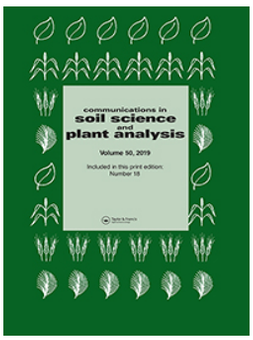

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Quantification of Poinsettia Root Growth by Image Scans or X-ray Computed Tomography Scans in Three Propagation Substrates at Varied Moisture Levels

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ABSTRACT

The objective was to refine protocols to quantify rooting of plant cuttings and the water/air microenvironment of substrates using x-ray computed tomography (CT). Poinsettia (*Euphorbia pulcherrima*) cuttings were propagated in three substrates (peat, rockwool, and phenolic foam) at varied moisture levels. In Experiment 1, adventitious rooting occurred in cuttings grown in rockwool or foam from 12% to 86% volumetric water content (VWC) and 12% to 80% volumetric air content (VAC). The highest root growth occurred in rockwool at 59% VWC and 33% VAC. There was an advantage to quantifying root growth by CT in rockwool and foam, in contrast to peat, because of clear differences in material density between root and substrate during image processing. In Experiment 2, root growth was quantified in peat by two-dimensional image scans under similar growth chamber environments, with rapid root growth from 52% to 63% VWC and 16% to 26% VAC. In Experiment 3, CT was used to quantify the substrate microenvironment at 0.5 cm slices to further describe the environment at the base of the plant cutting. Rapid rooting occurred in microenvironments above 56% VWC and 14% VAC, whereas the low 3% VAC in foam at high moisture may limit root growth.

Abbreviations: volumetric water content (VWC), volumetric air content (VAC), volumetric solid content (VSC)

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Adventitious roots; CT; peat; phenolic foam; rockwool; volumetric water content

Introduction

Vegetative cuttings are commonly used for clonal propagation in forestry and horticulture (Bellini, Pacurar, and Perrone 2014). Total sales for propagative floriculture material in the US were estimated at $394 million in 2015 (U.S Department of Agriculture 2016). Greenhouse propagation uses high misting frequency to maintain high relative humidity, between 90% and 99% (Monselise and Hagin 1955), to reduce transpiration in the cutting (Santos et al. 2011). These conditions affect the water and air balance in small propagation cells, resulting in high moisture and low aeration (oxygen) in the root substrate (Milks, Fonteno, and Larson 1989b). Because the diffusion of oxygen is 10,000 times slower in water than in air (Colmer 2003; Currie 1970), high substrate moisture impedes diffusion of oxygen by blocking the continuum of air-filled pores. Lack of oxygen in the root zone negatively affects mitosis, cell division, and root respiration rate of stem cuttings (Amoore 1961; Drew and Lynch 1980). Poor substrate aeration has been observed to enhance pathogenicity of *Phytophthora* on oxygen-limited roots (Filmer et al. 1986; Ownley, Benson, and Bilderback 1990).

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Management of the rooting environment is vital to rooting success, and annual losses were estimated in 1999 at $US50 million in The Netherlands from poor to slow rooting, or root decay (De Klerk, Van Der Krieken, and De Jong 1999)

Substrates have been designed to provide adequate air porosity at high moisture or at container capacity (Argo, Biernbaum, and Fonteno 1996; DeBoodt and Verdonck 1972; Gislerød 1982; Handreck and Black 2002). There are many substrate components used for young plant production including loose-filled components such as *Sphagnum* peat, perlite, and vermiculite. Commercial propagation increasingly uses "stabilized" substrates such as rockwool, phenolic foam (Oasis^{TM}), peat-polymer blends, and fabric-wrapped pots that are pre-formed and hold the substrate together as a transplantable unit. Because stabilized substrates do not require a root ball to hold loose-filled particles together, production time to produce a transplantable plug can be reduced (Huang and Fisher 2014).

During propagation, adventitious roots may form from shoots, stems (stem-borne), or leaves post-embryonically and therefore not originating from the radicle. The process of harvesting stem cuttings from stock plants results in a wound response by producing jasmonic acid, activating a cascade of signaling molecules increasing auxin at the base of the stem, and releasing sugars required for cell division and callus formation (Friend, Coleman, and Isebrands 1994; Steffens and Rasmussen 2016). The formation of adventitious roots has been described as (1) an induction phase occurring in cells near vascular tissue, (2) an initiation phase with cambial cell division and formation of the root meristem, and (3) an expression phase where root growth occurs (Bellini, Pacurar, and Perrone 2014). Species that form stem-borne roots must have the cell plasticity within cambial cells to undergo de-differentiation in order to form the new root meristem.

Root growth has been quantified using many different methods. These methods include two-dimensional (2D) image scans analyzed by root measurement programs following removal of the root substrate and floating the roots on water. In addition, x-ray computed tomography (CT) allows imaging and quantification of undisturbed roots *in situ* (Judd, Jackson, and Fonteno 2015; Pierret et al. 2003; Piñeros et al. 2016). Information is lost when roots were washed for 2D image scans, whereas CT preserves root architecture and spatial distribution within the root substrate. Three-dimensional imaging of roots by photography is limited to plants grown in transparent environments such as agar, gellan gum, hydroponics and aeroponics (Fang, Yan, and Liao 2009; Herdel et al. 2001). Magnetic resonance imaging (MRI) has been used to image roots within soil and to study the movement of water within plant organs by using the magnetic moment of nuclei such as H^+ (protons) that are abundant in water and biota (Stingaciu et al. 2013; van Dusschoten et al. 2016). Computed tomography (CT) uses an x-ray beam that passes through an object that slowly rotates whereby physical density results in attenuation (Metzner et al. 2015). Quantification of root growth in soils resulted in greater root spatial resolution by CT compared to MRI (Metzner et al. 2015) and has been used successfully to quantify root growth in soils (Tracy et al. 2015, 2013, 2012; Zappala et al. 2013).

