# Substrate Stratification Can Reduce Peat Requirement Associated with Young Plant Production

Maureen Thiessen[1] and Jeb S. Fields[2]

**Keywords. cuttings, growing media, liners, plugs, propagation, soilless culture**

**Abstract. Production of young plants from cuttings and seed relies heavily on peat and frequent, but light, irrigation. Interest in reducing peat usage as well as a propensity for short container heights to inhibit drainage have led to the exploration of alternative techniques to improve substrate airspace in young plant production. Substrate stratification has been shown to be effective for reducing excessive moisture content and improving root growth in the lower strata of larger containers. This research evaluated the effects of substrate stratification in 5.1-cm tall, 37-cm$^3$ cell plug trays using two plant propagation substrates: a bark-based vegetative cutting substrate (16% peat) and a peat-based seed germination substrate (65% peat). Each substrate was stratified by layering over either a commercially available wood fiber or horticultural grade perlite and was compared with an unstratified control. Substrate physical properties were measured on unplanted substrate treatments. Cuttings of two common bedding plants [coleus (*Solenostemon scutellarioides* 'Salsa Verde') and evolvulus (*Evolvulus glomeratus* 'Blue Daze')] were grown in the vegetative bark-based substrate treatments, and seeds of three common seed-started taxa [basil (*Ocimum basilicum* 'Thai Towers'), hibiscus (*Hibiscus moscheutos* 'Luna Pink Swirl'), and zinnia (*Zinnia elegans* 'Zesty Purple')] were grown in the seed peat-based substrate treatments. Finished plants were assessed for plug integrity and various growth parameters. Stratification with perlite increased airspace in the vegetative substrate only, not in the seed substrate. Stratification with wood fiber resulted in reduced airspace and increased container capacity in both substrate types. Stratification with perlite decreased plug integrity compared with nonstratified treatments, whereas wood fiber stratification resulted in similar or improved plug integrity, even in treatments in which root growth was reduced. Dry root biomass was greatest in both nonstratified substrates, with perlite stratification generally associated with the lowest root biomass. Perlite stratification was also associated with the lowest total root length and total root surface area, whereas wood fiber stratification resulted in values equivalent or greater than nonstratified treatments. Despite decreases associated with perlite stratification, however, both perlite- and wood fiber–stratified treatments produced quality plugs that established successfully post-transplant. The results demonstrate that using stratification in young plant production may provide growers with an opportunity to reduce peat consumption in propagation operations.**

The commercial production of young plants (e.g., seedling plugs and rooted cutting liners) relies heavily on the use of peatmoss, which is the primary organic component of propagation substrates. Peat-based substrates provide high water-holding capacity, low bulk density, uniformity, and other physiochemical and biological properties that make it optimal for plant production (Hartmann et al. 2002). The fine-particle texture of peat provides an added advantage to the small volumes of plug cells, thus it often accounts for 50% of the volume of propagation substrates (Hartmann et al. 2002). The physical properties of peat substrates coupled with the limited volumes of plug cells necessitate precision management of moisture levels. The lower portion of any container filled homogeneously with a single substrate stays wetter than the top of the container, often creating a zone of saturation at the base of frequently irrigated containers. As container height decreases, the saturation zone increases, further reducing available airspace (AS) and oxygen to the growing roots (Milks et al. 1989; Yafuso et al. 2019). Therefore, improper moisture management can inhibit full rooting or lead to disease resulting from elevated moisture content.

The demand for peatmoss is increasing steadily as horticultural production grows and soilless cultivation expands into new and existing sectors (Fields et al. 2021b; Jackson et al. 2022), including vertical farming, small fruit, and Christmas trees. Concerns have arisen surrounding the ecological and environmental impacts of peat extraction (Alexander et al. 2008; Cleary et al. 2005; Dunn and Freeman 2011), and these two factors lead many growers to seek the partial replacement of peat used in growing mixes.

A practice that can both alleviate excessive moisture and reduce peat use is substrate stratification, in which soilless substrates of differing physical properties are layered within a container to modify air- and water-holding capacities (Fields et al. 2021a). In larger containers (>2.5 L), stratification of fine-textured, greater water-holding-capacity substrates on top of coarser, more porous substrates led to improved root growth in *Petunia* (Fields and Criscione 2023). Work by Criscione et al. (2022) demonstrates that stratification is effective because it improves water availability in the top of the container where a germinating seed or transplanted plug or liner use water the most. This is important because water is lost quickly in these locations, where gravity and evapotranspiration cause can cause rapid water loss. Stratification also improves air-filled porosity in the lower portions of the container where water is used later in the growing period, and where gravity and container geometry cause excessive moisture (Fields et al. 2024). Previous substrate stratification research has shown reduced peat use and improved rooting in finished crops (Fields and Criscione 2023; Fields et al. 2022). Very little exploration of substrate stratification in smaller plug and liner containers has occurred (Thiessen and Fields 2024).

In large containers, coarse pine bark with greater air-filled porosity is generally used in the lower strata, but in smaller containers, fine-textured materials are also suitable. Perlite is already widely used in greenhouse substrates to improve air-filled porosity, but limited research exists on its use in stratification (Thiessen and Fields 2024). Wood fiber is an emerging, domestically sourced material that is gaining commercial traction as a soilless substrate component, and it has shown to be highly porous and to improve the air-filled porosity of substrates

(Dickson et al. 2022; Harris et al. 2020; Jackson 2018; Thiessen and Fields 2024). In previous work, Thiessen and Fields (2024) first explored the use of substrate stratification techniques on the production of woody transplant materials that were considered more difficult to root. The use of substrate stratification techniques in standard plug production for herbaceous cuttings and seedlings, which make up the majority of the greenhouse floriculture products in the industry, may have a greater impact on peat reduction and crop losses associated with moisture-related diseases and disorders.

The objective of our research was to explore the effects of substrate stratification in both bark-based and peat-based substrates in small-volume containers used in seeded plug and rooted liner production. We hypothesize that the use of stratification techniques will improve overall air-filled porosity within the cell and thus lead to improved rooting. Furthermore, with successful stratification techniques, reduction in peat reliance by young-plant producers can be achieved.

