Rhizometer root tracing techniques and digital image analysis for assessing and quantifying seedling root growth in substrates

B.E. Jackson, L.A. Judd and W.C. Fonteno

Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695, USA.

Abstract

The ability to observe and measure root growth of horticultural plants grown in pot culture is difficult to do without disturbing or damaging the roots of growing plants. The rhizometer was recently developed at North Carolina State University to observe plant root growth and measure the effect of root growth on undisturbed substrate physical properties over time. The clear cylinder design of the rhizometer allows for visible observations of the rhizosphere so that root data collection, such as root count, root length, and root hairs can be quantified without disturbance. The objective of this study was to measure several root system parameters on plants grown in the rhizometer using tracing techniques and digital image software. Root growth in three substrates were compared in this study; peat amended with 20% (v/v) of either perlite (PL), pine-wood-chips (PWC) or shredded-pine-wood (SPW). Both PWC and SW were produced by hammer-milling freshly chipped or shredded loblolly pine trees (*Pinus taeda*) through a 6.35 mm hammer mill screen. Twenty rhizometers were filled with each individual substrate and four species of seeds were individually planted directly into the rhizometers (one seed per rhizometer); bean (*Phaseolus vugaris* 'Gold Rush'), corn (*Zea mays* 'Jubilee'), tomato (*Solanum lycopersicum* L. 'Better Boy'), and marigold (*Tagetes erecta* 'Inca Orange') resulting in 60 rhizometers used. Three root measurements were taken once root tips were visible along the rhizometer cylinder; number of root tips (RT), number of roots with visible root hairs (RH), and cumulative root length (RL). Root length was measured by tracing the roots on a transparency sheet, taking a digital photograph, and using RootReader 2D software to select the traced roots and calculate total root length of the picture. Number of RT, RH, and RL measurements had linear responses over time for all four species. At 12 days after emergence (DE), corn had the largest total RL in the SPW substrate, and tomato had a higher total RL in PWC substrate at 12 DE compared to the other substrates. The higher total RL observed with these two species in SW and PWC substrates at 12 DE could possibly be attributed to structural particle differences (size and shape) of these three aggregates and the matrix of those particles in the container that facilitated faster or easier root growth through the substrate. As the roots of these plants grew into the substrates and likely altered the substrates' physical properties over time, there were no observable differences in RT, RH or RL among the substrates for each species at measurement dates after 12 DE. Root tracing and digital analysis were effective tools in further assessing root growth characteristics of seedlings in the rhizometer.

Keywords: pine tree substrate, pine wood chips, rhizosphere

INTRODUCTION

Rhizometers are an apparatus that allow for visual observations of a growing root system, and in situ measurements of substrate physical properties (Judd, 2013; Judd et al., 2015). The meaning of the term rhizometer is derived from *rhizo-*, meaning rhizosphere, and *-meter*, stemming from porometer (a device that measures physical properties of substrates). The rhizometer is made from a clear cylinder that is 7.6 cm tall × 7.6 cm inside diameter, which allows for visible observations of the rhizosphere so that root data, such as root count, root branching/architecture, quantifying root hairs, etc. can be quantified without disturbing the rootball (Figure 1). The rhizometer cores are designed to be fitted in the NCSU Porometer method to determine substrate physical properties in situ. The rhizometers have a 3.8 cm tall collar attached to the top to aid in packing the rhizometer with substrate and having extra space to plant a plug or seed. The rationale of this apparatus was to measure both the physical properties of substrates and the effects of growing roots on substrates, while also having the ability to observe and measure roots in situ (Judd et al., 2014). It is the clarity of this apparatus that offers potential in new ways of analyzing and characterizing root systems and root growth.

Digital imaging of the visible rhizometer walls could be a way to quantify root systems, beyond subjective assessments and ratings. Digital imaging includes photographs or videos, scanned images of exposed roots, or scanned root tracings. These images can be used by computer programs to evaluate root systems. Some of these programs include RootLM, RootReader 2D, EZ-Rhizo, WinRHIZO and WinRHIZO Tron. RootReader 2D was developed at Cornell University, and images of intact root systems can be uploaded into the program and root growth responses quantified from whole root systems or specific roots of interest (Clark et al., 2013). The objective of this study was to use root tracings and digital analysis technology to measure/quantify root growth parameters of seedlings using the rhizometer.