Yafuso (2019) quantified water and air relations in small propagation cells for peat, rockwool, and foam substrates. This study found that CT scanning of these root substrates provided estimates of water and air porosity that were consistent with traditional gravimetric methods, and allowed for quantification of the distribution of water and air through the soil profile. That study focused on scanning of substrates at container capacity (a state where the substrate is saturated and then drained), whereas substrate moisture level varies during horticultural production because of evapotranspiration.

For this study, we hypothesize there is a quantifiable range in water and air content in the microenvironment at the base of a stem cutting that is associated with rapid adventitious rooting by providing an adequate balance between hydration and oxygen supply. Our objective was to quantify rooting and the water/air microenvironment using x-ray computed tomography (CT) or two-dimensional scans of three substrates (peat, rockwool, and foam) that had widely differing physical properties, in order to develop protocols for future propagation studies. The effects of substrate type and moisture level were investigated by growing *Euphorbia pulcherrima* 'Prestige Red' (poinsettia)

cuttings in the three substrates (rockwool and foam in one experiment, and peat in a second experiment) at three moisture levels, with root growth quantified at day 14 of propagation in a growth chamber. Rockwool and foam are stabilized substrates that cannot easily be separated from roots but have material density which differs considerably from plant roots, thereby facilitating segmentation of substrate from roots during image analysis of CT scans. In contrast, CT scanning of plant roots in peat substrate was more challenging because of similar material density of roots and peat. Therefore, a separate experiment was conducted to quantify rooting in peat with three moisture levels using 2D image scans. To describe the rooting microenvironment for propagation experiments, the three substrates at each of the three experimental moisture levels were CT-scanned to quantify the balance between volumetric water and air contents at varied depths in propagation cells.

Materials and methods

Experiments were conducted at the University of Florida (UF) Environmental Horticulture Research Greenhouse Complex in Gainesville, FL. The water source for all experiments was municipal tap water, with an electrical conductivity (EC) of 0.4 mS/cm and 40 mg L^{-1} $CaCO_3$ alkalinity.

The substrates used during this study were *Sphagnum* peat (peat), rockwool, and phenolic foam. The particle size distribution of Lithuanian peat, measured by volume using the method from Huang and Fisher (2014) and Huang, Fisher, and Argo (2012a), resulted in 27.6% coarse (>2.0 mm), 69.1% medium (0.5 to 2.0 mm), 2.6% fine (150 μm to 0.5 mm), and 0.9% dust (<150 μm) (Von Post scale 2–3, Puustjarvi and Robertson 1975). The pH of peat was 5.7, and EC was 1.6 mS/cm. Peat were filled into propagation trays that contained cells of 55 mL volume. Gravimetric porosity analysis described by Yafuso (2019) for peat resulted in 22% volumetric solid content (VSC) and dry bulk density of 87.5 g L^{-1}. Manufactured substrates were rockwool cylindrical plugs of 40.5 mL ("rockwool", Grogan, the Netherlands) and phenolic foam cubes of 30.5 mL ("foam", Oasis^{TM}, Kent, OH). Rockwool is formed by heating limestone and basalt to 1600°C resulting in threads of 5 μm with pore size of 4.5 to 5 μm (Da Silva, Wallach, and Chen 1995). The foam substrate was a matrix of phenol-formaldehyde foam with monodispersed pores (Milks, Fonteno, and Larson 1989a). Gravimetric analysis of rockwool found 8% VSC and dry bulk density of 78.9 g $L^{-1,}$ whereas foam had 2% VSC and 20.3 g L^{-1} dry bulk density.

X-ray nano-CT (GE v|tome|x m 240, Wunstorf, Germany 240) was carried out at the University of Florida, Research Service Center (Gainesville, FL). Prior to scanning, plants were dried to approximately 10% volumetric water content (mL water/mL volume; VWC) in a drying oven at 33°C for 24 to 48 h. Aerial plant parts of stem and leaves were removed at the base of the stem. Up to six substrate samples were stacked in a clear plastic tube to allow scanning of multiple samples in a single scanning run. Rockwool samples were scanned at 80 kV with current of 175 μA, whereas foam samples were scanned at 60 kV with a current of 175 μA. Both substrates (rockwool or foam) had a total of 1,200 images per column with voxel resolution of 49.8 μm, and total scan time of 20 min. per sample. Peat samples were scanned at 70 kV and 250 μA with voxel resolution of 38.8 μm for total of 1700 images per column, and run time of 30 min. Image segmentation of roots in peat was difficult to achieve because of similar particle density, and the quality of CT scanning depends on density separation (Heeraman, Hopmans, and Clausnitzer 1997; Kaestner, Schneeneli, and Graf 2006).

Scanned images were processed (datos|x GE Sensing and Inspection Technologies, Wunstorf Germany) prior to image segmentation and three-dimensional (3D) visualization using 3D software (VG Studio Max 3.0, Heidelberg Germany). Segmentation of roots was achieved using the region-growing tool in VG Studio Max 3.0 by selecting acceptable threshold value that selected roots rather than substrate. Examples of scans in the three substrates at this point of image analysis are shown in Figure 1. The time required for slice-by-slice image segmentation of roots ranged from 15 to 35 min. for rockwool and foam where density of the substrate differed than that of roots, whereas segmentation in peat required more time (up to 3x) because residual water within peat strands were similar to the density of roots. For precision of root volume analysis, the open-close tool was used to fill the spaces