## Materials and methods

**Substrate treatments.** For vegetatively propagated species, substrate treatments consisted of 37 $cm^3$ (3.4-cm-wide × 5.1-cm-deep) cell trays (98-cell CN-PLG-098; T.O. Plastics, Clearwater, MN, USA) manually filled 1) entirely with a bark-based substrate of 58% bark fines (Phillips Bark, Brookhaven, MS, USA), 16% sphagnum peatmoss (Pure Canadian Sphagnum Peat Moss; Fertilome, Bonham, TX, USA), and 25% perlite [Aerosoil (10% of > 3.36 mm, 40% of > 2.38 mm, 75% of >1.19 mm, and 90% of > 0.595 mm); Dicaperl, Conshohocken, PA, USA), labeled vegetative nonstratified (VNS); or with either 2) vegetative stratified-Hydrafiber (VS-HF; EZ-Blend Hydrafiber, Profile Products, Buffalo Grove, IL, USA) or 3) vegetative stratified-perlite (VS-P; Aerosoil, Dicaperl) in the bottom half of the cells and the bark-based blend in the top half of the cells.

For seed-propagated species, substrate treatments consisted of 37 $cm^3$ (3.4-cm-wide × 5.1-cm-deep) cell trays (98-cell CN-PLG-098; T.O. Plastics) filled manually with 1) entirely with a commercial peat-based germinating mix comprised of 65% Canadian sphagnum peatmoss, 25% perlite, and 10% vermiculite (Jolly Gardener Pro-Line C/GP; Oldcastle APG, Atlanta, GA, USA), labeled seed nonstratified (SNS); or with either 2) seed stratified-Hydrafiber (SS-HF; EZ-Blend Hydrafiber, Profile Products) or 3) seed stratified-perlite (SS-P; Horticultural Grade, PVP Industries) in the bottom half of the cells and the peat-based mix in the top half of the cells (Fig. 1).

**Substrate physical properties.** After propagation trays were filled with substrate treatments, trays were thoroughly watered to drainage and allowed to equilibrate overnight. Four individual cells of each treatment were cut from the tray and used to determine total porosity (TP), AS, container capacity (CC), and bulk density ($D_b$) using a modified procedure (Thiessen and Fields 2024) based on the North Carolina State University Porometer method (Fonteno et al. 1995), in which no core is used. Instead, individual specific propagation cells were used to determine the actual air and water balance of the container. The height of the cells was 5.1 cm whereas that of a porometer core is 7.5 cm. Thus, although this assessment was considered adequate to estimate the air and water balance of the cell, the values cannot be compared and contrasted with other standardized research that uses cores. Cells were submerged slowly in tap water until the substrate surface appeared hydrated. The drainage hole at the cell base was covered while it was transferred to a funnel and beaker on top of a scale, where it was allowed to drain. All drainage was measured. The substrate was then dried at 105 °C for 48 h and weighed. Calculations for TP, AS, CC, and $D_b$ were performed according to the calculations detailed in Thiessen and Fields (2024) and Fonteno et al. (1995). We operated on the assumption that the

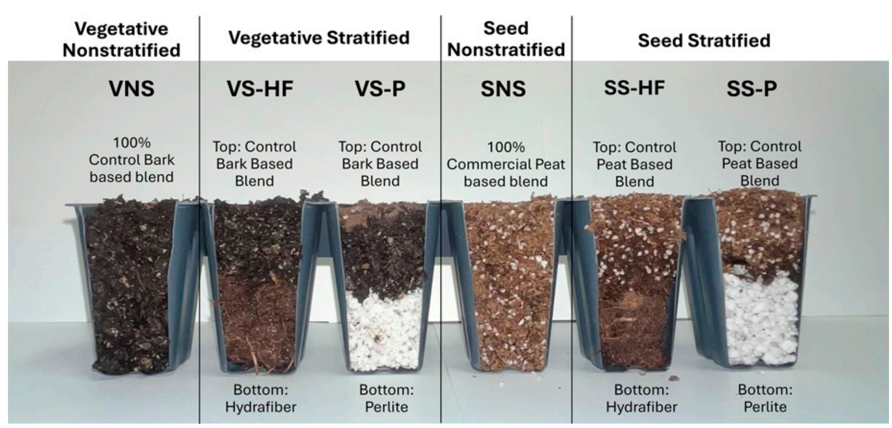


**Fig. 1. Cross sections of each propagation substrate treatment used in this propagation experiment, which explored the concept of stratifying (layering) substrate materials within an individual propagation cell. From left to right: Vegetative nonstratified (VNS) bark-based substrate, vegetative substrate stratified atop Hydrafiber (VS-HF), vegetative substrate stratified atop perlite (VS-P), seed nonstratified (SNS) peat-based substrate, seed substrate stratified atop Hydrafiber (SS-HF), and seed substrate stratified atop perlite (SS-P).**


Received for publication 12 Nov 2024. Accepted for publication 16 Jan 2025.

Published online 17 Mar 2025.



[1]Hammond Research Station, Louisiana State University Agricultural Center, 21549 Old Covington Highway, Hammond, LA 70403, USA

[2]Environmental Horticulture Department, University of Florida, Fifield Hall, 2550 Hull Road, Gainesville, FL 32611, USA



This work was funded in part through support from the Louisiana Board of Regents Research Competitiveness Fund [LEQSF(2023-26)-RD-A-02], the Floriculture & Nursery Research Initiative project 58-5082-3-024 A1, and the US Department of Agriculture National Institute of Food and Agriculture Specialty Crops Research Initiative Project Beyond Peat: Substrate Security for a Changing Climate (2024-51181-43290).

We thank Martha Milagros Vilchez Herrera for her assistance with propagation and data collection.


Mention of trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product and does not imply its approval nor the exclusion of other products that also may be suitable.


J.S.F. is the corresponding author. E-mail: jsfields@ufl.edu.





https://doi.org/10.21273/HORTTECH05570-24

reduced size of the cell would result in any perched water table being a greater proportion of the total volume than that found in a standard porometer analysis.

**Propagation.** On 22 Feb 2024, one unrooted vegetative cutting was placed in the center of each cell for the vegetative treatment, with 28 cells planted for each of the two species used [*Solenostemon scutellarioides* 'Salsa Verde' (coleus) and *Evolvulus glomeratus* 'Blue Daze' (evolvulus)] and substrate treatment combination, totaling 84 cells per species. Vegetative plants were maintained under a plastic tent with intermittent mist (4 s every 10 min between 7:00 AM and 7:00 PM daily) until roots emerged from the bottom of the tray. These plants were then removed from mist and hand-watered until cells were fully rooted.

