Stratified Cutting Propagation Substrates Reduce Herbaceous Plug Structural Stability but Facilitate Similar Root Growth to a Control

Benjamin K. Hoover[1], Martin J. De La Cruz[1], Emma C. Whitehead[1], and Annica Wu[1]

Keywords. ***Agastache*** **× 'Sunrise Red Improved', biochar,** ***Cuphea hyssopifolia*** **'Maria', parboiled rice hulls, perlite, plug production**

Abstract. Adventitious root development on cuttings may be promoted by oxygen availability in the rooting zone. Commercial propagation substrates are frequently peat-based, which can be problematic when used in propagation systems with intermittent misting due to excessive water holding. We tested top- or middle-stratifying a peat-based substrate with coarse amendments or a fine biochar. We had seven treatments, with a commercial peat-based propagation substrate as our control, then applying either a top (3 cm deep) or middle (2 cm deep) layer of coarse perlite, parboiled rice hulls, or fine coconut shell biochar in a pot with a top diameter of 6.3 cm, depth of 6.1 cm, and volume of 140 cm^3. We stuck unrooted cuttings of four herbaceous plants [hybrid hyssop (*Agastache* × 'Sunrise Red Improved'), false heather (*Cuphea hyssopifolia* 'Maria'), blanket flower (*Gaillardia ×grandiflora* 'Eclipse'), and Jame sage (*Salvia ×jamensis* 'Ignition Purple')]. We used completely randomized designs, with each plant species treated as a separate experiment. Each experiment had a replication of 7. We repeated the experiments in two series. After ~4 weeks, we evaluated plug stability with a subjective scale (0 = no visible roots, unstable; 1 = minimal roots, one or two roots visible, unstable; 2 = moderate roots, more than two roots but large spaces with no roots, partially unstable; 3 = acceptable roots, many roots with only small gaps, stable; 4 = optimum roots, many roots with full coverage, stable), dry root weight, total root area, and total root length. Stratification significantly reduced plug structural stability. Across experiments and series, there was not a clear stratification effect on root growth. Considering that stratification with perlite, parboiled rice hulls, and fine biochar reduced plug structural stability but did not provide clear growth advantages, growers should consider alternate stratification materials that provide sufficient binding to maintain plug stability.

Plant production from unrooted cuttings (URCs) is an important part of the US floriculture industry. US floriculture propagative materials accounted for sales of $513 million in 2023 (US Department of Agriculture, National Agricultural Statistics Service 2023). An additional 1.4 billion URCs, valued at $150 million, were imported in 2023 (World Bank 2023). Rooting of cuttings is often accomplished in peat-based substrates. Most propagation substrates used in commercial plug production are uniform blends of multiple components. Substrates are selected for their ability to support the propagule while providing adequate oxygen and water holding (Davies et al. 2018).

Recent research has identified benefits of stratified substrates in container-grown nursery crops, such as increased productivity and reduction of peat use (Fields and Criscione 2023). To date, limited research has been done with rooting URCs in stratified substrates. Thiessen and Fields (2024) used a fine bark and peat propagation mix bottom-stratified with coarse perlite for cutting propagation of seven woody plant species. Stratification did not increase root growth or plug quality. Thiessen and Fields (2025) successfully rooted coleus (*Solenostemon scutellarioides*) and evolvulus (*Evolvulus glomeratus*) in bark-based substrates bottom-stratified with perlite or wood fiber. The perlite-stratified plugs resulted in lower root growth means than the control, but the finished product was a quality plug. When a plant growth index was calculated for plugs 18 d after transplanting, the stratified plugs had the same mean as the nonstratified control. Compared with the control, the use of perlite increased airspace in this study, while wood fiber decreased airspace.

Oxygen in the rooting zone is critical for root development (Gislerod 1983). The use of overhead misting is common when rooting URCs and often results in overapplication of water during the critical rooting process (Santos et al. 2008). Using stratified substrates with coarse components with macropores that promote oxygen holding in the rooting zone might allow for superior root growth. Perlite is often used in propagation substrates to improve aeration (Fonteno and Dole 2006). Parboiled fresh rice hulls (PBHs) have been identified as a promising alternative to perlite in greenhouse production substrates. Peat substrates with perlite or PBH amendment showed similar plant growth outcomes when tested with four bedding plant species (Evans and Gachukia 2004), while PBH-amended substrates had higher levels of air-filled pore space than perlite-amended substrates (Evans and Gachukia 2007). Criscione et al. (2022) observed increased water holding in containers with a fine substrate layered over a coarse substrate in an experiment involving production of Chinese fringe flower (*Loropetalum chinense*). We propose to switch the order of the stratification components to bring the coarse layer to the top of the container, thus into the rooting zone.