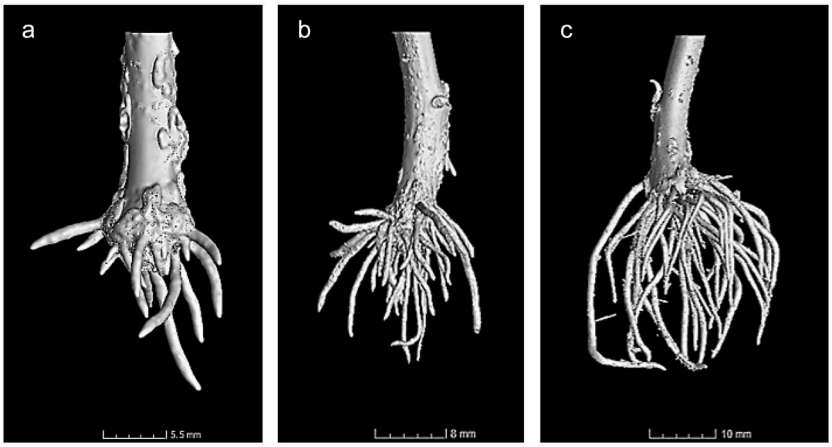

Figure 1. Examples showing image segmentation of poinsettia roots in three propagation substrates (foam, rockwool, and peat) by CT scans at day 14 after transplant. Root surface area and volume plant^{-1} for (A) foam was 3.2 cm^2 and 0.06 cm^3, (B) rockwool was 7.2 cm^2 and 0.11 cm^3, and (C) peat was 27.9 cm^2 and 0.59 cm^3 from these individual replicates in Experiment 1.

within roots. To capture the complete root surface, the edge refinement tool in VG Studio Max 3.0 was used, and to remove residual water and substrate clinging to roots, the software smoothing tool was used. The 3D polyline tool in VG Studio Max 3.0 was used to remove the base of the stem for accuracy of root quantification. Root spatial distribution was quantified by aligning roots to a measuring grid, followed by segmenting in sections 0.5 cm tall using the 3D polyline tool (as shown in Figure 2), which required an additional 10 to 15 min. processing time per sample.

Expt. 1. The effect of stabilized substrates at varied irrigation moisture on root growth of poinsettia quantified by CT

The first experiment aimed to quantify root growth of unrooted *Euphorbia pulcherrima* 'Prestige Red' by CT for the two stabilized substrates, rockwool, and foam, at three irrigation moisture levels. The experimental design was a split plot where the main plot was moisture level (low, medium, high)

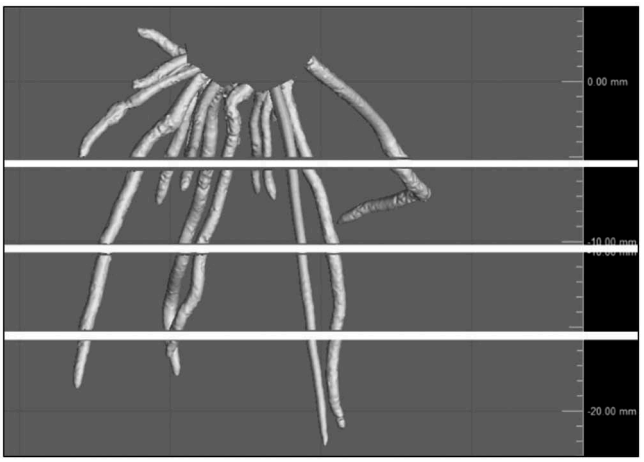

Figure 2. Example of spatial distribution analysis of root growth from three-dimensional CT scans as the next step after image segmentation. Each image was separated into 5 mm slices to analyze root surface area (mm^2) and volume (mm^3) within each slice.

and sub-plot was substrate (rockwool or foam). Substrate moisture levels were established by subirrigation of capillary mats at heights of 0, 2.5, and 5 cm relative to the surface of water and referred to as "high," "medium," or "low" moisture levels, respectively. Each moisture level were replicated four times. A replicate tray contained a substrate type and three sub-replicate plants. Additional plants were grown in this experiment in peat in order to refine CT-scanning methods in this substrate but were not included in statistical analysis.

The growth chamber used fog generated by an ultrasonic fogger to maintain relative humidity (RH) at 95%. Mist emitters of 69 μm-diameter droplet (Coolnet Pro Fogger, Netafim, Israel) were used to maintain cutting hydration and averaged 5 sec. duration at 30 min. intervals for day 1 to 5 and decreased to 5 sec. duration at 100 min. intervals for day 6 to 14, and night mist stopped by day 7. Light was supplemented with light-emitting diode (LED) lights that emitted 149 μmol m^{-2} s^{-1} at the canopy level. The initial photoperiod was 15 h that increased to 21 h by day 10 of propagation for a daily light integral of 8.1 to 12.2 mol m^{-2} day^{-1}, respectively. During the experiment, air temperature averaged 24.9°C and 98.4% RH. Plant leaf and substrate surface temperature were similar and averaged 22.9°C. Tray weights were measured gravimetrically over-time (3 days) during the experiment to quantify VWC and VAC.

Plants were CT-scanned at day 14 of propagation following the previously described method for quantification of total surface area, volume, and spatial root quantification. Roots were counted manually for total root count growth variable. Plant subreplicates were averaged by replicate tray prior to statistical analysis. Total root growth, root growth at each 0.5 cm vertical section, and volumetric water and air contents were separately analyzed by a two-way ANOVA using PROC GLIMMIX with fixed effects as substrate and moisture level at $p = .05$ in SAS (SAS Version 9.4, SAS Institute, Cary, NC).

Expt. 2. The effect of peat substrate at varied irrigation moisture on root growth of poinsettia quantified by image scans

The second experiment aimed to quantify root growth of poinsettia in peat at varied moisture levels by 2D image scans. The experimental design was a completely randomized design whereby substrate moisture levels (low, medium, and high) were replicated by using a tray. There were four replicate trays per treatment combination and three plant sub replicates per tray. Substrate moisture levels were established by subirrigation of capillary mats previously described in Experiment 1. Unrooted cuttings were transplanted into loose filled propagation trays (55 mL cells) with a fine peat substrate.