On 19 Feb 2024, two seeds were placed in a small indentation in the center of each cell for the seed treatment, with 28 cells planted for each of three species [*Ocimum basilicum* 'Thai Towers' (basil)], *Zinnia elegans* 'Zesty Purple' (zinnia), and *Hibiscus moscheutos* 'Luna Pink Swirl' (hibiscus)] and substrate treatment combination, totaling 84 cells per species. Seeded plants were maintained in a plastic-covered greenhouse with 25% shade, under a humidity dome until cotyledon emergence, then were watered twice daily, once in the morning between 8:00 AM and 12:00 PM, and once in the afternoon between 2:00 and 4:00 PM, with a mist nozzle to maintain adequate leaf turgor.

Beginning 4 weeks after sticking or sowing, plants were checked weekly for rooting by gently pulling plants from the cells. When five of five plants were pulled and maintained substrate integrity (i.e., did not fall part and were held together by the roots), that species and treatment was harvested, and days from sticking or sowing to fully rooted were recorded.

**Harvest.** At harvest, the growth index [GI = (Height + Widest width + Perpendicular width) ÷ 3] was measured for a random sample of six plants per treatment and species. These replicates then underwent a drop test, during which they were weighed, dropped from a height of 1 m, and weighed again to assess material lost during handling. The purpose of this measurement was to determine the susceptibility of plug quality to the transplanting process. Roots were then severed from the shoots, washed of all media, and dried for 2 d at 70 °C to determine dry weight. Shoots were also dried for 2 d at 70 °C, and dry weight used to calculate a root-to-shoot ratio from Root dry weight ÷ Shoot dry weight.

**Root morphology.** Another five random selected plugs were used to assess root system morphology according to the procedure outlined in Fields and Criscione (2023). Roots were severed from the shoots, washed of all substrate, and separated/teased apart on a standard sheet of white paper (21.6 × 27.9 cm) placed in a rectangular container and submerged in tap water to 1 cm. Photos of the roots were taken from a height of 32 cm using a light-inhibiting chamber and an iPhone 13 Mini (Apple, Cupertino, CA, USA) using a flash. Images were then preprocessed with the smartphone application Turbo-Scan (Piksoft, Inc., Piedmont, CA, USA) to remove glare and define roots. Images were then analyzed using RhizoVision software (ver. 2.0.3, Zenodo, Geneva, Switzerland) (Seethepalli and York 2021) to approximate total root length, average root diameter, and total root surface area using the following parameters: 230 DPI, image a thresholding level of 230, filtering of nonroot objects at 1 $mm^2$, a root pruning threshold of 1, and root diameter size classes defined as very fine (0–0.5 mm), fine (0.5–1.0 mm), medium (1.0–2.0 mm), and large (>2.0 mm). Lines of known lengths printed on 21.6 × 28-cm sheets of paper photographed at the same height as the root photos were used to calibrate RhizoVision between known lengths and approximations ($R^2 = 0.9996$).

**Crop finishing.** The remaining plugs for each species and treatment were transplanted into 8.9-$cm^2$ pots and grown in a substrate consisting of 58% bark fines (Phillips Bark), 16% sphagnum peatmoss (Pure Canadian Sphagnum Peat Moss; Fertilome), and 26% perlite (Horticultural Grade; PVP Industries) by volume and were grown for an additional 18 d. The GI was then measured on a random six replications per species and substrate treatment combination to assess any effects of plug treatment on crop finishing.

**Data analysis.** All measures were analyzed using JMP Pro 18 (SAS Institute, Cary, NC, USA). For substrate physical properties, a one-way analysis of variance (ANOVA) was used to analyze least squares means and to determine response levels according to substrate treatment within propagation method and with substrate treatment nested within propagation method. Least squares means of responses during the propagation experiment were analyzed using multiple-factor ANOVA, with substrate treatment, propagation type (vegetative vs. seed), and species nested within propagation type as main effects, as well as interactions of substrate with propagation type and species. When data residuals were not normal, a log transform was performed to achieve normality. ANOVAs were also performed within each species, with substrate treatment as the main effect. When the ANOVA was significant, means separation using Tukey's honestly significant difference ($\alpha = 0.05$) was performed.

## Results and discussion

**Substrate physical properties.** Total porosity was highest in the SNS substrate and lowest in the VNS substrate (Table 1). Stratification did not alter TP of either substrate treatment significantly, except in the SS-P substrate. AS ranged from 5.4 to 21.8 $cm \cdot cm^{-3}$ (Table 1), which meets or exceeds AS determined in previous research. In a study measuring substrate properties in several commercial propagation substrates in 25-$cm^3$ cells (Huang et al. 2012), AS ranged from 4.8 to 9.7 $cm \cdot cm^{-3}$. AS was greatest in the VS-P treatment at 21.8 $cm \cdot cm^{-3}$ ($P < 0.0001$) and lowest in the seed SS-P treatment at 5.4 $cm \cdot cm^{-3}$ ($P = 0.0192$). It was unexpected that AS decreased in the seed substrate with stratification with both perlite and HF; it is possible the fine particle size of the peat in this substrate allowed enough migration of substrate particles into the ASs of the HF and perlite layers, thus decreasing the proportion of larger pores, and therefore AS, in the substrate matrix. In the vegetative substrate, stratification with perlite increased AS by 86% compared with nonstratified. In previous work, Thiessen and Fields (2024) found a 39% increase in AS when stratifying a

Table 1. Physical properties of substrate treatments used in production of vegetative and seed propagated species.[i]

| Substrate[ii] | Static physical properties | | | |
|---|---|---|---|---|
| | Total porosity ($cm^3 \cdot cm^{-3}$) | Airspace ($cm^3 \cdot cm^{-3}$) | Container capacity ($cm^3 \cdot cm^{-3}$) | Bulk density ($g \cdot cm^{-3}$) |
| VNS | 0.81 a[iii] | 0.12 b | 0.69 ab | 0.20 a |
| VS-HF | 0.83 a | 0.09 b | 0.74 a | 0.18 b |
| VS-P | 0.84 a | 0.22 a | 0.62 b | 0.16 c |
| *P* value | 0.3956 | <0.0001 | 0.0068 | 0.0005 |
| SNS | 0.92 a | 0.13 a | 0.79 a | 0.09 b |
| SS-HF | 0.88 a | 0.07 ab | 0.81 a | 0.10 ab |
| SS-P | 0.82 b | 0.05 b | 0.77 a | 0.10 a |
| *P* value | 0.0014 | 0.0192 | 0.3445 | 0.0280 |

[i] Physical properties were determined using a modified procedure (Thiessen and Fields 2024) based on the North Carolina State University Porometer test (Fonteno et al. 1995).

