Novel Methods for Observing and Quantifying Root Growth of Horticultural Crops©

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INTRODUCTION

A large portion of the U.S. Green Industry is involved with growing plants in containers, including nursery crops, annual bedding plants and potted herbaceous perennials. With such a large portion of the industry in containers, it is important to understand the factors that influence root growth to attain optimal benefits from container production. Several factors that affect root growth include the physical and chemical properties of substrates. Physical properties include porosity and water holding capacity, percentage of fine particles and bulk density (Mathers et al., 2007). Chemical properties include pH, cation exchange capacity and soluble salts (Mathers et al., 2007). There are several known techniques used to measure these factors that affect root growth, but methods used to measure the whole root system or measure the growth of roots over time are not as widely available. It is also not well understood how roots change and affect the physical properties of substrates in the container over time. The most common root system measurements reported in scientific literature are: (1) subjective root ratings and (2) root dry weight measurements. Root ratings, while being non-destructive, are completely subjective to the person rating the root system and can vary person to person. The second method of root washing is widely accepted as a valid determination of root mass but it is well understood/assumed that a percent of root (particularly fine roots) mass is lost. Oliveira et al. (2000) reported that almost 20-40% of the original root weight is lost during root washing of certain plant species.

A non-destructive technique for measuring horizontal root growth (Horhizotron™) was developed at Auburn University and Virginia Tech that offers a simple, non-destructive technique to measure root growth over time (Wright and Wright, 2004). This Horhizotron™ is constructed out of eight panels of glass attached to an aluminum base to form four wedge-shaped quadrants. The Horhizotron™ was built to fit a plant removed from a 1-3-gal container and placed in the center so the quadrants extend away from the root ball. This technique is most appropriate for assessing/observing root growth from rootballs likely to study post-transplant root response. This technique does not allow for observations and study of small plant root development such as, herbaceous plugs and nursery liners. In order to study root growth of seeds, liners and plugs during production, new techniques need to be developed and evaluated.

The objectives of this work were: (1) design and testing of a small scale version of a Horhizotron™ suitable for small plant material and (2) design and testing of the Rhizometer, an in situ technique for determining the influence of plant roots on the physical root environment.

MATERIALS AND METHODS

Mini Horhizotrons

A small scale version of a Horhizotron™ was produced (mini Horhizotron) with a three quadrant configuration suitable for observing root growth of small plant material (Fig. 1A). The clear quadrants, similar to the quadrants of the original Horhizotron™, allow for visible access to the roots and transparent grids can be placed on them allowing for measurements to be taken. Potential measurements include root length, speed of root growth, presence and quantity of root hairs, and root branching/architecture. Shade panels were constructed to restrict light from the quadrant faces (rhizosphere) and three drainage holes drilled in the bottom of each quadrant to allow proper drainage (Fig. 1B). Three substrates were used in the initial testing of the mini Horhizotron; peat and perlite (70:30,

v/v), peat:pine-wood-chips, and peat:shredded-pine-wood. The wood was processed from delimbed loblolly pine trees (*Pinus taeda* L.), harvested in Jan 2012 and either chipped or shredded, then processed in a hammermill through a 6.35 mm screen. The substrates were mixed and amended with 3.86 kg·m^{-3} dolomitic limestone on 1 June 2012. Three mini Horhizotrons were filled with each individual substrate on 2 June 2012, tapped three times to settle substrate and then filled to the top with substrate again. One *Echinacea purpurea* 'Prairie Splendor' plug (162-plug flat) was planted into the center of each. One mini Horhizotron is considered a replication since all three quadrants contain the same substrate. Three substrates x three replications of each substrate made a total of nine mini Horhizotrons. Mini Horhizotrons were completely randomized on a greenhouse bench and fertigated with 200 ppm 20-10-20 Peters Professional® water soluble fertilizer. Root length measurements (cm) were taken on the three longest roots appearing on the face of each quadrant on 11, 25, and 39 days after planting (DAP). Each quadrant has two measurable faces giving a sum of six quadrant faces per mini Horhizotron. Measuring three roots per quadrant face × six quadrant faces per root box × three root box reps per substrate equals 54 data points. Data was analyzed using Linear Regression ($P \leq 0.05$) (SAS Institute version 9.2, Cary, North Carolina).

Fig. 1. (A) Design of the mini Horhizotron illustrating the three quadrant configuration, (B) removable shade panels, and (C) use in plant growth study.