The growth chamber environment for misting frequency and supplemental light were previously described in Experiment 1. The average temperature was 25.2°C and RH was 95.7%. Daily leaf and soil temperature were similar and averaged 24.7°C. Plants were destructively harvested on day 14 of propagation. After root washing, roots were scanned at 800 dpi (Epson Perfection 4990 PHOTO, Indonesia) and analyzed in root measurement software (winRHIZO^{TM} Pro 2017a, Regent Instruments, Inc., Canada) for total root length, surface area, and volume. Roots were counted manually for the total root count growth variable. Root growth variables were averaged for each replicate tray prior to statistical analysis. Root data were analyzed as a one-way ANOVA using PROC GLIMMIX with fixed effects of moisture level at $p = .05$ in SAS.

Expt. 3. Quantification of the substrate microenvironment by CT

A trial without plants grown was conducted to describe the microenvironment of VWC and VAC. The experiment was a randomized block design with the three substrates (peat, rockwool, and foam) at three moisture levels used in Experiments 1 and 2. There were three replicate cells for each treatment combination. Water was supplemented with nutrients (17.0 N, 1.7 P, 14.1 K at 200 mg L^{-1} N) where the pH and electric conductivity were measured at 6.7 and 1.8 mS/cm, respectively. Substrates were subirrigated to container capacity and allowed to equilibrate on capillary mats for 5 days. Substrates were then measured gravimetrically to quantify VWC, VAC, and VSC using the methods described by Huang

and Fisher (2014) and Huang, Fisher, and Argo (2012b). The average temperature was 22°C and RH was 65%. In addition to gravimetric measurements, substrates were scanned using CT at 80 kV and 175 μA with a voxel resolution of 59.5 μm. There were 1000 images per column averaging three images and total run time of 17 min.

Quantification of substrate components of water, air, or solid was carried out by image segmentation of air for peat or segmentation of water in rockwool and foam. The gravimetric analysis of VSC was used as a constant to calculate the (1) VWC in peat or (2) VAC in rockwool and foam using the method described by Yafuso (2019).

Data for VWC and VAC from gravimetric and CT analysis were compared using a two-way ANOVA by PROC GLIMMIX with fixed effects of substrate and method (gravimetric or CT) at α = 0.05 in SAS. Cell spatial distribution of VWC or VAC data was analyzed using a three-way ANOVA with fixed effects of substrate, moisture level, and cell depth at an α = 0.05 in SAS.

Results

Expt. 1. The effect of stabilized substrates at varied irrigation moisture on root growth of poinsettia quantified by CT

There were broad differences in the VWC and VAC for the different substrate and moisture level combinations, resulting in differences in root growth (Figure 3). Analysis of variance found that substrate and moisture level did not affect root count, but there were main effects at the $p < .05$ level of moisture level on root surface area and volume. In addition, there was an interaction effect of substrate and moisture level on root surface area.

In rockwool, higher root growth was observed at high moisture (59% VWC and 33% VAC) relative to low (12% VWC and 80% VAC) and medium moisture levels (22% VWC and 70% VAC) (Figure 3). Root growth in foam was similar across very different moisture levels of 17%, 33%, and 86% VWC corresponding to 79%, 65%, and 12% VAC (Figure 3).

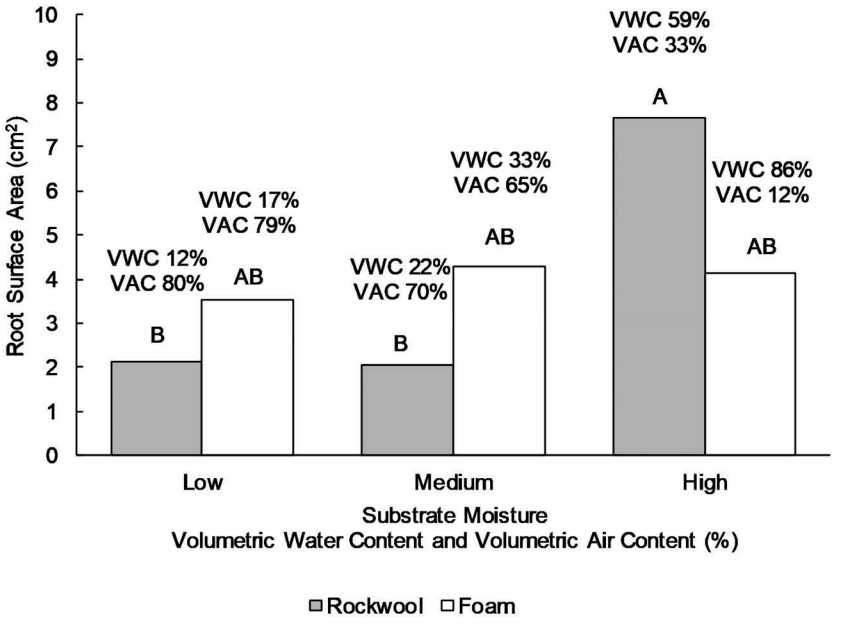

Figure 3. Effect of substrate (rockwool or foam) and varied moisture level (low, medium, or high) on root surface area plant^{-1} (poinsettia) at day 14 in Experiment 1. Least-square means were from 12 replicates per treatment combination of substrate and moisture level, compared using Tukey's honestly significant difference at α = 0.05. Labels in parentheses show the volumetric water content (VWC) and volumetric air content (VAC).