[ii] VNS = nonstratified (control) substrate for vegetatively propagated species that was comprised of 58% bark fines (Phillips Bark, Brookhaven, MS, USA), 16% sphagnum peatmoss (Pure Canadian Sphagnum Peat Moss; Fertilome, Bonham, TX, USA), and 25% perlite (Horticultural Grade; PVP Industries, Bloomfield, OH, USA); VS-HF = stratified treatment with bark-based blend layered on top of Hydrafiber (EZ-Blend Hydrafiber; Profile Products, Buffalo Grove, IL, USA); VS-P = stratified treatment with bark-based blend layered on top of perlite (Horticultural Grade; PVP Industries); SNS = nonstratified (control) commercial substrate for seed-propagated species that was comprised of 65% Canadian sphagnum peatmoss, 25% perlite, and 10% vermiculite (Jolly Gardener Pro-Line C/GP; Oldcastle APG, Atlanta, GA, USA); SS-HF = stratified treatment with commercial substrate layered on top of Hydrafiber (EZ-Blend Hydrafiber; Profile Products); SS-P = stratified treatment with commercial substrate layered on top of perlite (Horticultural Grade; PVP Industries).

[iii] Lowercase letters represent similarities assessed through means separation analysis by Tukey's honestly significant difference at $\alpha$ = 0.05, wherein dissimilar letters represent statistical differences of means.

very similar bark-based propagation substrate atop horticultural perlite, with a very similar overall AS (25%) to that found in our work (22%). Unlike Thiessen and Fields (2024), wood fiber did not increase overall AS in either the seed or vegetative substrates; however, the previous work incorporated an extruded wood fiber, whereas our work stratified with a disk-refined wood fiber (HF). Still, it is unexpected that stratification with HF did not increase overall AS, given that previous work has determined AS of pure HF to be from ~32 $cm \cdot cm^{-3}$ (Dickson et al. 2022) to ~33 $cm \cdot cm^{-3}$ (Thiessen et al. 2023), which is greater than that of both nonstratified substrates used in our study. In our study, the method of fiber combination may be an important explanation. Disk-refined wood fibers (e.g., HF) have a propensity to aggregate when separated manually (as opposed to mechanical separation using specialized equipment likely found in commercial growing operations). It is possible that the large aggregate sizes compared with the small volume of the plug cells cause AS within the aggregates to become more dominant in the overall plug space, as opposed to AS between aggregates that would dominate in larger containers. Furthermore, the blending process could better separate wood fibers and allow the increased AS that wood fiber offers to be conferred to the overall mix (Jackson 2018).

CCs ranged from 62 to 81 $cm \cdot cm^{-3}$ (Table 1) and were within ranges (57–86 $cm \cdot cm^{-3}$) found in the survey by Huang et al. (2012) of commercial propagation substrates. Substrate CC values were similar across the seed substrates at ~80 $cm \cdot cm^{-3}$ ($P = 0.3445$). CCs were lower with the vegetative substrate treatments ($P < 0.0001$), with HF slightly increasing CC and perlite significantly lowering CC by 11% ($P = 0.0068$; Table 1). The VNS treatment had the greatest $D_b$ (0.20 $g \cdot cm^{-3}$; $P < 0.0001$), more than double that of the SNS substrate. Bark-based substrates often have greater $D_b$ values than peat-based substrates. Stratifying low-$D_b$ wood fiber and perlite materials decreased the overall $D_b$ of the vegetative substrate while slightly increasing that of the seed substrate (Table 1).

**Plug growth.** Full rooting occurred in coleus, evolvulus, and zinnia crops at 34 d after sticking or sowing, and at 46 d for basil and hibiscus. It was visually observed that less rooting occurred in the bottom layer of the VS-P and SS-P treatments compared with other treatments. Although these plugs could be removed from the trays without noticeable particle loss, many

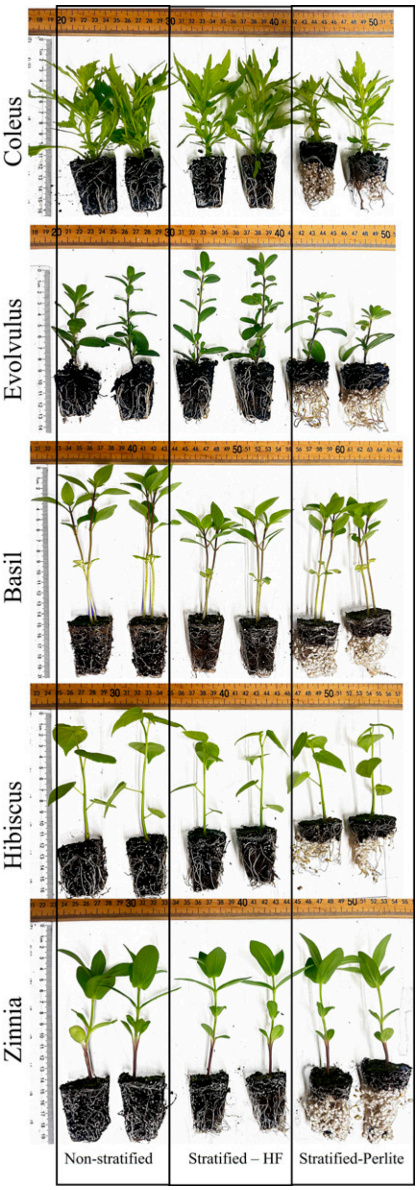


**Fig. 2. Finished plugs upon harvest of experiment exploring the concept of stratifying (layering) different substrate materials within an individual cell. Pictured are two replications from each substrate treatment side by side. (left to right) Nonstratified (propagation substrate), propagation substrate over Hydrafiber, and propagation substrate over perlite. The coleus and evolvulus were rooted asexually, whereas the basil, hibiscus, and zinnia were seed-germinated.**

disintegrated during the harvest and measuring processes (Fig. 2). In both vegetative and seed substrates, nonstratified and stratified-HF treatments exhibited increased rooting throughout the entire plug than in stratified-perlite. Stratified-HF improved total root length when compared with the

