

Mini-Horhizotron: A Novel Technique for Observing and Quantifying Root Systems of Plants in Pot Culture

Lesley A. Judd, Brian E. Jackson, and William C. Fonteno
Department of Horticultural Science, North Carolina State University,
Campus Box 7609, Raleigh, NC 27606

lajudd@ncsu.edu

Index Words: Rhizosphere, rhizometrics, root architecture, root growth, substrate

Significance to Industry: The mini-Horhizotron was developed to observe root growth and development, and root growth over time of small seedlings, herbaceous plugs, or woody liners in container production. The results of this study indicate faster root growth/root establishment of plants grown in substrates containing fresh pine wood chip (PWC) compared to plants grown in the peat:perlite substrate. For the second objective, only root growth of plants grown in PWC substrate were significantly different between mini-Horhizotrons filled completely with one substrate and mini-Horhizotrons with a different substrate in each quadrant. These experiments: 1) further test the usefulness of the mini-Horhizotron as a research tool in studying root growth; 2) show differences in root growth as influenced by different substrates; and 3) test the feasibility of utilizing a different experimental design in studying root growth across three treatments simultaneously.

Nature of Work: A large portion of the horticultural industry is involved with growing plants in pot culture. Root growth is a central element in overall plant performance, and therefore it is important to understand the factors that influence root growth. Quantifying root growth has ranged over the last several decades, including methods like root drawings, pin boards, rhizotrons, and minirhizotrons. More recently, computer programs have increased in use as technology advances and measuring root growth becomes easier.

Two of the most common methods that are accepted by the scientific community used for quantifying root growth are 1) subjective ratings and 2) root washing for determining dry biomass. Subjective root ratings can be a method to quantify root systems; however this method is completely dependent on the rater. Rating is a subjective measurement, and the person rating must first understand how to accurately rate the size of the root system (5). It is common to wash roots of container-grown plants to be able to view the root structure and assess root dry mass. Washing roots will reveal the root system to be viewed; however this removes the roots from their natural position/architecture and up to 20-40% of the fine roots (including root hairs) are often lost in the washing process (2, 4). This creates a need for new non-destructive methods that can measure the whole root system in situ and root growth/development over time.

In an effort to overcome some of the drawbacks on the techniques mentioned above, new root measuring methods have been created. The Horhizotron™, a non-destructive technique, was developed at Auburn University and Virginia Tech to measure horizontal root growth from the root ball of a container-grown nursery plant, allowing for post-transplant root growth assessment (6). The Horhizotron™ is constructed out of eight panels of glass attached to an aluminum base to form four quadrants and fits a range of nursery stock root balls. The substrate in each quadrant can be modified in various ways in order to examine the effects of different rhizosphere conditions (6). However, the size of the Horhizotron™ is restricted to large sized root balls, the glass panels are not permanent and can move and crack and the shade box does not restrict all light from the root system. More recently, a large-volume rhizotron was developed to observe root growth in an environment closer to natural soil conditions. This apparatus is aboveground which allows for relatively easy data collection (3). However, this design is even larger than the Horhizotron™ and is intended for woody plants, with large root balls, to imitate post-transplant conditions.

A new technique, the mini-Horhizotron, was developed in the Horticultural Substrates Laboratory at North Carolina State University to study root growth of seeds, plugs and woody liners during production (1). The mini-Horhizotron is a root box designed with three quadrants extending away from the center of the box, allowing for different substrates to be placed in the separate quadrants. Quadrants are clear sided which allows visible measurements to be taken from a plant/seed planted in the center, such as root length, speed of root growth, presence and quantity of root hairs, and root architecture/branching. Shade panels were constructed to fit snugly against the plexiglass quadrants and completely block sunlight from the rhizosphere. The shade panels do not cover the substrate surface, allowing for irrigation to be directly applied to the substrate surface, same as normal watering practices of container-grown plants.

The quadrants allow for root growth observations in each separate quadrant, and the possibility of filling each quadrant with a different substrate in order to measure the effects of different substrates on one plant or lessen the number of replications needed. The objectives of this study were to use the mini-Horhizotron to: 1) observe and quantify the effects of different substrates on root growth of *Rudbeckia hirta* and 2) test the influence of experimental design on root growth of plants grown in mini-Horhizotrons that have either: A) all quadrants filled with the same substrate or B) each quadrant filled with a different substrate.

