Effect of Substrate Stratification Without Fine Pine Bark Particles on Growth of Common Nursery Weed Species and Container-grown Ornamental Species

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ABSTRACT. Substrate stratification is a new research area in which multiple substrates, or the same substrate with differing physical properties, are layered within a container to accomplish a production goal, such as decreasing water use, nutrient leaching, or potentially reducing weed growth. Previous research using stratification with pine (*Pinus* sp.) bark screened to ≤1/2 or 3/4 inch reduced the growth of bittercress (*Cardamine flexuosa*) by 80% to 97%, whereas liverwort (*Marchantia polymorpha*) coverage was reduced by 95% to 99%. The objective of this study was to evaluate substrate stratification with pine bark screened to remove all fine particles as the top strata of the substrate and determine its effect on common nursery weeds and ornamental plants. Stratified treatments consisted of pine bark screened to either 1/8 to 1/4 inch, 1/4 to 1/2 inch, or 3/8 to 3/4 inch, applied at depths of either 1 or 2 inches on top of a standard ≤1/2-inch pine bark substrate. An industry-standard treatment was also included in which the substrate was not stratified but consisted of only ≤1/2-inch pine bark throughout the container. A controlled-release fertilizer was incorporated at the bottom strata in all stratified treatments (no fertilizer in the top 1 or 2 inches of the container media), whereas the industry standard treatment had fertilizer incorporated throughout. Compared with the nonstratified industry standard, substrate stratification decreased spotted spurge (*Euphorbia maculata*) counts by 30% to 84% and bittercress counts by 57% to 94% after seeding containers. The shoot dry weight of spotted spurge was reduced by 14% to 55%, and bittercress shoot dry weight was reduced by 71% to 93% in stratified treatments. Liverwort coverage was reduced by nearly 100% in all the stratified substrate treatments. Compared with the industry standard substrate, stratified treatments reduced shoot dry weight of ligustrum (*Ligustrum japonicum*) by up to 20%, but no differences were observed in growth index, nor were any growth differences observed in blue plumbago (*Plumbago auriculata*).

Weed management in container nursery production is challenging due to various factors, including increased weed competition in the restricted rooting environment of a container (Berchielli-Robertson et al., 1990; Fretz 1972), lack of herbicide options for controlling many taxa, and high costs associated with labor (i.e., hand weeding) and herbicides (Ingram et al., 2016, 2017). Additionally, with the higher cost of hand weeding and various challenges associated with an herbicide-only management strategy, there is a need for integrated and sustainable weed management strategies. The primary component of container nursery substrate consists of pine (*Pinus* sp.) bark, comprising 60% to 80% of most substrate mix (Lu et al., 2006). This control over the substrate and fertilizer can be modified to control weeds. At present, cultural or nonchemical practices, such as mulching (Altland et al., 2016; Bartley et al., 2017; Marble et al., 2019; Richardson et al., 2008) and strategic fertilizer placement (Fain et al., 2003; Khamare et al., 2020; Saha et al., 2019) have garnered a renewed interest from researchers for developing weed management strategies that could be combined with herbicides to develop an integrated program (Yu and Marble, 2022).

An additional cultural or nonchemical method that could have potential as a weed management tool is "layering" or stratified substrates (Khamare et al., 2022). This is a new area of research in which multiple substrates, or the same substrate with differing physical properties, are layered within a container to accomplish a production goal, such as decreasing water use or nutrient leaching, or potentially reducing weed growth (Criscione et al., 2022; Fields et al., 2021; Khamare et al., 2022). Because of the inherent moisture gradient in a container filled uniformly with the same substrate, the upper portion dries quickly, which requires more frequent irrigation to provide enough moisture for recently potted plants to establish, especially soon after transplanting and before roots are fully developed (Fields et al., 2020, 2021; Owen and Altland, 2008). The use of stratified substrates as described by Fields et al. (2020, 2021), in which a finer or water-retentive substrate is added on top of a coarser or freely draining substrate, creates a more uniform moisture gradient throughout the container (Criscione et al., 2022). Fields et al. (2021) reported that by using substrates with a high level of moisture and nutrient retention characteristics placed on top of a coarse, freely draining substrate, fertilizer rates could be reduced by 20% without

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Units

Units To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.3048	ft	m	3.2808
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
28.3495	oz	g	0.0353
1.7300	oz/inch^3	g·cm^{-3}	0.5780
(°F − 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

negative effects on the growth or quality of 'Megalpio' (Red Drift®) rose (*Rosa*) compared with an industry-standard substrate. In another study, stratified substrates improved the growth and quality of 'Ruby' loropetalum (*Loropetalum chinense*) compared with a traditional homogenous substrate system in which the entire container was filled uniformly with a single substrate throughout the container (Criscione et al., 2022).