**Table 2. Measurements of plug integrity and growth parameters at plug harvest and at 4 weeks after transplanting.**

| Substrate[i] | Drop test loss (g)[ii] | GI (cm)[iii] | Dry root mass (g) | Dry shoot mass (g)[iv] | Root:shoot ratio[iv] | Total root length (mm)[v] | Total root surface area ($mm^2$)[v] | Avg. root diam (mm)[v] | GI at 18 d |
|---|---|---|---|---|---|---|---|---|---|
| Coleus (vegetatively propagated) | | | | | | | | | |
| VNS | 1.91 ab[vi] | 11.1 a | 0.047 a | 0.148 a | 0.310 a | 15,224 a | 29,996 a | 0.636 a | 15.06 a |
| VS-HF | 1.22 b | 10.3 a | 0.047 a | 0.129 a | 0.354 a | 17,072 a | 30,670 a | 0.582 a | 16.06 a |
| VS-P | 5.03 a | 8.94 a | 0.033 a | 0.088 ab | 0.367 a | 8,973 b | 15,911 b | 0.573 a | 15.7 a |
| Evolvulus (vegetatively propagated) | | | | | | | | | |
| VNS | 11.4 b | 5.72 ab | 0.043 a | 0.097 a | 0.435 a | 3,621 b | 8,161 ab | 0.716 a | 15.7 a |
| VS-HF | 7.04 c | 6.36 a | 0.042 a | 0.104 a | 0.392 a | 6,972 a | 14,058 a | 0.645 b | 15.0 a |
| VS-P | 15.4 a | 5.22 b | 0.037 a | 0.068 b | 0.513 a | 2,452 b | 5,380 b | 0.695 ab | 10.9 a |
| Basil (seed propagated) | | | | | | | | | |
| SNS | 1.63 b | 9.33 a | 0.038 a | 0.151 a | 0.250 b | 12,158 a | 20,208 a | 0.540 a | 18.44 a |
| SS-HF | 0.55 b | 8.00 b | 0.033 a | 0.094 b | 0.350 a | 8,589 b | 12,126 b | 0.458 b | 17.39 a |
| SS-P | 5.32 a | 8.19 b | 0.028 a | 0.104 b | 0.263 ab | 7,191 b | 12,200 b | 0.550 a | 17.83 a |
| Hibiscus (seed propagated) | | | | | | | | | |
| SNS | 1.83 b | 8.33 a | 0.052 a | 0.095 a | 0.536 a | 5,035 a | 8,150 a | 0.521 b | 17.3 a |
| SS-HF | 1.87 b | 6.86 b | 0.037 b | 0.066 b | 0.543 a | 4,544 a | 7,360 ab | 0.521 b | 14.8 a |
| SS-P | 4.30 a | 6.85 b | 0.045 ab | 0.062 b | 0.702 a | 3,703 b | 6,458 b | 0.561 a | 14.8 a |
| Zinnia (seed propagated) | | | | | | | | | |
| SNS | 1.71 b | 8.28 a | 0.020 a | 0.098 a | 0.186 a | 7,888 a | 12,767 a | 0.525 a | 14.6 a |
| SS-HF | 1.61 b | 8.00 ab | 0.017 a | 0.067 b | 0.235 a | 10,233 a | 15,489 a | 0.490 a | 14.8 a |
| SS-P | 8.36 a | 7.39 b | 0.017 a | 0.058 b | 0.274 a | 7,645 a | 12,761 a | 0.536 a | 14.7 a |
| $P$ value$_{substrate}$ | <0.0001 | <0.0001 | 0.0028 | <0.0001 | 0.0172 | <0.0001 | <0.0001 | <0.0001 | 0.0465 |
| $P$ value$_{PropType}$ | <0.0001 | 0.8038 | <0.0001 | <0.0001 | 0.0115 | <0.0001 | <0.0001 | <0.0001 | 0.0097 |
| $P$ value$_{species[PropType]}$ | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0003 |
| $P$ value$_{PropType \times Substrate}$ | 0.0623 | 0.0666 | 0.0870 | 0.0003 | 0.4229 | <0.0001 | <0.0001 | 0.0217 | 0.1801 |
| $P$ value$_{Substrate \times Species[PropType]}$ | <0.0001 | 0.1839 | 0.3904 | 0.5726 | 0.1510 | <0.0001 | <0.0001 | 0.1633 | 0.1102 |

[i] VNS = nonstratified (control) substrate for vegetatively propagated species that was comprised of 58% bark fines (Phillips Bark, Brookhaven, MS, USA), 16% sphagnum peatmoss (Pure Canadian Sphagnum Peat Moss; Fertilome, Bonham, TX, USA), and 25% perlite (Horticultural Grade: PVP Industries, Bloomfield, OH, USA); VS-HF = stratified treatment with bark-based blend layered on top of Hydrafiber (EZ-Blend Hydrafiber; Profile Products, Buffalo Grove, IL, USA); VS-P = stratified treatment with bark-based blend layered on top of perlite (Horticultural Grade; PVP Industries). SNS = nonstratified (control) commercial substrate for seed-propagated species that was comprised of 65% Canadian sphagnum peatmoss, 25% perlite, and 10% vermiculite (Jolly Gardener Pro-Line C/GP; Oldcastle APG, Atlanta, GA, USA); SS-HF = stratified treatment with commercial substrate layered on top of Hydrafiber (EZ-Blend Hydrafiber; Profile Products); CS-P = stratified treatment with commercial substrate layered on top of perlite (Horticultural Grade; PVP Industries).

[ii] The difference in plug mass before and after dropping from a height of 1 m.

[iii] Growth index (GI) = [(Widest width + Perpendicular width + Height of plant) ÷ 3].

[iv] When data were not normal, means and analyses were based on a log transform of data.

[v] Root length, surface area, and diameter measures were determined using RhizoVision software (ver. 2.0.3, Zenodo, Geneva, Switzerland) (Seethepalli and York 2021).

[vi] Data represent least square means of six replicates (five for root morphology traits) within each species. Lowercase letters represent similarities assessed through means separation analysis by Tukey's honestly significant difference at $\alpha = 0.05$, wherein dissimilar letters represent statistical differences of means. Statistical assessment was only conducted within a species.

nonstratified treatment in coleus, evolvulus, and zinnia, but not in basil or hibiscus ($P < 0.0001$; Table 2). Total root length and total root surface area were greater in nonstratified and stratified-HF treatments than stratified-perlite treatments ($P < 0.0001$; Table 2), with less observable differences in the seed-propagated species. This potentially reflects the difference in AS between the two stratified-perlite treatments. It may be that AS was increased in the vegetative substrate to the point of growth inhibition. In the seed substrate, fine-particle movement from the top strata to the bottom strata may have reduced the perlite effect on AS, thereby reducing root growth inhibition compared with the VS-P treatment. Root dry biomass values were generally higher in the nonstratified substrates and lower in the stratified, with perlite more often lower than HF (Table 2 and Fig. 3). Hibiscus was an exception, with root dry biomass in stratified-perlite between that in nonstratified and stratified-HF treatments. The lack of rooting in the lower strata and decreases in root length and dry biomass in stratified-perlite vs. nonstratified systems were also found in previous propagation work on woody species by Thiessen and Fields (2024).

