THE RELATIONSHIP BETWEEN WATER AND OXYGEN IN PLANT PROPAGATION

By

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To my parents for their unchanging love and devotion
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LIST OF ABBREVIATIONS

2D Two-dimension
3D Three-dimension
ANOVA Analysis of variance
CC Container capacity (same as field capacity)
CT Computed tomography
DO Dissolved oxygen
EC Electrical conductivity
h Hours
HSD Honestly significant difference
Min Minute
MRC Moisture retention curve; moisture characteristic curve
MRI Magnetic resonance image
PAR Photosynthetically active radiation
s Seconds
VAC Volumetric air content (relative to cell volume)
VSC Volumetric solid content (relative to cell volume)
VWC Volumetric water content (relative to cell volume)
Greenhouse propagation of vegetative plant cuttings combines high moisture level and small container size, which could potentially limit oxygen supply for adventitious rooting. The objectives were to 1) evaluate supersaturated water by injecting pure oxygen (oxygenate) during propagation and continued growth of transplants, 2) describe the water and air relations in three propagation substrates, and 3) quantify root growth by two or three-dimensional imaging during propagation of poinsettia in substrates and varied moisture levels. Irrigating with oxygenated water (DO of 31.1 mg·L$^{-1}$) did not benefit root or plant growth during mist propagation or continued growth of transplants compared to ambient water (DO of 7.1 mg·L$^{-1}$). Substrates used during propagation have differing water retention properties. Stabilized substrates, rockwool and foam, had low matric potential (< 30 cm), whereas peat had high matric potential (~ 300 cm). Comparison of methods (frozen column or tensiometer) were used to describe moisture retention curves (MRCs) resulted in similarities for substrates with low matric potential and differed in peat where column height constrained the measured tensions. Comparison of methods [X-ray computed tomography (CT) or gravimetric] were used to describe volumetric water (VWC) and air content (VAC) resulted in similarities for rockwool, foam, and differed in peat. This difference may be due to a limitation of the CT scanning resolution of 59 µm resulting in lower
VAC. However, CT provides visualization and spatial distribution of water and air within substrate. Unrooted poinsettia cuttings were propagated in a growth chamber in three different substrates at three substrate moisture levels. Root growth was quantified by CT in rockwool and foam, whereas two-dimensional image scans were accurate for peat. Propagules rooted under a wide range of VWC and VAC, however high root growth was observed in rockwool and peat between a range of 52 to 63% VWC and 16 to 33% VAC. Under high moisture conditions, foam had low VAC (3%) at the base of the plant cutting, which may limit oxygen supply for root growth. This study emphasizes the need to supply adequate oxygen supply through selection of a substrate with high air porosity and by avoiding water-logged conditions.
CHAPTER 1
INTRODUCTION

Young plant production in horticulture includes seedlings and vegetative propagation (Bellini et al., 2014). In 2015, propagative floriculture material reported sales of $394 million dollars in the US (USDA, 2016). During propagation, environmental conditions of high relative humidity and misting frequency aids to maintain cutting hydration (Santos et al., 2011). Consequently, excess moisture in container substrate may result in slow or no rooting increasing the time for crop production (De Klerk et al., 1999). In addition, high substrate moisture increases the risk of disease, such as Pythium (Chérif et al., 1997). Quantification of the water and air (oxygen) microenvironment in container substrate that results in rapid rooting can be used to provide growers with a range in water and air aiding in best irrigation management during propagation.

The overall thesis objective was to quantify the balance between water and oxygen (in air or dissolved in water) and effect on rooting of vegetative cuttings. In container substrate, oxygen (O₂) can be supplied to plants roots through air-filled pores or by dissolved oxygen (DO) in water-filled pores. Research focused on evaluating a technology to supersaturate DO in irrigation water, quantification of water and air relations in substrates, and effect of substrates and varied moisture level on root growth during propagation.

In Chapter 2, unrooted cuttings were mist propagated with supersaturated water or ambient tap water to study the effect on root growth. There was potential to evaluate the benefits of irrigating with supersaturated water compared to ambient tap water on plant growth at differing phenology.

Chapter 3, water and air relations of three propagation substrates were quantified by moisture retention curves where the frozen column ‘alternative’ method was compared to the
industry standard method by tensiometers. Substrate porosity (by volumetric: water, air, solid) were quantified using newly developed methodology by x-ray computed tomography (CT) that was compared to gravimetric measurements or conventional method. There was potential to compare and develop new methods to quantify water-air relations in propagation substrates.

Chapter 4, root growth was quantified by three dimensional (3D) x-ray CT or two-dimensional (2D) image scans to study the effect of substrates and varied moisture levels on rooting of poinsettia cuttings. There was potential to develop methods for root quantification in different substrates of peat, rockwool, and foam using CT. In addition, CT scans of substrates at varied moisture level can be used to describe the microenvironment similar to propagation experiments. Understanding of substrate water and air relations is foundational for best irrigation management that will aid in reduced losses during propagation.
CHAPTER 2
OXYGENATION OF IRRIGATION WATER DURING PROPAGATION AND CONTAINER PRODUCTION OF BEDDING PLANTS

In container substrates, moisture level and substrate porosity affect oxygen supply to roots. Oxygen can be supplied to roots either as a gas present in air-filled pores or as DO in water. In atmospheric air, oxygen is at 20.9%, equivalent to 274 mg·L$^{-1}$ at 1 atm and 25 °C. Within the root zone, van Iersel and Dove (2014) pointed out that in a well-irrigated substrate, the relative humidity is likely to be close to 100%, resulting in reduced partial pressure of oxygen because of water vapor (19.1-20.6 kPa), and root and microbial respiration could further lower O$_2$ concentration. Oxygen saturation concentration in water decreases as temperature increases, with 8.3 mg·L$^{-1}$ saturated DO at 25 °C, compared with 11.3 mg·L$^{-1}$ at 10 °C. The diffusion rate of oxygen in water is ≈10,000 times slower than the diffusion in air (Colmer, 2003; Wegner, 2010). Consequently, transport of O$_2$ in a substrate and availability for root uptake are dependent on factors that affect diffusion and advection (bulk flow) of oxygen, including the surface area of substrate particles and water that are in contact with air, the continuum of gas-filled pores, and substrate moisture level (Currie, 1970). Peat-based substrates are designed to avoid complete saturation and provide air-filled porosity for oxygen supply to roots, even when irrigated to CC (Argo et al., 1996; DeBoodt and Verdonck, 1971). In a typical peat-based substrate, air porosity can be up to 32% by volume at CC in a 1 L container, but this percentage can decrease to 5% in small cells used for propagation of seedlings and unrooted cuttings (Argo et al., 1996; Gislerød, 1982; Handreck and Black, 2002). Lower substrate oxygen conditions are likely to occur with fine substrate particles, small container size, and high moisture. These conditions may also occur during mist propagation of plant cuttings because high substrate moisture is needed to prevent plant wilting, there are frequent irrigation events, and plants are grown in small cells (Santos et al., 2011).
Oxygen supply to and within roots involves several processes and is vital for root physiological function. Diffusion and bulk flow of oxygen into container substrate often occurs rapidly, replacing oxygen used in respiration by roots and microbes (van Iersel and Dove, 2014). Oxygen from air-filled pores can diffuse passively into the root tip (Lemon, 1962; Luxmoore et al., 1970) or DO can enter through water pathways (Luxmoore et al., 1970). Apoplastic porosity through contiguous intercellular spaces allows for the movement of oxygen within the root (Armstrong and Drew, 2002; Colmer, 2003). Channels in membranes or aquaporins also facilitate cell-to-cell symplastic transport of oxygen (Herrera and Garvin, 2011; Hub et al., 2009). These oxygen transport processes in the root allow the vital functions related to respiration and nutrient uptake to be carried out (Drew, 1988). Respiration rates have been estimated at 300–500 ng·cm⁻³·s⁻¹, 0.3–0.5 ng·L⁻¹·s⁻¹ for apical regions of the root at 23 °C (Armstrong et al., 2000). Over-irrigation fills substrate pores with water rather than air, and oxygen demand from respiring roots and microbes can further result in low (hypoxic) to no oxygen (anoxic) levels (Armstrong and Drew, 2002; Drew, 1983; Morard and Silvestre, 1996; Naasz et al., 2009). Plant stress has been observed in hydroponic production conditions under low DO concentrations from 0.1 to 2 mg·L⁻¹ (Goto et al., 1996; Zheng et al., 2007). Stress responses include decreased root metabolism and nutrient uptake, root death, and wilting (Armstrong and Drew, 2002; Drew, 1983; Ehret et al., 2010; Handreck and Black, 2002; Morard and Silvestre, 1996). Low oxygen at the root zone also increases the risk of diseases from microbial pathogens such as Pythium (Chérif et al., 1997).

In some studies, oxygenation of irrigation water has increased plant biomass. Dissolved oxygen in water can be increased with turbulence, bubbling of air or purified oxygen, and the addition of chemicals such as hydrogen peroxide (Schröder and Lieth, 2002). Oxygen injecting
technology can increase DO above oxygen-saturated levels in irrigation water (Schröder and Lieth, 2002; Zheng et al., 2007). Lei et al. (2016) grew corn in vermiculite substrate under completely saturated conditions. Irrigating plants with oxygenated water (two aeration systems) increased the DO from 3.5 to 6.5 mg·L$^{-1}$, resulting in higher corn yield and biomass compared with those grown with ambient irrigation water with 0.3–4.5 mg·L$^{-1}$ DO. In a hydroponic system, when DO ranged from ambient of ≈8.5 mg·L$^{-1}$ to supersaturated 30 mg·L$^{-1}$, there were no effects on tomato dry mass (Zheng et al., 2007). In the Zheng et al. (2007) study, plant growth decreased and roots appeared stunted and thick when DO was further increased to ≈40 mg·L$^{-1}$.

Although reports on oxygen injecting technology of irrigation water in propagation or container production of bedding plants are lacking, potential positive effects could include increased root or total growth and root health.

Our objective was to evaluate whether oxygenation of irrigation water affected plant growth and substrate DO levels during 1) mist propagation of unrooted cuttings and 2) subsequent growth in containers after transplant. Greenhouse experiments were run with mist propagation of vegetative cuttings of *Calibrachoa × hybrida* ‘Aloha Kona Dark Red’ and *Lobelia erinus* ‘Bella Aqua’ in plug trays. Plants of these two species and *Pelargonium × hortorum* ‘Patriot Red’ were subsequently grown to flowering in 10.2 cm diameter containers.

Supplemental experiments were conducted without plants to provide additional details on DO under the experimental growing conditions.

**Materials and Methods**

Experiments were conducted in computer-controlled heated greenhouses at the University of Florida (UF) Environmental Horticulture Research Greenhouse Complex in Gainesville, FL. Atmospheric pressure used to calculate the DO solubility ([http://water.usgs.gov/software/DOTABLES/](http://water.usgs.gov/software/DOTABLES/)) was provided by the UF Department of Physics
Weather Station (http://www.phys.ufl.edu/weather/). The water source for all experiments was greenhouse tap water, with an electrical conductivity (EC) of 0.4 mS·cm$^{-1}$ and 40 mg·L$^{-1}$ CaCO$_3$ alkalinity. The main water types were either ambient tap water (not oxygenated) or oxygenated tap water with injection of purified oxygen (Mazzei Aerous-8, San Luis Obispo, CA) as water passed through a bypass manifold at 6–7 L·min$^{-1}$ into a contact tank to increase oxygen dissolution. Dissolved oxygen was measured with a multipoint factory-calibrated optical oxygen sensor (oxygen pressure) and temperature probe (Ocean Optics, Dunedin, FL).