In a previous study, we applied the stratification strategy described by Fields et al. (2021) inversely to evaluate weed control benefits (Khamare et al., 2022). The larger coarse particles were used as top strata containing no fertilizer, and a fine-textured, highly moisture-retentive substrate comprised the bottom strata. The principle was to use two of the most efficient nonchemical weed management methods of strategic fertilizer placement combined with a mulch-like layer. Results of our previous study revealed that substrate stratification reduced the growth of bittercress (*Cardamine flexuosa*) by 80% to 97%, and liverwort (*Marchantia polymorpha*) coverage was reduced by 95% to 99%. Additionally, there was no difference in the growth of ligustrum (*Ligustrum japonicum*) and blue plumbago (*Plumbago auriculata*) in stratified substrates compared with industry-standard substrates (Khamare et al., 2022). Substrate stratification with larger coarse particles as the top strata provides a mulch-like layer that holds less moisture and has no nutrient available for weed seeds to germinate and establish. Although more research is needed, we hypothesized that weed control benefits were derived due to the larger particle substrate retaining less moisture and the fact that the larger particle pine bark resulted in weed seeds being flushed deep into the substrate, reducing their chances of germinating (Keddy and Constabel, 1986). Additionally, the top substrate strata contained no fertilizer, which would simulate a dibbling or subdressing fertilization regime, both of which can be effective for weed management (Stewart et al., 2018). In our previous study, the top coarse strata consisted of pine bark screened through 3/8-, 1/2-, or 3/4-inch sieves, and contained all particles less than or equal to the screen sizes. While weed growth was reduced and crop growth was unaffected, additional evaluation is warranted to determine how various pine bark screening techniques could be used to further reduce weed growth and what effect these techniques have on ornamental plant growth. Therefore, the objective of this study was to evaluate the effect of the substrate stratification method reported by Khamare et al. (2022) but with further screening to remove more fine pine bark particles from the substrate used as the top strata and determine what impact this would have on common nursery weeds and ornamental plants.

Materials and methods

Experiments were conducted in a shadehouse and nursery pad at the Mid-Florida Research and Education Center in Apopka, FL, in Mar 2020 and repeated in Jun 2020. Aged pine bark (screened to ≤1 inch) was purchased from a local supplier and further screened by hand through different soil sieves to yield three size ranges including 1/8 to 1/4 inch, 1/4 to 1/2 inch, and 3/8 to 3/4 inch. To create a standard substrate, pine bark was screened to pass through a 1/2-inch screen and included all fines (≤1/2 inch). Stratified treatments were constructed by applying the 1/8- to 1/4-inch, 1/4- to 1/2-inch, and 3/8- to 3/4-inch pine bark as the top strata with the bottom strata consisting of ≤1/2-inch bark. The top strata were applied at a depth of either 1 or 2 inches, resulting in six stratified substrate treatments (abbreviated as top substrate size: screen size: S for stratification: top depth in inches or 1/8–1/4:S:1, 1/8–1/4:S:2, 1/4–1/2:S:1, 1/4–1/2:S:2, 3/8–3/4:S:1, and 3/8–3/4:S:2). An industry-standard treatment was also included in which the substrate was not stratified but consisted of only the ≤1/2-inch pine bark used throughout (≤1/2:TO) the container. In all treatments, a controlled-release fertilizer [Osmocote 17N–2.2P–9.1K (8 to 9 months); ICL Specialty Fertilizers, Dublin, OH, USA] was applied at a rate of 35 g per container. However, fertilizer was incorporated only in the bottom strata in all stratified treatments (no fertilizer in the top 1 or 2 inches of the container medium) while the industry standard treatment had fertilizer incorporated throughout.