Rhizometers

Physical properties of substrates, including total porosity (TP), container capacity (CC) and air space (AS), can be measured with the NCSU Porometer method (Fonteno et al., 1995). The NCSU Porometer method uses aluminum 7.6 cm cores to measure physical properties. Altland et al. (2011) reported using these aluminum cores to grow nursery crops in pumice to test the changes in air space and porosity over time. Based on the work of these authors, an apparatus was designed (Rhizometer) to allow for both viewing a growing root system and in situ measurement of substrate physical properties. The Rhizometer is made from a clear cylinder which allows for visible observations for data collection, including root count, root branching/architecture, quantifying root growth, etc. Clear cylindrical plexiglass tubes were cut to the measurement of 7.6 cm tall × 7.6 cm inside diameter to make a core the same dimensions as the aluminum NCSU Porometer cores, and a 3.8 cm tall × 7.6 cm inside diameter collar which is attached to the top of the core with parafilm (Fig. 2A). The collar extension is attached to the top of the core to aid in packing the core and allows extra space for planting a plug. A 20-mesh fiberglass screen was cut to fit the bottom of the core and attached with a hose clamp (Fig. 2B).

Dark colored foil was used to restrict light and held in place with rubber bands. Rhizometers were filled on 18 May 2012 with a peat, perlite, and vermiculite (60:20:20, by vol.) substrate and tapped 5 times to achieve similar bulk density in every core, mimicking the porometer process. Marigold (*Tagetes erecta* 'Inca Orange') plugs (288 plug tray) were planted into the packed core which was then wrapped with foil for light restriction. Ten Rhizometers were harvested at 7, 14, 21 and 28 DAP, with five to be used in the porometer method to determine physical properties and the other five used to determine root mass. Rhizometers were completely randomized in the greenhouse and fertigated as needed with 200 ppm N 20-10-20 Peters Professional® water soluble fertilizer. To prepare the Rhizometer for the porometer method, shoots were severed and the collar was removed, revealing 1-2 cm of substrate. This substrate and any roots above the 7.6-cm long core were removed such that the substrate surface within the core was level with the top of the core. The bottom screen was removed, leaving a level core ready for the porometer method. From the porometer, TP, CC, and AS was measured and compared to the root dry mass from every harvest. Data was analyzed using least significant difference ($P \leq 0.05$) (SAS Institute version 9.2, Cary, North Carolina).

Fig. 2. (A) Design of Rhizometer illustrating the clear sided plexiglass allowing for root observations and measurements, (B) the screened bottom, and (C) a complete planted Rhizometer.

RESULTS

Mini Horhizotrons

As the first plant growth trial using the mini Horhizotron, root growth was easily visible

(similar to the original Horhizotron™) and the potential of data collection was possible as was hoped during the design phase of this apparatus. At 11 and 25 DAP, root growth among the three substrates were similar, however at 39 DAP root growth/length was greater in the shredded wood substrate (Fig. 3). These data prove the mini Horhizotron can be used to show treatment effects on root growth. Based on this trial experiment, it seems that data can be collected on root growth of small plants in the same manner the large Horhizotron™ is used with large rootballs.

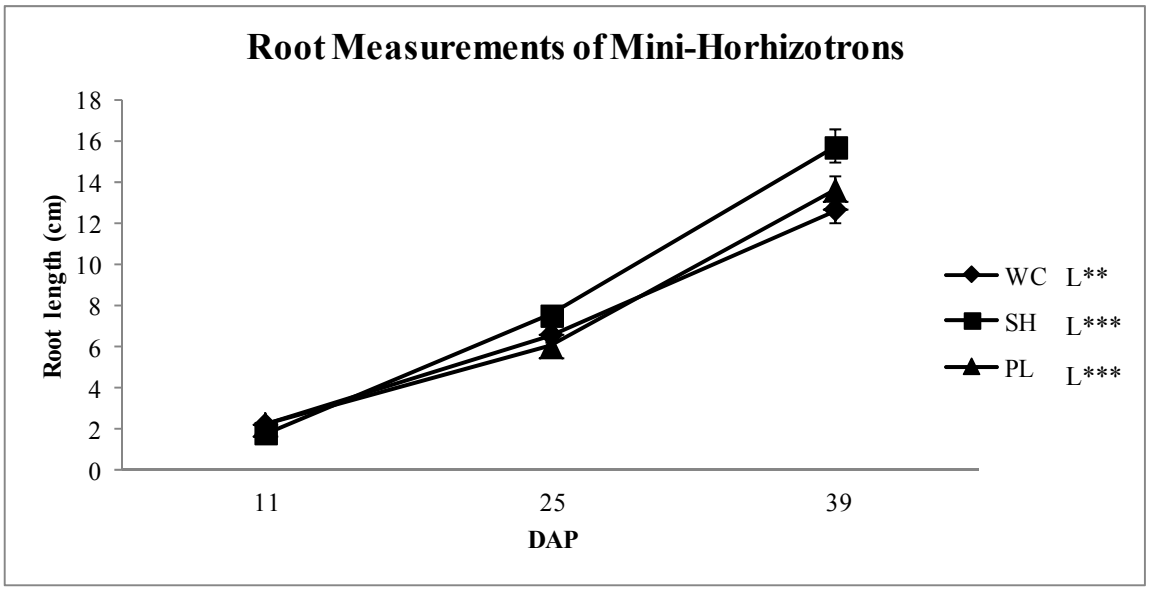

Fig. 3. Root growth of *Echinacea* measured 11 to 39 days after transplanting (DAP) when grown in peat amended with three components at 30% (v/v). The three components are wood chips (WC), shredded wood (SH) and perlite (PL). Each point represents the means ± SE indicated by SE bars (n=54). L=linear, significant at $P \leq 0.05$ (*), 0.01 (**), or 0.001 (***), respectively.