Amending peat-based substrates for herbaceous cuttings with fine coconut shell biochar has been observed to have a slightly positive effect on herbaceous cutting root growth at rates up to 20% (v/v) when tested with six herbaceous perennial species. Fine coconut shell biochar at rates of 40% or 80% resulted in decreased root area and root length compared with a control, while promoting many short primary root initials (Hoover 2017).

We hypothesize that using coarse components that promote airspace

Received for publication 4 Aug 2025. Accepted for publication 4 Sep 2025.

Published online 14 Oct 2025.

[1]Department of Plant Sciences, California Polytechnic State University, 1 Grand Avenue, San Luis Obispo, CA 93407, USA

We thank Griffin for donating unrooted cuttings and Wendy Robinson for technical assistance. Mention of a trademark, proprietary product, or vendor does not constitute an endorsement.

B.K.H. is the corresponding author. E-mail: bkhoover@calpoly.edu.

https://doi.org/10.21273/HORTTECH05739-25

around the base of the cutting stem might lead to increased root initiation when cuttings are grown under mist systems. Additionally, we hypothesize that a layer of fine coconut shell biochar in propagation substrate might promote root initiation.

When rooted plugs are transplanted, plug structural integrity is critical. This integrity is influenced by substrate components and root growth (Wang et al. 2020). Rooted cuttings in stratified treatments that contained perlite had significantly lower plug integrity after a drop test than nonstratified plugs or those stratified with wood fiber (Thiessen and Fields 2025). The use of stratified coarse components might put plug structural integrity at risk, since fine particles, such as peat or coir, often serve to unify substrate structure.

Our objective in this study was to test top- and middle-stratification effects on herbaceous cutting rooting. We stratified a commercial, peat-based propagation substrate with two coarse amendments, perlite and PBH, as well as fine coconut shell biochar. We then measured plug structural integrity and root growth outcomes.

Materials and methods

Experimental design. We conducted these experiments in two repetitions, which we refer to as series. In the first series, we set up four separate completely randomized design experiments with the independent factor of substrate stratification, which contained seven levels. Each of the four experiments contained one plant species. An experimental unit was one pot containing one cutting; each experiment had 49 experimental units and 7 replicates for each treatment ($N = 49$, $n = 7$). Series 1 began 16 Apr 2024, and in series 2, we repeated every experiment beginning 7 May 2024.

Substrate treatments. We filled small round pots [top diameter 6.3 cm, depth 6.1 cm, volume 140 cm^3 (1269; Anderson Die and Manufacturing, Portland, OR, USA)] with our substrate treatments. Our control was a commercial, peat-based propagation substrate (Seeding Gold, 0-10 mm; Pindstrup Mosebrug A/S, Ryomgård, Denmark). We amended the control mix to create six additional stratified substrates (Fig. 1). Three were top-stratified, with a top layer (3 cm deep) of coarse perlite (NorCal, Richmond, CA, USA), parboiled rice hulls (PBH, Rice Hulls, PowerGrow Systems, Vineyard, UT, USA), or fine coconut shell biochar (Bay Area Biochar, Concord, CA, USA) with the control substrate as the bottom 3-cm layer. We created three middle-stratified substrates with three layers, each 2 cm deep. The top and bottom layers were the control substrate, with the center consisting of coarse perlite, PBH, or fine coconut shell biochar.

Before sticking cuttings, we tested substrate pH and electrical conductivity (EC) using a 2:1 dilution method for sample preparation. A 50-cm^3 substrate sample was diluted in 100 mL of distilled water for 1 h and then measured with probes (IQ150 and 2265FS; Spectrum Technologies Inc., Aurora, IL, USA). We tested the control, the blended substrates, and the raw amendments with three replications each ($n = 3$).

Cutting propagation. We acquired URCs for four herbaceous perennial plants [hybrid hyssop (*Agastache* × 'Sunrise Red Improved'), false heather (*Cuphea hyssopifolia* 'Maria'), blanket flower (*Gaillardia* ×*grandiflora* 'Eclipse'), Jame sage (*Salvia* ×*jamensis* 'Ignition Purple')] from a commercial supplier (Vivero North America Corp., Homestead, FL, USA). The false heather cuttings were ~3 cm long, the hybrid hyssop and Jame sage cuttings were ~4 cm long, and the blanket flower cuttings were ~6 cm long. We stuck the cuttings ~1 cm deep in the substrate. We placed the cuttings under intermittent mist, controlled by a vapor pressure deficit sensor (Water Pro VPD; MicroGrow Control Systems Inc., Temecula, CA, USA), for 24 h in a greenhouse with an approximate 60% light reduction and bench bottom heat at 70 °F. We then removed the cuttings from the intermittent misting for foliar auxin application with indole-3-butryic acid (Advocate®;

Fig. 1. Cross-sections of the substrate treatments used in a stratification experiment with herbaceous cuttings. The seven treatments, left to right, were as follows: control with a commercial peat-based blend, top-stratified perlite on the control blend (TP), top-stratified rice hulls on the control blend (TR), top-stratified fine coconut shell biochar on the control blend (TB), middle-stratified perlite between layers of the control blend (MP), middle-stratified rice hulls between layers of the control blend (MR), and middle-stratified fine coconut shell biochar between layers of the control blend (MB).