Particle size and substrate physical properties. Substrate physical properties and particle size analysis were evaluated for each substrate particle size. Particle size analysis was determined by passing 100-g oven-dried samples of each of the three sizes of pine bark through 12.5-, 6.3-, 4-, 2.8-, 2.0-, 1.4-, 1.0-, 0.71-, 0.50-, 0.35-, 0.25-, 0.18-, 0.106-mm soil sieves. Particles ≤0.106 mm were collected in a pan. The sieves and pans were shaken for 5 min with a sieve shaker (Ro-Tap Rx-29; W.S. Tyler, Mentor, OH, USA). The residues at each sieve were collected, weighed, and recorded individually to determine particle size distribution. Particle sizes were then combined and classified into coarse (>2.0 mm), medium (0.50 to 2.0 mm), and fine (<0.50 mm) (Altland et al., 2018). Three replicate samples for each substrate were analyzed. Substrate physical properties including air space (AS), total porosity (TP), container capacity (CC), and bulk density (D_b) were determined on three replicates of each substrate using the North Carolina State University porometer method following procedures described by Fonteno and Harden (2010).

Effect of substrate stratification on the growth of ornamental plants. Ornamental plant and weed growth were evaluated separately in separate sets of containers to avoid having any weed competitive effect be a confounding factor. To evaluate the growth of common ornamental plants in the stratified substrate treatments, uniform 2-inch plug tray liners of ligustrum and blue plumbago were obtained from a local nursery. Liners were transplanted into a 1-gal (7 inches height, 7 3/4 inches diameter) nursery container using the above-mentioned substrates. The root ball of ornamental plants was planted into the top strata of all stratified substrate treatments as the containers were filled. As root ball depth was 2 inches, root balls were either covered ~50% in the larger stratified substrate and 50% in the industry standard (1 inch depth) or were placed on top of the industry standard (2 inches depth). Following potting, all plants were placed on a full sun nursery pad, irrigated 1/2 inch per day via overhead irrigation (Xcel-Wobbler™; Senninger Irrigation, Clermont, FL, USA) via two irrigation cycles (7:00 AM and 2:45 PM) and evaluated for 24 weeks after planting (WAP). Data collection included plant growth index [(height + width at widest point + perpendicular width) ÷ 3] measured every 8 WAP, in addition to root and shoot dry weights at the study conclusion (24 WAP). The experiment was a completely randomized design with eight single-container replications for each treatment and each ornamental species. The first experimental run was initiated on 11 Mar 2020, and the second on 12 May 2020.

Effect of substrate stratification on the growth of common nursery weed species. A separate set of experiments were conducted to assess weed establishment and growth. Twenty-five seeds of spotted spurge (*Euphorbia maculata*) and bittercress were surface sown onto separate sets of 1-gal containers filled with the aforementioned substrates without ornamental plants. The containers with spotted spurge were placed on a full sun nursery pad, irrigated 1/2 inch per day by the same overhead irrigation system described previously. Containers with bittercress were placed inside a shade house (60% shadecloth, average photosynthetic active radiation of 1385 $\mu mol \cdot m^{-2} \cdot s^{-1}$ outside vs. 530 $\mu mol \cdot m^{-2} \cdot s^{-1}$ inside) and irrigated 0.3 inch per day via overhead irrigation (Xcel-Wobbler). Data collection included counts of emerged spotted spurge and bittercress at 4 WAP and spotted spurge and bittercress with one or more true leaves at 10 WAP (cotyledon stage plants were not counted). Shoot dry weight was collected at the trial conclusion (10 WAP) by clipping plants at soil level and placing shoots in a forced-air oven at 60 °C for 7 d, reaching a constant weight. The experiment was a completely randomized design with eight single-container replications per treatment. The first experimental run for spotted spurge and bittercress was initiated in Apr 2020 with spotted spurge being repeated in May 2020 and bittercress in Dec 2020.

