Stratified Substrates Can Reduce Peat Use and Improve Root Productivity in Container Crop Production

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Abstract. Peat use in horticulture continues to be scrutinized as consumers are becoming increasingly aware of the environmental sustainability concerns associated with peat. Thus, the horticultural industry is driven to search for peat alternatives. Substrate stratification (i.e., vertical layering of unique media atop another in a singular container) has been studied in nursery substrates and has demonstrated improved resource efficiency with regard to water and fertilizer inputs. However, minimal research has evaluated using the concept of stratified substrates as an attempt to reduce peat inputs in greenhouse production. Hence, the objective of this study was to identify if stratifying costly floriculture media atop of low-cost pine bark can reduce peat use, reliance, and cost within the floriculture industry. A floriculture crop, Petunia hybrid ‘Supertunia Honey’, was grown in two distinct substrate treatments: 1) nonstratified (commercial peat-based floriculture substrate) and 2) stratified peat-based substrate layered atop aged pine bark (1:1 by volume) under two different irrigation schedules. Crop growth was evaluated, including growth indices, shoot physiological responses, and root growth measurements. Substrate hydraulic properties such as matric potential and volumetric water content were monitored over time. The results demonstrated that a petunia crop can be produced in stratified substrate systems and yield similarly sized and quality crops as traditionally grown plants. Furthermore, the stratified substrate-produced crop had improved root productivity, yet less bloom, when compared with nonstratified-grown crops.

The nursery and floriculture industries generate substantial annual revenue ($10 billion) and have drastically increased in sales since the beginning of the 20th century (US Department of Agriculture 1998, 2020). Most of these crops are produced in container systems (US Department of Agriculture 2020) that have been shown to be advantageous in production when compared with traditionally grown in-field crops (Whitcomb 1984). Currently, there is a growing transition in fruit and vegetable production from soil-based to soilless culture (Fields et al. 2021a), primarily due to efficient use of resources, limitations on fumigant use, and improved access. There is also a humanitarian aspect in the shift to soilless production. Container crop production does not require arable land, allowing this cost-effective production practice to be implemented near urban areas and can be used to meet growing nutritional needs of economically stressed neighborhoods and combat food deserts. Recent research has indicated that staple crops such as potatoes have explored transitioning their production practices to soilless culture (Brocio et al. 2022). This alone demonstrates the importance of substrate materials toward sustaining the global population, not just through horticultural crop production, but through staple crop agriculture as a whole.

It is evident that the future of horticulture and agriculture will rely heavily on soilless substrates (Raviv and Leith 2008), and the demand for substrate materials is rapidly increasing. Blok et al. (2021) provide informative models that estimate that by 2050, there will be a 490% and 260% increase in global ornamental and vegetable production, respectively. This is a result of several factors: 1) an estimated growth in human population (i.e., + 2 billion; FAO 2014), 2) an increase in global income tends to equate with higher standards of living and calls for more agricultural commodities (Fraiture and Wichelns 2009), 3) global shifts from rural living to urbanization are expected to increase from 50% to 70% by 2050 (FAO 2009), which 4) pushes for the increase in ornamental crop production due to urban resident desire to live in aesthetically pleasing environments (i.e., street views, parks, container terrace gardens, etc.). As such, to sustain these intense demands of horticultural crop and food production, there will be considerable increase in requirements for soilless substrates, especially in peat materials, which is estimated at a 200% increase (Blok et al. 2021).

Peat moss has been the primary substrate component for >60 years due to its desirable physical and chemical properties (i.e., enhanced porosity, water retention, and cation exchange capacity) for containerized crop production (Baker 1957). However, peat use in horticulture continues to be scrutinized, as many European countries have restricted or limited peat inputs (Barrett et al. 2016), and there is public criticism on the sustainability of harvesting peat moss. Moreover, as climate change worsens, unpredictable weather patterns can limit peat harvest yield (Bragazza 2008), which can result in downstream supply chain shortages, affecting growing operations worldwide. In addition, peat substrates can be costly compared with other soilless substrates (Raviv and Leith 2008). The economic, ecologic, and social consequences of peat reliance justify identifying peat-alternative substrate materials in horticulture.