**Experiment 1A. Propagation Plant Trial**

This experiment ran from 30 Mar. to 14 Apr. 2016, and aimed to measure the effect of two factors (water types and plant species) on rooting of vegetative plant cuttings. The experimental design was a split-plot design with water type as the main plot and plant species as the subplot. The water type (either ambient or oxygenated water) was randomly located in two irrigation zones on a bench and was replicated over four benches. Within an irrigation zone (a particular water type delivered onto a particular bench), there were two randomly positioned 51 count plug strip trays for each of the two species (*Calibrachoa* or *Lobelia*). To measure growth, root and shoot data were collected at two time periods (day 7 and 14). During the trial, ambient and oxygenated water flowed into unpressurized 18.9 L source tanks every 1.75 h for 5 min (sufficient to completely replace the stored solution). Water was then pumped at 310 kPa from the source tank to irrigation lines using a subpump to a computer-controlled solenoid valve that allowed mist to be applied to the bench through 69 µm diameter propagation nozzles (Coolnet Pro Fogger, Netafim, Israel). There were three propagation nozzles per irrigation zone, and the nozzles were spaced 61 cm between each nozzle and 61 cm above the bench.

*Calibrachoa ×hybrida* ‘Aloha Kona Dark Red’ and *Lobelia erinus* ‘Bella Aqua’ were supplied as unrooted cuttings by Dümmen Orange (Las Mercedes, El Salvador). The unrooted
cuttings were transplanted into 102 count plug trays with 25 mm diameter cells (20.3 mL volume per cell) filled with a 60 peat:40 perlite (v/v) substrate. A porosity test on three replicate trays each with 18 cells resulted in an average of 8% air porosity, 69% water porosity, 23% solid, bulk density of 114 g·L⁻¹, and water-holding capacity of 14 mL/cell. A surfactant (Capsil; Aquatrols, Paulsboro, NJ) was applied to plant foliage at 0.31 mL·L⁻¹ to transplanted plant cuttings to reduce surface tension for more even distribution of water on the leaf surface and thereby reduced water loss in the cutting. Irrigation frequency averaged at 26 min for 10 s during week 1 and decreased to 2 h for 10 s during week 2 from 7:00 am to 7:00 pm. At night, irrigation frequency averaged at 23 min for 10 s on days 1 to 3 and decreased to 1.3 h for 10 s on days 4 to 7, with no night irrigation during week 2.

The average DO and temperature spot-checked during daylight hours from the source tank for ambient water equaled 7.0 and 31.1 mg·L⁻¹ for oxygenated water at an average water temperature of 23 °C. Air temperature averaged to 21.4 °C, relative humidity was 72%, and daily light integral photosynthetically active radiation (PAR) was 9.9 mol·m⁻²·d⁻¹. Six plants per replicate tray were destructively sampled on day 7 and 14. Total root length per plant (representing the sum of primary adventitious roots emerging from the stem but not including secondary branched roots) was measured with a ruler. An average total root length per plant was calculated for each replicate tray. Root and shoot dry mass per plant were also averaged for each replicate tray. The bench and bench × water type interaction were considered as random effects, and water type, species, and time, and their interactions were considered as fixed effects and were analyzed using PROC GLIMMIX in SAS (SAS Version 9.4; SAS Institute, Cary, NC), with Tukey’s honestly significant difference at $P = 0.05$ for mean separation.
Experiment 1B. Persistence of Supersaturated DO in Water over Time

A factorial experiment was run to study the effect of water type and water movement on DO level over time. The water type was either ambient tap or oxygenated water held in an unpressurized 18.9 L container. Water movement was either controlled by a submersible pump at 378.5 L·h⁻¹ or water was not stirred. There were three replicate containers for each factorial combination of water type and water movement. Dissolved oxygen was measured at 4 cm depth from the surface over time (0, 30, 90, 150, 210, and 270 min). Data were analyzed by three-way analysis of variance (ANOVA) using PROC GLIMMIX, with Tukey’s honestly significant difference for mean separation in SAS.

Experiment 1C. Dissolved Oxygen Measured Under Propagation Trial Conditions

The change in DO was measured as water flowed through the irrigation system used in Expt. 1A. There were two factors a) water type, which included either ambient or oxygenated water, as described in Expt. 1A, and b) the irrigation location, which was a point within the irrigation line where the DO was measured including 1) the initial 18.9 L “source tank,” 2) “bench no nozzle,” where water was pumped from the source tank through 1.9 cm diameter tubing and then 1.3 cm diameter tubing into a collection container on the bench, and 3) a “bench with nozzle,” where water was pumped from the source tank through 1.9 cm tubing and then through the 69 µm propagation nozzles and into a collection container on the bench. The experiment was carried out in a completely randomized order of the factorial combinations of water type (main plot) and irrigation location (subplot), and the experiment (“block”) was repeated four times. Dissolved oxygen was measured immediately after the water collected on the bench. Data were analyzed by two-way ANOVA using PROC GLIMMIX with water type, irrigation location, and their interactions as fixed effects, and block × water type as a random effect, with Tukey’s honestly significant difference for mean separation in SAS.
Experiment 1D. Dissolved Oxygen Measured In Propagation Cells

The effect of two factors (two water types and five applied volumes of water) on DO was quantified in the root substrate described for Expt. 1A in propagation cells. The water type was either ambient or oxygenated water, as described in Expt. 1A, averaging 7.1 mg·L$^{-1}$ at 22.2 °C or 31.5 mg·L$^{-1}$ at 22.7 °C, respectively. The applied volume of water added to the substrate was based on percent container volume from 0% (0 mL/cell), 25% (5 mL), 50% (10 mL), 100% (20 mL), and 200% (40 mL). The highest applied volume of water was 40 mL per cell, which represented 285% of the water holding capacity (14 mL/cell). The experiment was a factorial design where each of the 10 combinations of water type and applied water volume were measured in random order, and the experiment (“block”) was carried out five times. Before applying a water type, the container substrate was subirrigated to saturation and allowed to drain for 15 min to reach CC. The water type was then poured onto the substrate surface. A toothpick was used to indent the substrate before inserting the oxygen sensor. The DO was measured immediately at 1 cm depth from the surface of the substrate with the oxygen sensor and temperature probe, allowing the oxygen sensor to stabilize (minimum of 40 s) before recording a measurement. Data were analyzed by two-way ANOVA using PROC GLIMMIX where water type, applied water volume, and their interactions were fixed effects, block × water type and block × applied water volume were random effects, and Tukey’s honestly significant difference was used for mean separation.

Experiment 2A. Container Plant Trial

This trial, conducted from 13 Apr. to 12 May 2016, aimed to measure the effect of water type and irrigation delivery methods on rooting, and plant growth was tested for each of three plant species. The water types (ambient and oxygenated water) were supplemented with nutrients (17.0N–1.7P–14.1K at 150 mg·L$^{-1}$ N). The irrigation delivery method was either a) top watering,
where 180 mL of nutrient solution was poured onto the container substrate surface or b) subirrigation, where 180 mL of nutrient solution was applied in a 12.7 cm diameter saucer holding the pot. Plant species included the same *Calibrachoa* and *Lobelia* cultivars from Expt. 1A., and *Pelargonium × hortorum* ‘Patriot Red’. The experiment was a randomized complete block design with a total of four blocks (benches) and two replicate pots per block for each combination of water type, irrigation delivery method, and plant species. Rooted cuttings of each species were transplanted into 10.2 cm diameter pots (425 mL/pot) filled with peat:perlite (60:40 v/v) substrate. Weight of six randomly selected pots was continuously logged using a weight scale and was manually checked several times each day. The plants were irrigated at 45% of CC measured gravimetrically, resulting in a total of 11 irrigation events during the trial. Irrigation at this moisture level is a typical research practice to provide water to bedding plants before wilting point, for example Johnson et al. (2013). Plant chlorophyll content was measured on the second fully expanded leaf with the average of three measurements per plant using a Minolta SPAD 502 m (Konica Minolta, Osaka, Japan). The plants were destructively sampled after four weeks, with eight replicate plants per water type, irrigation delivery, and species combination. The total root length was measured on a root scan using a root image analysis program (WinRHIZO Pro v. 2007a; Regent Instruments, Quebec, Canada), and dry mass of root and shoot were measured. Data were analyzed separately for each species by two-way ANOVA using PROC GLIMMIX with water type, irrigation delivery, and their interactions analyzed as fixed effects.

A porosity test for six replicate substrate-filled pots resulted in an average 19% air porosity, 57% water porosity, 24% solid, bulk density of 110 g·L⁻¹, and water holding capacity of 243 mL. The average DO for the ambient water source was 6.0 mg·L⁻¹ at 26.3 °C, pH was 6.8, and EC was 1.4 μS·cm⁻¹. The average DO for the oxygenated water source was 27.7 mg·L⁻¹.
at 26.5 °C, the pH was 7.0, and EC was 1.4 μS·cm⁻¹. Daily averages of data collected each minute for air temperature was 22.7 °C, relative humidity averaged 78%, and daily light integral of PAR was 17.1 mol·m⁻²·d⁻¹.

**Experiment 2B. Subirrigation with Pelargonium at Medium and High Moisture Level**

To test whether oxygenated water would affect plant growth at high moisture level, two factors (two water types and two moisture levels) were varied to assess the effect on root and plant growth of *Pelargonium*. This trial was carried out simultaneously with Expt. 2A, and the water types were either ambient or oxygenated water described in Expt. 2A. The two moisture levels were managed by subirrigating when the average weight of six pots weighed the equivalent of 45% of CC measured gravimetrically to provide a medium moisture level or at 80% CC for the high moisture level. The experiment was a completely randomized design with eight replicate pots per treatment combination of water type and moisture level. The plants were subirrigated with 180 mL of nutrient solution per irrigation event as described in Expt. 2A. There were 11 or 18 irrigation events for the medium or high moisture levels, respectively. The same plant data were collected as in Expt. 2A and were analyzed by two-way ANOVA using PROC GLIMMIX where water type, moisture level, and their interactions analyzed as fixed effects.

**Experiment 2C. Dissolved Oxygen in Substrate with Top Watering**

To assess the persistence of DO in substrate without plants, a three-factor (two water types, five volumes of applied water, and two measured depths) experiment was carried out. The water types were either ambient or oxygenated tap water. The average DO for ambient water and oxygenated water sources was 6.8 mg·L⁻¹ at 22.5 °C and 31.0 mg·L⁻¹ at 22.9 °C, respectively. The volume of applied water poured onto the substrate surface was 0% (0 mL), 25% (106 mL), 50% (212 mL), 100% (425 mL), or 200% (850 mL) of container volume. The highest volume of 850 mL represented 350% of CC. The experiment was a factorial experiment where all treatment
combinations of water type (main plot), applied volume of water (subplot), and DO were measured at two depths in each pot (sub-sub-plot) in a randomized order, and the experiment was repeated five times. The container substrate was subirrigated and drained for 15 min to bring to CC before applying the water treatment. The DO was immediately measured in the substrate at 2 and 4 cm depth from the surface of the substrate with an oxygen sensor and temperature probe using the procedure in Expt. 1D. Data were analyzed by three-way ANOVA using PROC GLIMMIX with water type, volume, depth, and their interactions as fixed effects and block × water type × volume as a random effect, and Tukey’s honestly significant difference was used for mean separation.