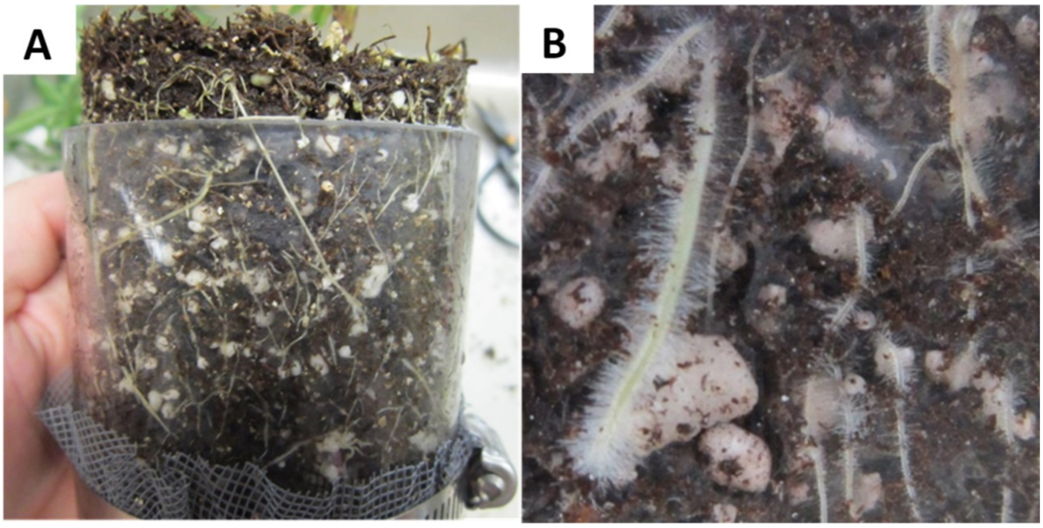

Figure 1. (A) Design of rhizometer illustrating the clear sided plexiglass allowing for root observations and measurements, (B) an up close view of root hairs that can be easily seen and/or measured in situ when the foil covering (to restrict sunlight) is removed from the rhizometer.

MATERIALS AND METHODS

Three substrates were compared in this study, peat amended with 20% (by volume) of either perlite (PL), pine wood chips (PWC), or shredded-pine-wood (SPW). On 19 Dec. 2012, eight-year-old loblolly pine trees (*Pinus taeda* L.) were harvested at ground level, delimbed, and stored under shelter from weather. On 2 Jan. 2013 the delimbed pine logs were chipped in a DR Chipper (18 HP DR Power Equipment, model 356447; Vergennes, VT, USA) to produce coarse wood chips. The coarse pine wood chips were then processed in a hammermill through a 6.35 mm screen to produce PWC. To produce SPW: on 18 Jan. 2013, delimbed loblolly pine logs were shredded through a Wood Hog shredder (Morbark® model 3800; Winn, MI, USA), yielding coarse shredded-pine-wood which was then processed in a hammermill through a 6.35 mm screen yielding the more fibrous SPW end product (Jackson and Fonteno, 2013).

On 19 Jan. 2013, the substrates were blended and amended with dolomitic limestone at 5.04 kg m^{-3} to achieve a target pH of 5.8. On 20 Jan. 2013, a total of 60 rhizometers were filled; 20 with each individual substrate. The weight of each rhizometer was recorded and 115 g of substrate was added to each rhizometer. At time of planting, the substrates had a mass wetness of 1.5 (g/g), which assured similar packing and substrate volume in the rhizometers, and prevented potential hydrophobicity or swelling when watered. Each rhizometer was tapped five times by dropping the filled rhizometers from a height of 10 cm on a flat surface to achieve similar BD in every core. Four species of seeds were planted directly into the rhizometers; bean (*Phaseolus vugaris* 'Gold Rush') and corn (*Zea mays* 'Jubilee') seeds (Livingston Seed Co., Columbus, OH) were planted at a depth of 2 cm; and tomato (*Solanum lycopersicum* 'Better Boy') and marigolds were planted at a depth of 1 cm. These species were chosen for their fast germination rate and variability in root types. Planted rhizometers were completely randomized within species on a greenhouse bench in Raleigh, NC (Figure 2) and watered as needed without fertilizer until germination occurred, then the rhizometers were fertilized at each watering with 150 mg L^{-1} nitrogen derived from 20N-4.4P-16.6K and injected at 1:100 ratio by Dosatron injector [(D14MZ2); Dosatron International, Inc., Clearwater, FL].