Considering soilless substrates (i.e., peat) are in high demand and there is increasing evidence that warrants reducing peat usage, the horticultural industry must identify new and alternative options for soilless production. Recent substrate research has indicated unique approaches to integrate sustainability (e.g., water and mineral nutrient conservation) within soilless cultivation and may further provide opportunities to relieve the anticipated substrate exigencies with regard to horticultural peat reliance, cost, and sustainability. Soilless substrate stratification, a developing substrate management strategy, involves laying two unique media atop another within a singular container and it continues to gain interest nationally and internationally (Fields et al. 2021b). The potential use of stratified substrates has been shown to improve natural resource efficiency by modifying the physical nature of the container-substrate profile (Fields et al. 2021b). Preceding stratified research has layered fine-textured substrates (e.g., to enhance water and mineral nutrient retention) in the upper container proportions, whereas in the lower proportions, coarse substrates were used to encourage drainage and reduce deleterious water table effects (Criscione et al. 2022a, 2022b; Fields et al. 2021b). Promising results have demonstrated that substrate stratification may support possibilities for nursery growers to reduce water and fertilizer inputs by 25% and 20%, respectively, and continue producing equivalent or better crops than traditionally used nursery production methods (Criscione et al. 2022a; Fields et al. 2021b). Evidence has also shown improved containerized crop rooting in stratified systems when compared with conventional growing practices (Fields et al. 2022). Moreover, others have
Stratified substrate concepts are used to stratify controlled-release-fertilizer placement (Ammons et al. 2022) and to reduce weed germination and associated herbicide application costs in container systems (Khamare et al. 2022). As substrate stratification research continues, additional benefits for this practice in greenhouse crop production systems will arise.

Some cropping systems (e.g., floriculture and greenhouse crops) depend on peat-based media for crop productivity to reach peak crop quality and quick salability. Stratifying high-performance peat-based substrates atop of low-cost pine bark may present benefits for growers in the form of reducing peat use and subsequently lowering substrate-related expenditures. To expand, a high-performance peat-based substrate may cost upward of 10 times that of a pine bark substrate (by volume) for growers in the southeastern United States. Therefore, reducing the volume of peat-based substrates required may subsequently result in decreased associated costs. Dennis et al. (2010) found that growers felt the largest barrier to incorporating more sustainable practices in their production was compatibility with their current production systems and infrastructure. Recent on-farm trials that stratified peat atop bark have indicated that incorporating stratified substrate management practices was an easy and quick transition. More research is warranted to identify any added time and resources (i.e., equipment and labor) it requires to transition in implementing stratification techniques within floriculture production.

The objective of this research study was to identify cost-effective alternative substrate management practices for direct application into floriculture production. Specifically, the goal was to identify if crops can be produced in a peat-reduced substrate system. This goal was accomplished by evaluating crop performance and monitoring substrate hydraulic properties in situ throughout a production cycle when high-quantity peat-based substrates are stratified atop relatively inexpensive aged pine bark. Based on preliminary evaluations, the authors hypothesize crops grown in stratified peat-based substrates will be of similar or superior quality when compared with nonstratified substrate grown crops.

### Materials and Methods

**Substrate preparation.** Aged pine bark (*P. taeda*; Phillips Bark Processing Co., Brookhaven, MS, USA) was limed with 1.77 kg m\(^{-3}\) of dolomitic lime (Lime-Rite Pelletized Dolomitic Lime, Roswell, GA, USA) and 0.89 kg m\(^{-3}\) of micronutrients (Micromax G90505; ICL Specialty Fertilizer, Dublin, OH, USA). A commercial floriculture substrate (50% Canadian Sphagnum Peat Moss, Aged Pine Bark, Perlite, and Vermiculite; Jolly Gardner Pro-Line C/P, Atlanta, GA, USA) was also procured. Porometer analysis was conducted to measure substrate static physical properties (air space, container capacity, total porosity, and bulk density) as described by Fonteno and Harden (2010). Particle size distribution was measured via porometer analysis. Total porosity was calculated from the lysimeter data to attain more precise values depicting these tensiometers in the root zone. Water potential values were obtained from the lysimetry data. Shoot fresh weight was measured at five separate times (one container replicate within each zone) throughout the study. Within each treatment, shoot fresh weight was plotted against days after irrigation initiation. Regression analysis was used to predict shoot fresh weight of each substrate placed on lysimeters. Shoot fresh weight was measured after 48 h at 105°C. The substrates were then weighed to attain a dry substrate. Substrate dry weight + container weight was subtracted from the lysimeter data. Shoot fresh weight was measured on all plants fertigated every other week (600 mL) with 200 ppm N liquid fertilizer (20N–20P–20K; Peters Professional Fertilizer, Summerville, SC, USA).

