HortScience 54(11):2024–2030. 2019. https://doi.org/10.21273/HORTSCI14145-19

Water and Air Relations in Propagation Substrates

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Additional index words. CT, moisture retention, peat, phenolic foam, rockwool, tomography

***Abstract*. Greenhouse propagation of unrooted plant cuttings is characterized by short container cell height and high irrigation frequency. These conditions can result in high moisture level and low air content in soilless container substrates ("substrates"), causing delayed growth of adventitious roots and favoring root disease. The objective of this study was to quantify and compare substrate water and air relations for three propagation substrates (peat, rockwool, and phenolic foam) that varied widely in physical characteristics using four methods: 1) evaporation method with a tensiometer, 2) frozen column method, 3) gravimetric analysis, and 4) X-ray computed tomography (CT) analysis. Moisture retention curves based on evaporation (1) and the frozen column (2) resulted in differences for peat, but similar curves for rockwool and foam. The frozen column method was simple and low cost, but was constrained by column height for peat, which had a higher water potential compared with the other two substrates. Substrate porosity analysis at container capacity by gravimetric or CT methods were similar for volumetric water and air content (VWC and VAC) in rockwool and foam, but differed for peat for VWC and VAC. Gravimetric analysis was simple, rapid, and low cost for whole-cell analysis, but CT further quantified spatial water and air relations within the cell and allowed visualization of complex water and air relations in an image. All substrates had high water content at container capacity ranging from 67% to 91% VWC with 5% to 11% VAC in the short propagation cells, emphasizing the need for careful irrigation management.**

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, overwatering 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 propagule hydration and oxygen supply for root respiration, both of which are requirements for rapid root growth, development, and subsequent plant health (Bilderback and Lorscheider, 1995; Reisch, 1967).

The combination of short container height and fine substrate particles in propagation increases risk of inadequate gas exchange in the substrate. A wide range of substrates and amendments is used during young plant production, including peat (sphagnum), bark, 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 to evenly fill small container cells, resulting in decreased pore size and higher water retention (da Silva et al., 1993). Container size modifies the ratio of water and air within the substrate, because as column height decreases, there is an increased proportion of water and corresponding reduction in air (Argo et al., 1996; Handreck and Black, 2002; Milks et al., 1989b, 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 to quantify VWC, VAC, volumetric solid content (VSC), and dry bulk density (Fonteno, 1993). The porometer method (Fonteno, 1993) uses a 348 cm^3 standard volume that can be modified for propagation plug cells with a shorter column height (Milks et al., 1989c). The maximum and minimum 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 volume pressure plate extractor (Milks et al., 1989a) in 348-cm^2 containers (7.3-cm diameter × 7.6-cm height). However, water and air balance is highly dependent on container size and shape, 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 based on a modeled peat-vermiculite substrate (Caron and Nkongolo, 1999). Gravimetric measurements also can be made directly in propagation trays, or with individual stabilized cells that do not easily conform to a standard porometer shape and volume (Huang and Fisher, 2014). 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. In contrast, stabilized substrates had a VWC from 37% to 91% and VAC between 1.9% and 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).

Water potential of a substrate, and the relationship between VWC and plant available water, are usually measured as a 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 may not be 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

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Received for publication 19 Apr. 2019. Accepted for publication 29 July 2019.

This research is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, "Clean WateR^3—Reduce, Remediate, Recycle," #2014-51181-22372. We thank our industry partners in the Floriculture Research Alliance (FloricultureAlliance.org) for their financial support and supply of materials. We thank James C. Colee from University of Florida Agriculture Statistics for providing the statistical models and analytical methods to compare nonlinear curves.

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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 (Gislerød, 1983; Healy, 2008), and gradually changes to wet-dry cycles following the emergence of adventitious roots to provide aeration (Loach, 1988). In propagation, many cells are also shorter than 5 cm (Huang and Fisher, 2014; Milks et al., 1989c), and differences in moisture and air level at low tensions are therefore of great importance.