Rhizometers

Rhizometer data show that marigold roots have no effect on substrate CC over four weeks (all measurement dates), but that a slight decrease in TP does occur (Table 1). The decrease in TP from 7 to 28 DAP can be attributed to the decrease in AS. The decrease in AS can likely be explained by the increase in root mass over time (Fig. 4). As roots grew, it is possible that they filled the pore space therefore causing a decrease in the substrate AS. These data suggest that even though AS decreases over time (slightly), few changes occur to a substrate as a result of marigold root growth. The effect that different species and root types have on changes to physical properties during crop production is unknown and needs further investigation.

Table 1. Changes in physical properties of a peat-based greenhouse substrate as influenced by root growth when measured in situ by the Rhizometer technique at 7, 14, 21, and 28 days after planting (DAP) in 2012[z].

DAP	Total porosity[y] (% vol)	Container capacity[x] (% vol)	Air space[w] (% vol)
7	90.68 a[v]	74.06 b	16.62 a
14	91.70 a	77.16 a	14.58 ab
21	89.92 ab	74.84 b	15.08 ab
28	88.78 b	75.58 ab	13.16 b

[v]Means were separated within column between weeks by least significance difference at $P \leq 0.05$.
[w]Air space is the volume of water drained from the sample ÷ volume of the sample.
[x]Container capacity is (wet weight – oven dry weight) ÷ volume of the sample.
[y]Total porosity is equal to container capacity + air space.
[z]Data collected from five Rhizometers every week and represented as means. Analysis performed using the North Carolina State University Porometer method (Fonteno et al., 1995).

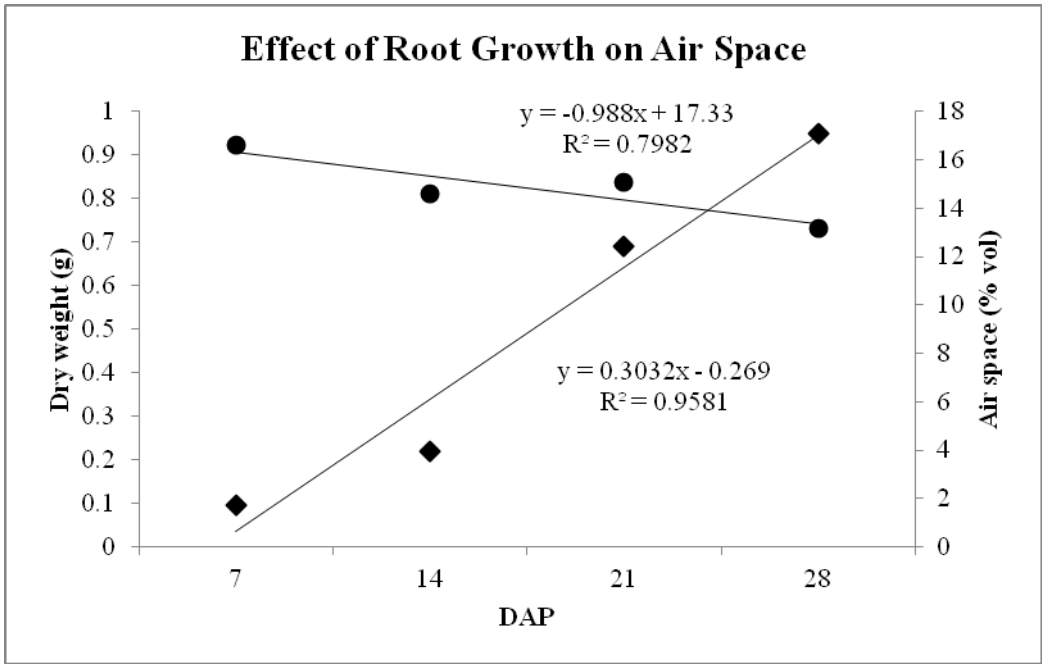

Fig. 4. Effect of increasing root growth (♦) on air space (●), with physical properties measured on Rhizometer apparatus containing roots by NCSU Porometer method at 7, 14, 21, and 28 days after planting (DAP) for four weeks (Fonteno et al., 1995).

DISCUSSION

These initial experiments testing the usefulness of two new techniques for assessing and quantifying undisturbed root growth have yielded promising results. The mini Horhizotron has endless potential of studying numerous factors affecting root growth of greenhouse plugs and nursery liners during production. The ability to visualize, observe and measure the growth of small plants in a non-destructive way will further expand root growth research and understanding. Both the mini Horhizotron and Rhizometer offer potential as techniques to study undisturbed root systems which most importantly includes fine root mass (root hairs) which is often lost during traditional root washing methodology.

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