### Monitoring substrate moisture status

Lysimeter data were used to calculate the substrate system’s volumetric water content. This was conducted on culminations of the study, where the substrates placed on lysimeters were dried for 48 h at 105°C. The substrates were then weighed to attain a dry weight, and the substrate dry weight + container weight was subtracted from the lysimeter data. Shoot fresh weight was measured five separate times (one container replicate within each zone) throughout the study. Within each treatment, shoot fresh weight was plotted against days after irrigation initiation of the study and a regression line was fitted using JMP Pro (16.1.0; SAS Institute, Inc., Cary, NC, USA). The equation attained for nonstratified regular irrigation, nonstratified deficit irrigation, stratified regular irrigation, and stratified deficit irrigation (Eqs. [1] to [4], respectively) was used to predict shoot fresh weight each day of the study and the predicted fresh shoot weight was subtracted from the lysimeter data to attain more precise

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Container capacity cm(^3) cm(^{-3})</th>
<th>Air space (%)</th>
<th>Total porosity (%)</th>
<th>Bulk density g cm(^{-3})</th>
<th>Extra large (&gt;6.3 mm)</th>
<th>Large (6.3–2.0 mm)</th>
<th>Medium (2.0–0.7 mm)</th>
<th>Fine (&lt;0.7 mm) g cm(^{-3})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional bark</td>
<td>0.55</td>
<td>0.29</td>
<td>0.83</td>
<td>0.17</td>
<td>28.8</td>
<td>41.8</td>
<td>18.0</td>
<td>12.1</td>
</tr>
<tr>
<td>Peat-based media</td>
<td>0.67</td>
<td>0.16</td>
<td>0.83</td>
<td>0.15</td>
<td>&lt;0.0001</td>
<td>0.0594</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>P value</td>
<td>0.0002</td>
<td>0.0002</td>
<td>0.5370</td>
<td>0.0633</td>
<td>4.3</td>
<td>35.4</td>
<td>33.9</td>
<td>27.0</td>
</tr>
</tbody>
</table>

*Measured via porometer analysis. Total porosity = minimum air space (AS); minimum air-filled porosity after free drainage + maximum water holding (container capacity; CC; maximum water holding capacity after free drainage).%

*Percent of particle dry weight occupying extra large > 6.3 mm, large > 2.00 mm, medium > 0.71 mm, and fine < 0.71 mm.

*Percent overall treatment effects using analysis of variance with a significance value of α = 0.05.
calculations.

Fresh shoot biomass $= \frac{5/C_0}{3/C_2}$ (days after transplant) $[1]$

Fresh shoot biomass $= \frac{-10.499 + 1.708}{1.025}$ (days after transplant) $[2]$

Fresh shoot biomass $= \frac{1.619 + 1.061}{(days \text{ after transplant})}$ $[3]$

Fresh shoot biomass $= \frac{-3.586 + 1.061}{(days \text{ after transplant})}$ $[4]$

Harvests. A total of four harvests occurred throughout the study starting 8 d after irrigation initiation. Each additional harvest occurred every subsequent 15 ± 1 d. Culmination of the study was 50 d after irrigation initiation (10 May 2022). Every harvest consisted of randomly selecting a single plant per zone. For each harvest, representative crop photos were captured, along with plant growth index [(plant height + width + perpendicular width) ÷ 3], flower count, chlorophyll content (SPAD 502 Plus; Spectrum Technologies, Inc., Aurora, IL, USA), shoot fresh weight, and root morphological traits were measured. Shoot and root dry weight were measured by drying the plant samples at 70°C for 7 d. For the final harvest, all substrate root systems were partitioned in half (at the stratified interface; 8.25 cm) and dry root biomass of the top and lower half of the container was measured.