Table 1. Chemical properties of substrates used in stratified substrate experiments with herbaceous cuttings (n = 3).[i]

	Substrates[ii]									
Chemical properties	Control	TP	TR	TB	MP	MR	MB	Perlite	Rice hulls	Biochar
pH	5.5 fg[iii]	5.7 def	5.5 g	5.9 bc	5.6 efg	5.5 fg	5.7 cde	9.0 a	5.8 cd	6.0 b
EC (ms/cm)	0.23 c	0.12 ef	0.24 bc	0.25 bc	0.17 de	0.24 bc	0.22 cd	0.08 f	0.28 ab	0.31 a

[i] Substrate pH and electrical conductivity (EC) were determined using a 2:1 dilution method for sample preparation. A 50-cm^3 substrate sample was diluted in 100 mL of distilled water for 1 h and then tested with probes.

[ii] Control was a commercial propagation substrate. MB = middle-stratified treatment with top and bottom layers of control substrate and center 2 cm consisting of fine coconut shell biochar; MP = middle-stratified treatment with top and bottom layers of control substrate and center 2 cm consisting of coarse perlite; MR = middle-stratified treatment with top and bottom layers of control substrate and center 2 cm consisting of parboiled rice hulls; TB = top-stratified treatment with 3 cm of fine coconut shell biochar above the control; TP = top-stratified treatment with 3 cm of coarse perlite above the control; TR = top-stratified treatment with 3 cm of parboiled rice hulls above the control.

[iii] Means in a row that do not share a letter are significantly different according to Tukey's honestly significant difference test ($P \leq 0.05$).

Fine Americas Inc., Walnut Creek, CA, USA) at 400 ppm at the rate of 0.2 L/m^2. The cuttings were returned to intermittent misting 45 min after application, when the foliage was dry. After 14 d, we removed the cuttings from the intermittent misting, placing them in a greenhouse to be hand-irrigated as needed. The plants were watered to container capacity with 150 ppm N of 20N–4.4P–16.6K 21 d after sticking. Atmospheric temperature, substrate temperature, atmospheric humidity, and photosynthetically active radiation data were collected every 30 min by a weather station on the greenhouse bench (Watchdog 2450; Spectrum Technologies Inc., Aurora, IL, USA). The mean atmospheric temperatures in series 1 were 75.6/69.3 °F (day/night), the mean substrate temperatures were 69.1/64.3 °F (day/night), the mean atmospheric relative humidity during the misting phase was 65.7%, and the mean daily light intensity was 86.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The mean atmospheric temperatures in series 2 were 78.7/72.3 °F (day/night), the mean substrate temperatures were 76.1/71.1 °F (day/night), the mean atmospheric relative humidity during the misting phase was 85.4%, and the mean daily light intensity was 123.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Harvest. Three weeks after sticking, the cuttings were evaluated by gently removing the plug from the container without disturbing the roots. We harvested the cuttings when we observed three control plugs, and each had root growth sufficient for transplanting. Slight variations in harvest timing occurred due to schedule availability and the time required for the harvesting process. In the first series, we harvested blanket flower 31 d after sticking and hybrid hyssop, false heather, and Jame sage 33 d after sticking. In the second series, we harvested blanket flower and Jame sage 25 d after sticking and hybrid hyssop and false heather 32 d after sticking.

We harvested the rooted cuttings by removing them from the containers and assessing the stability of the plugs outside the containers. Each rooted cutting was removed from its container and placed on the laboratory bench. After this handling, we used a subjective plug structural stability rating to qualify the plug stability and root presence on the outer portion of the plug (0 = no visible roots, unstable; 1 = minimal