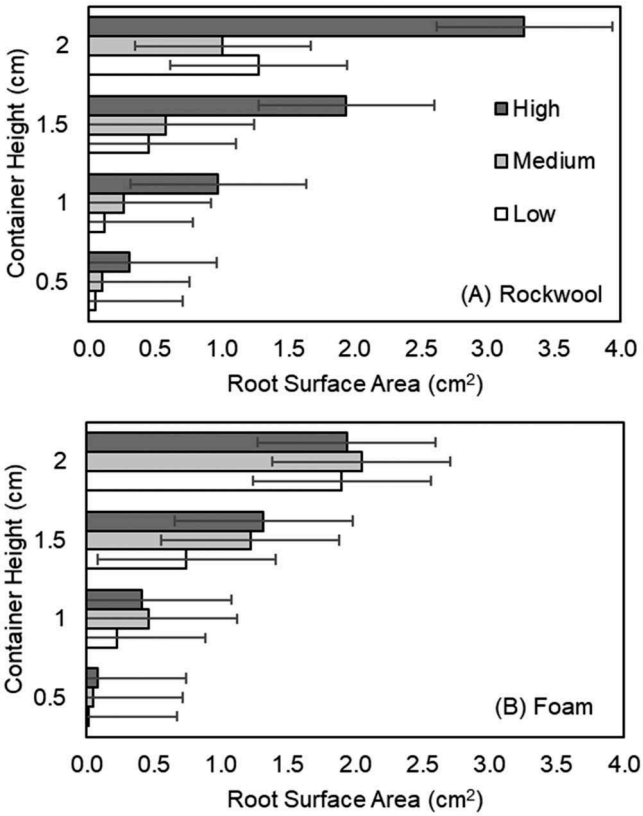

Figure 4. Quantification of spatial distribution of root surface area plant^{-1} at different moisture levels in rockwool and foam from Experiment 1 for 0.5 cm sections from the bottom to top of the propagation cell. Bars represent the least-squared mean root surface area of 12 replicates per moisture level with error bars representing Tukey's least significant difference at $\alpha = 0.05$.

Root spatial distribution in 0.5 cm sections from the bottom of the cell is shown in Figure 4. At this early stage of propagation (after 14 days), roots were continuing to emerge from the stem and callus. Therefore, the highest root surface area was near the base of the stem, which was 2 cm above the bottom of the container. The high moisture level resulted in greater root surface area than lower moisture levels in rockwool between 2 and 1.5 cm above the cell base. Foam had a similar root distribution across moisture levels within each vertical segment.

Expt. 2. The effect of peat substrate at varied irrigation moisture on root growth of poinsettia quantified by image scans

In peat, VWC was estimated to be 52%, 58%, and 63%, corresponding to VAC of 26%, 20%, and 16% at the low, medium, and high moisture levels, respectively. The VWC of peat at the lowest two moisture levels was higher than VWC for rockwool and foam under the same experimental conditions in Experiment 1, which is the result of a higher water retention for peat compared with the other two substrates (Yafuso 2019).

After roots were washed and separated from peat substrate, roots were then quantified in 2D image scans using root measurement software. There was similar and rapid root growth, with no effect of moisture level on root length, surface area, or root count. The average root length was 46.3 cm plant^{-1} ± 25.8 (mean ± 95% confidence intervals), root volume was 0.35 cm^3 plant^{-1} ± 0.21, and root count was 18 plant^{-1} ± 7.6, and root surface area was 14.4 cm^2 plant^{-1} ± 8.2.

Expt. 3. Quantification of the substrate microenvironment by CT

Comparison of gravimetric and CT methods to estimate VWC and VAC found similar results between methods at each moisture level in rockwool and foam (Table 1). However, there were differences between the VWC and VAC estimated by the two methods in peat. Yafuso (2019) also found close agreement in gravimetric and CT estimation of VWC and VAC in rockwool and foam when substrates were at container capacity. The VAC may have been underestimated by CT due to the CT-scanning resolution of 59.5 µm since internal pores in peat were approximately 15 µm (Carey, Quinton, and Goeller 2007).

The VWC and VAC measured gravimetrically in rockwool and foam during Experiment 1 were similar to the VWC and VAC measured gravimetrically in Experiment 3 for these substrates. However, peat had a lower VWC and higher VAC in Experiment 3 compared with Experiment 2. The difference in moisture level for peat between Experiments may have resulted from a combination of variability from small samples, and slight differences in environmental conditions (substrates were under mist irrigation during Experiment 2 but not in Experiment 3).

The CT images for substrate and moisture level combinations in Experiment 3 (Figure 5) show different patterns of water and air distribution. Within peat, water and air-filled pores were observed throughout the vertical profile. As previously noted, peat had a higher moisture content at the lowest two moisture levels compared with the other substrates, and this water was distributed throughout the vertical profile (Figure 6). Rockwool was composed of thin threads of solid fibers, and the CT scan showed vertical channels of water and air (Figure 5). Foam at high moisture showed near-saturation of pores (Figure 5), and nearly all air-filled pores were at the top of the substrate (Figure 6). However, as the foam substrate dried, there was a more even distribution of water and air-filled pores.

The sectioning of the CT scans allowed analysis of VWC and VAC to describe the microenvironment at the base of the cutting (2 cm from the upper surface of the cell) (Table 2). This microenvironment can be compared with the level of rooting measured in Experiments 1 and 2. For Experiment 1, the highest level of rooting occurred with rockwool at the high moisture level, with 58% VWC and 34% VAC. Other substrate and moisture level combinations in Experiment 1 had much lower VWC and higher VAC, with the exception of foam at the highest VWC (95%) and lowest VAC (3%). As noted above, there are challenges with estimating the exact VWC and VAC in peat using CT scanning. However, the estimates for peat in Experiment 2 for VWC were between 56% to 64% and VAC between 14% to 22%, and no differences in root growth were observed in Experiment 2.

Table 1. Comparison of volumetric water (VWC) or air (VAC) content measured by gravimetric or CT scanning in three substrates and three moisture levels in Experiment 3. Least-square means were from three replicates cells per treatment combination, compared using Tukey's honestly significant difference at $\alpha = 0.05$. Substrate volumetric solid content for peat was 22%, rockwool was 8%, and foam was 2%.