RhizoVision. Root system morphology was assessed at each harvest event. The total root length, the quantity of root tips and branching points, average root diameter, and total root surface area were estimated via RhizoVision software (Seethepalli et al. 2021). In preparation for root imaging analysis, roots were cleaned completely from substrate debris and segmented to fit in a square container (8244 cm$^3$) on standard white printer paper (21.6 cm × 27.9 cm). The container was filled with 400 mL of deionized water.

Fig. 1. Particle size distribution curve of conventional pine bark and peat-based (50% Canadian Sphagnum Peat Moss, Aged Pine Bark, Perlite, and Vermiculate) substrates. Each error bar is constructed using a 95% confidence interval estimate of the mean.

Fig. 2. Plant growth index. Crops were grown in one of two experimental substrate treatments [nonstratified (100% floriculture mix) and stratified (50% floriculture mix layered atop conventional bark)] irrigated under two different irrigation schedules [regular (400 mL/2 d) and deficit (400 mL/3 d)]. Error bars represent 1 standard error from the mean. The NS, *, **, *** values represent nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.
Nonstratified Substrate Irrigation Shoot biomass (g)
Nonstratified Regular 4.0 fg a 0.4 c 1.734.6 d 1.488.6 e 2.470.9 d 0.23 abc 122.5 c
Stratified Regular 3.9 fg 0.8 bc 1.923.4 cd 1.749.9 cde 3.078.3 cd 0.21 abcd 124.1 c
Nonstratified Deficit 4.2 fg 0.6 bc 2.038.0 cd 1.659.8 cde 2.803.9 cd 0.24 a 148.1 bc
Stratified Deficit 2.9 g 0.8 bc 1.994.4 cd 1.686.1 d 3.164.5 cd 0.23 ab 142.8 bc
P value Substrate - 0.4418 0.0697 0.8636 0.7087 0.4882 0.1436 0.9531
P value Irrigation - 0.6963 0.5554 0.6594 0.8885 0.7611 0.0732 0.4764
P value Sub×Irr - 0.5492 0.4140 0.7837 0.7599 0.8577 0.3146 0.9094

Stratified Substrate - 0.0013 0.9312 0.6978 0.4175 0.6619 0.1819 0.8086
P value Substrate - 0.0047 0.0358 0.3133 0.1192 0.2996 0.1382 0.3782
P value Irrigation - 0.6581 0.9312 0.8666 0.5178 0.7958 0.2338 0.9605

Harvest 1

Nonstratified Regular 10.9 cde 1.3 ab 3.602.5 bcd 3.280.8 bcde 5.946.9 abcde 0.21 bcd 230.3 bc
Stratified Regular 7.3 cde 1.3 ab 3.847.2 bcd 3.823.6 bcde 6.090.5 abcde 0.20 d 238.6 bc
Nonstratified Deficit 8.7 ef 1.1 abc 3.219.0 bcd 2.902.3 bcde 4.887.2 bcd 0.21 bcd 205.9 bc
Stratified Deficit 5.7 cde 1.1 abc 3.316.4 bcd 2.965.5 bcde 5.442.6 bcd 0.21 bcd 211.4 bc
P value Substrate - 0.0013 0.9312 0.6978 0.4175 0.6619 0.1819 0.8086
P value Irrigation - 0.0239 0.3558 0.3133 0.1192 0.2996 0.1382 0.3782
P value Sub×Irr - 0.6581 0.9312 0.8666 0.5178 0.7958 0.2338 0.9605

Harvest 2

Nonstratified Regular 19.6 a 1.5 ab 4.581.1 ab 4.381.7 abc 7.188.0 ab 0.20 d 283.9 ab
Stratified Regular 14.2 bcd 1.4 ab 4.592.3 ab 4.267.7 bcd 7.152.8 ab 0.20 d 283.5 ab
Nonstratified Deficit 10.3 cde 1.2 abc 3.057.8 bcd 2.968.5 bcd 4.434.5 bcd 0.20 cd 191.6 bc
Stratified Deficit 7.5 cde 1.2 abc 3.594.9 bcd 3.666.4 bcd 5.578.4 bcd 0.20 d 218.3 bc
P value Substrate - 0.0001 0.8361 0.0334 0.1878 0.0175 0.9571 0.0258
P value Irrigation - 0.2545 0.6879 0.6072 0.5777 0.4387 0.2515 0.6516