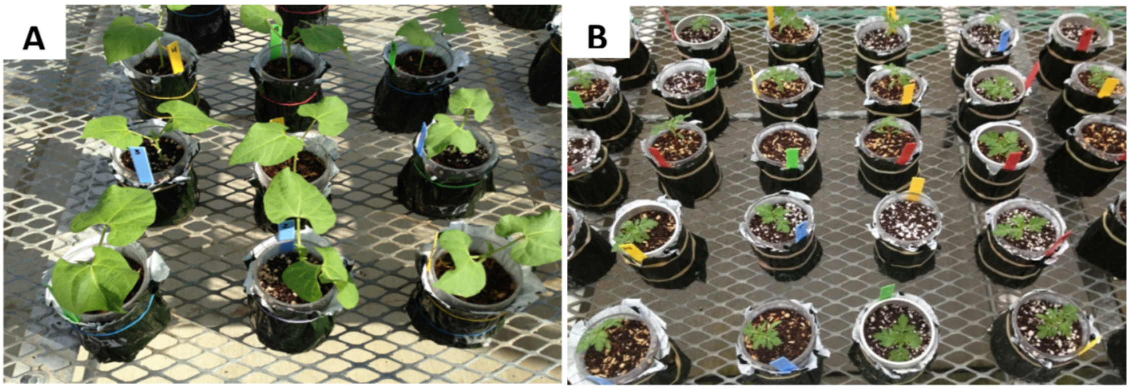

Figure 2. (A) Beans growing in the rhizometer. Notice the foil wrappers that are secured to the outside of the apparatus to restrict light during plant growth, (B) randomization of substrate treatments in rhizometers containing marigold seedlings.

For each species, three root measurements were taken once root tips were visible on the rhizometer walls/surface and these measurements were continued every four days, for a total of 13 days (four measurement dates). Root measurements included: 1) number of root tips (RT), 2) number of roots with visible root hairs (RH), and 3) cumulative root length (RL) of rhizometer surface (root coverage). Number of root tips and roots with hairs were measured by counting the visible roots against the clear core of the rhizometers. A transparency (27.9×7.6 cm transparency film; 3M Visual Systems Division, Austin, TX, USA) was cut to the dimensions of the rhizometer, wrapped around and held in place with a rubber band. Cumulative root length was measured by tracing the roots on the transparency sheet (Figure 3) with a thin-tip, wet-erase transparency marker, taking a digital photograph of the traced roots, and uploading the image to root reading software (RootReader 2D version 4.3.1; Cornell University, USDA-ARS, Ithaca, NY, USA). The RootReader 2D software selected the traced roots and measured total root length of the traced roots. The tracing on the transparency sheet was then erased to remove the ink and the sheet was used for the next tracing. Root measurement data were analyzed using repeated measures and analysis of variance (SAS Institute version 9.2, Cary, NC, USA). Means separation was determined using LSD ($P \leq 0.05$).