An alternative method to generate an MRC is the use of the frozen column method, whereby the substrate is brought to field capacity, frozen, and then sectioned to quantify VWC and VAC within each vertical section (Altland et al., 2010; Dane and Hopmans, 2002; Owen and Altland, 2008). The VWC using the frozen column method allows a comparison between the water potential from the column height to the water potential 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 pressure plate or frozen column (core) methods, with the pressure plate 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 CT, the root zone microenvironment of water and air relations can be quantified and visualized (Daly et al., 2015; Nimmo, 2004) in addition to root morphology (Tracy et al., 2013, 2015a, 2015b). Daly et al. (2015) CT-scanned clay and sand substrates at different moisture levels and found that estimating or modeling VWC and VAC and other physical properties by CT provided a complementary method to using a ceramic plate or gravimetric measurement.

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 of this study 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 by using four methods: 1) MRCs by evaporation, 2) frozen column, 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 peatmoss (“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 1-L 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 had a dry bulk density of 87.5 g·L^{-1}, and 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 (Grodan, Roermond, the Netherlands) were composed of basalt and limestone heated to 1600 °C, forming threads of 5 µm, and the average pore size was 4.5 to 5.0 µm (da Silva et al.,1995), with a dry bulk density of 78.9 g·L^{-1}. Phenol-formaldehyde foam [Oasis, Kent, OH (“foam”)] root cubes with a dry bulk density of 20.3 g·L^{-1} were composed of a foam matrix with monodispersed pore distribution (Milks et al., 1989b).

Method 1. MRCs using evaporation. The evaporation method (Hyprop; UMS, Munich, Germany) was used for each substrate described by Fields et al. (2016) to plot MRCs. Samples were subirrigated to saturation for 24 h in a basin. Cores of 250-mL volume (8-cm diameter and 5-cm height) were 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 ≈10 d (ranging between experimental runs between 295.8 and 440.6 cm for peat, 39.5 and 45.2 cm for rockwool, and 25.0 and 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. [1]) where VWC (θ) is a function of pressure (h, in cm).

Parameters were fitted with nonlinear regression (PROC NLIN in SAS Version 9.4, SAS Institute, Cary, NC).

$$\theta = \theta r + (\theta s - \theta r)/[(1 + (h/X_0)^n] \qquad [1]$$

The parameter θ_s represented the VWC at saturation (zero tension), θ_r was the residual VWC (lower asymptote), h was the column height (relative to the bottom), 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 evaporation method were compared between substrates using a paired-sample t test of the nonlinear fitted Eq. [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 nonlinear regression were tabulated and compared between substrates.

Method 2. Moisture retention curve by frozen column method. The frozen column method was used to generate MRCs 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 2 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. VWC and VAC were calculated for each 1-cm section. VWC as a function of column height data were fitted to the nonlinear model Eq. [1], and obtained the fit parameters θ_s, θ_r, n, and X_0. Differences between substrates for the frozen column method were compared using the same analytical approaches described for evaporation (t test and comparing parameter estimates). In addition, within each substrate there was a comparison between evaporations and the frozen column methods using these statistical approaches.

Method 3. Gravimetric whole-cell analysis. Porosity was measured for the original container dimensions and shape of the propagation cells. Substrates were saturated by adding water to a basin with propagation substrate up to 1 cm to the top 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, VAC, and 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 (v|tome|x M 240; GE, Boston, MA) at UF, Research Service Centers (1041 Center Drive, P.O. Box 116621, Gainesville, FL 32611). An additional three replicate cells of peat were on capillary mats and equilibrated to 51% VWC and were also scanned. The scanned settings were a voltage of 80 kV, current of 175 µA, and 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, Wunstorf, Germany) before 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, volume of the 3D representation of a cell was defined by removing border sections that included edge areas outside the solid propagation cell, and 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: 1) the air volume and 2) the volume of the combined matrix of water plus solid substrate. Second, gravimetric analysis of VSC from gravimetric whole-cell analysis was used as a constant to subtract the solid portion from (2) 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 VAC was calculated by dividing the segmented volume of air by the segmented total volume. Percent VWC 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 analysis of variance (ANOVA) by PROC GLIMMIX with fixed effects of the three substrates and two methods (gravimetric or CT) at α = 0.05 in SAS. 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.