**Experiment 2D. Dissolved Oxygen in Substrate with Subirrigation over Time**

To assess the irrigation delivery of subirrigation on the substrate DO, a three-factor (two water types, three measured depths, and four time periods) experiment was carried out. The water types were either ambient or oxygenated tap water. The average DO measured for ambient water and oxygenated water sources was 6.1 mg·L⁻¹ at 26.8 °C and 25.1 mg·L⁻¹ at 26.5 °C, respectively. The measured depths were 2, 4, or 6 cm below the substrate surface and time periods were 0, 30, 60, or 120 min (after an initial 15 min subirrigation). All combinations of water type (main plot) and three DO measurement depths (subplot) in each container were measured in a random order at each of the four time periods. Each container was only measured at one time period (destructive sampling). There were five experimental runs (“blocks”). DO was measured in a slightly different manner from other experiments to avoid damaging the oxygen sensor, by surrounding the probe with a plastic cylinder that was open at the measurement point. Data were analyzed by ANOVA using PROC GLIMMIX with water type, time, depth, and their interactions analyzed as fixed effects and block × water type × time as a random effect, with Tukey’s honestly significant difference for mean separation.
Results

Experiment 1A. Propagation Plant Trial

Oxygenation of irrigation water did not affect root length, shoot dry mass, root dry mass, or total dry mass compared with ambient water. There were no significant differences within species for water type and interaction of water type by species. All plants were observed to have roots by day 7, with total root length averaging 8.8 cm/plant for Calibrachoa and 7.3 cm/plant for Lobelia. Calibrachoa had greater total dry mass of 0.052 g/plant at day 7 compared with Lobelia with 0.019 g/plant ($P = 0.0001$). By day 14, total root length had increased to 125 cm for Calibrachoa with a total dry mass of 0.092 g/plant and a shoot to root ratio of 4.3. Lobelia had a total root length of 75 cm on day 14, a total dry mass of 0.038 g/plant, and a shoot to root ratio of 2.3. Total dry mass of Calibrachoa was greater by 142.1% than Lobelia, resulting in a species difference ($P = 0.0001$). Plants appeared vigorous and healthy in all treatments.

Expt. 1B. Persistence of supersaturated DO in water over time.

Experiment 1B. Persistence of Supersaturated DO in Water over Time

There were significant main effects for water type, water movement, time, and interaction effects of water type × water movement and water type × time ($P < 0.01$). For ambient tap water, the DO level was unaffected by time or water movement and averaged to 7.1 ± 0.05 mg·L$^{-1}$ (mean ± standard error). For oxygenated water that was not stirred, DO was initially 28.3 mg·L$^{-1}$, and the measured level of 26.5 mg·L$^{-1}$ after 270 min was not significantly lower than the initial DO. For oxygenated water that was stirred, DO decreased from an initial 26.8–16.9 mg·L$^{-1}$ (a 37% decrease) after 270 min. Oxygenated water that was stirred had a greater decrease in DO over time than oxygenated water that was not stirred. However, both stirred and nonstirred treatments remained supersaturated at 208% and 324% oxygen saturation, respectively, after 270 min at 24 °C.
Experiment 1C. Dissolved Oxygen Measured Under Propagation Trial Conditions

The DO measured in irrigation water at different points in the propagation system (Fig. 2-1) had a significant interaction between water type and irrigation point ($P = 0.0001$). Oxygenated water decreased slightly from the source tank (26.0 mg·L$^{-1}$) to the “bench no nozzle” (24.6 mg·L$^{-1}$), and greatly decreased to 8.7 mg·L$^{-1}$ at the “bench with nozzle.” Ambient water did not differ in DO between the source tank (6.3 mg·L$^{-1}$) and “bench no nozzle” (7.0 mg·L$^{-1}$) but increased to 8.7 mg·L$^{-1}$ at the “bench with nozzle.” Dissolved oxygen was ≈100% of saturation at the “bench with nozzle” measurement point, regardless of the water type, which is the likely reason that water oxygenation did not affect plant growth in Expt. 1A. The mist nozzle (with a 69 µm diameter orifice reported by the manufacturer) produced fine water droplets, thereby increasing the surface area exposed to air. As a result, we hypothesize that increased DO levels in ambient water and off-gassing of oxygen in oxygenated water occurred. In an unpublished study, we measured DO at a commercial greenhouse in North Carolina and similarly found that although well water at the source tank was 74% of saturation, after water passed through a 200 µm mist nozzle (XR 11002; TeeJet, Wheaton, IL), the DO increased to ≈100% of saturation. Irrigation nozzles have previously been reported to increase DO concentration by increasing the droplet surface area exposure and movement in air (Schröder, 1994; Schröder and Lieth, 2002; Vestergaard, 1984; Wever et al., 2001).

Experiment 1D. Dissolved Oxygen Measured In Propagation Cells

In plug cells, the addition of oxygenated water increased the measured substrate DO (Fig. 2-2) from 8.5 to 12.3 mg·L$^{-1}$ (a 46% increase in oxygen) as the applied volume of water increased from 0% to 200% of container volume. There was a positive relationship between increasing applied volume of oxygenated water and the substrate DO in plug cells. The addition
of ambient water did not change the substrate DO, which averaged 7.5 mg·L\(^{-1}\) across the applied volumes of water.

**Experiment 2A. Container Plant Trial**

Overall, oxygenated water did not increase the root or plant growth in any of the three bedding plants (Table 2-1). There was an increase measured in shoot dry mass of 8.3% \((P = 0.032)\) and 17% root dry mass \((P = 0.012)\) for *Lobelia* with ambient water compared with oxygenated water. Top-watered plants had greater root growth by 20.4% increase in total root length \((P = 0.047)\) and 21.8% increase for root dry mass \((P = 0.035)\) compared with subirrigation delivery method for *Calibrachoa*.

**Experiment 2B. Subirrigation with Pelargonium at Medium and High Moisture Level**

There were no effects of water type on plant growth (Table 2-2). Similarly, there was no interaction between water type and substrate moisture level on plant growth. A 26.6% increase in root length was observed in plants grown at high substrate moisture (80% CC) compared with plants grown at medium moisture (45% CC) level \((P = 0.005)\). There were no differences measured in leaf chlorophyll (SPAD) within plant species in Expt. 2A and 2B (data not shown) and all plants appeared healthy.

**Experiment 2C. Dissolved Oxygen in Substrate with Top Watering**

The addition of oxygenated water increased the substrate DO measured at 2 and 4 cm depths (Fig. 2-3) from 8.6 to 14.5 mg·L\(^{-1}\) (68% increase) and 6.5 to 11.6 mg·L\(^{-1}\) (78% increase), respectively, as the applied volume of water increased from 0% to 200% of container volume \((P = 0.0001)\). Similar results were observed for the substrate DO measured in plug cells (Expt. 1D.). When a large volume of oxygenated water was applied with top watering, sufficient substrate solution was displaced, resulting in a measurable increase in the substrate DO. The application of ambient water did not increase the substrate DO, which averaged 8.5 and 7.2
mg·L$^{-1}$ for 2 and 4 cm depths, respectively. The overall substrate DO for both water types decreased by 1.8 mg·L$^{-1}$ from the 4 cm depth compared with the 2 cm depth ($P = 0.0001$). Decreased oxygen concentration has been measured at increasing depths in container substrate because there is likely to be decreased diffusion of gas from the substrate upper surface, and lower depths contain a greater proportion of water-filled pores (Argo et al., 1996; Dresbøll and Thorup-Kristensen, 2010; van Iersel and Dove, 2014; Wever et al., 2001).

**Experiment 2D. Dissolved Oxygen in Substrate with Subirrigation over Time**

Following subirrigation, the substrate DO ranged from 7.3 to 7.7 mg·L$^{-1}$ and averaged 87.4% of saturation regardless of the water type (oxygenated or ambient), time after irrigation (0, 30, 60, and 120 min), or measurement depth (2, 4, or 6 cm). The substrate DO was similar between water types measured after subirrigation because there was less displacement of the substrate solution compared with increased substrate DO when top watered in Expt. 2C.

**Discussion**

Some published studies have found limited responses to oxygenation of irrigation water. This may have occurred when root zone conditions with ambient irrigation water were not sufficiently hypoxic to affect root respiration, or because an elevated DO was not maintained throughout the irrigation system and substrate. Watermelon grown in 40 L perlite (<5 mm diameter) bags were irrigated with oxygenated or ambient nutrient solution (Bonachela et al., 2005) and showed no differences in plant biomass, fruit yield, or fruit quality. In that study, although the DO measured at the dripper averaged 13.5 or 5.9 mg·L$^{-1}$ for oxygenated or ambient water, respectively, DO in the substrate solution was similar (4.5 or 3.7 mg·L$^{-1}$ for the two water sources). Bonachela et al. (2010) grew greenhouse tomatoes in rockwool with 40% air-filled porosity and irrigated with either oxygenated or ambient treated wastewater with average DO of 14.6 or 4.5 mg·L$^{-1}$, respectively. Dissolved oxygen measured in the center of the rockwool slab
was similar for both irrigation treatments (5.1 or 4.8 mg·L\(^{-1}\), respectively, for oxygenated or ambient irrigation water), and similar plant biomass and yield was observed with both water types. The similar DO levels in the substrate solution and lack of plant response in both Bonachela studies may have resulted from using substrates with high air porosity. Ehret et al. (2010) conducted a series of experiments with pepper and cucumber, in cedar sawdust, perlite, and pumice and with DO of the nutrient solution ranging from 2 to 31.6 mg·L\(^{-1}\). Pepper fruit yield was unaffected by water oxygenation and cucumber fruit yield was increased by oxygenated water in one of three trials.

However, in some other published studies, oxygenation of nutrient solution has resulted in improved yield compared with plants grown in hypoxic to anoxic conditions, in addition to reduced root necrosis (Marfà et al., 2005), increased leaf area and root mass (Holtman et al., 2005), increased macronutrient uptake (Marfà et al., 2005), and increased shelf life in cucumber (Ehret et al., 2010) and pepper (Ehret et al., 2010; Marfà et al., 2005). For example, Lei et al. (2016) found that corn yield increased by oxygenation of irrigation water for plants grown in a completely water-logged vermiculite substrate under warm water temperature (averaging 27.7 \(^{\circ}\)C), where DO in the ambient irrigation water at times decreased to 1 mg·L\(^{-1}\) in the substrate. These completely saturated conditions differed from plant growth in peat–perlite substrate with moisture levels at or below CC in our studies.

Although, there were no effects on plant growth or rooting observed in the propagation trial (Expt. 1), we found that water held in unpressurized containers maintained supersaturated DO levels after 4.5 h for oxygenated water, although DO level dropped by 37% with constant stirring. Water that passed through fine mist nozzles increased the droplet surface area in contact with air, leading to an increase in DO for ambient tap water and off-gassing of oxygen in
oxygenated water. As a result, misting brought both water types to the DO saturation point. The substrate DO increased (Expt. 1C) when a high volume of oxygenated water was applied to plug cells, whereas ambient tap water did not increase the substrate DO.

Plant stress symptoms for hypoxia were not observed in the container plant trial (Expt. 2A) or when *Pelargonium* was grown at a high moisture level (Expt. 2B), which is the likely reason that irrigating with oxygenated nutrient supplemented water did not benefit plant growth. Oxygen supply to roots can be affected by substrate porosity and irrigation management. Root substrates and growing containers are designed to have adequate air porosity for root respiration, even at CC (Argo et al., 1996; DeBoodt and Verdonck, 1971; Gislerød, 1982; Handreck and Black, 2002), and oxygen has far greater diffusion rate in air than DO in water (Colmer, 2003; Wegner, 2010). The average air porosity was 19% (Expt. 2A and 2B) for pots at CC and the lowest substrate DO was measured at 6.5 mg·L\(^{-1}\) (Expt. 2C and 2D).