A separate set of nursery containers were used to evaluate liverwort growth on stratified substrates in Dec 2020. Ten weeks before initiating the experiment and filling containers, four to five pieces of liverwort were transplanted onto the surface of 1.68-L (5 1/4 inches height, 5 1/4 inches diameter) square nursery containers that had been previously filled with a pine bark: peat substrate (80:20 v:v) amended with the same controlled-release fertilizer via incorporation as described above. Containers were then placed inside the same shade house mentioned previously and irrigated 1/2 inch per day via overhead irrigation (Xcel-Wobbler). Containers remained in the shade house until the surface of the containers was filled with liverwort (no visible substrate upon visual inspection) and gemmae cups had formed. At this time (~10 weeks after transplanting), these containers were used as inoculum to sporulate the treatments naturally because liverwort can spread asexually through the splashing of gemmae or sexually via airborne spores (Newby et al., 2007). Square 1.68-L nursery containers were filled and fertilized with the stratified and industry-standard treatments mentioned previously and placed inside the same shade house. To initiate the experiment, the inoculum containers were placed around each replication at a distance of 0.2 inches so that the experimental containers had an inoculum container on all four sides. Liverwort surface coverage was assessed at 16 WAP by taking digital photos of each treatment using a smartphone (iPhone 8 Plus; Apple, Cupertino, CA, USA) from a height of 3 ft. Images were cropped using a software program (Microsoft Paint; Microsoft Corp, Redmond, WA, USA) so that only the surface of the substrate and liverwort was visible in the image. Liverwort coverage was then determined using the color threshold tool (hue, saturation, and brightness) in Image processing software [Abramoff et al., 2004 (ImageJ; U.S. National Institutes of Health, Bethesda, MD, USA)] to provide a quantitative value of liverwort coverage. Due to the seasonality of liverwort growth in Florida in which growth and spread can significantly decrease due to heat, both experimental runs were initiated in Dec 2020.

Statistical analysis. Data were subjected to analysis of variance using statistical software (JMP® Pro ver. 14; SAS Institute Inc., Cary, NC, USA). Before analysis, all data were inspected to ensure the assumptions of analysis of variance were met. When appropriate, post hoc means comparisons were performed using Tukey's honestly significant difference test at a 0.05 significance level. There was no treatment-by-experimental run interactions; therefore, results were combined across both experimental runs.

Results and discussion

Particle size and substrate physical properties. Particle size distribution showed that the 1/4 to 1/2-inch and 3/8 to 3/4-inch substrates had the highest percent of coarse particles (>2.0 mm; 93.4% and 96.3%, respectively) and the lowest percent of medium and fine particles compared with other substrates (Table 1). The 1/8 to 1/4-inch substrate consisted of 35.9% medium particles (2.0 to 0.50 mm), higher than other substrates evaluated and followed by the ≤1/2-inch industry standard (29.6%). Both the <1/2-inch industry standard and the 1/8 to 1/4-inch substrate had the greatest amount of fine particles, but both were still below the recommended level of 10% to 15% for adequate water-

Table 1. Particle size distribution of pine bark screened to retain 1/8 to 1/4-inch, 1/4 to 1/2-inch, 3/8 to 3/4-inch, and ≤1/2-inch particles.

Substrate size (inch)[i]	Pine bark particle size distribution (% wt)[ii]		
	Coarse (>2.0 mm)[iii]	Medium (2.0–0.50 mm)	Fine (<0.50 mm)
1/8 to 1/4	56.8 c[iv]	35.9 a	7.2 a
1/4 to 1/2	93.4 a	2.4 c	4.1 b
3/8 to 3/4	96.3 a	1.2 c	2.4 b
≤1/2	61.7 b	29.6 b	8.6 a

[i] Particle size distribution of 1/8 to 1/4-inch, 1/4 to 1/2-inch, 3/8 to 3/4-inch, and ≤1/2-inch pine bark. Substrate was air-dried until a constant weight was achieved, and then 100-g (3.53-oz) samples were passed through sieves in descending order. Means show weights (grams) collected in each sieve (n = 3); 1 g = 0.0353 oz, 1 inch = 2.54 cm.

[ii] Sieve sizes used to determine particle size included 12.5-, 6.3-, 4.0-, 2.8-, 2.0-, 1.4-, 1.0-, 0.71-, 0.50-, 0.35-, 0.25-, 0.18-, 0.106-mm screens and a pan at the bottom to collect all materials that passed through the smallest sieve. Particles sizes were combined and further classified into coarse (>2.0 mm), medium (0.50 to 2.0 mm), and fine (<0.50 mm); 1 mm = 0.0394 inch.

[iii] Pine bark was thoroughly screened to achieve 1/8 to 1/4-inch, 1/4 to 1/2-inch, and 3/8 to 3/4-inch particle size pine bark. The standard substrate of ≤1/2-inch consisted of pine bark equal or smaller than 1/2 inch (including fine particles).