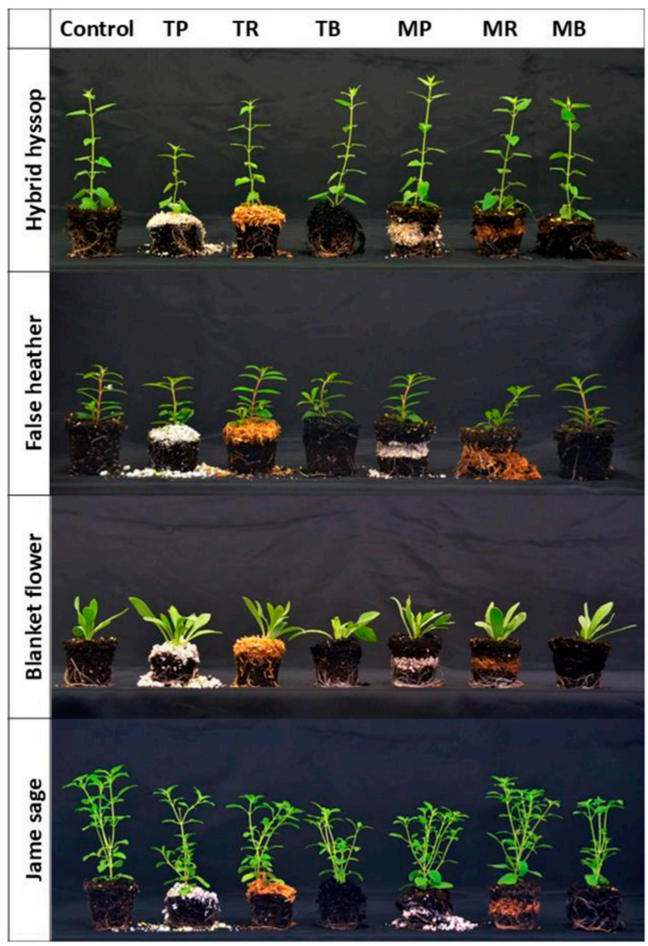

Fig. 2. Plug structural integrity photos from a stratification experiment with herbaceous cuttings. Pictures were taken at the time of harvest, using representative samples. The seven treatments, left to right, were as follows: control with a commercial peat-based blend, top-stratified perlite on the control blend (TP), top-stratified rice hulls on the control blend (TR), top-stratified fine coconut shell biochar on the control blend (TB), middle-stratified perlite between layers of the control blend (MP), middle-stratified rice hulls between layers of the control blend (MR), and middle-stratified fine coconut shell biochar between layers of the control blend (MB).

roots, one or two roots visible, unstable; 2 = moderate roots, more than two roots but large spaces with no roots, partially unstable; 3 = acceptable roots, many roots with only small gaps, stable; and 4 = optimum roots, many roots with full coverage, stable). Unstable plugs separated into pieces or lost more than 25% of their volume. Partially unstable plugs remained in one piece but had small sections, less than 25% of their volume, that fell away. Stable plugs remained intact throughout the process. All plug ratings were assigned by the same reviewer. We then photographed representative plants from each treatment to show plug stability status. We used a spray nozzle to clean the substrate from the roots, and then we photographed representative root systems. We excised the roots of each plant onto an acetate sheet and scanned them at 600 dpi on a white background (Perfection V19; Epson America, Long Beach, CA, USA). The roots were then oven-dried at 72 °C for 48 h and weighed (g).

Root imaging analysis. We processed root scans with ImageJ (version 1.54g; National Institutes of Health, Bethesda, MD, USA) using the enhance local contrast filter, with blocksize set to 130, histogram bins at 200, and the maximum slope at 5.00. The scans were saved as binary images and then analyzed in RhizoVision Explorer (version 2.0.3; Zenodo, Geneva, Switzerland) (Seethepalli and York 2021) to calculate total root length (mm) and total root surface area (mm^2). We used the Broken Roots analysis mode, with thresholding set at 200 (filter nonroot objects = 1; enable edge smoothing: threshold = 2; feature extraction: root pruning = 2).

Data analysis. Substrate chemical property data were analyzed with JMP Pro 18 (SAS Institute, Cary, NC, USA), using a one-way analysis of variance (ANOVA) for the main effect of substrate, with a post hoc Tukey honestly significant differences test. Residuals for each data set were checked to confirm homogeneity of variance and normal distribution. Root growth data were analyzed with JMP Pro 18, using a two-way mixed model ANOVA with restricted maximum likelihood to estimate variance components. We set series as a random effect and stratification as a fixed effect. Residuals for each data set were checked to confirm homogeneity of variance and normal distribution; if these assumptions were not met, the data were log transformed. Each species was analyzed separately. When an effect was detected, Tukey's honestly significant difference test was used to compare means. The threshold for rejecting a null hypothesis for all tests was 0.05.

Results and discussion

Substrate chemical properties. The pH means of all substrates except perlite used in this experiment were within favorable ranges (5.5 to 6.5) for herbaceous cuttings at the start of the experiment (Table 1) (Davies et al. 2018). The pH of perlite was higher than the target range (mean, 8.9), but it can be used as a propagation medium despite its high pH due to its low buffering capacity (Davies et al. 2018). The EC means of all substrates and raw components were within favorable ranges at the start of the experiment (Table 1) (Davies et al. 2018). Differences in nutrient holding likely occurred between substrates when the plants were irrigated with 20N–4.4P–16.6K, based on the expected cation exchange capacities of the substrates (Davies et al. 2018). These differences were not great enough to produce observed growth effects. At the time of harvest, some variability in stem growth occurred within each species, but it did not appear to be associated with treatments (Figs. 2 and 3). We did