Drop test results were consistent with these observations (Table 2). Stratified-perlite treatments lost significantly ($P < 0.0001$) more plug material ($\bar{x} = 8.1$ g) than nonstratified ($\bar{x} = 4.2$ g) and stratified-HF ($\bar{x} = 2.7$ g) treatments (Fig. 4), thus indicating more vulnerability to damage during the transplanting process. Stratified-HF treatments demonstrated greater plug integrity through similar or lower losses after dropping when compared with the nonstratified treatments ($P < 0.0001$). Drop test losses were greatest overall in evolvulus ($P < 0.0001$), suggesting all treatments within that species could have benefited from more rooting time before harvest. Despite this, HF stratification showed a significantly lower evolvulus plug loss than nonstratified. In basil, where total root length was lower in VS-HF than VNS, drop test losses were still 66% lower with VS-HF (Table 2). Previous work (Thiessen et al. 2023; Thiessen and Fields 2024) has shown that HF tends to aggregate, likely conferring this increase in plug integrity. Thus, HF shows the potential to increase the handling ability of unfinished plugs. This characteristic can benefit the industry by preserving plug quality during shipment, and by allowing plug orders to be filled on time or earlier despite weather or other production factors that may cause delayed rooting.

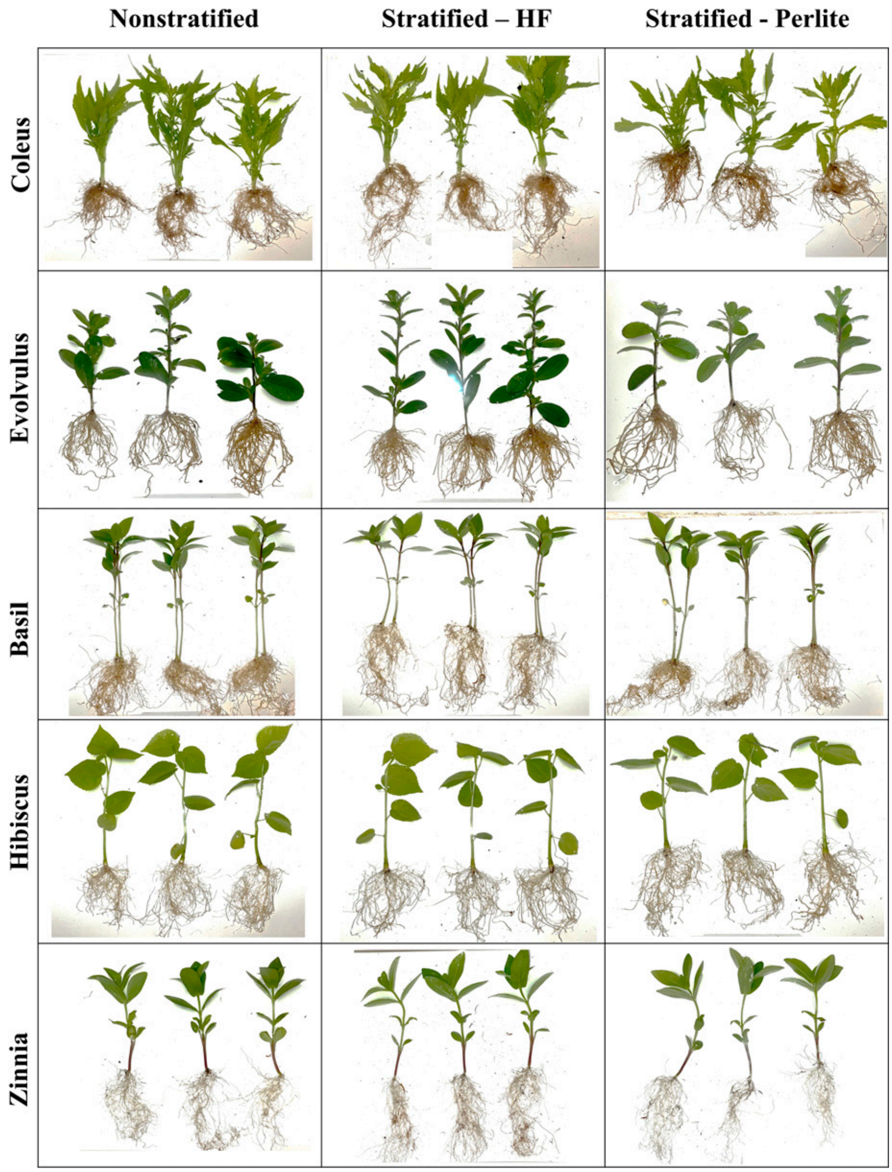


**Fig. 3. Finished plugs upon harvest after vegetatively rooting or seeded production in each substrate treatment exploring the use of stratifying (layering) different substrate materials in an individual cell. Three replications of each treatment were washed of all substrate and roots were teased apart. Treatments included nonstratified (propagation substrate), propagation substrate over Hydrafiber (Stratified-HF), and propagation substrate over perlite (Stratified-Perlite). The coleus and evolvulus were rooted asexually, whereas the basil, hibiscus, and zinnia were seed-germinated.**

Across both species, the VS-P treatment had a significantly lower GI ($\bar{x} = 7.1$ vs. 8.3 and 8.4 cm; $P = 0.0666$) and shoot dry weight ($\bar{x} = 0.08$ vs. 0.12 g; $P = 0.0003$) at harvest than VNS and VS-HF (Table 2). However, the plugs from the VS-P treatment were still considered viable for production. Compact plugs are often considered desirable, with many operations opting for smaller plug cells with greater plant density; therefore, the ability to remain compact can be considered a benefit. In fact, perlite stratification may be useful when environmental conditions favor shoot growth, causing undesirable stretching, such as high humidity, low light, and