[iv] Means followed by the same letter within a column are not significantly different according to Tukey's honestly significant difference test at $P < 0.05$.

Table 2. Total porosity, air space, container capacity, and bulk density of pine bark screened to retain 1/8 to 1/4-inch, 1/4 to 1/2-inch, 3/8 to 3/4-inch, and ≤1/2-inch particles.

	Physical properties[ii]			
Substrate size (inch)[i]	Total porosity (% vol)[iii]	Air space (% vol)[iv]	Container capacity (% vol)[v]	Bulk density ($g \cdot cm^{-3}$)[vi]
1/8 to 1/4	77.8 a[vii]	42.8 a	35.0 b	0.15 b
1/4 to 1/2	73.9 a	42.8 a	31.1 b	0.15 b
3/8 to 3/4	68.8 a	51.2 a	17.5 c	0.14 b
≤1/2	78.4 a	36.1 a	42.3 a	0.17 a

[i] Substrate consisting of 1/8 to 1/4-inch, 1/4 to 1/2-inch, 3/8 to 3/4-inch, and ≤1/2-inch inch pine bark. Pine bark was thoroughly screened to achieve 1/8 to 1/4-inch, 1/4 to 1/2-inch, 3/8 to 3/4-inch-particle size pine bark. The standard substrate of ≤1/2-inch consisted of pine bark equal to or smaller than 1/2 inch (including fine particles); 1 inch = 2.54 cm.

[ii] Analysis for physical properties was performed using the North Carolina State University porometer method (Fonteno and Harden, 2010). Sample means are presented (n = 3).

[iii] Total porosity is equal to container capacity + air space.

[iv] Air space is the volume of water drained from the sample ÷ volume of the sample.

[v] Container capacity is (wet weight – oven dry weight) ÷ volume of the sample.

[vi] Bulk density after forced air drying at 105 °C (221.0 °F) for 48 h; 1 $g \cdot cm^{-3}$ = 0.5780 $oz/inch^3$.

[vii] Means followed by the same letter within a column are not significantly different according to Tukey's honestly significant difference test at $P < 0.05$ level.

holding properties (Goodwin 1980). Presumably, the 1/8 to 1/4-inch, 1/4 to 1/2-inch, and 3/8 to 3/4-inch substrates should have consisted entirely of coarse particles (≥2.0 mm) with a small percentage of fine particles, which was the case for the 1/4 to 1/2-inch and 3/8 to 3/4-inch substrates which contained more than 90% coarse particles. For the 1/8 to 1/4-inch substrate, ∼43% of particles fell below that level, most likely because the substrate was not fully dry before sifting while the particle size distribution reported in this article was conducted with fully dried samples and thus more accurate. Some commercial bark suppliers provide screened pine bark similar to the treatments described in this work. These commercial vendors are often using moist pine bark from large piles exposed to rain and weather. Therefore, the differences between the actual particle size distribution and the nominal particle size descriptions of the treatments used in this paper will likely reflect real-world commercial products. Although there were distinct differences in the particle size distribution of the substrates evaluated, no differences were observed in TP or AS, with TP ranging from 69% to 78% and AS ranging from 36% to 51% (Table 2). Although all substrates fell within the recommended TP range 50% to 85% (Bilderback et al., 2013), all substrates exceeded the recommended 10% to 30% for AS, but the ≤1/2-inch standard substrate was numerically closest to the desired range. Although different particle sizes had little to no effect on TP as has been observed previously (Altland et al., 2011; Fields et al., 2021), the AS/CC ratio changed within the substrates with the industry standard ≤1/2-inch substrate having the highest CC (42%) and the 3/8 to 3/4-inch substrate having the lowest (17.5%), with no difference being observed between the 1/8 to 1/4-inch and 1/4 to 1/2-inch substrates. Similar to results with AS, all of the substrates were below the recommended 45% to 65% CC, but again the industry standard (42%) was closest to the recommended range. Overall, physical property analysis showed that the <1/2-inch substrate had the greatest ability to retain water, followed by the 1/8 to 1/4-inch and 1/4 to 1/2-inch substrates while the 3/8 to 3/4-inch substrate had the least.