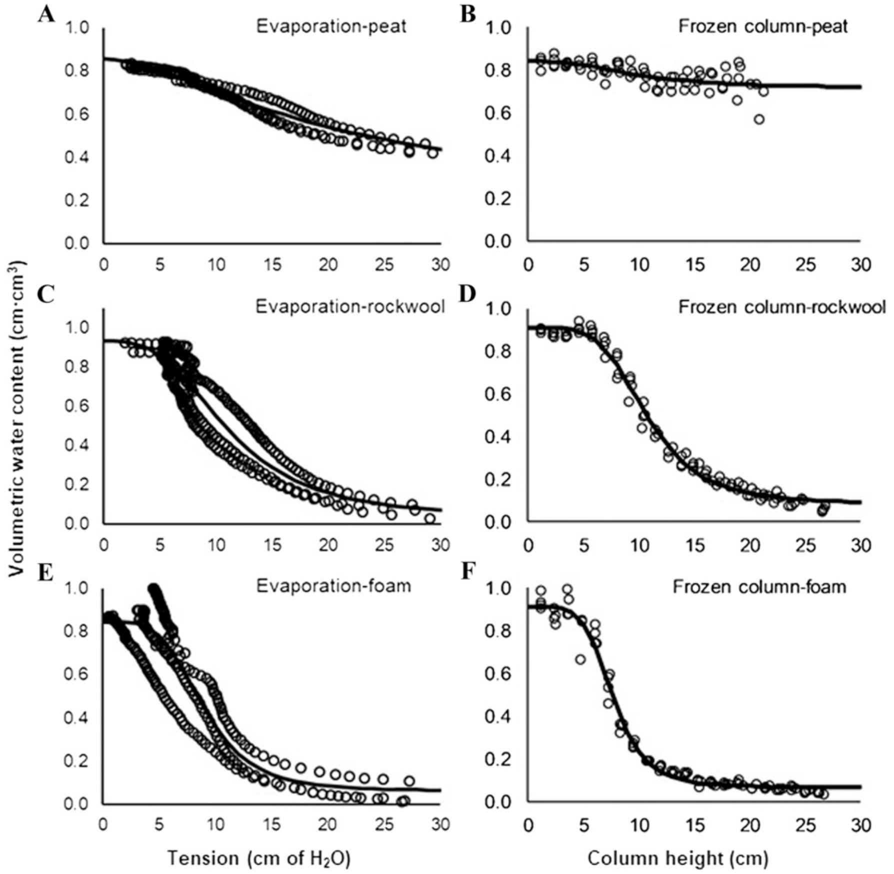

Fig. 1. Moisture retention curves by evaporation or frozen column methods for peat (**A**, **B**), rockwool (**C**, **D**), and foam (**E**, **F**). Evaporation analysis was the result of n = 3 runs per substrate. Frozen column analysis was the result of n = 4 per substrate.

Results and Discussion

Method 1. MRCs by evaporation method. At no tension (saturation), peat held a similar amount of water to the other two substrates (as shown by comparisons of Fig. 1A, C, and E, and estimates of θ_s in Table 1). However, peat had a much higher water potential, meaning more water was retained as moisture tension increased (represented by the rate parameter *n* and residual VWC parameter θ_r in Table 1). The MRC resulted in acceptable R^2 values ranging from 0.44 to 0.99 to the model fit for all substrates (Table 1). Peat at saturation was estimated at 86% VWC (θ_s in Table 1). As substrate dried, the inflection point was near 58% VWC or tension of 19 cm and decreased to residual VWC at 23% (θ_r in Table 1). At tensions above 200 cm (beyond the data displayed in Fig. 1A), measured VWC in peat was within 5% of the estimated θ_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

Table 1. Model estimates using a four-parameter log-logistic model for moisture retention curves of three substrates and two methods of evaporation or frozen column substrate. Evaporation 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 θ_s = water content at saturation, θ_r = residual water content, n = is a rate parameter, and X_0 = tension due to curve change from convex to concave or inflection point.