**Summary**

Injecting oxygen into irrigation water increased DO to ≈300% of the equilibrium saturation level of oxygen solubility in water in our study. Water in unpressurized containers held relatively stable DO levels over a 4.5 h period of time at room temperature of 24 °C for both oxygenated and tap water. However, passing irrigation water through fine propagation nozzles increased the droplet surface area, allowing for greater diffusion of oxygen. Dissolved oxygen therefore increased in ambient tap water and off-gassed in oxygenated water to bring both water types to saturation, and no difference in plant growth was observed in the propagation trial.

Oxygenating irrigation water did not benefit plant growth during subsequent growth in containers because the plants were not grown in completely water-logged and hypoxic conditions but rather had adequate air porosity in the container substrate. We conclude that the best method to provide adequate oxygen to roots is through a substrate with air-filled porosity and avoidance
of overwatering. Adding oxygenated water to an already saturated container substrate is not a recommended approach to water or oxygen management.
Table 2-1. Results of the container trial showing the effect of water type (ambient water or oxygenated water) and water delivery method (top watered or subirrigation) on root length and plant dry mass (Expt. 2A). Data were analyzed separately for each of the three species. The least square means are from eight replicates. Analysis of variance (ANOVA) summary for treatment effects at a significant difference of $\alpha = 0.05$.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Water Type</th>
<th>Water Delivery</th>
<th>Root Length (cm)</th>
<th>Shoot Dry Mass (g)</th>
<th>Root Dry Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrachoa</td>
<td>Ambient</td>
<td>Top watered</td>
<td>2277</td>
<td>2.98</td>
<td>0.22</td>
</tr>
<tr>
<td>Calibrachoa</td>
<td>Oxygenated</td>
<td>Top watered</td>
<td>2320</td>
<td>3.08</td>
<td>0.21</td>
</tr>
<tr>
<td>Calibrachoa</td>
<td>Ambient</td>
<td>Subirrigated</td>
<td>1896</td>
<td>2.47</td>
<td>0.16</td>
</tr>
<tr>
<td>Calibrachoa</td>
<td>Oxygenated</td>
<td>Subirrigated</td>
<td>1923</td>
<td>2.84</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Summary of ANOVA analysis for Calibrachoa
- Water Type: NS NS NS
- Water Delivery: * NS *
- Water Type*Water Delivery: NS NS NS

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Water Type</th>
<th>Water Delivery</th>
<th>Root Length (cm)</th>
<th>Shoot Dry Mass (g)</th>
<th>Root Dry Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lobelia</td>
<td>Ambient</td>
<td>Top watered</td>
<td>2917</td>
<td>2.14</td>
<td>0.30</td>
</tr>
<tr>
<td>Lobelia</td>
<td>Oxygenated</td>
<td>Top watered</td>
<td>2685</td>
<td>1.90</td>
<td>0.26</td>
</tr>
<tr>
<td>Lobelia</td>
<td>Ambient</td>
<td>Subirrigated</td>
<td>2748</td>
<td>1.99</td>
<td>0.28</td>
</tr>
<tr>
<td>Lobelia</td>
<td>Oxygenated</td>
<td>Subirrigated</td>
<td>2361</td>
<td>1.91</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Summary of ANOVA analysis for Lobelia
- Water Type: NS * *
- Water Delivery: NS NS NS
- Water Type*Water Delivery: NS NS NS

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Water Type</th>
<th>Water Delivery</th>
<th>Root Length (cm)</th>
<th>Shoot Dry Mass (g)</th>
<th>Root Dry Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelargonium</td>
<td>Ambient</td>
<td>Top watered</td>
<td>1424</td>
<td>5.56</td>
<td>0.33</td>
</tr>
<tr>
<td>Pelargonium</td>
<td>Oxygenated</td>
<td>Top watered</td>
<td>1544</td>
<td>5.87</td>
<td>0.34</td>
</tr>
<tr>
<td>Pelargonium</td>
<td>Ambient</td>
<td>Subirrigated</td>
<td>1431</td>
<td>5.63</td>
<td>0.35</td>
</tr>
<tr>
<td>Pelargonium</td>
<td>Oxygenated</td>
<td>Subirrigated</td>
<td>1408</td>
<td>5.54</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Summary of ANOVA analysis for Pelargonium
- Water Type: NS NS NS
- Water Delivery: NS NS NS
- Water Type*Water Delivery: NS NS NS

NS = no significance, * = significant at p=0.05 level
Table 2-2. Results of subirrigation with *Pelargonium* showing the effect of water type (ambient water or oxygenated water) and moisture level ("medium" plants were irrigated when dried to 45% CC or "high" plants were irrigated at 80% CC) on root length and plant dry mass (Expt. 2B). The least square means of eight replicates. Analysis of variance (ANOVA) summary for treatment effects at a significant difference of $\alpha = 0.05$.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Water Type</th>
<th>Moisture Level</th>
<th>Root Length (cm)</th>
<th>Shoot Dry Mass (g)</th>
<th>Root Dry Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pelargonium</em></td>
<td>Ambient</td>
<td>Medium</td>
<td>1273</td>
<td>4.97</td>
<td>0.41</td>
</tr>
<tr>
<td><em>Pelargonium</em></td>
<td>Oxygenated</td>
<td>Medium</td>
<td>1236</td>
<td>4.91</td>
<td>0.42</td>
</tr>
<tr>
<td><em>Pelargonium</em></td>
<td>Ambient</td>
<td>High</td>
<td>1608</td>
<td>6.03</td>
<td>0.50</td>
</tr>
<tr>
<td><em>Pelargonium</em></td>
<td>Oxygenated</td>
<td>High</td>
<td>1566</td>
<td>5.12</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Summary of ANOVA analysis

<table>
<thead>
<tr>
<th></th>
<th>Water Type</th>
<th>Moisture Level</th>
<th>Water Type*Moisture Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>**</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = no significance, * = significant at p=0.05 level
Figure 2-1. The effect of water type (ambient water or oxygenated water) and irrigation points (source tank, a bench with no propagation nozzle, or a bench with a mist propagation nozzle) on dissolved oxygen concentration in Expt. 1C. Data are least square means of four replicates. Mean separation letters using Tukey’s honestly significant difference at $\alpha = 0.05$ and error bars indicate $\pm 95\%$ confidence intervals.
Figure 2-2. The effect of water type (ambient water or oxygenated water) and volume of water added (0%, 25%, 50%, 100%, or 200% of container volume) on substrate oxygen measured at 1 cm depth in plug cells (25 mm diameter) in Expt. 1D. Data are least square means of five replicates. Mean separation letters using Tukey’s honestly significant difference at $\alpha = 0.05$ and error bars indicate $\pm 95\%$ confidence intervals.
Figure 2-3. The effect of water type (ambient water or oxygenated water) and volume of water added (0%, 25%, 50%, 100%, or 200% of container volume) on substrate oxygen compared at 2 or 4 cm measured depth in pots (10.2 cm diameter) in Expt. 2C. Data are least square means of five replicates. Significant difference was at $\alpha = 0.05$ and error bars indicate $\pm 95\%$ confidence intervals.
CHAPTER 3
WATER AND AIR RELATIONS IN PROPAGATION SUBSTRATES

During propagation of plant cuttings, high humidity and frequent mist irrigation are provided to hydrate unrooted cuttings, encourage callus development, and stimulate adventitious root formation (Santos et al., 2011). However, over-watering can potentially delay rooting and increase disease risk (Chérif et al., 1997; Heiskanen, 1995; Leakey, 2004). An appropriate combination of substrate selection and irrigation practices is therefore needed to balance adequate supply of water for plant hydration and oxygen supply for root respiration, both of which are requirements for rapid root growth and plant health (Bilderback and Lorscheider, 1995; Reisch, 1967).

The combination of small container size and fine substrate particles in propagation increases risk of excess moisture content in the substrate. A wide range of substrates and amendments is used during young plant production including peat (Sphagnum), coco coir, wood fiber, vermiculite, perlite, phenolic foam, and rockwool (Fonteno and Nelson, 1990; Handreck and Black, 2002; Milks et al., 1989a). Fine particle sizes of these components are often required in order to evenly fill small container cells, resulting in decreased pore size and higher water retention (da Silva et al., 1993b). Container size modifies the volume of water and air within the substrate such that, as column height decreases there is an increased proportion of water and a reduction in air that is proportional to total porosity (Argo et al., 1996; Handreck and Black, 2002; Milks et al., 1989b; Milks et al., 1989c; Rivière and Caron, 2001). Physical properties of propagation substrates are further modified by the use of “stabilized” substrates, which include phenolic foam, peat-polymer blends, fabric-wrapped cells, and other materials that hold the substrate together negating the need for a complete root ball and allowing for a shorter crop cycle and a reduction in transplanting stress (Huang and Fisher, 2014).
For gravimetric analysis of physical properties, substrates at a standardized volume and level of compaction are weighed at full saturation, after drainage, and after drying in order to quantify volumetric water (VWC), volumetric air (VAC), volumetric solid content (VSC), and dry bulk density (Fonteno et al., 1993). The Fonteno et al. (1993) method with a 348 cm$^3$ standard volume can be modified for propagation plug cells with a shorter column height (Milks et al., 1989c), by measuring directly in propagation trays, or with individual stabilized cells (Huang and Fisher, 2014). The ratio of water to air at container capacity (which is the field capacity within a particular container type) describes the substrate water-air relations, with typical levels of 45% to 65% water and 10% to 30% air space with a variety of substrates (Bilderback et al., 2005) using the North Carolina method (Milks et al., 1989a) in 348 cm$^2$ containers (7.3 cm diameter x 7.6 cm height). However, water and air balance is highly dependent on container size, with air-filled porosity decreasing from 19% to less than 1% as substrate height decreased from a 15 cm-tall pot to a 1.3 cm-tall seedling plug tray with a peat-vermiculite substrate (Caron and Nkongolo, 1999). A survey of commercial propagation substrates found widely differing physical properties, with loose-filled products having VWC from 57% to 86% and VAC ranging from 4.8% to 9.7% in 25 cm$^3$ cells. Whereas, stabilized substrate contained VWC from 37% to 91% and VAC between 1.9% to 5.9% in 10 to 28 cm$^3$ cells (Huang and Fisher, 2014). A limitation of gravimetric analysis at one moisture level is that it ignores the dynamic change in air and water as moisture level changes during crop production (Caron and Nkongolo, 1999).

Matric potential of a substrate, and the relationship between VWC and plant available water, are usually measured as substrate dries from saturation over time by measuring the tension of water using a ceramic-tip tensiometer (Wallach, 2008). Moisture retention curves (MRCs)
describe water availability for uptake by plant roots. Water held in substrate below 50 cm of tension has been defined as easily plant-available water, 50 to 100 cm describes water buffering capacity that is available to plants during periods of rapid transpiration, whereas tension above 100 cm is not available for plant root uptake (DeBoodt and Verdonck, 1972; Naasz et al., 2005). In horticultural production in containers, the tension at which wilting occurs depends on the plant species and growing conditions (DeBoodt and Verdonck, 1972). For example, Kiehl et al. (1992) found that potted chrysanthemum grown under moist conditions wilted above 10 kPa (102 cm), and recommended automatic irrigation triggered at a tension of 5 kPa (51 cm).

Because plant cuttings initially have limited or no roots, the moisture level is typically maintained close to container capacity during callus formation (Healy, 2008) and gradually changes to wet-dry cycles following the emergence of adventitious roots in order to provide aeration. In propagation, many cells are also shorter than 5 cm (Milks et al., 1989c; Huang and Fisher, 2014), and differences in moisture and air level at low tensions are therefore of great importance.