Substrate	Moisture	Method	VWC	Tukey's	VAC	Tukey's
Peat	Low	Gravimetric	38%	G	40%	D
Peat	Low	CT	55%	ED	23%	F
Peat	Medium	Gravimetric	46%	F	32%	E
Peat	Medium	CT	61%	CB	17%	G
Peat	High	Gravimetric	53%	E	25%	F
Peat	High	CT	65%	B	13%	G
Rockwool	Low	Gravimetric	14%	J	78%	A
Rockwool	Low	CT	14%	J	79%	A
Rockwool	Medium	Gravimetric	22%	I	70%	C
Rockwool	Medium	CT	21%	I	71%	BC
Rockwool	High	Gravimetric	59%	CD	33%	E
Rockwool	High	CT	59%	CD	33%	E
Foam	Low	Gravimetric	22%	I	76%	BA
Foam	Low	CT	19%	I	79%	A
Foam	Medium	Gravimetric	32%	H	66%	C
Foam	Medium	CT	31%	H	67%	C
Foam	High	Gravimetric	81%	A	17%	G
Foam	High	CT	80%	A	18%	G

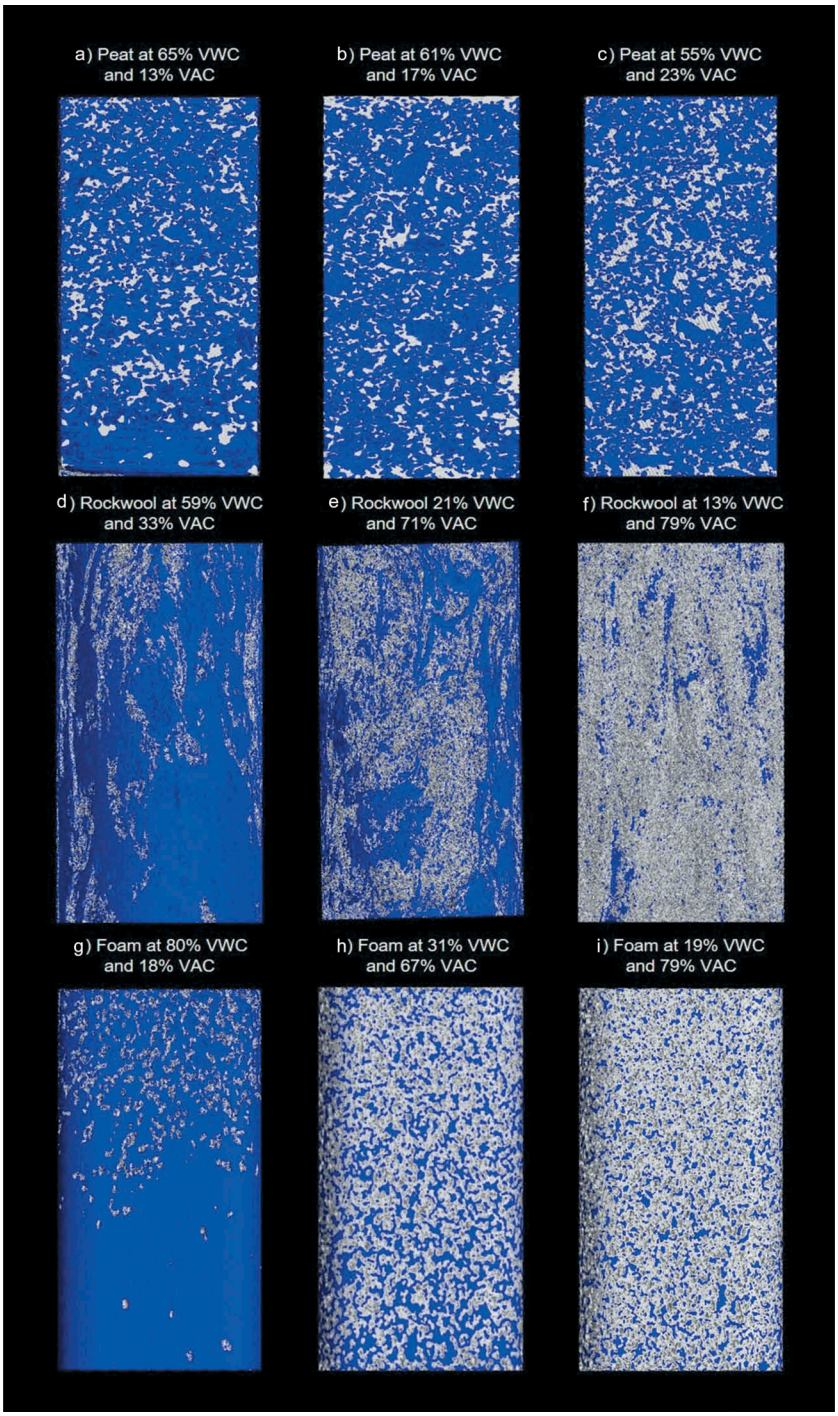

Figure 5. Visualization of water and air relations for three substrates at three moisture levels (left to right for high, medium, and low) from Experiment 3. Volumetric water (VWC) and air (VAC) content labels represent the least-square means of three replicate cells per treatment combination estimated by CT (from Table 1). The blue (dark) color represents segmented water-solid matrix in peat or water in rockwool and foam, and white to light gray represents segmented air. Images were not in scale between substrates, and the actual volume for peat was 55 mL, rockwool 40.5 mL, and foam 30.5 mL. Color online only.