Harvest 3

Nonstratified Regular 20.2 a 1.3 ab 4.058.0 bc 3.511.9 bcd 6.386.0 abc 0.21 abcd 263.8 abc
Stratified Regular 15.6 abc 1.9 a 6.429.2 a 6.983.5 a 9.603.2 a 0.20 d 392.1 a
Nonstratified Deficit 16.2 ab 1.5 ab 4.058.0 bc 3.847.4 bcd 6.019.8 abc 0.21 bcd 263.5 abc
Stratified Deficit 9.8 de 1.4 ab 3.861.4 bcd 4.670.0 ab 5.354.9 bcd 0.20 d 234.6 bc
P value Substrate - 0.0025 0.0835 0.0152 0.0041 0.0928 0.0531 0.1032
P value Irrigation - 0.0051 0.2368 0.0066 0.1045 0.0087 0.0852 0.0193
P value Sub×Irr - 0.5026 0.0405 0.0056 0.0398 0.0198 0.9159 0.0197

Harvest 4

*Plant tissue was dried at 70°C for 7 d and weighed.
**RhizoVision output data measuring root morphology traits.
***Measures of overall treatment effects using analysis of variance with a significance value of α = 0.05 within each harvest across two substrate and irrigation treatments.
****Letters denote detected differences among means using Tukey’s highly significant difference test (α = 0.05) within entire columns.

To ensure each photo was taken at identical water to spread apart individual roots. Once the roots were laid carefully and separated to the paper, a photo was taken with an iPhone XR (Apple, Cupertino, CA, USA). To ensure each photo was taken at identical heights in darkness, a light inhibiting root imaging chamber apparatus was constructed with a hole at the top for camera placement. Root images were scanned with TurboScan (Piksoft Inc, Piedmont, CA, USA) and converted to a PDF format. This was required because a flash from the photo created a glare in the image and RhizoVision was not able to correctly analyze the photo. Thereafter, images were cropped to the paper dimensions. The new files were uploaded to the RhizoVision software, and the dots per inch were calibrated to 326 in RhizoVision (iPhone XR camera specifications). Root systems were larger than the square container used; thus, roots were divided into segments and analyzed. Therefore, measured output from RhizoVision required summing the values within each sample besides average diameter.

Data analysis. The data presented in tables and figures with associated statistics were analyzed in JMP Pro (16.1.0; SAS Institute, Inc., Raleigh, NC, USA) using analysis of variance (ANOVA) to identify any significant statistical differences across the means of the responses previously described. If significant, post hoc Tukey’s honestly significant difference test (α = 0.05) was used to separate means across two substrates and crop growth performance among irrigation schedules, such as growth index, flower count, chlorophyll content, dry weights, and...
root growth (total length, number of root tips, number of branching points, average diameter, and total surface area).

All tensiometer and lysimetry data were analyzed in JMP Pro (16.1.0). Weekly mean substrate matric potential values were calculated in the upper and lower strata and $\Theta$. In addition, an ANOVA was used to identify any significant statistical differences in means of matric and $\Theta$ values within irrigation schedules across substrate treatments. Pearson’s pairwise correlation was used to determine the strength of these linear relationships.

**Results and Discussion**

**Substrate physical properties.** There are unique relationships between substrate particle diameter classifications and their respective physical and hydraulic properties (Nkongolo and Caron 1999). For peat-based substrates, 60% of the particle diameters were < 2.0 mm, whereas 70% of the bark substrate particle diameters were > 2.0 mm (Table 1; Fig. 1). Decreasing substrate particle diameters generally exhibits negative and positive relationships to substrate water and air storage capacities, respectively (Kiran et al. 2021). Due to capillary pore proportions (Puustjarvi and Robertson 1975), large particle substrates such as bark have greater pore sizes (Drzal et al. 1999), which increases substrate drainage (Hoskins et al. 2014) in comparison with other soilless substrates. The bark media retained significantly less water ($P = 0.0002$) than the peat-based media; however, peat substrates are inherently fibrous and have increased microporosity, which resulted in lower air-filled porosity values than bark ($P = 0.0002$; Table 1). Altland et al. (2011) and Fields et al. (2021b) described that manipulating substrate particle diameters will often change AS/CC balances, while having little to no effect on substrate TP values as observed herein (Table 1).