An alternative method to generate a MRC is the use of the frozen column substrate method, whereby the substrate is brought to field capacity, frozen, and then sectioned in order to quantify VWC and VAC within each vertical section (Altland et al., 2010; Owen and Altland, 2008). The VWC using the frozen column method allows a comparison between the gravitational tension from the column height to the moisture tension measured using a tensiometer. In this study, Altland et al. (2010) found similar, but statistically different, MRCs for bark-based substrates tested with either the tensiometer or frozen column methods, with the tensiometer method estimating a higher water content at saturation, slightly lower moisture levels at tensions
below 10 cm, and similar moisture levels at higher tensions compared with the frozen column method.

Through a more recently developed method, x-ray computed tomography (CT) the substrate microenvironment of water and air relations can be quantified and visualized (Daly et al., 2015; Nimmo, 2004) in addition to the soil effects on root morphology (Tracy et al., 2013; Tracy et al., 2015a). Within soils, quantification by CT of total porosity, tortuosity, VWC, VAC, and hydraulic conductivity has been previously reported. Daly et al. (2015) CT-scanned clay and sand substrates at different moisture levels and found that estimating VWC and VAC by CT provided an alternative method to using a ceramic plate and gravimetric measurement, and allowed for visualization of water and air distributed in a column.

Because of the small container size, specialized materials, and the high moisture conditions in propagation, standard testing protocols need to be modified for quality control testing in propagation substrates (Huang and Fisher, 2014). The objective was to quantify and compare substrate water and air relations of three propagation substrates (peat, rockwool, and phenolic foam) that varied widely in physical characteristics using four methods: (1) moisture retention curves by tensiometer, (2) frozen column methods, (3) gravimetric analysis, and (4) CT analysis. The goal was to identify strengths and weaknesses of each method for quality control testing for propagation substrates and to inform substrate selection and irrigation management for plant propagation.

**Materials and Methods**

Experiments were conducted in laboratories at the University of Florida (UF) in Gainesville, FL. Substrates consisted of peat, rockwool, and phenol-formaldehyde foam. The *Sphagnum* peat moss (“peat”) was a 100% sod peat sourced from Lithuania (Von Post scale 2-3, Puustjarvi and Robertson 1975) with a pH of 5.7 and an electric conductivity of 1.6 mS·cm⁻¹.
The particle size distribution of the peat was tested with three 1L replicates, resulting in (by volume) 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) based on the methodology and description of particle size categories from Huang et al. (2012a) and Huang and Fisher (2014). Peat characteristics were described by particle density of 1.3 g·cm³ and dry bulk density of 87.5 g·L⁻¹. The organic matter was 90.8%, with 52.7% carbon and 1.1% nitrogen, and a C/N ratio of 46.6 (QAL, Panama City, FL). Rockwool plugs (Grogan, the Netherlands) were comprised of basalt and limestone heated to 1600°C, forming threads of 5 µm and the average pore size was 4.5 to 5 µm (da Silva et al., 1995). The particle density of rockwool was 2.8 g·cm³ and dry bulk density was 78.9 g·L⁻¹. Phenol-formaldehyde foam [Oasis™, Kent, OH (“foam”)] root cubes were comprised of a foam matrix with monodispersed pore distribution (Milks et al., 1989b). In foam, the particle density was 2.8 g·cm³ and dry bulk density was 20.3 g·L⁻¹.

**Method 1. Moisture Retention Curves Using Tensiometer**

Tensiometers (Hyprop, UMS, Munich, Germany) were used for each substrate with the evaporative method described by Fields et al. (2016) in order to plot moisture retention curves. Samples were subirrigated to saturation for 24 hours in a basin. Cores of 250 mL volume (8 cm diameter and 5 cm height) drained for 15 min and weighed to determine the initial water holding capacity. With rockwool samples, two holes were bored at two depths (3.8 cm and 1.3 cm from the base) using an auger positioning tool. The tensiometer base, equipped with two tensiometers (1.3 cm and 3.8 cm tall), was fixed to each beveled core with substrate, fitting tensiometers precisely into the bored holes. For peat and foam samples, the tensiometers were pushed through to create their own holes. Water potential from the two tensiometers and weight of the assembly were recorded every 10 min. (Tensioview software, Munich, Germany). Measurements
continued until water in the upper tensiometer cavitated after approximately 10 days (ranging between experimental runs between 295.8 to 440.6 cm for peat, 39.5 to 45.2 cm for rockwool, and 25.0 to 27.2 cm for foam). Substrates were removed from the core, dried in a forced-air oven at 105°C for 48 h, and weighed.

Data were analyzed using a modified van Genuchten (1980) four-parameter log-logistic model (Eq. [3-1]) where volumetric water content ($\theta$) is a function of pressure ($h$, in cm). Parameters were fitted with non-linear regression (PROC NLIN in SAS Version 9.4, SAS Institute, Cary, NC).

$$\theta = \theta_r + (\theta_s - \theta_r)/[(1+(h/X_0)^n)] \quad [1]$$ (3-1)

The parameter $\theta_s$, represented the VWC at saturation (zero tension), $\theta_r$ was the residual VWC (lower asymptote), $X_0$ was the inflection point in the sigmoid curve, and $n$ was a rate parameter (Altland et al., 2010). The MRCs generated using the tensiometer method were compared between substrates using a paired-sample t-test of the non-linear fitted equation [3-1], with a separate t-test for each possible paired comparison of substrates. In addition, the estimates and 95% confidence intervals for the four parameters from non-linear regression were tabulated and compared between substrates.

**Method 2. Moisture Retention Curve By Frozen Column Method**

The frozen column method was used to generate moisture retention curves of the three substrates (peat, rockwool, and foam) at container capacity, with four replicates per substrate (Altland et al., 2010; Owen and Altland, 2008). Peat was filled into 3.8 cm diameter clear plastic tubes of 30.5 cm length and dropped at a height of 6 cm three times to provide consistent compaction. Rockwool and foam plugs were 3.4 cm diameter and 34 cm in height. The
substrates were subirrigated to a water level of 25 cm to provide complete saturation for 4 h.
followed by drainage for 30 min. Substrate samples were stored at -6.6°C for two days, and the
frozen substrate was then sectioned at 1 cm heights using a horizontal band saw. For each
section, the width and height were measured with a digital caliper. Gravimetric measurements
were recorded when sectioned samples defrosted and dried. Volumetric water and air content
were calculated for each 1 cm section.

Volumetric water content as a function of column height data was fitted to the non-linear
model Eq. [3-1], obtained the fit parameters $\theta_s$, $\theta_r$, n, and $X_0$. Differences between substrates for
the frozen column method were compared using the same analytical approaches described for
tensiometer (t-test and comparing parameter estimates). In addition, within each substrate there
was a comparison between tensiometers and the frozen column methods using these statistical
approaches.

**Method 3. Gravimetric Whole-Cell Analysis**

Substrates were saturated by subirrigating overnight followed by draining for 30 min to
achieve container capacity. Gravimetric measurements were recorded at saturation, container
capacity, and after drying to calculate VWC and VAC, VSC, using the methods described by
Huang et al. (2012b) and Huang and Fisher (2014), with direct measurements of porosity within
propagation trays. There were three replicate cells for each substrate at container capacity. The
55 mL cells were filled with peat, whereas rockwool plugs were 40.5 mL, and the foam root
cubes were 30.5 mL.

**Method 4. X-ray Tomography (CT) Whole-Cell Analysis**

Each substrate was brought to container capacity, as described for the gravimetric whole-
cell analysis (Method 3), and were scanned using CT with a 240-kV x-ray tube with a tungsten
target (GE v\text{tome}\text{x M 240, Boston, USA}) at the University of Florida, Research Service Centers
(1041 Center Drive, P.O. Box 116621, Gainesville, FL 32611). An additional three replicate cells of peat were dried to 51% VWC and were also scanned. The scanned settings were as follows: voltage of 80 kV, current of 175 µA, a 200 ms detector time, averaging three images with a skip of one image per rotation with total of 1000 images, and a voxel resolution of 59.5 µm. Raw two dimensional projections were processed (datos|x software v. 2.3, GE Sensing Inspection Technologies GmbH, Germany) prior to importing into VG StudioMax 3.0 (Volume Graphics, Heidelberg, Germany) to perform segmentation and three-dimensional (3D) visualization.

A combination of image segmentation from CT and results from gravimetric analysis of the solid component was used to quantify VWC and VAC for substrates in propagation cells. First, a subsection of the 3D representation of a volume cell was defined by removing, for example, small sections that included edge areas outside the solid propagation cell. This subsection was then segmented by creating regions of interest by intensity (density) threshold or using the region-growing tool within VG StudioMax 3.0. This allowed the volume to be differentiated into two densities: (a) the air volume and (b) the volume of the combined matrix of water plus solid substrate. Second, gravimetric analysis of volumetric solid content (VSC) from gravimetric whole-cell analysis was used as a constant to subtract the solid portion from (b) water. This approach was necessary because image segmentation of water was not possible in peat because water enters internal pores of peat resulting in a similar density between water and the combined water/peat matrix. Percent volumetric air content (VAC) was calculated by dividing the segmented volume of air by the segmented total volume. Percent volumetric water content was calculated by subtracting VAC and VSC from 1.
Data for VWC and VAC from the gravimetric and CT whole-cell analysis were compared using a two-way ANOVA by PROC GLIMMIX with fixed effects of the three substrates and two methods (gravimetric or CT) at $\alpha=0.05$ in SAS. A representative scan was reported for each substrate to visualize the quantified water and air content.

Spatial distribution of water and air content within each cell was quantified by sectioning the vertical profile by 0.5 cm increments using the 3D-polyline tool in parallel view (VG Studio Max 3.0). There were three replicates per substrate at container capacity, plus peat at 51% VWC. Data for the three substrates at container capacity were analyzed statistically by a two-way ANOVA, with fixed effects of substrate and cell depth and their interaction.

**Results and Discussion**

**Method 1. Moisture Retention Curves Using Tensiometer**

At zero tension (saturation), peat held a similar amount of water to the other two substrates (as shown by comparisons of Fig. 3-1a, 3-1c, and 3-1e, and estimates of $\theta_s$ in Table 3-1), however peat had a much higher matric potential meaning more water was retained as moisture tension increased (represented by the rate parameter $n$ and residual VWC parameter $\theta_r$ in Table 3-1). The MRC resulted in acceptable $R^2$ values to the model fit for all substrates (Table 3-1). Peat at saturation was estimated at 86% VWC ($\theta_s$ in Table 3-1). As substrate dried, the inflection point was near 58% VWC or tension of 19 cm and decreased to residual VWC at 23% ($\theta_r$ in Table 3-1). At tensions above 200 cm (beyond the data displayed in Fig. 3-1a), measured VWC in peat was within 5% of the estimated $\theta_r$. As substrate dried, plant easily available water (below 50 cm) for peat ranged from 86% to 34% VWC, and plant available water (50 to 100 cm) ranged from 34% to 27% VWC. Water below 27% VWC was beyond 100 cm of tension and unavailable to plants, based on the model. Within peat substrates, water at high tension is largely bound within internal pores in peat fibers or granules whereas freely available water is in larger
pores between peat particles (Rivière and Caron, 2001). Peat has high water buffering capacity that facilitates plant available water over a range of moisture tensions and resist water-loss by environmental conditions (Naasz et al., 2005; Rivière and Caron, 2001). Peat is the most widely-used component in container propagation substrates in the U.S. (based on the survey of propagation substrates by Huang and Fisher, 2014), and while high water buffering capacity reduces risk of dehydration of cuttings the substrate can easily become waterlogged unless mist irrigation is carefully controlled.