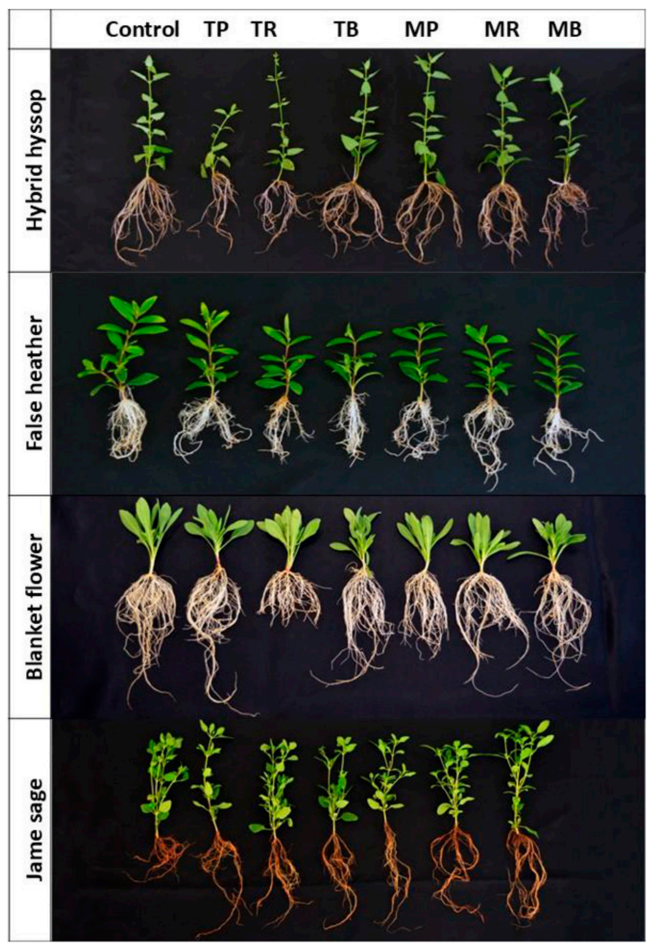

Fig. 3. Plug washed root system photos from a stratification experiment with herbaceous cuttings. Pictures were taken at the time of harvest, using representative samples. The seven treatments, left to right, were as follows: control with a commercial peat-based blend, top-stratified perlite on the control blend (TP), top-stratified rice hulls on the control blend (TR), top-stratified fine coconut shell biochar on the control blend (TB), middle-stratified perlite between layers of the control blend (MP), middle-stratified rice hulls between layers of the control blend (MR), and middle-stratified fine coconut shell biochar between layers of the control blend (MB).

Table 2. Mixed-model analysis of variance probability values for plug stability rating and root growth means for herbaceous cuttings at harvest in stratified substrate experiments repeated in two series.

Species	Source of variation	Plug structural stability rating (0 to 4 scale)[i]	Dry root wt (g)	Total root length (mm)[ii]	Total root surface area (mm^2)
Hybrid hyssop	Stratification	<0.0001	0.0926	0.0405	0.0664
	Stratification × series	0.0268	0.0706	0.3905	0.3765
False heather	Stratification	<0.0001	0.0040	<0.0001	<0.0001
	Stratification × series	0.0213	0.2600	0.1546	0.1732
Blanket flower	Stratification	<0.0001	0.0016	<0.0001	<0.0001
	Stratification × series	0.0045	0.3510	0.9682	0.9701
Jame sage	Stratification	<0.0001	0.0241	0.0970	0.0619
	Stratification × series	0.0430	0.1317	0.1219	0.0573

[i] 0 = no visible roots, unstable; 1 = minimal roots, one or two roots visible, unstable; 2 = moderate roots, more than two roots but large spaces with no roots, partially unstable; 3 = acceptable roots, many roots with only small gaps, stable; 4 = optimum roots, many roots with full coverage, stable.
[ii] Total root length and total root surface area were calculated with RhizoVision software (version 2.0.3; Zenodo, Geneva, Switzerland) (Seethepalli and York 2021).
Series was treated as a random effect, and stratification was treated as a fixed effect. Each species was analyzed separately.

not collect stem growth measurements, but no consistent differences in leaf color, stem height, or stem diameter were observed.

Plug structural stability. All four plant species had variability in plug stability rating means between stratification and series, which resulted in interactions (Table 2). Each plant species achieved a mean plug structural stability rating of 3, equating a stable root system, in the control substrate by the harvest period (Figs. 2 and 4). None of the stratified substrates consistently met this standard. In most outcomes, the stratified substrates produced a significantly lower mean plug stability rating than the control (Fig. 4; $P \leq 0.05$). No top-stratified substrates had a mean plug stability rating equal to the control in both series of the experiment (Fig. 4). In five instances, a middle-stratified substrate had a mean plug stability rating that was the same as the control in both series of the experiment. Hybrid hyssop had the same mean plug stability rating in MP and MR as the control, false heather and blanket flower both had the same mean plug stability rating in MP as the control, and Jame sage had the same mean plug stability rating in MR as the control (Fig. 4). In these cases, root development on the outer part of the plug was sufficient to hold it together; there was not substantial root development within the internal part of the middle layer. The use of coarse components for stratification, such as perlite and PBH, often had a negative effect on plug stability, which aligns with the findings of Thiessen and Fields (2025). Stratified treatments containing biochar often appeared stable initially but would break apart when handled. This was caused by poor root development in the exclusively biochar regions, but the fine particles would initially bind when wet.