							Model	
Substrate	Method	θ_s	θ_r	X_0	n	*F*	*P* > *F*	R^2
Peat	Evaporatiom	0.86 ± 0.02	0.23 ± 0.01	19.63 ± 0.85	1.69 ± 0.13	3721.06	<0.0001	0.981
Peat	Frozen column	0.84 ± 0.04	0.71 ± 0.05	9.85 ± 6.8	2.47 ± 3.6	18.66	<0.0001	0.436
Rockwool	Evaporation	0.93^z	0.03 ± 0.06	11.16 ± 0.68	3.02 ± 0.32	4145.34	<0.0001	0.821
Rockwool	Frozen column	0.91 ± 0.02	0.08 ± 0.02	10.75 ± 0.26	4.43 ± 0.46	2476.43	<0.0001	0.989
Foam	Evaporation	0.84 ± 0.03	0.06 ± 0.05	8.91 ± 0.52	4.22 ± 0.85	2263.82	<0.0001	0.789
Foam	Frozen column	0.91 ± 0.02	0.07 ± 0.01	7.67 ± 0.19	5.25 ± 0.59	2019.64	<0.0001	0.986

[z] The parameter θ_s was set as a constant of 0.93 for rockwool for the evaporation 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.

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 is a widely used component in container propagation substrates in the United States, and although high water-buffering capacity reduces risk of dehydration of cuttings, a peat-based 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 MRCs resulted in a high correlation (R^2 = 0.82 and 0.79 for rockwool and foam, respectively). Based on the measured data from the evaporative method, the saturated parameter for rockwool in Eq. [1] was set as a parameter constant value of 93% during model fitting because the predicted parameter θ_s exceeded 100%. The VWC declined to near the estimated θ_r of 3% at low tension of 30 cm (Fig. 1B; Table 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. 1E). Similar to rockwool at 30 cm of tension, the VWC of foam was close to θ_r of 6%. In comparison, the MRC for foam by Milks et al. (1989a) also declined to nearly 3% VWC at ≈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 method. The estimated MRCs from the frozen column method for peat (Table 1) had a lower R^2 value (0.44) compared with other substrates and methods because 1) the column height was only 21 cm, whereas the evaporation data indicated θ_r was approached at much higher tensions, and 2) some variability in data points in the 0- to 30-cm range (Fig. 1B) occurred due to column diameter (frozen column diameter was 3.8 cm compared with 8 cm core diameter for tensiometer). This uncertainty also was reflected in the broader confidence intervals for peat with the frozen column method than the evaporation method (Table 1). Peat averaged 82% VWC in the lower 10 cm of the column, similar to the θ_s estimate of 84% ± 4% by evaporation, and only decreased slightly to 75% in the upper half of the column (10 to 21 cm) (Fig. 1B).

The frozen column method for 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 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. 1D). Similarly, foam held 91% VWC at saturation, which decreased

Table 2. 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.

Method	Information provided	Equipment cost ($)	Operator skill level
Evaporation	Dynamic and static characteristics Moisture retention curve Hydraulic conductivity Water-holding capacity Air-filled porosity Bulk density	Moderate	High
Frozen column	Dynamic and static characteristics Moisture retention curve Water-holding capacity Air-filled porosity Bulk density	Low	Moderate
Gravimetric	Static characteristics Water-holding capacity Air-filled porosity Bulk density	Low	Moderate
Computed tomography	Static characteristics Visualization of water and air Spatial distribution of water and air Water-holding capacity Air-filled porosity	High	Very high

Table 3. Summary of whole-cell analysis by gravimetric or computed tomography (CT) for volumetric water content (VWC) and volumetric air content (VAC) 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. Least-square means for gravimetric and CT analysis were the result of n = 3 cells for each treatment combination, and letters after VWC and VAC represent mean separation using Tukey's honestly significant difference at α = 0.05.