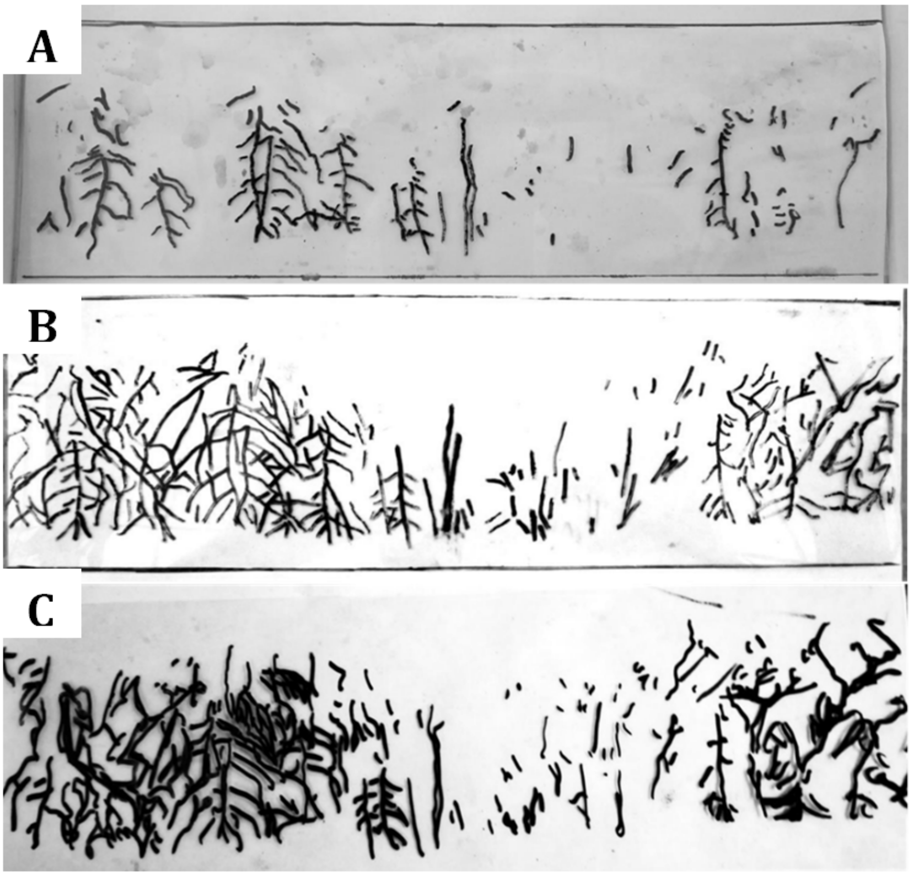

Figure 3. Tracings of bean root systems at (A) four days, (B) eight days, and (C) 12 days after seedling emergence and root appearance on the rhizometer walls.

RESULTS AND DISCUSSION

The marigold seeds were the first to emerge, however there was no visible root growth against the clear rhizometer in any of the three substrates until 12 days after emergence (DE). Corn and bean seeds emerged next, and root growth was visible against the rhizometer after 4 DE. The tomato seeds were the last to emerge, and root growth was visible against the rhizometer starting at 8 DE. The three root measurements discussed above were taken on both the corn and bean rhizometers, starting at 4 DE until 16 DE. At 16 DE, most of the visible area of the rhizometer's clear sides were covered with roots and it was decided that further measurements were not needed.

The corn seedlings grown in the rhizometers had significant differences among the substrates for the RT, RH and RL measurements at 12 DE (Table 1). There were more corn root tips and roots with root hairs when grown in the SPW substrate than the corn grown in the PWC substrate. At 12 DE, corn grown in the SPW substrate had more root tips and root hairs compared to the PWC substrate. Number of root tips, RH and RL measurements had linear responses over time for all four species (Table 1), showing increasing root growth in all species. At 12 DE, corn had the longest total root length in the SPW substrate compared to PWC and PL substrates (Table 1).

Tomato had a higher total root length in PWC substrate on 12 DE, whereas marigold had the lowest total root length in PWC substrate. This observed event shows species specific root growth; the tomato roots grown in PWC substrate had enhanced total root length although the marigold root system grown in PWC substrate did not. The rest of the measurement dates showed no difference in RL among the substrates (Table 1).

Table 1. Root system measurements of rhizometers planted with four plant species in three different substrates with measurements taken every four days after date of emergence (DE) of the seedlings.