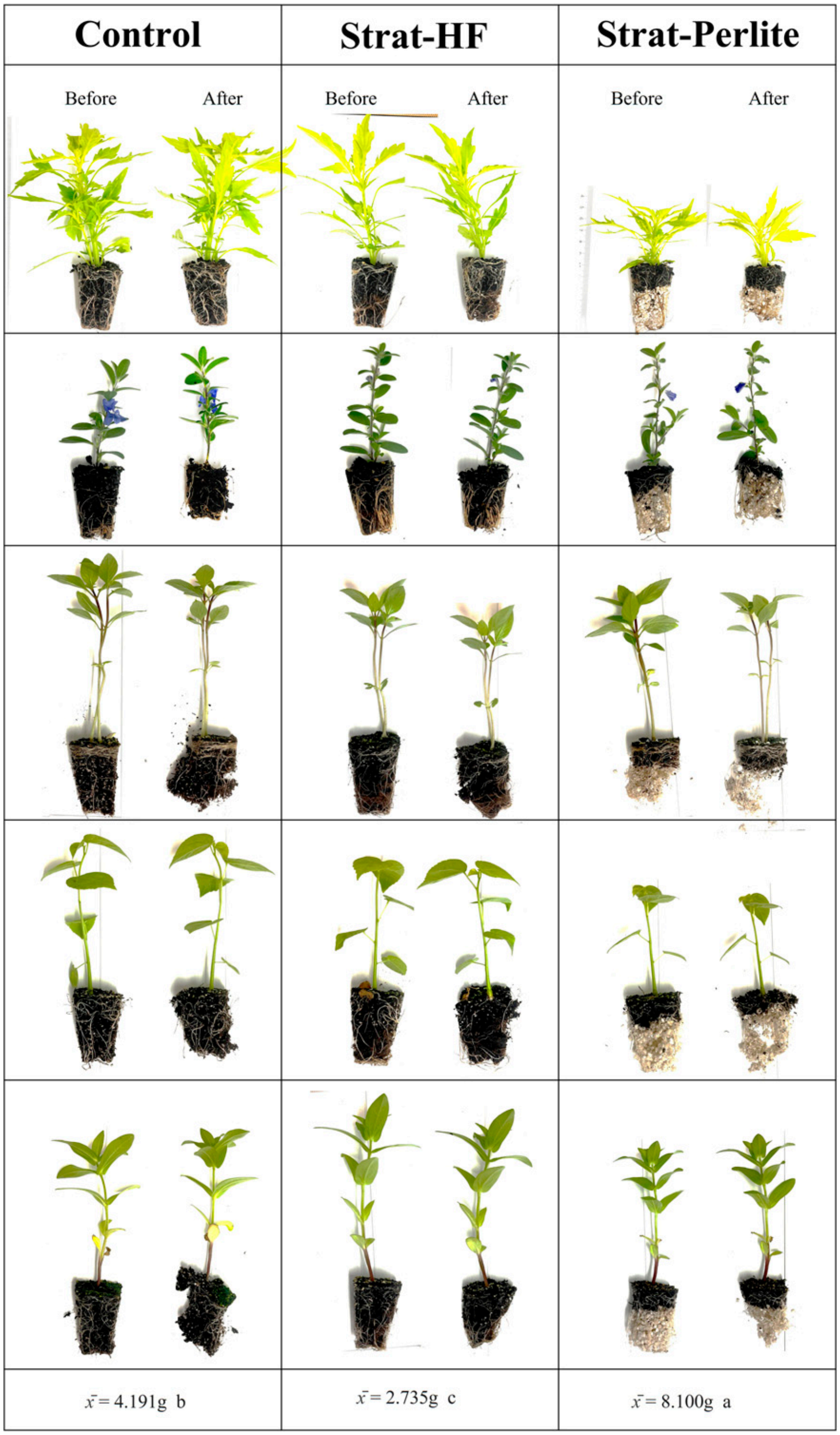


**Fig. 4. Representative vegetative and seeded plugs before (right) and after (left) performing a drop test from a height of 1 m (i.e., holding plug at 1 m and dropping onto table) to test plug integrity. The experiment explored the concept of applying stratification (layering) techniques to small propagation cells. Treatments included nonstratified (Control; propagation substrate), propagation substrate over Hydrafiber (Strat-HF), and propagation substrate over perlite (Strat-Perlite). The coleus and evolvulus were rooted asexually; the basil, hibiscus, and zinnia were seed-germinated.**

other factors (Styer 2002). Thus, perlite stratification could potentially offer an alternative practice to plant growth regulator use in some cases. In seed-propagated varieties, the GIs at harvest and shoot dry weight were highest in the SNS treatment, and similar in the SS-HF and SS-P treatments (GI, $P$ = 0.0666; shoot dry weight, $P$ = 0.0003). Root-to-shoot ratios, which reflect the partitioning of resources by the growing plant, were similar among all treatments ($P$ = 0.4229) except between the SNS treatment ($\bar{x}$ = 0.29) and the VS-P treatment ($\bar{x}$ = 0.43), where partitioning of resources toward the roots was significantly greater, and consistent with the results of lower shoot growth in stratified-perlite treatments. Again, despite lower overall root growth, the slightly higher R-to-shoot ratios (although not statistically significant) in stratified-perlite treatments within each species demonstrates the potential for this practice when the production environment favors shoot stretching. The loss of plug integrity when using perlite stratification may be alleviated using stabilizing structures such as biodegradable inserts or wraps. Future research could evaluate improving plug integrity with perlite stratification by blending the perlite with HF in the bottom strata, possibly combining the plug-handling benefits of HF with the growth-regulating effects of perlite.

**Transplant performance.** Across species, the GI of 18-d transplants showed the same results, with SNS treatments ($\bar{x}$ = 16.8 cm) showing the highest growth, VS-P treatments ($\bar{x}$ = 13.3 cm) showing the lowest growth, and all other treatments similar ($P$ = 0.1801; Table 2). However, within each species, all treatments showed a similar GI 18 d after transplant ($P$ = 0.1102), indicating that no deleterious effects on root or shoot development from stratification with any material were conferred in the overall production process. Thus, both stratification materials can be used to achieve successful plug production in both seed and vegetative systems with half the peat.

