.

Wraith, J.M. and C.K. Wright. 1998. Soil water and root growth. *HortScience* 33:951–959.

Wright, A.N. and R.D. Wright. 2004. The Horhizotron™: A new instrument for measuring root growth. *HortTechnology* 14:560–563.

Wright, R.D. and J.F. Browder. 2005. Chipped pine logs: A potential substrate for greenhouse and nursery crops. *HortScience* 40: 513–515.