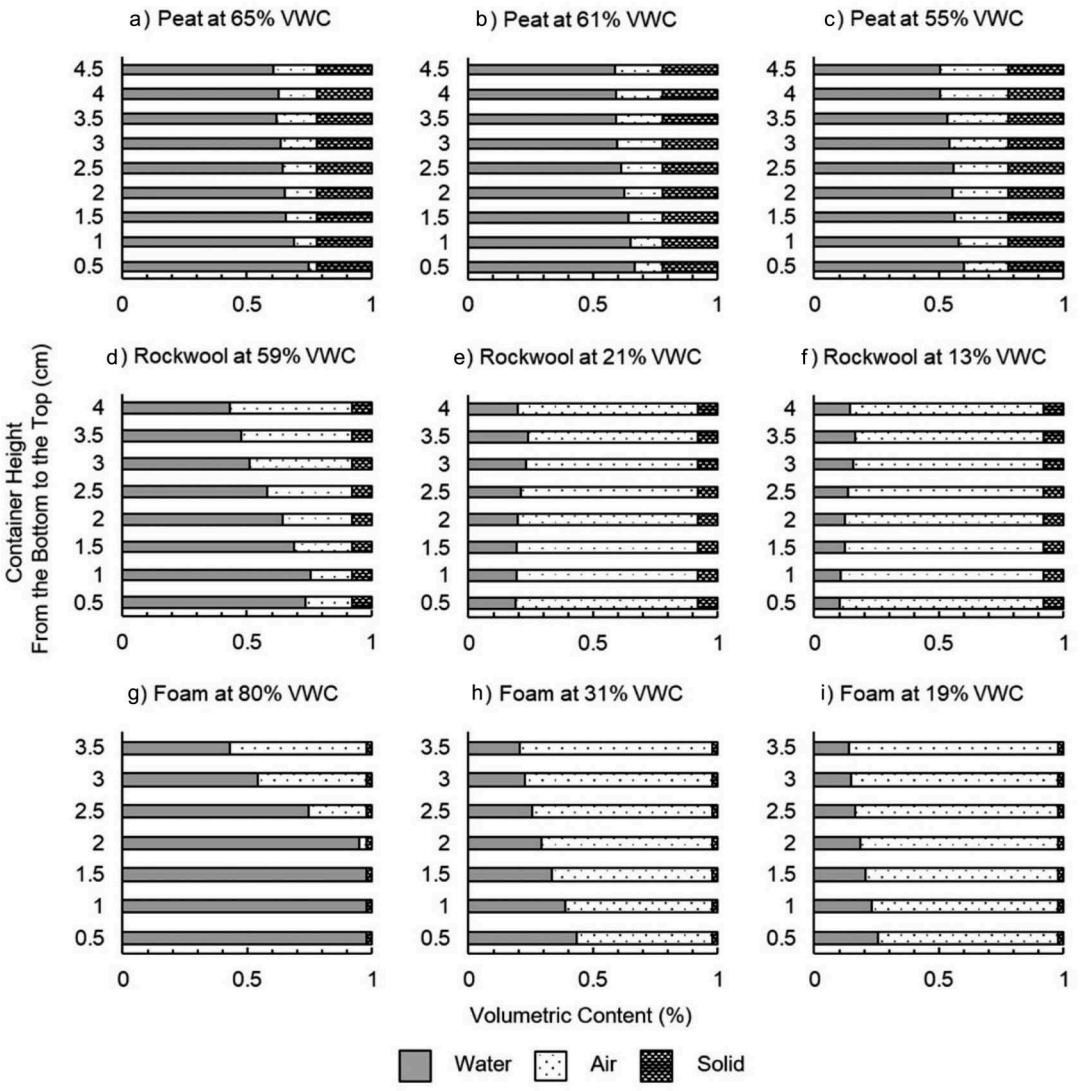

Figure 6. Cell spatial distribution of volumetric water and air content for three substrates at three moisture levels and cell depths by 0.5 cm sections (from the cell bottom to the top) from Experiment 3. Least-square means were of three replicates cells per treatment combination. Substrate volumetric solid content for peat was 22%, rockwool was 8%, and foam was 2%.

Discussion

Commercial propagation substrates vary widely in VWC and VAC, VWC from 57% to 86% and VAC from 4.8% to 9.7% in a survey of loose-filled and stabilized substrates in 128-count propagation trays by Huang, Fisher, and Argo (2012b). The lack of precise agreement for container media from the many studies that evaluate the effect of VWC, VAC, and other substrate physical properties on adventitious rooting can be attributed to differences in plant species, irrigation, and environmental factors, and methods used to measure VWC and VAC (Bunt 1988). In addition, VWC and VAC alone are not adequate to describe the root zone conditions because of differences in matric potential of substrates and resulting water availability at a given VWC, and because oxygen diffusion rate can be as important as VAC as a measure of oxygen supply during propagation (Gislerød 1983).

Table 2. Volumetric water (VWC) and air (VAC) content for the three substrates at three moisture levels from experiment 3 at a cell depth of 2 cm relative to the upper surface of the cell, which represents the position of the base of the poinsettia stem. Least-square means were of three replicates cells per treatment combination, with 95% confidence intervals using Tukey's honestly significant difference at $\alpha = 0.05$. Substrate volumetric solid content for peat was 22%, rockwool was 8%, and foam was 2%. The relative root growth column summarizes results from experiments 1 and 2, where root growth was consistently high across moisture levels for peat in experiment 2, and differences in root growth between substrate and moisture level combinations for rockwool and foam in experiment 1 (as shown in Figure 3).

Substrate	Moisture	VWC	VAC	Relative Root Growth
Peat	Low	56% ± 4%	22% ± 4%	High (Expt 2)
Peat	Medium	61% ± 4%	17% ± 4%	High (Expt 2)
Peat	High	64% ± 4%	14% ± 4%	High (Expt 2)
Rockwool	Low	13% ± 4%	79% ± 4%	Low (Expt 1)
Rockwool	Medium	21% ± 4%	71% ± 4%	Low (Expt 1)
Rockwool	High	58% ± 4%	34% ± 4%	High (Expt 1)
Foam	Low	19% ± 4%	80% ± 4%	Medium (Expt 1)
Foam	Medium	29% ± 4%	69% ± 4%	Medium (Expt 1)
Foam	High	95% ± 4%	3% ± 4%	Medium (Expt 1)

No plant wilting was observed in our study, despite a wide range in VWC from 12% to 86%, because high air humidity within the plant canopy was provided through fog and mist irrigation. To prevent wilting of leafy cuttings by evapotranspiration, water must be freely available to the cutting (Leakey 2004). In Experiment 1, although there was low VWC (12% to 33%), in some moisture treatments, rockwool, and foam, substrate water would still be available for root uptake based on the moisture retention curves because there was a low matric potential of <5 kPa at these moisture levels (Yafuso 2019). In addition, at low VWC water exists as a thin film that may coat the cutting surface and aid in hydration. Water vapor is also near saturation within air-filled pores unless substrate is extremely dry (van Iersel and Dove 2014; Wallach 2008). However, there was more rapid root growth in rockwool when VWC increased from 33% to 59%. In addition, rapid root growth was observed in the experimental range of 52% to 63% VWC in peat. We hypothesize that although the water present in rockwool and foam at the low and medium moisture levels was plant-available in Experiment 1, it was less than optimal for root growth.