**Evaluating crop production.** On study initiation, all crops were equivalent in size in both the control ($P = 0.7046$) and deficit ($P = 0.2842$) irrigation schedules (Fig. 2). Plant shoot growth increased similarly across treatments throughout the study, where midway through production, it appears that crops grown in nonstratified systems were slightly larger under both irrigation treatments (Fig. 2). However, there were no detectable differences in the final crop growth index on study culmination under either irrigation regimen or substrate system (Fig. 2). Plants grown with more frequent irrigation events had a greater final growth index than plants grown with less frequent irrigation ($P = 0.0291$), regardless of substrate system ($P = 0.7607$; Fig. 2). Crop growth in a nonstratified system irrigated regularly appeared to stop growing in the final two growth index measurements, whereas crops grown in a stratified system irrigated regularly continued to grow. Criscione et al. (2022a) received similar results, although in a deficit irrigation, traditionally
grown crops near the end of the study began to decrease in growth while all stratified treatments continued to outperform crops grown in non-stratified systems. There was a significant main effect on shoot biomass for both substrate ($P < 0.0025$) and irrigation ($P < 0.0051$), where crops grown in stratified substrate systems under deficit irrigations weighed less than all other treatment combinations in the final harvest (Table 2). Nevertheless, despite statistical differences in shoot biomass, there were few differences in crop marketability and growth, providing insight that growers can possibly shift their substrate systems and still produce salable crops (Fig. 3).

Crop chlorophyll count was greater in both deficit treatments on 58 d after production (Fig. 4). Thereafter, chlorophyll count remained relatively similar across treatments throughout the remainder of the study (Fig. 4). This was not hypothesized considering drought stress typically reduces chlorophyll count (Yang et al. 2021). Plants grown in nonstratified substrate systems consistently exhibited more blooms and had greater total flower count than crops grown in stratified media (Fig. 4). This was detected in both the control ($P = 0.0300$) and deficit ($P = 0.0600$) irrigation schedules (Fig. 4). Drought or water-stressed conditions have been shown to induce reproductive and physiological traits, such as flowering in some fruit crops (Wu et al. 2017) and in petunias (Hatamifar and Samani 2017). In addition, fine roots, which are responsible for water and mineral nutrient absorption (McCormack et al. 2015), have been observed in some container crops to contain greater concentrations in the shallowest substrate depths (Bauerle et al. 2013).

Greater flower count in nonstratified-grown crops may be attributed to several factors. Stratified root production visually appears to be greater in the upper strata and takes longer to explore the lower strata than that of crops grown in nonstratified systems (Fig. 5). Moreover, nonstratified substrate systems, regardless of the type of media used, contain a moisture content depth profile, resulting in drier upper layers and wetter lower layers (Owen and Allland 2008). Although not measured directly, it is possible that in the upper layers stratified roots contained greater quantities of fine roots, which enabled better water absorption even under stressful conditions (Cuneo et al. 2021), and resulted in less water-stress-induced flowering (Figs. 4 and 5). Thus, crops grown in stratified substrate systems possibly reduced water-stress...
symptoms (i.e., increased flowering) as observed in nonstratified-grown plants.

**Root growth and development.** Root morphological characteristics were estimated through RhizoVision software (Seethepalli et al. 2021) and are displayed in Table 2. There were no treatment effects for any morphological trait until the third crop harvest, where irrigation had strong effects on total root length ($P < 0.0334$), branch point count ($P < 0.0175$), and total root surface area ($P < 0.0258$; Table 2). In the final harvest, there were several statistical differences across treatments. Crops growing in a stratified substrate under the control irrigation contained greater root morphology characteristic values than the nonstratified system in total root length ($P < 0.0056$) and root tip count ($P = 0.0398$; Table 2). Balliu et al. (2021) described that increasing irrigation frequency generally promotes root development and health. Crops grown under the control irrigation, regardless of substrate system, typically contained more morphologically defined root traits than those grown under irrigated deficit (Table 2). The lack of differences in average root diameter may indicate that any increase in root mass was driven by increased root lengths (Table 2).