Substrate	Methods	VWC, %	VAC, %
Peat	Gravimetric	65 b	11 a
	CT	70 a	6 b
Rockwool	Gravimetric	87 a	5 a
	CT	87 a	5 a
Foam	Gravimetric	91 a	7 a
	CT	93 a	5 a

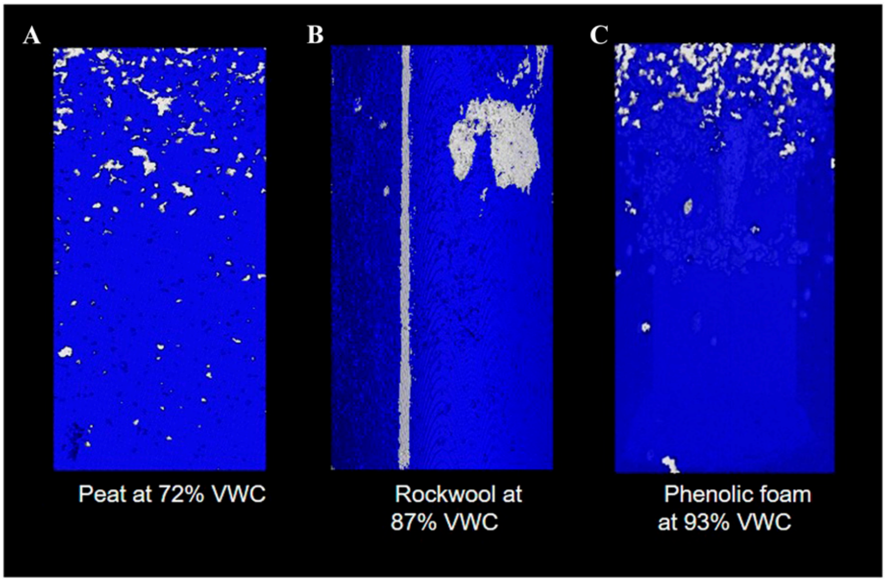

Fig. 2. Images of computed tomography–scanned substrate showing the water and air distribution in substrates at container capacity in propagation-sized cells. Images show one example scan per substrate (**A**–**C**). Container capacity was achieved by overnight subirrigation of substrates, followed by draining for 30 min. Substrate with dark gray 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.

to 58% at the inflection point (7.7 cm), and further decreased to 7% at 27-cm tension (Fig. 1f). In both substrates, the frozen column method clearly demonstrated low water 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 water 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 θ_r, X_0, and n did not overlap between methods (Table 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 4 h 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 previously, the θ_s parameter was set as a constant when fitting Eq. [1] for the evaporation data (Table 1). In foam, although curve-fitting parameters of θ_s and X_0 did not overlap, the *t* test comparison did not show significant differences between methods. Comparison of MRCs by paired *t* test within a method (evaporation or frozen column) for all possible paired comparisons resulted in differences across substrates that describe their unique water-retention properties.

The evaporation method allowed quantification of VWC at a higher soil tension than the frozen column method, and was therefore more accurate for quantifying θ_r and plant unavailable water for peat. However, for the low water 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 skill and has increased equipment cost compared with the frozen column substrate method (Table 2). We also noted greater variability in VWC data at low tensions with the tensiometer in rockwool and foam (Fig. 1). In addition to MRCs, the evaporation method has been used to describe hydraulic conductivity and substrate porosity (Fields et al., 2016, 2017; Naasz et al., 2005; Owen and Altland, 2008; 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). 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 VAC of <10% has been shown to have greater severity in root rot of toyon compared with VAC of 10% to 20% (Filmer et al., 1986).