Rockwool and foam held water at low tensions (i.e., had low water buffering capacity as residual water content was less than 50 cm) and essentially all water was available for plant uptake. Model fit for MRC’s resulted in a high correlation ($R^2 = 0.82$ and 0.79 for rockwool and foam, respectively). Based on the measured data from the Hyprop™ the saturated parameter for rockwool in Eq. [3-1] was set at 93% during model fitting because the predicted parameter $\theta_s$ exceeded 100%. The VWC declined to near the estimated $\theta_r$ of 3% at low tension of 30 cm (Fig. 3-1b; Table 3-1). Similar studies by da Silva et al. (1995) with rockwool resulted in saturation at 95% VWC but rapidly decreased to nearly zero water content at a tension of 51 cm (5 kPa). Foam at saturation was estimated to have 84% VWC, however there was high variability in the data at low tensions (Fig. 3-1e). Similar to rockwool at 30 cm of tension the VWC of foam was close to $\theta_r$ of 6%. In comparison, the MRC for foam by Milks et al (1986a) also declined to nearly 3% VWC at approximately 50 cm of tension. Low water buffering capacity of rockwool and foam may require more frequent irrigation under high evapotranspiration conditions (da Silva et al., 1995; Fonteno and Nelson, 1990).

**Method 2. Moisture Retention Curve By Frozen Column Substrate**

The estimated MRCs from the frozen column method for peat (Table 3-1) had a lower $R^2$ value (0.44) compared with other substrates and methods because (a) the column height was only
21 cm, whereas the tensiometer data indicated $\theta_r$ was approached at much higher tensions, and (b) there was some variability in data points in the 0 to 30 cm range (Fig. 3-1b) possibly due to column diameter (frozen column diameter was 3.8 cm compared to 8 cm core diameter for tensiometer). This uncertainty was also reflected in the broader confidence intervals for peat with the frozen column method than the tensiometer method (Table 3-1). Peat averaged 82% VWC in the lower 10 cm of the column, similar to the $\theta_s$ estimate of 84±4% by tensiometer, and only decreased slightly to 75% in the upper half of the column (10 to 21 cm) (Fig. 3-1b).

The frozen column method in rockwool and foam resulted in higher $R^2$ values (0.99) because the entire function describing the MRC fell within the range of measured column heights (Table 3-1). Rockwool held 91% VWC at saturation, then decreased to 76% at the inflection point (10.8 cm), further decreasing to 8% at 27 cm tension (Fig. 3-1d). Similarly, foam held 91% VWC at saturation, that decreased to 58% at the inflection point (7.7 cm), and further decreased to 7% at 27 cm tension (Fig. 3-1f). In both substrates, the frozen column method clearly demonstrated low matric potential, with VWC less than 10% at the top of the column (less than 30 cm in height). In a short propagation cell, this may not lead to high stratification of moisture, but this low matric potential would have a large impact on vertical water and air distribution in taller containers.

The two methods for MRCs resulted in significant differences for peat ($P<0.001$) but not for rockwool and foam based on the paired sample t-test. In peat, confidence intervals for the curve parameters $\theta_r$, $X_0$, $n$ did not overlap between methods (Table 3-1). Experimentally for the frozen column method, using a column height of 100 cm, use of a piezometer, and possibly additional time for soaking the substrate beyond 4h would improve the resolution of the MRC for peat and better describe plant available water. In rockwool, confidence intervals for curve
parameters overlapped between methods with the exception of the rate parameter \((n)\), although as noted above the \(\theta_s\) parameter was set as a constant when fitting Eq. [3-1] for the tensiometer data (Table 3-1). In foam, although curve-fitting parameters of \(\theta_s\) and \(X_0\) did not overlap, the t-test comparison did not show significant differences between methods. Comparison of MRC’s by paired t-test within a method (tensiometer or frozen column) for all possible paired comparisons resulted in differences across substrates that describes their unique water retention properties.

The tensiometer method allowed quantification of VWC at a higher soil tension than the frozen column method, and was therefore more accurate for quantifying \(\theta_r\) and unavailable water for peat. However, for the low matric potential substrates rockwool and foam, both methods were adequate. Similar information and statistical methods can be used with both methods, however Altland et al. (2010) noted that the use of tensiometers requires a higher level of technical skills and has increased equipment cost compared with the frozen column substrate method (Table 3-4). We also noted greater variability in VWC data at low tensions with the tensiometer in rockwool and foam (Fig. 3-1). In addition to MRCs, tensiometers have been used to describe hydraulic conductivity and substrate porosity (Deboodt and Verdonck, 1972; Naasz et al., 2005; Schindler, 1980; Schindler et al., 2016).

**Method 3. Gravimetric Whole-Cell Analysis**

In small propagation cells, substrates held high VWC relative to VAC at container capacity using the gravimetric method, and there was a lower estimate in VWC and higher VAC in peat compared with the other two substrates (Table 3-2). Similar findings of high water and low air content in 2.2 cm-tall seedling plug cells were observed at container capacity in several peat:vermiculite ratios with different levels of compaction (Milks et al., 1989c). High moisture and low VWC of <10% has been shown to have greater severity in root rot of toyon compared to VAC of 10% to 20% (Filmer, 1986). Increasing container height results in increased air porosity
as gravity increases drainage in a given substrate, thus providing greater stratification of water throughout the vertical column. For example, foam at container capacity had a VAC of 30% in 15 cm-tall containers, but this decreased to 2% VAC in 2.2 cm-tall plug cells (Milks et al., 1989c). There was an overestimate of VSC by 7% and underestimate of total porosity in small propagation cells for peat. It is likely that these discrepancies were due to compaction. Slowly increasing the height of water during subirrigation of peat in a basin and allowing at least two saturation/drainage cycles would improve water uptake as pockets of air exist in peat strands (Naasz et al., 2008). Gravimetric whole-cell analysis describes physical properties in small propagation cells, but does not represent the vertical distribution of water and air content within a cell (Table 3-4).

**Method 4. X-ray Tomography (CT) Whole-Cell Analysis**

Quantification of VWC and VAC by gravimetric versus CT scanning methods differed for peat but not for the other two substrates (Table 3-2). In our analysis, the solid content (and therefore total porosity) for the CT scans was based on the gravimetric analysis and was therefore not independent between methods. However, the division between VWC and VAC within total porosity was estimated by analysis of the CT digital image.

Representative images of a tomography slice for each substrate is shown in Figure 3-2. The peat image shows small air-filled pores more evenly distributed through the column compared with rockwool or foam. The rockwool shows an air-filled vertical line that arises from a planting slit designed during manufacturing to allow the cutting to be inserted. The large air-filled pore at the top right of the rockwool image shows that even though this is a manufactured substrate, there can be some variability in wettability and pore size. The foam image shows most of the air-filled pores occurring in the top of the cell.
Spatial quantification of the propagation cells by 0.5 cm sections found that air-filled pores were located mainly toward the top of the cell but were absent toward the bottom, and that water filled most pore spaces in all substrates (Fig. 3-3). In rockwool, air was mostly present in the vertical planting slit, which is part of the design of this propagation (Fig. 3-3b). The scan resolution was 59 µm, which means that VAC smaller than this resolution might exist but were not quantified using this method and this may explain the lower estimate for VAC by CT for peat [internal pore size ~ 15 µm from Carey et al., (2007)] compared with the gravimetric method. A previous study by Fonteno (1989) described the spatial distribution of water and air in a tall container (16.9 cm) with volume of 3.9 L. The spatial profile ranged from 1 to 16.9 cm in height by applying increasing tensions from 3.8, 10, 20, 40, 50, 75, 100, 200, and 300 cm. The tall container had 58% VWC and 28% VAC (Fonteno, 1989) whereas small cells (55 mL and 4.5 cm height) in this study had 77% VWC and 1% VAC relative to the bottom (1 cm) of the container. This comparison further emphasizes the importance of quantifying the spatial distribution of water and air for a specific substrate and container size combination.

The CT image in Figure 3-4 for peat at container capacity (72% VWC) provides a visual representation that high moisture level is likely to block the path of air infiltration, whereas allowing the substrate to dry to 51% VWC increases the continuity of air-filled pores for gas exchange and oxygen supply to roots. This has implications for irrigation management, because providing wet-dry cycles would increase VAC to >20% throughout the cell profile in peat (Fig. 3-4 and Table 3-3), which is in the range of air content that would not be limiting for root growth (DeBoodt and Verdonck, 1972; Gislerød, 1982; Handreck and Black, 2002).

Gravimetric whole cell analysis was used to describe static substrate properties, and using the gravimetric estimate of VSC greatly simplified quantitative analysis by CT. Tracy et al.
(2015b) segmented all three components (solid, water, and air) in sandy and clay loam soils. However, with a substrate that absorbs water into internal pores, which is typical of propagation substrates, it becomes very difficult to segment the solid/water matrix. Gravimetric whole cell analysis was simple and quick in contrast to CT that requires specialized equipment and image segmentation software that were costly, complicated, and time consuming (Table 3-4). Estimated time from start to finish for CT quantitative analysis of one sample was 1.5 to 3 h, not considering protocol development and software competence. The CT analysis allowed for spatial stratification and visualization of complex water and air relations within substrates. Both gravimetric and CT methods provide static characteristics, but by analyzing substrates at different moisture levels, such as shown in Figure 3-4, these methods can be combined with tensiometer data to generate MRCs (Daly et al., 2015).

Summary

Water and air relations of three propagation substrates were quantified by tensiometers or frozen column methods. Moisture retention curves described low matric potential in rockwool and foam compared with peat. There was also more consistency in MRC between the two methods for rockwool and foam compared with peat. Gravimetric and CT methods resulted in similar estimates of volumetric water and air content at container capacity in rockwool and foam, but VWC estimates differed in peat between methods. In propagation cells, all substrates held high water content and most pores were filled with water at container capacity, meaning that waterlogging would be possible with all substrates under poor control of mist irrigation. Peat had an even distribution of water as it dried from container capacity to 51% VWC. It would be useful to quantify water distribution in the three substrates under a range in moisture conditions. Peat had higher water buffering capacity than rockwool or foam, which on the one hand would aid in cutting hydration because the substrate would dry slowly, but also increases the risk of over-
watering. Irrigation strategy based on substrate matric potential is necessary to ensure adequate balance of water and air appropriate to the production phase.
Table 3-1. Model estimates using a four-parameter log-logistic model for moisture retention curves of three substrates and two methods of tensiometer or frozen column substrate. Model fit resulted in F statistic values and curve fit resulted in adjusted $R^2$. Tensiometer analysis was the result of n=3 runs per substrate. Frozen column analysis was the result of n=4 per substrate. Confidence intervals (±) were set at 0.05. The parameters for the log-logistic model were $\theta_s =$ water content at saturation, $\theta_r =$ residual water content, $n =$ is a rate parameter, and $X_0 =$ tension due to curve change from convex to concave or inflection point.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Method</th>
<th>$\theta_s$</th>
<th>$\theta_r$</th>
<th>$X_0$</th>
<th>$n$</th>
<th>F</th>
<th>P &gt; F</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peat</td>
<td>Tensiometer</td>
<td>0.86 ± 0.02</td>
<td>0.23 ± 0.01</td>
<td>19.63 ± 0.85</td>
<td>1.69 ± 0.13</td>
<td>3721.06</td>
<td>&lt;.0001</td>
<td>0.981</td>
</tr>
<tr>
<td>Peat</td>
<td>Frozen column</td>
<td>0.84 ± 0.04</td>
<td>0.71 ± 0.05</td>
<td>9.85 ± 6.8</td>
<td>2.47 ± 3.6</td>
<td>18.66</td>
<td>&lt;.0001</td>
<td>0.436</td>
</tr>
<tr>
<td>Rockwool</td>
<td>Tensiometer</td>
<td>0.93</td>
<td>0.03 ± 0.06</td>
<td>11.16 ± 0.68</td>
<td>3.02 ± 0.32</td>
<td>4145.34</td>
<td>&lt;.0001</td>
<td>0.821</td>
</tr>
<tr>
<td>Rockwool</td>
<td>Frozen column</td>
<td>0.91 ± 0.02</td>
<td>0.08 ± 0.02</td>
<td>10.75 ± 0.26</td>
<td>4.43 ± 0.46</td>
<td>2476.43</td>
<td>&lt;.0001</td>
<td>0.839</td>
</tr>
<tr>
<td>Foam</td>
<td>Tensiometer</td>
<td>0.84 ± 0.03</td>
<td>0.06 ± 0.05</td>
<td>8.91 ± 0.52</td>
<td>4.22 ± 0.85</td>
<td>2263.82</td>
<td>&lt;.0001</td>
<td>0.789</td>
</tr>
<tr>
<td>Foam</td>
<td>Frozen column</td>
<td>0.91 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td>7.67 ± 0.19</td>
<td>5.25 ± 0.59</td>
<td>2019.64</td>
<td>&lt;.0001</td>
<td>0.863</td>
</tr>
</tbody>
</table>