Illustrated in Fig. 6A are rhizosphere profiles partitioned in half, demonstrating that crops growing in stratified systems had increased lower root biomass under both regular ($P = 0.0061$) and deficit ($P < 0.0001$) irrigation schedules than nonstratified-grown crops. Savvas and Gruda (2018) stated that substrates that contain greater aeration in the root zone typically result in improved root growth. Moreover, the substrate moisture content depth distribution influences root spatial distribution (Wallach 2008), where root growth typically mimics the substrate moisture profile (Ismail and Ozawa 2009). Considering the stratified system was developed to retain more moisture in the upper strata, it is likely that in the nonstratified system, moisture infiltrated into the lower proportions more readily due to gravitational effect (Owen and Altland 2008). This would subsequently increase aeration and gas diffusivity at the container base of the stratified substrates (Cannavo and Michel 2013); therefore, root growth likely was encouraged in the substrate with increased aeration, resulting in greater biomass (Fig. 6A) and total root length (Table 2; Fig. 6B). Root growth appeared to have occurred at different spatial
rates in substrate systems, where stratified-grown crops seemed to have explored the upper portion of the profile longer than nonstratified-grown roots (Fig. 5). However, once stratified roots explored into the lower half, root growth flourished (Figs. 5 and 6).

Particle and pore distribution play fundamental roles in water redistribution, hydraulic conductivity, and water retention and availability for plants (Hille 2004). Improved soilless substrate unsaturated hydraulic conductivity has been shown to improve plant vigor and water acquisition, especially in deficit conditions (Fields et al. 2017). Fields et al. (2018) stated that finer materials such as sphagnum peat have greater unsaturated hydraulic conductivity values than larger substrates like bark. Moreover, Bartley et al. (2022) reviewed particle densities of horticultural substrates and concluded that peat-based substrates have greater particle densities than bark. Although nonstratified systems contained more peat-based substrate (by volume), the container base may have experienced oxygen deficit conditions (Cannavo and Michel 2013). Thus, the greater particle density and improved unsaturated hydraulic conductivity in the upper strata helped promote more root growth under deficit conditions in stratified systems, whereas the decrease particle density and less water storage in the lower strata (e.g., bark) promoted more root growth (Fig. 6A).

Monitoring substrate hydraulic properties. Substrate profile water potential (ψ) and Θ measurements were averaged weekly after irrigation scheduling began and are plotted in Figs. 7 and 8. There were no statistical differences detected across substrate treatments within either irrigation schedule for ψupper and ψlower (Fig. 7); however, there were differences detected among Θ across each substrate system and irrigation schedule (Fig. 8). Substrate as a main effect had no influence, but irrigation scheduling had an effect on Θ values for most of the study, where substrates irrigated more frequently had greater Θ (Fig. 8).

Nonetheless, there were moderate correlations between substrate hydraulic measurements and plant tissue traits. To expand, root biomass was more correlated with Θ than shoot biomass (r = −0.6370 and −0.4856, respectively; Table 3), which can be attributed to root water uptake in the substrate. In addition, root biomass was more strongly correlated with ψlower than ψupper (r = −0.3199 and −0.1815, respectively; Table 3).

These results align with the data depicted in Fig. 6A, where all crop roots had significantly greater root biomass in the lower container portion than upper portions except for nonstratified-grown crops under regular irrigation. Although not measured directly, roots growing in peat-based substrates may have contained greater fine root proportions, whereas roots growing in bark substrates may have contained larger roots due to the larger substrate pore diameters. Thus, the greater root biomass in the lower stratum may be attributed to larger roots. These results contrast the results observed by Fields et al. (2022), where there were greater root biomass values measured in the upper strata than lower. However, the system used fine bark particles layered over coarse bark; thus, there were different pore diameters and distributions (peat-based substrate vs. screened fine bark particles; unscreened bark vs. screened coarse bark).