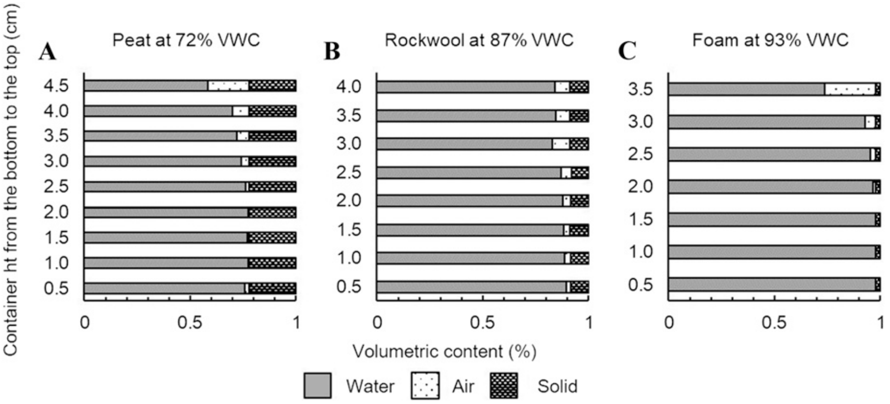

Fig. 3. Spatial quantification for (**A**) peat, (**B**), rockwool, and (**C**) foam using computed tomography for volumetric water and air content at container capacity. Volumetric solid content was measured gravimetrically and used as a constant. Horizontal bars represent least squares means from n = 3 replicates per substrate. X-axis is the total percent volumetric content. Y-axis is the container depth from the bottom to top (cm).