The study was executed on July 30, 2012 in greenhouses at North Carolina State University, Raleigh, NC. Three substrates were used, 70% (v/v) peat moss amended with either 30% perlite (PL), pine wood chips (PWC), or shredded-pine-wood (SW). Eight-year-old loblolly pine trees (*Pinus taeda* L.) were harvested on 4 July, 2012 at ground level and delimbed in Chatham County, NC and subsequently stored under shelter for protected from the weather. The delimbed pine logs were then chipped in a DR Chipper (18 HP DR Power Equipment, model 356447; Vergennes, VT) to produce small wood chips. The pine logs destined for shredding were processed in a Wood Hog

shredder (Morbark® model 4600XL; Winn, MI). Both the chipped and shredded wood was then processed in a hammermill through a 6.35 mm (0.25 in.) screen (Meadows Mills, North Wilkesboro, NC) to produce two end products, PWC and SW. The substrates were mixed on 28 July 2012, all substrates were tested for initial pH and then amended with dolomitic limestone at $4.45 \text{ kg} \cdot \text{m}^{-3}$ ($7.5 \text{ lb} \cdot \text{yd}^{-3}$) to achieve a desired pH of 5.8. On 30 July 2012 three mini-Horhizotrons were filled with an individual substrate and tapped three times, by lifting the mini-Horhizotron 10 cm (4 in.) from a hard surface and dropping, to settle the substrate and then filled to the top with substrate again, to accommodate for substrate settling which occurs after initial irrigation events during the beginning of normal crop production. Six mini-Horhizotrons were divided in the center with a cardboard divider and each quadrant was filled with one of the three substrates in random order and the same tapping and refilling procedure occurred. The cardboard divider was then removed, and one plug of *Rudbeckia hirta* 'Becky Yellow' (288-tray; C. Raker & Sons, Inc., Litchfield, MI) was planted into the center of all mini-Horhizotrons. Each of the mini-Horhizotrons that contained the same substrate in all quadrants was considered a single treatment. The six mini-Horhizotrons with a different substrate in each quadrant was considered a triple treatment. A total of 15 mini-Horhizotrons were used in this experiment. Plants in each substrate were over-head watered as needed depending on weather conditions, and never showed symptoms of water stress. Plants were fertilized at each watering with 200 ppm nitrogen with Peters Professional 20-10-20 Peat-Lite Special (The Scotts Co., Marysville, OH). Root length measurements were taken on the three longest roots appearing on the face of each quadrant on 15, 19, 23, 27, 31, and 35 days after planting (DAP). Each quadrant has two measureable faces giving a sum of six quadrant faces per mini-Horhizotron. Measurements were taken by placing a transparent sheet (3M Visual Systems Division, Austin, TX) with a printed on cm^2 ($0.39 \times 0.39 \text{ in.}$) grid on each face, and roots were measured from the start of the gridlines, which was at the center of one of the three sides, to the end of the gridlines, which reached the end of the quadrants. Data were subjected to the general linear model procedures and regression analysis (SAS Institute version 9.2, Cary, NC). Means were separated by least significant differences at $P \leq 0.05$.

Results and Discussion: Data taken from the single treatment mini-Horhizotrons showed that *Rudbeckia* roots grew faster in the SW and PWC substrates compared to plants grown in the PL substrate, until the fourth measurement date (27 DAP; Fig. 1). After 27 DAP, plants grown in SW had longer root lengths compared to plants grown in PWC or PL substrates. This study indicates there is enhanced root growth in substrates amended with pine components, and that the mini-Horhizotrons provide an effective method/technique for measuring root growth of small herbaceous plants in situ. These experiments provide results showing the ability to easily manipulate the root environment (rhizosphere) in the mini-Horhizotron, and to also have the capacity to detect and quantify the influence of the rhizosphere on root growth of plants. Root system development can easily be measured in the mini-Horhizotrons, as well as observations of root architecture/branching and root hairs.

The second objective of comparing the two experimental designs (mini-Horhizotron as a single versus triple treatment) by substrate, root growth in the SW and PL substrates were not significantly different across all measurement dates (Table 1). Root growth in the PWC substrate was different between the experimental designs from 15 DAP through 27 DAP, and it was observed that plants grown in PWC substrates in the replication design had greater root growth. At the end of the study, plants grown in PWC substrate did not have significant root growth differences between the experimental designs (Table 1).

Literature Cited

1. Judd, L.A. 2013. Rhizometrics: Novel Techniques to Observe and Measure Root Growth of Container-Grown Crops. North Carolina State University, North Carolina. M.S. Thesis.
2. 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: A.L. Smit, A.G. Bengough, C. Engels, M. van Noordwijk, S. Pellerin, and S.C. van de Geijn (eds.). Root methods: A handbook. Springer-Verlag, Berlin, DE.
3. 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.
4. van Noordwijk, M. and J. Floris. 1979. Loss of dry weight during washing and storage of root samples. Plant Soil 53:239-243.
5. 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.
6. 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 *Rudbeckia* root growth^z in different experimental designs using the mini-Horhizotrons grown in peat amended with either perlite, pine-wood-chips or shredded-pine-wood.