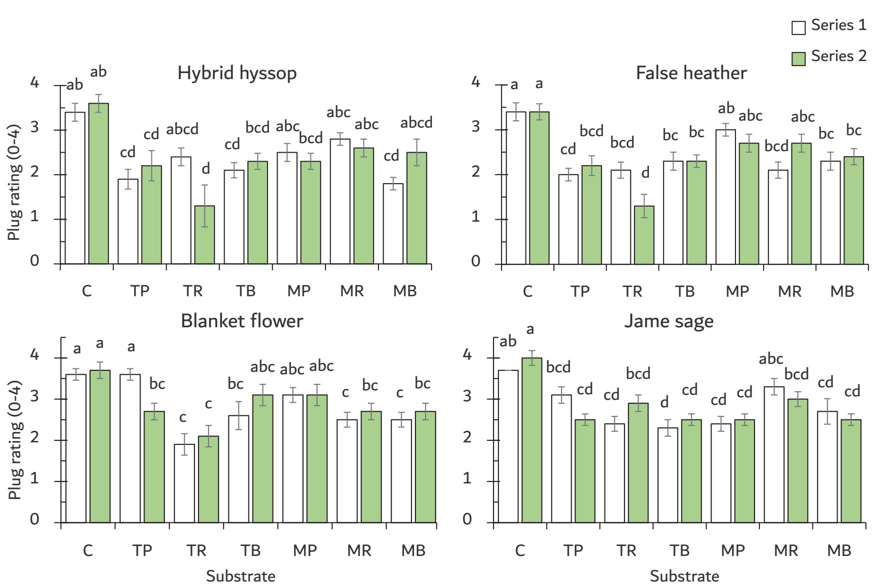

Fig. 4. Plug stability rating least squared means with *SE* for herbaceous cuttings at harvest in stratified substrate experiments. The rating scale was as follows: 0 = no visible roots, unstable; 1 = minimal roots, one or two roots visible, unstable; 2 = moderate roots, more than two roots but large spaces with no roots, partially unstable; 3 = acceptable roots, many roots with only small gaps, stable; 4 = optimum roots, many roots with full coverage, stable. Unstable plugs separated into pieces or lost more than 25% of their volume. Partially unstable plugs remained in one piece but had small sections, less than 25% of their volume, that fell away. Stable plugs remained intact throughout the process. Means for each species that do not share a letter are significantly different according to Tukey's honestly significant difference test ($n = 7$, $P \leq 0.05$). C = control of commercial propagation substrate; MB = middle-stratified treatment with top and bottom layers of control substrate and center 2 cm consisting of fine coconut shell biochar; MP = middle-stratified treatment with top and bottom layers of control substrate and center 2 cm consisting of coarse perlite; MR = middle-stratified treatment with top and bottom layers of control substrate and center 2 cm consisting of parboiled rice hulls; TB = top-stratified treatment with 3 cm of fine coconut shell biochar above the control; TP = top-stratified treatment with 3 cm of coarse perlite above the control; TR = top-stratified treatment with 3 cm of parboiled rice hulls above the control.

Root growth. Cuttings rooted in all substrates. Root growth in the control and stratified treatments had similar morphology after washing (Fig. 3). Before washing, more root development was observed in the regions of the substrate that were the control

Table 3. Root growth means with *SE* for herbaceous cuttings at harvest in stratified substrate experiments repeated in two series.