*The parameter $\theta_s$ was set as a constant of 0.93 for rockwool for the tensiometer curves based on the measured total porosity in the Hyprop system, because otherwise the nonlinear regression estimates were not close to the observed porosity for this substrates. The data set for the tensiometer method was contributed by Dr. James Altland (USDA-ARS).*
Table 3-2. Summary of whole cell analysis by gravimetric or CT for volumetric water content and volumetric air content for the three propagation substrates at container capacity. Substrate solid content was estimated by gravimetric analysis and solid values (22% for peat, 8% for rockwool, and 2% for foam) were treated as a constant for each substrate.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Methods</th>
<th>Water</th>
<th>Tukey's HSD</th>
<th>Air</th>
<th>Tukey's HSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peat</td>
<td>Gravimetric</td>
<td>67%</td>
<td>D</td>
<td>11%</td>
<td>A</td>
</tr>
<tr>
<td>Peat</td>
<td>CT</td>
<td>72%</td>
<td>C</td>
<td>6%</td>
<td>B</td>
</tr>
<tr>
<td>Rockwool</td>
<td>Gravimetric</td>
<td>87%</td>
<td>B</td>
<td>5%</td>
<td>B</td>
</tr>
<tr>
<td>Rockwool</td>
<td>CT</td>
<td>87%</td>
<td>B</td>
<td>5%</td>
<td>B</td>
</tr>
<tr>
<td>Foam</td>
<td>Gravimetric</td>
<td>91%</td>
<td>AB</td>
<td>7%</td>
<td>AB</td>
</tr>
<tr>
<td>Foam</td>
<td>CT</td>
<td>93%</td>
<td>A</td>
<td>5%</td>
<td>B</td>
</tr>
</tbody>
</table>

LS means for gravimetric and CT analysis were the result of n=3 cells for each treatment combination. Tukey’s HSD was calculated at $\alpha = 0.05$. 
Table 3-3. Cell spatial quantification by CT for volumetric water or air content in peat at container capacity (72% VWC) and when substrate dried to medium moisture (51% VWC), represented visually in Fig. 3-4. Volumetric water or air content by depth represents the LS means of n=3 replicates per moisture level and Tukey’s HSD at α 0.05. Volumetric solid content was estimated at 22%.

<table>
<thead>
<tr>
<th>Moisture level</th>
<th>Depth (cm)</th>
<th>VWC</th>
<th>Tukey's</th>
<th>VAC</th>
<th>Tukey's</th>
</tr>
</thead>
<tbody>
<tr>
<td>Container capacity</td>
<td>0.5</td>
<td>58%</td>
<td>C</td>
<td>20%</td>
<td>B</td>
</tr>
<tr>
<td>Container capacity</td>
<td>1</td>
<td>70%</td>
<td>B</td>
<td>8%</td>
<td>C</td>
</tr>
<tr>
<td>Container capacity</td>
<td>1.5</td>
<td>72%</td>
<td>AB</td>
<td>6%</td>
<td>CD</td>
</tr>
<tr>
<td>Container capacity</td>
<td>2</td>
<td>74%</td>
<td>AB</td>
<td>4%</td>
<td>CD</td>
</tr>
<tr>
<td>Container capacity</td>
<td>2.5</td>
<td>76%</td>
<td>AB</td>
<td>2%</td>
<td>CD</td>
</tr>
<tr>
<td>Container capacity</td>
<td>3</td>
<td>77%</td>
<td>A</td>
<td>1%</td>
<td>D</td>
</tr>
<tr>
<td>Container capacity</td>
<td>3.5</td>
<td>77%</td>
<td>A</td>
<td>1%</td>
<td>D</td>
</tr>
<tr>
<td>Container capacity</td>
<td>4</td>
<td>77%</td>
<td>A</td>
<td>1%</td>
<td>D</td>
</tr>
<tr>
<td>Container capacity</td>
<td>4.5</td>
<td>76%</td>
<td>AB</td>
<td>2%</td>
<td>CD</td>
</tr>
<tr>
<td>Medium</td>
<td>0.5</td>
<td>53%</td>
<td>CD</td>
<td>25%</td>
<td>AB</td>
</tr>
<tr>
<td>Medium</td>
<td>1</td>
<td>51%</td>
<td>D</td>
<td>27%</td>
<td>A</td>
</tr>
<tr>
<td>Medium</td>
<td>1.5</td>
<td>49%</td>
<td>D</td>
<td>29%</td>
<td>A</td>
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<td>50%</td>
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<tr>
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<td>2.5</td>
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<tr>
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<tr>
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<tr>
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<td>54%</td>
<td>CD</td>
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</table>
Table 3-4. Summary of the information provided, the cost for laboratory equipment, and the necessary level of technical operator skill for the four different substrate analysis methods evaluated in this article, along with published research.

<table>
<thead>
<tr>
<th>Method</th>
<th>Information Provided</th>
<th>Equipment Cost ($)</th>
<th>Operator Skill Level</th>
</tr>
</thead>
</table>
| Tensiometer  | Dynamic and static characteristics  
Hydraulic conductivity  
Water holding capacity  
Air-filled porosity  
Bulk density  
Dynamic and static characteristics  
Moisture retention curve | Moderate             | High                |
| Frozen Column| Water holding capacity  
Air-filled porosity  
Bulk density  
Static characteristics  
Water holding capacity  
Air-filled porosity  
Bulk density  
Static characteristics  
Visualizations of water and air  
Moisture retention curve | Low                  | Moderate            |
| Gravimetric  | Water holding capacity  
Air-filled porosity  
Bulk density  
Static characteristics  
Visualization of water and air  
Moisture retention curve | Low                  | Moderate            |
| CT           | Spatial distribution of water and air  
Water holding capacity  
Air-filled porosity | High                | Very high           |
Figure 3-1. Moisture retention curves by tensiometer or frozen column methods for peat, rockwool, and foam. Tensiometer analysis was the result of n=3 runs per substrate. Frozen column analysis was the result of n=4 per substrate. Non-linear regression, model defined as: $\theta_r + (\theta_s - \theta_r)/[1+(c m \text{ of } H_2O \text{ or } \text{Height}/X_0)^n]$, $\theta_r$ = residual water content, $\theta_s$ = water content at saturation, $n$ = is a rate parameter, and $X_0$ = tension due to curve change from convex to concave.
Figure 3-2. Images of CT-scanned substrate showing the water and air distribution in substrates at container capacity in propagation sized cells. Images are of one representative substrate. Container capacity was achieved by overnight subirrigation of substrates, followed by draining for 30 minutes. The blue color represents segmented water-solid matrix in peat or water in rockwool and foam, and white to light gray represents segmented air. Images are not to scale, actual volume of peat was 55 mL, rockwool was 40.5 mL, and foam was 30.5 mL.
Figure 3-3. Spatial quantification by CT for volumetric water and air content at container capacity. Volumetric solid content was measured gravimetrically and used as a constant. Sub-figures represent LS means from n=3 replicates per substrate. X-axis is the total percent volumetric content. Y-axis is the container depth from the bottom to top (cm).
Figure 3-4. CT images of peat and spatial quantification from container capacity or 72% VWC (a-b) and as substrate dried to medium moisture or 51% VWC (c-d). Spatial quantification represents the LS means of n=3 replicates per moisture level.
Vegetative cuttings are commonly used for clonal propagation in forestry and horticulture (Bellini et al., 2014). Total sales for propagative floriculture material in the US was estimated at $394 million in 2015 (USDA, 2016). Greenhouse propagation uses high misting frequency to maintain high relative humidity, between 90% to 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 et al., 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 et al., 1990). 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 et al., 1999).

Substrates have been designed to contain adequate air porosity at high moisture or at container capacity (Argo et al., 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™), 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 et al., 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 et al., 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 et al., 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 et al., 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
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., 2012; Tracy et al., 2013; Tracy et al., 2015a; Zappala et al., 2013).

In Chapter 3, water and air relations were quantified 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⁻¹ and 40 mg·L⁻¹ CaCO₃ 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 et al. (2014) and Huang and Fisher (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 in Chapter 3 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™, 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 et al., 1995). The
foam substrate was a matrix of phenol-formaldehyde foam with monodispersed pores (Milks et al., 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 analysis by 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 hours. 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 et al., 1997; Kaestner et al., 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 4-1. 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 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 Fig. 4-2), which required an additional 10 to 15 min. processing time per sample.

**Experiment 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) 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·m²·s⁻¹ at the canopy level. The initial photoperiod was 15 hr. that increased to 21 hr. by day 10 of propagation for a daily light integral of 8.1 to 12.2 mol·m²·day, 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=0.05 in SAS (SAS Version 9.4, SAS Institute, Cary, NC).

**Experiment 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™ 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=0.05 in SAS.

**Experiment 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⁻¹, 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 et al. (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 in Chapter 3.

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

Experiment 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 (Fig. 4-3). Analysis of variance found that substrate and moisture level did not affect root count, but there were main effects at the p < 0.05 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) (Fig. 4-3). Root growth in foam was similar across very different moisture levels of 17%, 33%, and 86% VWC corresponding to 79%, 65%, and 12% VAC (Fig. 4-3).

Root spatial distribution in 0.5 cm sections from the bottom of the cell is shown in Figure 4-4. 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.
Experiment 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 (Chapter 3).

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 ± 25.8 (mean ± 95% confidence intervals), root volume was 0.35 cm³ ± 0.21, and root count was 18 ± 7.6, and root surface area was 14.4 cm² ± 8.2.

Experiment 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 4-1). However, there were differences between the VWC and VAC estimated by the two methods in peat. In Chapter 3, there was a 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 et al., 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 (Fig. 4-5) 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 (Fig. 4-6). Rockwool was composed of thin threads of solid fibers, and the CT scan showed vertical channels of water and air (Fig. 4-5). Foam at high moisture showed near-saturation of pores (Fig. 4-5), and nearly all air-filled pores were at the top of the substrate (Fig. 4-6). 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 4-2). 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.