## Conclusion

All substrate treatments produced both seeded and vegetative plugs successfully. Substitution of 50% of the total volume of propagation substrate via stratification can have economic benefits. HF stratification did not improve AS in the substrates used in our experiment, but was associated with increased total root length in coleus, evolvulus, and zinnia, which may provide further economic benefit even in low-cost, bark-based substrates, as this can improve plug quality and shorten finish time. Stratification with HF reduced the total volume of peat-based germination substrates required, while improving plug integrity and maintaining plug health, and can be achieved through adequate separation with specialized mechanical equipment and multiple passes through tray-filling machines. In addition, the added plug integrity can help hasten plug finish time, increasing the number of crop turnovers achieved in a given time frame. In our research, stratification with perlite increased AS in bark-based vegetative substrates and reduced AS in peat-based seed substrates, while reducing plug handling and root and shoot growth. However, acceptable-quality plugs with similar transplant success as other treatments were still achieved. With the lack of plug integrity observed with perlite stratification, additional components such as binding polymers, or blending with more fibrous material such as wood fiber, may be needed to use perlite in stratification in young-plant production. However, stratification with a fibrous material such as HF maintained plug integrity while producing similar or higher measures in root and shoot development. Further research exploring stratification with materials of varying textures and properties is needed, such as a perlite and wood fiber blend. With the need to reduce the cost associated with substrate use, our research may provide a foundation for continued exploration into stratification practices in young-plant production.

## References cited


Alexander PD, Bragg NC, Meade R, Padelopoulos G, Watts O. 2008. Peat in horticulture and conservation: The UK response to a changing world. Mires Peat. 3:1–10.

Cleary J, Roulet NT, Moore TR. 2005. Greenhouse gas emissions from Canadian peat extraction, 1990–2000: A life cycle analysis. Ambio. 34(6):456–461.

Criscione KS, Fields JS, Owen JS Jr, Fultz L, Bush E. 2022. Evaluating stratified substrates effect on containerized crop growth under varied irrigation strategies. HortScience. 57(3):400–413. https://doi.org/10.21273/HORTSCI16288-21.

Dickson RW, Helms KM, Jackson BE, Machesney LM, Lee JA. 2022. Evaluation of peat blended with pine wood components for effects on substrate physical properties, nitrogen immobilization, and growth of *petunia* (*Petunia* ×*hybrida Vilm.*-Andr.). HortScience. 57(2):304–311. https://doi.org/10.21273/HORTSCI16177-21.

Dunn C, Freeman C. 2011. Peatlands: Our greatest source of carbon credits? Carbon Manag. 2(3):289–301. https://doi.org/10.4155/cmt.11.23.

Fields JS, Criscione KS. 2023. Stratified substrates can reduce peat use and improved root productivity in container crop production. HortScience. 58(3):364–372. https://doi.org/10.21273/HORTSCI-17019-22.

Fields JS, Criscione KS, Edwards AE. 2022. Single-screen bark particle separation can be used to engineer stratified substrate systems. HortTechnology. 32(5):391–397. https://doi.org/10.21273/HORTTECH05018-22.

Fields JS, Criscione KS, Owen JS Jr. 2024. Assessment of substrate physical properties in bark- and peat-based stratified substrate systems. HortScience. 59(12):1823–1827. https://doi.org/10.21273/HORTSCI18188-24.

Fields JS, Owen JS Jr, Altland JE. 2021a. Substrate stratification: Layering unique substrates within a container increases resource efficiency without impacting growth of shrub rose. Agron J. 11:1454. https://doi.org/10.3390/agronomyu11081454.

Fields JS, Owen A, Lamm J, Altland B, Jackson Y, Zheng L, Oki K, Fontenot J, Samtani J, Campbell B. 2021b. Soilless substrate science: A North American needs assessment to steer soilless substrate research into the future. Acta Hortic. 1317:313–318. https://doi.org/10.17660/ActaHortic.2021.1317.36.

Fonteno WC, Hardin CT, Brewster JP. 1995. Procedures for determining physical properties of horticultural substrates using the NCSU Porometer. Horticultural Substrates Laboratory, North Carolina State University, Raleigh, NC, USA.

Harris CN, Dickson RW, Fisher PR, Jackson BE, Poleatewich AM. 2020. Evaluating peat substrates amended with pine wood fiber for nitrogen immobilization and effects on plant performance with container-grown petunia. HortTechnology. 30(1):107–116. https://doi.org/10.21273/HORTTECH04526-19.

Hartmann HT, Davies FT, Kester DE, Geneve RL. 2002. Plant propagation: Principles and practices (7th ed). Prentice-Hall, Upper Saddle River, NJ, USA.

Huang J, Fisher P, Argo BR. 2012. Quality control in growing media: Porosity for plug and liner mixes. Ohio Florist's Assoc Bull 934, 2–7. https://www.mydigitalpublication.com/publication/?m=4456&i=119682&p=2&ver=html5. [accessed 7 Nov 2022].

Jackson BE. 2018. Substrates on trial: Wood fiber in the spotlight. https://www.greenhousemag.com/article/substrates-on-trial-wood-iber-in-the-spotlight/. [accessed 7 Nov 2022].

Jackson BE, Fields JS, Owen JS, Altland J. 2022. Soilless substrate science for current and future growers. Grower Talks. https://www.growertalks.com/Article/?articleid=25658. [accessed 29 Nov 2023].

Milks RR, Fonteno WC, Larson RA. 1989. Hydrology of horticultural substrates: III. Predicting air and water content of limited-volume plugs. J Am Soc Hortic Sci. 114(1):57–61. https://doi.org/10.21273/JASHS.114.1.57.

Seethepalli A, Dhakal K, Griffiths M, Guo H, Freschet GT, York LM. 2021. RhizoVision Explorer: Open-source software for root image analysis and measurement standardization. AoB Plants. 13(6):plab056.

Seethepalli A, York LM. 2021. RhizoVision Explorer: Interactive software for generalized root image analysis designed for everyone (version 2.0.3). https://zenodo.org/records/5121845.

Styer R. 2002. Using PGRs on plugs. https://gpnmag.com/article/using-pgrs-plugs/. [accessed 8 Nov 2024].

Thiessen ME, Fields JS. 2024. Improving air-filled porosity in woody propagation at the Hammond Research Station. Combined Proc Southern Region IPPS 73:319–331.

Thiessen M, Fields JS, Abdi D, Beasley J. 2023. Sugarcane bagasse is an effective soilless substrate amendment in quick-turn *Osteospermum* production. HortScience. 58(10):1170–1177. https://doi.org/10.21273/HORTSCI17286-23.

Yafuso EJ, Fisher PR, Bohorquez AC, Altland JE. 2019. Water and air relations in propagation substrates. HortScience. 54(11):2024–2030. https://doi.org/10.21273/HORTSCI14145-19.