Volumetric water content measurements were more strongly correlated with ψlower than ψupper, further validating moisture content distributions due to gravity in a substrate system (Owen and Alltland 2008). Among the root morphology traits, root diameter had the strongest correlation with Θ (r = 0.5936). This may indicate that more fine roots (smaller diameters) present in a system can have greater effect on substrate moisture status (i.e., more fine roots = more water uptake = decreased Θ; McCormack et al. 2015). It is possible that crops responded more to Θ than ψ because of the accuracy of the lysimeter systems vs. the elbow tensiometers, although the literature discusses that plants tend to respond more to Θ because of the amount of “work” or “energy” required to extract moisture from the substrate matrix (Cassel and Nielsen 1986; Hillel 2004).

### Table 3. Pairwise Pearson correlations between plant tissue measurements (shoot and root biomass, total root length, quantity of root tips and branching points, average root diameter, and total root surface area) and substrate hydraulic measurements (upper and lower matric potential values and volumetric water content), regardless of treatment. Values were measured from rootstocked containers. Crops were grown in one of two experimental substrate treatments [nonstratified (100% floriculture mix) and stratified (50% floriculture mix layered atop conventional bark)] and irrigated under two different irrigation schedules [regular (400 mL/2 d) and deficit (400 mL/3 d)].

<table>
<thead>
<tr>
<th></th>
<th>Biomassshoot</th>
<th>Biomassroot</th>
<th>Roottip count</th>
<th>Rootbranch point count</th>
<th>Total root length</th>
<th>Rootdiam</th>
<th>Rootsurface area</th>
<th>Upper ψ</th>
<th>Lower ψ</th>
<th>Θ</th>
</tr>
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<td>Biomassshoot</td>
<td>1.0000</td>
<td>0.6784</td>
<td>0.5583</td>
<td>0.6451</td>
<td>0.6517</td>
<td>−0.3926</td>
<td>0.6456</td>
<td>0.0243</td>
<td>−0.1780</td>
<td>−0.4856</td>
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<td>0.6761</td>
<td>0.6811</td>
<td>−0.5347</td>
<td>0.6488</td>
<td>−0.1815</td>
<td>−0.3199</td>
<td>−0.6370</td>
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<tr>
<td>Roottip count</td>
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<td>0.8759</td>
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<td>−0.6916</td>
<td>0.8891</td>
<td>−0.2068</td>
<td>−0.1892</td>
<td>−0.4786</td>
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<td>Rootbranch point count</td>
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</tr>
</tbody>
</table>

\( ^{\dagger} \) Plant tissue was dried at 70 °C for 7 d and weighed.

\( ^{\dagger\dagger} \) RhiizoVision output data measuring root morphology traits.

\( ^{\ddagger} \) Matric potential (kPa) of the upper 50% substrate profile. Elbow tensiometer was installed 25% below the container top.

\( ^{\dagger\ddagger} \) Matric potential (kPa) of the lower 50% substrate profile. Elbow tensiometer was installed 75% below the container top.

\( ^{\ast} \) Volumetric water content (cm\(^{-3}\) cm\(^{-3}\)) calculated gravimetrically through lysimetry.

### Conclusion

This study evaluated the potential of reducing the use of peat in greenhouse and floriculture production, thereby reducing production costs and material dependence. Costly peat-based media was stratified atop of inexpensive pine bark, reducing peat inputs by nearly 50%. This study further examined if crops of equivalent quality can be produced with reduced irrigation inputs, conserving water resources and increasing water use efficiency. The results herein demonstrated that a popular floriculture crop, petunia, can be grown in stratified peat-based media and produce similarly sized and quality crops as traditional, single-substrate-grown greenhouse plants. Moreover, stratifying peat-based substrates above pine bark enhanced crop rooting (biomass and morphological traits), yet decreased flowering (a primary objective in greenhouse crops). This study opens new routes of stratified substrate research, in which stratified-grown plants may experience less water stress than traditionally grown greenhouse container crops (due to enhanced rooting in locations where stressful conditions prevail) and as a result, may give growers more control over flowering time; thus, creating tighter flowering schedules and possibly improving production timing efficiency. These results suggest stratifying expensive peat-based media on top of low-cost pine bark can produce salable greenhouse crops such as Petunia, while reducing peat use by upward of 50% (by volume).

### References Cited


FAO. 2009. How to feed the world in 2050. FAO, Rome, Italy.