**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 et al. (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).

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 (Chapter 3). 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 (Fig. 4-3). However, foam at the highest moisture level had only 3% VAC at the base of the stem cutting (Table 4-2). 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 4-2) is an example of the importance of quantifying the microenvironment within a propagation cell where callusing and adventitious rooting occurs. Tracy et al. (2015b) 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 (Figs. 4-5 and 4-6). 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 cm² ± 8.2) was almost twice the highest amount of rooting in Experiment 1, which occurred in rockwool at high moisture (7.7 cm², Fig. 4-3). 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., 2013; Tracy et al., 2015a).

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 et al., 1993; Heiskanen, 1995), although it has advantages of a more even vertical distribution of VWC and VAC (Figs. 4-5 and 4-6) 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 et al, 1995; Fonteno and Nelson, 1990). 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 (Figs. 4-5 and 4-6). 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).

**Summary**

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 was 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.
Table 4-1. Comparison of volumetric water or air content measured by gravimetric or CT scanned 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%.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Moisture</th>
<th>Method</th>
<th>VWC</th>
<th>Tukey’s</th>
<th>VAC</th>
<th>Tukey’s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peat</td>
<td>Low</td>
<td>Gravimetric</td>
<td>38%</td>
<td>G</td>
<td>40%</td>
<td>D</td>
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<tr>
<td>Peat</td>
<td>Low</td>
<td>CT</td>
<td>55%</td>
<td>DE</td>
<td>23%</td>
<td>F</td>
</tr>
<tr>
<td>Peat</td>
<td>Medium</td>
<td>Gravimetric</td>
<td>46%</td>
<td>F</td>
<td>32%</td>
<td>E</td>
</tr>
<tr>
<td>Peat</td>
<td>Medium</td>
<td>CT</td>
<td>61%</td>
<td>BC</td>
<td>17%</td>
<td>G</td>
</tr>
<tr>
<td>Peat</td>
<td>High</td>
<td>Gravimetric</td>
<td>53%</td>
<td>E</td>
<td>25%</td>
<td>F</td>
</tr>
<tr>
<td>Peat</td>
<td>High</td>
<td>CT</td>
<td>65%</td>
<td>B</td>
<td>13%</td>
<td>G</td>
</tr>
<tr>
<td>Rockwool</td>
<td>Low</td>
<td>Gravimetric</td>
<td>14%</td>
<td>J</td>
<td>78%</td>
<td>A</td>
</tr>
<tr>
<td>Rockwool</td>
<td>Low</td>
<td>CT</td>
<td>14%</td>
<td>J</td>
<td>79%</td>
<td>A</td>
</tr>
<tr>
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<td>Gravimetric</td>
<td>22%</td>
<td>I</td>
<td>70%</td>
<td>C</td>
</tr>
<tr>
<td>Rockwool</td>
<td>Medium</td>
<td>CT</td>
<td>21%</td>
<td>I</td>
<td>71%</td>
<td>BC</td>
</tr>
<tr>
<td>Rockwool</td>
<td>High</td>
<td>Gravimetric</td>
<td>59%</td>
<td>CD</td>
<td>33%</td>
<td>E</td>
</tr>
<tr>
<td>Rockwool</td>
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<td>CT</td>
<td>59%</td>
<td>CD</td>
<td>33%</td>
<td>E</td>
</tr>
<tr>
<td>Foam</td>
<td>Low</td>
<td>Gravimetric</td>
<td>22%</td>
<td>I</td>
<td>76%</td>
<td>AB</td>
</tr>
<tr>
<td>Foam</td>
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<td>CT</td>
<td>19%</td>
<td>I</td>
<td>79%</td>
<td>A</td>
</tr>
<tr>
<td>Foam</td>
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<td>Gravimetric</td>
<td>32%</td>
<td>H</td>
<td>66%</td>
<td>C</td>
</tr>
<tr>
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<td>H</td>
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<td>81%</td>
<td>A</td>
<td>17%</td>
<td>G</td>
</tr>
<tr>
<td>Foam</td>
<td>High</td>
<td>CT</td>
<td>80%</td>
<td>A</td>
<td>18%</td>
<td>G</td>
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</tbody>
</table>
Table 4-2. 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, compared using Tukey’s honestly significant difference at α=0.05. Confidence intervals (c.i.) were at α=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 4-3).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Moisture</th>
<th>VWC</th>
<th>VAC</th>
<th>Relative Root Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peat</td>
<td>Low</td>
<td>56%  ± 4%</td>
<td>22%  ± 4%</td>
<td>High (Expt 2)</td>
</tr>
<tr>
<td>Peat</td>
<td>Medium</td>
<td>61%  ± 4%</td>
<td>17%  ± 4%</td>
<td>High (Expt 2)</td>
</tr>
<tr>
<td>Peat</td>
<td>High</td>
<td>64%  ± 4%</td>
<td>14%  ± 4%</td>
<td>High (Expt 2)</td>
</tr>
<tr>
<td>Rockwool</td>
<td>Low</td>
<td>13%  ± 4%</td>
<td>79%  ± 4%</td>
<td>Low (Expt 1)</td>
</tr>
<tr>
<td>Rockwool</td>
<td>Medium</td>
<td>21%  ± 4%</td>
<td>71%  ± 4%</td>
<td>Low (Expt 1)</td>
</tr>
<tr>
<td>Rockwool</td>
<td>High</td>
<td>58%  ± 4%</td>
<td>34%  ± 4%</td>
<td>High (Expt 1)</td>
</tr>
<tr>
<td>Foam</td>
<td>Low</td>
<td>19%  ± 4%</td>
<td>80%  ± 4%</td>
<td>Medium (Expt 1)</td>
</tr>
<tr>
<td>Foam</td>
<td>Medium</td>
<td>29%  ± 4%</td>
<td>69%  ± 4%</td>
<td>Medium (Expt 1)</td>
</tr>
<tr>
<td>Foam</td>
<td>High</td>
<td>95%  ± 4%</td>
<td>3%   ± 4%</td>
<td>Medium (Expt 1)</td>
</tr>
</tbody>
</table>
Figure 4-1. Examples showing 3D image reconstruction of roots in three propagation substrates (foam, rockwool, and peat) by CT scans. Root surface area and volume, respectively, were: a) foam 3.2 cm$^2$ and 0.06 cm$^3$; b) rockwool 7.2 cm$^2$ and 0.11 cm$^3$; and c) peat 27.9 cm$^2$ and 0.59 cm$^3$ from these individual replicates in Experiment 1.
Figure 4-2. 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.
Figure 4-3. Effect of substrate (rockwool or foam) and varied moisture level (low, medium, or high) on root surface area of 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 $\alpha=0.05$. Labels in parentheses show the volumetric water content (VWC) and volumetric air content (VAC).
Figure 4-4. Quantification of spatial distribution of roots 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 (4 cm). 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 $\alpha=0.05$. 
Figure 4-5. Visualization of water and air content 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 color represents segmented water-solid matrix in peat or water in rockwool and foam, and white to light gray represents segmented air. Images are not in scale between substrates, with the actual volume for peat being 55 mL, rockwool 40.5 mL, and foam 30.5 mL.
Figure 4-6. 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%.
CHAPTER 5
CONCLUSIONS

During propagation of vegetative plant cuttings water loss is prevented by establishing high humidity through misting. In container substrate, there is a relationship between water and air (oxygen) that is favorable for adventitious rooting. Evaluation of irrigating using two water types (oxygenated or ambient tap water) during mist propagation of unrooted cuttings and continued growth of transplants. The technology used to supersaturate water by injecting pure oxygen (oxygenated) into tap water was being promoted to growers. Research showed there were no differences in root or plant dry mass during mist propagation. Water that passed through fine mist emitters either off-gassed in oxygenated water or increased in ambient tap water to 100% DO saturation. Because of this finding, water types (oxygenated or ambient tap water) were delivered by pouring water (top-water) or subirrigation of plants in 10 cm pots. In addition, plants were maintained approximately above 80% moisture (relative to container capacity) or above 45% moisture. Irrigating with oxygenated water did not benefit or negatively affect growth of three bedding plants in porous peat substrate where presumably there was adequate aeration through air-filled pores. All plants grew vigorously throughout both trials.

Substrate water retention was unique for peat, rockwool, or foam by comparison of moisture retention curves. The ‘alternative’ frozen column method was similar to the industry standard method by tensiometers for low matric potentials (<30 cm) in rockwool and foam. However, within peat the matric potential was high (> 300 cm), there was a difference in MRCs compared within methods (frozen column or tensiometer) where the column height restricted the measured tensions. To improve the frozen column method for high matric potentials, increase the column height to 100 cm for better resolution of MRCs and description of plant available water. Water retention within substrates can be used to aid in irrigation strategies during propagation.
For example, substrates with low water retention or low matric potential may require frequent misting to maintain cutting hydration. Whereas, substrates with high water retention or high matric potential require careful mist management to prevent overwatering at early stages of propagation. Description of substrate porosity in small propagation cells by CT was similar to gravimetric measurements for rockwool and foam, where density separation of water, air, and substrate did not overlap and resulted in image segmentation of either water or air. In contrast, porosity measured in peat differed when compared within methods (CT or gravimetric) possibly due to the scanning resolution of 59 µm compared to internal pore size of peat ~ 15 µm thus underestimating air content. In addition, CT provided visualization and spatial distribution of water and air relations within substrates in small cells at container capacity. Peat at container capacity contained high moisture (72% VWC) and low VAC at 6%, but when substrate dried to 51% VWC the VAC increased to > 20% throughout the vertical column. This accurately describes irrigating by wet-dry cycles where there was adequate water for plant demand and aeration for root growth and nutrient mobilization.

Development of methodology for CT scanning of roots in three substrates (peat, rockwool, and foam) was achieved. There was greater density separation between air, roots, and substrate for rockwool and foam when water was removed that allowed for accurate and quicker image segmentation of roots. Whereas, residual water in peat strands had similar density to roots that negatively affected the precision of root image segmentation and was time consuming. In Experiment 1, poinsettia cuttings rooted in rockwool and foam under a wide range of VWC and VAC, however high root growth was observed in rockwool at 59% VWC and 33% VAC. Root growth in foam were similar across a wide range from 17% to 86% VWC and 12% to 79% VAC. This may be due to the imbalance of VWC and VAC, where chamber conditions of high
humidity and misting aided in cutting hydration and subsequent rooting. In Experiment 2, root growth in peat was high across a range of 52% to 63% VWC and 16% to 26% VAC. This description of VWC and VAC was in agreement to the microenvironment quantified at the approximate base of the stem cutting that resulted in high rooting. In foam at high moisture, spatial quantification at the base of the cutting had 95% VWC and low VAC at 3% that may be limiting for root growth but aid in callus formation. The relationship between water and air (oxygen) was quantified at 56% to 64% VWC and 14% to 34% VAC at the base of the stem cutting for rapid rooting of poinsettia. This range can be used for best irrigation management to provide the cutting with adequate water for hydration and adequate air for rapid root growth. The methods developed for CT scanning of roots and substrate is not limited by species or substrate and can be used to further research in soil plant atmosphere continuum.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Erin Joy Yafuso was born in Honolulu, Hawaii to Gary S. Yafuso and Doreen FumieLei Yafuso. She grew up on Molokai and later moved to Honolulu where she graduated from Moanalua High School in 2000. She attended the University of Hawaii at Manoa and graduated with a B.S. in plant and environmental biotechnology in 2005 followed by a M.S. in molecular biosciences and bioengineering in 2007. She continued at the University of Hawaii as a research associate from 2008 to 2009 and lectured courses in natural sciences from 2009 to 2015. She was admitted to the University of Florida for a PhD in horticultural sciences from 2015 to 2019. She plans to continue to work in plant sciences.