Substrate[i]	Dry root wt (g)	Total root length (mm)[ii]	Total root surface area (mm^2)[ii]
Hybrid hyssop			
Control	0.042 ± 0.003	3,290.3 ± 284.2	9,985.2 ± 965.8
TP	0.031 ± 0.004	2,203.7 ± 345.3	7,147.3 ± 943.0
TR	0.025 ± 0.004	2,000.6 ± 375.7	6,412.2 ± 1,106.3
TB	0.032 ± 0.004	2,567.0 ± 366.8	8,135.3 ± 1,137.2
MP	0.034 ± 0.004	2,600.4 ± 318.2	8,178.6 ± 991.1
MR	0.033 ± 0.004	3,299.8 ± 333.0	9,989.4 ± 1,114.0
MB	0.032 ± 0.004	2,909.7 ± 400.8	8,802.0 ± 1,255.6
False heather			
Control	0.025 ± 0.001 a[iii]	3,953.7 ± 174.3 a	8,336.6 ± 366.3 a
TP	0.025 ± 0.002 a	2,710.6 ± 191.1 b	6,510.8 ± 468.3 abc
TR	0.018 ± 0.002 b	1,460.5 ± 147.0 c	3,863.3 ± 394.0 d
TB	0.020 ± 0.001 ab	2,546.2 ± 213.1 b	5,413.0 ± 487.6 bcd
MP	0.020 ± 0.001 ab	2,412.4 ± 126.1 b	4,994.5 ± 296.3 cd
MR	0.025 ± 0.002 ab	3,224.7 ± 281.8 ab	7,158.5 ± 610.7 ab
MB	0.024 ± 0.002 ab	3,142.4 ± 290.6 ab	6,529.2 ± 577.6 abc
Blanket flower			
Control	0.080 ± 0.006 a	7,959.3 ± 825.0 a	21,830.9 ± 2,159.6 ab
TP	0.089 ± 0.011 a	9,379.3 ± 815.4 a	27,315.0 ± 2,446.6 a
TR	0.045 ± 0.007 b	3,770.2 ± 723.3 b	11,572.2 ± 2,058.3 c
TB	0.072 ± 0.008 ab	8,022.6 ± 1,114.6 a	21,562.3 ± 3,018.4 ab
MP	0.090 ± 0.007 a	9,793.5 ± 1,062.0 a	26,437.8 ± 2,772.8 ab
MR	0.064 ± 0.006 ab	7,686.5 ± 816.6 a	20,551.7 ± 2,337.4 abc
MB	0.067 ± 0.008 ab	5,969.2 ± 697.1 ab	16,658.5 ± 1,833.1 bc
Jame sage			
Control	0.029 ± 0.003 b	4,912.0 ± 329.0	12,017.4 ± 950.4 ab
TP	0.035 ± 0.003 ab	5,279.2 ± 451.3	13,706.8 ± 1,476.1 ab
TR	0.030 ± 0.004 ab	4,151.8 ± 499.6	10,962.5 ± 1,368.3 b
TB	0.035 ± 0.003 ab	4,704.1 ± 439.2	12,441.5 ± 1,350.6 ab
MP	0.032 ± 0.003 ab	4,741.7 ± 455.5	12,343.3 ± 1,316.5 ab
MR	0.040 ± 0.004 a	5,454.4 ± 558.7	14,442.1 ± 1,699.7 a
MB	0.035 ± 0.004 ab	4,527.6 ± 506.1	12,374.8 ± 1,532.7 ab

[i] Control was a commercial propagation substrate. MB = middle-stratified treatment with top and bottom layers of control substrate and center 2 cm consisting of fine coconut shell biochar; MP = middle-stratified treatment with top and bottom layers of control substrate and center 2 cm consisting of coarse perlite; MR = middle-stratified treatment with top and bottom layers of control substrate and center 2 cm consisting of parboiled rice hulls; TB = top-stratified treatment with 3 cm of fine coconut shell biochar above the control; TP = top-stratified treatment with 3 cm of coarse perlite above the control; TR = top-stratified treatment with 3 cm of parboiled rice hulls above the control.

[ii] Total root length and total root surface area were calculated with RhizoVision software (version 2.0.3; Zenodo, Geneva, Switzerland) (Seethepalli and York 2021).

[iii] Means for each species in a column that do not share a letter are significantly different according to Tukey's honestly significant difference test (n = 14, $P \le 0.05$).

Series was treated as a random effect, and stratification was treated as a fixed effect. According to a mixed-model analysis of variance, there was no interaction between series and stratification in these data. Stratification was analyzed as a main effect, with a post hoc Tukey's honestly significant differences test. Each species was analyzed separately.

than in the stratification amendments. We hypothesize that the use of coarse substrate stratification components might have reduced root-to-substrate contact, affecting water and nutrient availability. Orman-Ligeza et al. (2018) reported reduction of herbaceous plant branching in substrate air spaces, while Fields and Criscione (2025) saw improved root development in petunia (*Petunia* hybrid) when grown in moderately packed peat-based substrates with high levels of root-to-substrate contact.

The use of coarse stratification components or biochar did not result in consistent growth differences compared with the control (Table 3). Dry root weight means were affected by stratification treatment for three of the plant species (Table 2). False heather and blanket flower cuttings in top-stratified rice hulls on the control blend (TR) had lower dry root weight means than the control (Table 3; $P \le 0.05$). Jame sage had a higher dry root mean in middle-stratified rice hulls between layers of the control blend (MR) than in the control (Table 3; $P \le 0.05$). In most cases, top-stratified root systems had poor growth in the amendment and high growth rates in the lower control substrate.