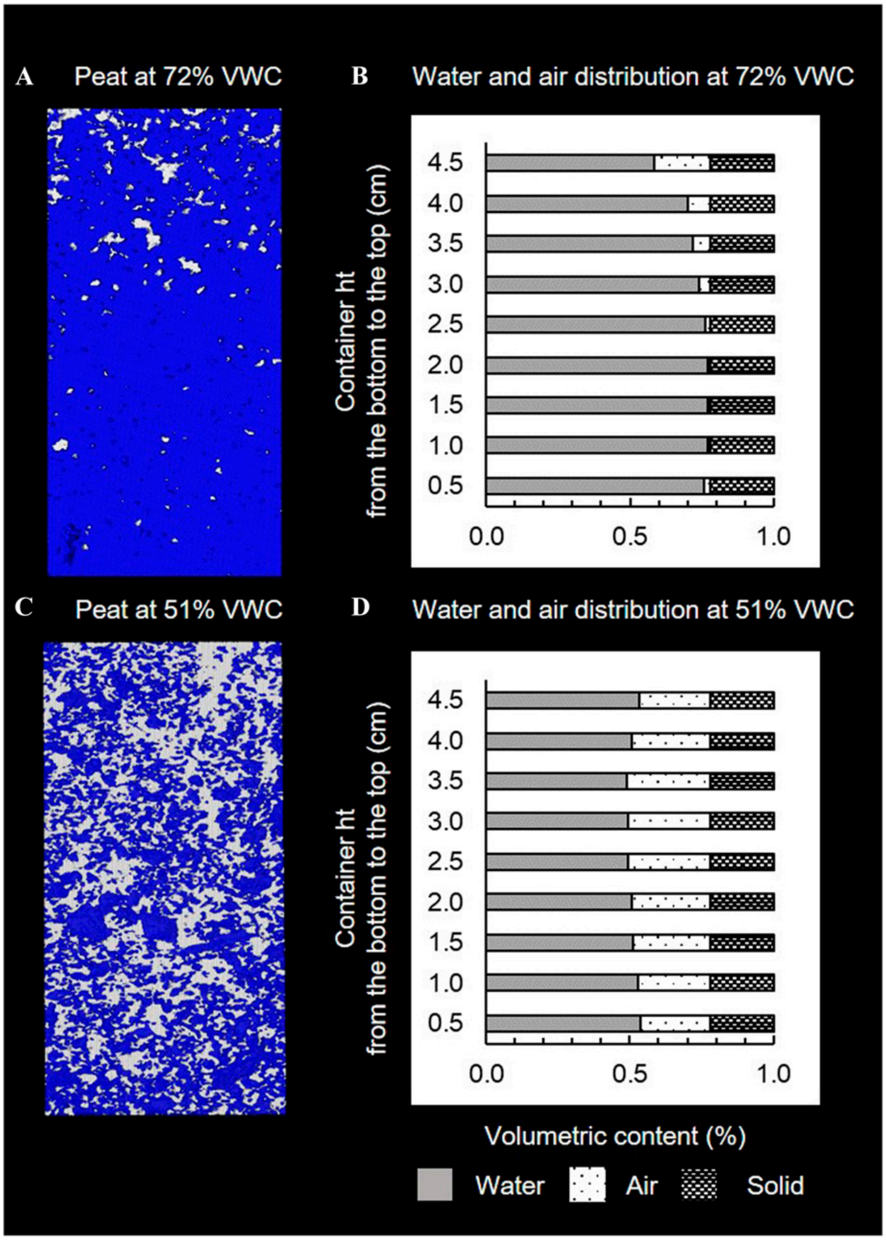

Fig. 4. CT images of peat and spatial quantification from container capacity (**A**, **B**) and as substrate dried to 51% VWC (**C**, **D**). Spatial quantification represents the least squares means of n = 3 replicates per moisture level.

Increasing container height results in increased air porosity, as gravity increases drainage in a given substrate, thus providing greater gradient 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 because 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 2).

Method 4. X-ray tomography (CT) whole-cell analysis. Quantification of VWC and VAC by gravimetric vs. CT scanning methods differed for peat but not for the other two substrates (Table 3). 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 Fig. 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). In rockwool, air was mostly present in the vertical planting slit, which is part of the design of this propagation substrate to allow insertion of the plant cutting (Fig. 3B). The scan resolution was 59 μm, which means that VACs 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). In contrast, 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 Fig. 4 for peat at container capacity (72% VWC) provides a visual representation that high moisture level is likely to block the movement of air, whereas allowing the substrate to dry to 51% VWC would increase 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. 4 and Table 4), 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).

Table 4. Cell spatial quantification by computed tomography (CT) for volumetric water content (VWC) or volumetric air content (VAC) in peat at container capacity (72% VWC) and when substrate dried to medium moisture (51% VWC), represented visually in Fig. 4. VWC or VAC by depth represents the least-square means of n = 3 replicates per moisture level and letters after VWC and VAC represent mean separation using Tukey's honestly significant difference at $\alpha = 0.05$. Volumetric solid content was estimated at 22%.

Moisture level	Depth (cm)	VWC, %	VAC, %
Container capacity	0.5	58 c	20 b
	1	70 b	8 c
	1.5	72 ab	6 cd
	2	74 ab	4 cd
	2.5	76 ab	2 cd
	3	77 a	1 d
	3.5	77 a	1 d
	4	77 a	1 d
	4.5	76 ab	2 cd
Medium	0.5	53 cd	25 ab
	1	51 d	27 a
	1.5	49 d	29 a
	2	50 d	28 a
	2.5	49 d	29 a
	3	51 d	27 a
	3.5	51 d	27 a
	4	53 d	25 ab
	4.5	54 cd	24 ab

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. (2015a, 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, which requires specialized equipment and image segmentation software that is costly, complicated, and time-consuming (Table 2). Estimated time from start to finish for CT quantitative analysis of one sample was 1.5 to 3.0 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 Fig. 4, these methods can be combined with evaporation data to generate MRCs (Daly et al., 2015).

Conclusion

Water and air relations of three propagation substrates were quantified by evaporation or frozen column methods. MRCs described low water 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 water potential is necessary to ensure adequate balance of water and air appropriate to the production phase.

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