Plant	Substrate	RT[1]	RH[2]	RL[3]	RT	RH	RL	RT	RH	RL	RT	RH	RL
		Days after emergence											
		4			8			12			16		
Corn[4]	PL[5]	6.0 a[6]	3.2 a	12.1 a	29.2 a	21.6 a	47.7 a	116.0 ab	104.0 ab	126.5 b	174.6 a	136.6 a	257.4 a
	PWC[7]	2.2 a	1.2 a	19.1 a	20.4 a	15.6 a	36.5 a	97.0 b	88.4 b	140.3 b	161.4 a	128.2 a	292.4 a
	SPW[8]	7.0 a	5.2 a	13.3 a	35.2 a	27.4 a	78.8 a	145.0 ab	133.4 ab	213.7 a	179.0 a	153.4 a	335.8 a
Bean[9]	PL	7.2 a	5.6 a	18.0 a	48.8 a	19.6 a	101.3 a	160.4 a	134.8 a	280.3 a	270.2 a	238.0 a	369.6 a
	PWC	8.2 a	5.6 a	13.9 a	76.6 a	25.0 a	158.7 a	176.2 a	149.6 a	249.7 a	284.8 a	220.8 a	320.2 a
	SPW	5.0 a	3.5 b	16.5 a	64.3 a	21.0 a	119.4 a	166.0 a	145.5 a	243.4 a	278.5 a	242.3 a	296.9 b
		8			12			16			20		
Tom.[10]	PL	0.3 a	0.3 a	0.3 a	16.5 a	16.0 a	16.6 b	19.0 a	7.25 a	12.2 a	58.0 a	47.0 a	75.3 a
	PWC	1.6 a	1.4 a	3.7 a	10.0 a	10.0 a	19.0 a	15.2 a	13.6 a	21.5 a	88.2 a	79.0 a	71.3 a
	SPW	0.6 a	0.6 a	1.5 a	14.6 a	14.4 a	16.0 b	18.8 a	16.8 a	19.8 a	58.8 a	47.6 a	46.3 a
		12			16			20			24		
Mar.[11]	PL	4.0 a	3.6 a	12.0 a	12.6 a	11.0 a	26.0 a	27.2 a	22.2 a	49.1 a	48.2 a	33.6 a	100.1 a
	PWC	2.0 a	1.4 a	12.8 b	12.2 a	12.0 a	19.6 a	28.8 a	22.8 a	40.7 a	52.8 a	39.8 a	193.3 a
	SPW	3.0 a	0.6 a	16.9 a	14.3 a	13.8 a	26.8 a	35.8 a	29.8 a	49.1 a	60.0 a	46.3 a	114.0 a

[1]RT is number of root tips visible inside the rhizometer.

[2]RH is number of roots with roots hairs visible on any point along the root inside the rhizometer.

[3]RL is total root length (cm) = the sum of the lengths of all visible roots, calculated by RootReader 2D (Cornell University).

[4]Corn (*Zea mays* ‘Jubilee’).

[5]PL = peatlite composed of 80 peat:20 perlite (v/v).

[6]Means separated across substrates for each plant species using least significant difference (LSD), $P \leq 0.10$. Means followed by the same letter are not significantly different.

[7]PWC = 80 peat:20 pine-wood-chip (v/v), PWC produced by chipping and hammer-milling loblolly (*Pinus taeda* L.) pine logs through a 6.35-mm screen.

[8]SPW = 80 peat:20 shredded-pine-wood (v/v) SPW produced by shredding and hammer-milling loblolly pine logs through a 6.35-mm screen.

[9]Bean (*Phaseolus vugaris* ‘Gold Rush’).

[10]Tom. = tomato (*Solanum lycopersicum* L. ‘Better Boy’).

[11]Mar. = marigold (*Tagetes erecta* ‘Inca Orange’).

CONCLUSIONS

With such a large portion of the industry involved with growing plants in containers, it is important to understand how root growth influences substrates used in container production. The clear cylinder of the rhizometer increased the functionality, and allowed for many different root observations and measurements to be made to further our understanding and study of growing root systems and the influence of various parameters on root growth in containers. The time for roots to become visible (4 to 12 days from seed) and the round cylinder could limit the number of visible measurements to be made in the system. This methodology is likely not appropriate for assessing total root growth of seedlings/plants due to the inability to see or measure roots in the substrate itself (that are not on the outside against the wall of the rhizometer). However, options for analyzing root development and anatomical/morphological characteristics could be further investigated with this method.

Literature cited

Clark, R.T., Famoso, A.N., Zhao, K., Shaff, J.E., Craft, E.J., Bustamante, C.D., McCouch, S.R., Aneshansley, D.J., and Kochian, L.V. (2013). High-throughput two-dimensional root system phenotyping platform facilitates genetic analysis of root growth and development. Plant Cell Environ. *36* (*2*), 454–466. PubMed http://dx.doi.org/10.1111/j.1365-3040.2012.02587.x

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

Judd, L.A. (2013). Rhizometrics: Novel techniques to observe and measure root growth of container-grown crops. M.S. Thesis (North Carolina, USA: North Carolina State University).

Judd, L.A., Jackson, B.E., and Fonteno, W.C. (2014). Rhizometrics: a review of three in situ techniques for observations and measurement of plant root systems in containers. Acta Hortic. *1034*, 389–397. https://doi.org/10.17660/ActaHortic.2014.1034.48

Judd, L.A., Jackson, B.E., and Fonteno, W.C. (2015). Rhizometer: an apparatus to observe and measure root growth and its effect on container substrate physical properties over time. HortScience *50*, 288–294.