Waterlogged conditions can result in low VAC and oxygen level, limiting root growth and respiration (Amoore 1961), impairing nutrient mobilization (Drew 1988), and favoring root pathogens (Filmer et al. 1986). Many studies have found that VAC can be limiting to root growth. For example, Bunt (1988) found that when tomatoes were grown at high irrigation frequency in soilless substrates with a broad range of VAC levels, root growth was not impeded when VAC was 10% or above, presumably because of adequate oxygen supply and air exchange. Gislerød (1983) varied VWC and VAC in peat and rockwool substrates during poinsettia propagation. Cuttings at container capacity (0 cm of tension) had less than 5% VAC, which resulted in reduced rooting and lower oxygen diffusion rate compared with tensions resulting 7.5% VAC or above in the Gislerød (1983) study. In our study, we found greater than 12% VAC in all treatments on a whole-cell basis (Figure 3). However, foam at the highest moisture level had only 3% VAC at the base of the stem cutting (Table 2). Overall, we conclude that a VWC of 52% or higher and a VAC of 12% or higher were not limiting to root growth.

The observation of low VAC at the stem base in foam at high moisture (Table 2) is an example of the importance of quantifying the microenvironment within a propagation cell where callusing and adventitious rooting occurs. Tracy et al. (2015) developed detailed three-dimensional images of sand and clay substrates at different moisture levels and combined this spatial data with matric potential measurements to quantify moisture release curves and hydraulic conductivity. A study by Baas and Gislerød (1997) in rockwool used a different approach to quantify VWC and VAC at different substrate heights and matric potentials using pycnometry and by providing pressure heads of 0, 3.3, 6.5, or 10 cm. The VAC at the base of the rose stem cutting (lower 2.75 cm) ranged from 20% to 25% resulted in rooting success whereas the upper portions of the block contained 37% to 42% VAC. Similarly, in Experiment 3 of our study, rockwool at high moisture at the top of the cell (from 2.5 to

4 cm in height) ranged from 34% to 49% VAC, whereas the bottom 2 cm of the cell ranged from 17% to 27% VAC (Figures 5 and 6). Visualization of the spatial distribution of water and air and its effect on root architecture has many potential applications, such as identifying phenotypes with drought resistance in agronomic crops (Lynch 1995).

Because of limitations in our experimental design, it was not possible to directly compare rooting between the three substrates. However, the root surface area in peat in Experiment 2 at day 14 (14.4 cm^2 $plant^{-1}$ ± 8.2) was almost twice the highest amount of rooting in Experiment 1, which occurred in rockwool at high moisture (7.7 cm^2 $plant^{-1}$, Figure 3). Differences between the experiments include different batches of cuttings, and also different root quantification methods (CT for rockwool and foam in Experiment 1 and 2D image scans in peat for Experiment 2). Previous research has, however, found that 2D and 3D root quantification methods can have high correlation (Tracy et al. 2015, 2013).

These results have implications for horticultural management, including irrigation, substrate selection, and transplanting method. Quantification of the substrate microenvironment with three widely different substrates (peat, rockwool, and foam), provided a target range of VWC and VAC levels that could aid in irrigation management for poinsettia. For example, it would be possible to measure weights of propagation trays under mist and relate this to gravimetric estimates of water and air levels. Under the same subirrigation conditions, peat absorbed more water and had less air than rockwool and foam, which is a result of the higher matric potential of peat. Peat therefore requires careful mist irrigation management to avoid overwatering during propagation (Da Silva, Wallach, and Chen 1993; Heiskanen, 1995; Yafuso 2019;), although it has advantages of a more even vertical distribution of VWC and VAC (Figures 5 and 6) if these levels can be maintained in a suitable range. In contrast, rockwool and foam have low matric potentials that may require frequent misting to maintain cutting hydration (Da Silva, Wallach, and Chen 1995; Fonteno and Nelson 1990; Yafuso 2019). The depth of inserting plant cuttings also has a great effect on water and air balance and subsequent root growth (Handreck and Black 2002). This is particularly important for rockwool and foam because of stratification in the vertical distribution of VWC and VAC (Figures 5 and 6). For example, foam at high moisture level had no quantifiable VAC in the bottom 2 cm, which would be favorable conditions for callus production but not rooting in poinsettia cuttings (Gislerød 1983).

Conclusion

Although CT can be used to quantify root distribution in all three substrates tested, rockwool and foam had advantages of a large difference in particle density compared with plant roots that resulted in successful image segmentation of roots. Therefore, rockwool or foam substrates provide a useful model system where the research aim is to quantify the architecture of undisturbed roots. In contrast, root growth in peat was easily quantified by 2D image scans because it is a "loose" substrate where root washing was possible. Adventitious rooting occurred across a wide range from 12% to 86% VWC and 12% to 80% VAC; however, the highest root growth occurred in rockwool with 59% VWC and 33% VAC or in peat in the range from 52% to 63% VWC and 16% to 26% VAC. When the VWC and VAC were quantified in the 0.5 cm slices at the base of the cutting using CT scans, a low VAC (3%) was identified for foam at high moisture, which could limit oxygen supply for root growth.

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