Substrate	Exp. Design ^x	DAP ^y							
		15	19	23	27	31	35		
PL ^s	Block ^w	1.7 a ^u	2.9 a	5.2 a	6.7 a	8.5 a	10.9 a	L ^{***t}	Q ^{***}
	Rep ^v	1.4 a	2.7 a	5.7 a	7.4 a	8.9 a	10.0 a	L ^{***}	Q ^{***}
PWC ^r	Block	1.4 b	2.6 b	5.3 b	6.9 b	9.7 a	11.2 a	L ^{***}	Q ^{***}
	Rep	2.9 a	4.1 a	6.7 a	8.3 a	10.3 a	12.3 a	L ^{***}	Q ^{***}
SW ^q	Block	2.6 a	3.6 a	7.0 a	8.6 a	11.9 a	13.0 a	L ^{***}	Q ^{***}
	Rep	2.8 a	4.4 a	7.5 a	9.4 a	12.6 a	14.1 a	L ^{***}	Q ^{***}
Significance		Exp. Design		***	Substrate		***	Interaction	NS

^zRoot growth in cm (1 cm = 0.394 in.).

^yDAP is day after planting.

^xExperimental design was either block or replication of mini-Horhizotrons.

^wBlock was the experimental design with each quadrant of mini-Horhizotrons filled with an individual substrate.

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

^uMeans separated by substrate within column by Duncan's multiple range test, $P \leq 0.05$. Means followed by the same letter are not significantly different.

^tNS, L, and Q represent no significant response, linear, and quadratic response, respectively, over time of individual substrates and experimental design type, *, **, *** represent significant effects when $P \leq 0.05$, 0.01, and 0.001, respectively.

^sPL substrate is 70:30 peat:perlite (v/v).

^rPWC substrate is 70:30 peat:pine-wood-chips (v/v).

^qSW substrate is 70:30 peat:shredded-pine-wood (v/v).

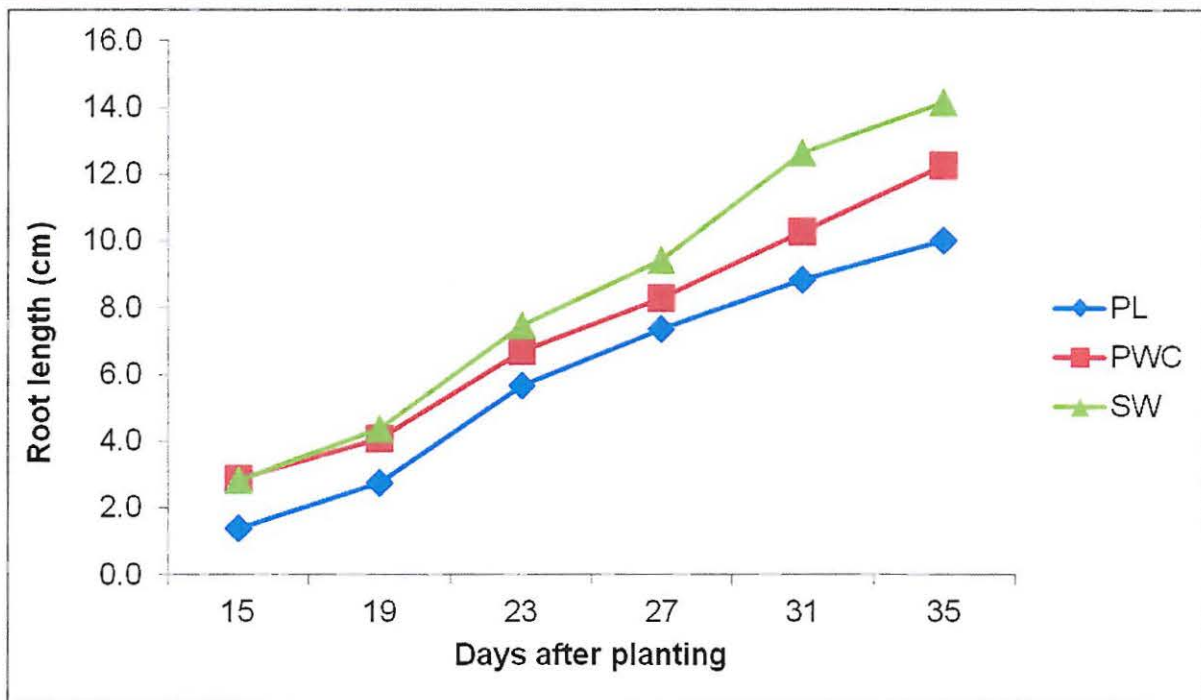


Figure 1. Root length measurements (1 cm = 0.394 in.) of *Rudbeckia* grown in 70% (v/v) peat amended with either 30% perlite (PL), pine-wood-chip (PWC), or shredded-pine-wood (SW).