Total root length and total root surface area outcomes mostly aligned with root dry weight outcomes. False heather and blanket flower had significantly lower total root length and total root surface area means than the control, which matched the root dry weight outcome (Table 3; $P \le 0.05$). False heather root growth was most affected by stratification, with total root length being reduced compared with the control in the top-stratified perlite on the control blend (TP), TR, top-stratified fine coconut shell biochar on the control blend (TB), and middle-stratified perlite between layers of the control blend (MP) treatments (Table 3; $P \le 0.05$). False heather roots were observed to be thinner and more finely textured than the other plants in this study, which might have contributed to this response. In contrast, hybrid hyssop and Jame sage had no reduction in root growth means compared with the control in this study (Table 3).

In these experiments, we did not see a clear stratification effect on root growth. This suggests that stratified substrates can produce comparable growth to a control, which supports earlier findings that stratification could contribute to reductions in peat use (Fields and Criscione 2023; Thiessen and Fields 2025). In this experiment, the top-stratified blend had ~54% amendment and 46% peat-based substrate per container (by volume), while the middle-stratified mix had ~35% amendment and 65% peat-based substrate per container (by volume).

Considering that stratification did not benefit root growth and resulted in reduced plug structural stability, using uniform blends of peat-based substrates mixed with cost-effective amendments may be a more attractive option to explore to reduce peat use than stratification. Alternately, Thiessen and Fields (2025) reported more plug integrity with wood fiber stratification of peat-based substrate than with perlite. In the selection of a stratification material, consideration should be given to its ability to bind and maintain plug stability.

References cited

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.

Davies FT Jr, Geneve RL, Wilson SB. 2018. Hartmann and Kester's plant propagation principles and practices (9th ed). Pearson Education, Upper Saddle River, NJ, USA.

Evans MR, Gachukia M. 2004. Fresh parboiled rice hulls serve as an alternative to perlite in greenhouse crop substrates. HortScience. 39(2):232–235. https://doi.org/10.21273/HORTSCI.39.2.232.

Evans MR, Gachukia M. 2007. Physical properties of sphagnum peat-based root substrates amended with perlite or fresh parboiled rice hulls. HortTechnology. 17(3):312–315. https://doi.org/10.21273/HORTTECH.17.3.312.

Fields JS, Criscione KS. 2023. Stratified substrates can reduce peat use and improve root productivity in container crop production. HortScience. 58(3):364–372. https://doi.org/10.21273/HORTSCI17019-22.

Fields JS, Criscione KS. 2025. Moderate increases in substrate packing density can improve petunia root development. HortTechnology. 35(5):636–638. https://doi.org/10.21273/HORTTECH05660-25.

Fonteno WC, Dole JM. 2006. Media, p 39–50. In: Dole JM, Gibson JL (eds). Cutting propagation: A guide to propagating and producing floriculture crops. Ball Publishing, Batavia, IL, USA.

Gislerod HR. 1983. Physical conditions of propagation media and their influence on the rooting of cuttings. Plant Soil. 75(1):1–14. https://doi.org/10.1007/BF02178609.

Hoover BK. 2017. Adventitious root development of herbaceous cuttings in biochar-amended substrates (abstr). HortScience. 52(9S):S148.

Orman-Ligeza B, Morris EC, Parizot B, Lavigne T, Babé A, Ligeza A, Klein S, Sturrock C, Xuan W, Novák O, Ljung K, Fernandez MA, Rodriguez PL, Dodd IC, De Smet I, Chaumont F, Batoko H, Périlleux C, Lynch JP, Bennett MJ, Beeckman T, Draye X. 2018. The xerobranching response represses lateral root formation when roots are not in contact with water. Curr Biol. 28(19):3165–3173.e5. https://doi.org/10.1016/j.cub.2018.07.074.

Santos KM, Fisher PR, Argo WR. 2008. A survey of water and fertilizer management during cutting propagation. HortTechnology. 18(4):597–604. https://doi.org/10.21273/horttech.18.4.597.

Seethepalli A, York L. 2021. RhizoVision Explorer: Interactive software for generalized root image analysis designed for everyone, version 2.0.3. Zenodo. http://doi.org/10.5281/zenodo.4095629.

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. 2025. Substrate stratification can reduce peat requirement associated with young plant production. HortTechnology. 35(2):233–240. https://doi.org/10.21273/HORTTECH05570-24.

US Department of Agriculture, National Agricultural Statistics Service. 2023. Quick stats. https://quickstats.nass.usda.gov/results/EEFC0483-60D9-36D8-9731-0C7911887B8C. [accessed 1 Aug 2025].

Wang G, Qu P, Huang H, Wu G, Yan H. 2020. Evaluation of coconut coir dust/modified urea–formaldehyde resins as a growing medium for pepper seedlings. HortTechnology. 30(3):322–330. https://doi.org/10.21273/HORTTECH04542-19.

World Bank. 2023. World Integrated Trade Solution, US unrooted cuttings and slips imports by country in 2023. https://wits.worldbank.org/trade/comtrade/en/country/USA/year/2023/tradeflow/Imports/partner/ALL/product/060210. [accessed 1 Aug 2025].