Effect of substrate stratification on the growth of ligustrum and plumbago. Ligustrum growth was similar among the substrate treatments except the growth with 1/8 to 1/4:S:2 substrate that was slightly smaller compared with ≤1/2:TO at 8 WAP (Table 3). Ligustrum growth differences were observed at 16 WAP where plants grown in the ≤1/8 to 1/4:TO had a higher growth index than plants grown in 1/8 to 1/4:S:2 and 1/4 to 1/2:S:2. However, at the trial conclusion at 24 WAP, no growth differences were observed among substrate treatments. Although growth index was similar among all treatments, shoot and root dry weights showed that stratification resulted in biomass reduction. Compared with the standard substrate of ≤1/2:TO, stratified treatments which resulted in lower shoot biomass included 1/8 to 1/4:S:2 (20% reduction), 1/4 to 1/2:S:1 (17% reduction) and 1/4 to 1/2:S:2 (15% reduction). These stratified treatments also resulted in a decrease in root growth, with a reduction of 25%, 27%, and 25% for the 1/8 to 1/4:S:2, 1/4 to 1/2:S:1, and 1/4 to 1/2:S:2 substrates, respectively. In contrast to results observed with ligustrum, no differences in growth indices were observed in plumbago throughout the experiment (Table 4). Similarly, by 24 WAP, there was no difference in the shoot and root dry weight of plumbago among stratified substrates, and they were similar to the plants grown in the standard substrate of ≤1/2:TO.

Table 3. Growth index and biomass of container-grown ligustrum [1 gal (3.8 L)] for 24 weeks in stratified and nonstratified pine bark substrates.

	Growth index (cm)[ii]			Biomass[iii]	
Substrate[i]	8 WAP	16 WAP	24 WAP	Shoot wt (g)	Root wt (g)
≤1/2:TO	20.3 a[iv]	34.5 a	43.5 a	101.9 a	46.7 a
1/8 to 1/4:S:1	18.7 ab	30.7 abc	43.4 a	92.6 abc	38.9 ab
1/8 to 1/4:S:2	15.4 b	24.8 c	37.8 a	81.6 c	35.0 b
1/4 to 1/2:S:1	17.8 ab	28.9 abc	37.3 a	84.6 bc	34.0 b
1/4 to 1/2:S:2	16.8 ab	26.8 bc	40.8 a	87.1 bc	35.0 b
3/8 to 3/4:S:1	17.8 ab	31.1 ab	46.4 a	95.9 abc	37.5 ab
3/8 to 3/4:S:2	18.9 ab	32.6 ab	45.3 a	98.9 ab	39.2 ab

[i] Substrate consisted of 1/8 to 1/4-inch pine bark (PB) at 1 inch depth (1/8–1/4:S:1), 1/8 to 1/4-inch PB at 2 inches depth (1/8–1/4:S:2), 1/4 to 1/2-inch PB at 1 inch depth (1/4–1/2:S:1), 1/4 to 1/2-inch PB at 2 inches depth (1/4–1/2:S:2), 3/8 to 3/4-inch PB at 1-inch depth (3/8–3/4:S:1), 3/8 to 3/4-inch PB at 2 inches depth (3/8–3/4:S:2), ≤1/2-inch PB throughout (≤1/2:TO); 1 inch = 2.54 cm.

[ii] Growth index was determined by calculating [(height + width at widest point + perpendicular width) ÷ 3] from 0 to 24 weeks after planting (WAP); 1 cm = 0.3937 inch.

[iii] Shoot and root dry weight at 24 WAP; 1 g = 0.0353 oz.

[iv] Means followed by the same letter within a column are not significantly different according to Tukey's honestly significant difference test at $P < 0.05$.

Table 4. Growth index and biomass of container-grown blue plumbago [1 gal (3.8 L)] for 24 weeks in stratified and nonstratified pine bark substrates.

Substrate[i]	Growth index (cm)[ii] 8 WAP	16 WAP	24 WAP	Biomass[iii] Shoot wt (g)	Root wt (g)
≤1/2:TO	35.8 a[iv]	42.4 a	49.7 a	77.0 a	29.9 a
1/8 to 1/4:S:1	36.2 a	41.9 a	45.8 a	75.6 a	28.5 a
1/8 to 1/4:S:2	36.1 a	42.3 a	45.3 a	73.0 a	28.1 a
1/4 to 1/2:S:1	36.6 a	43.2 a	49.0 a	79.4 a	29.6 a
1/4 to 1/2:S:2	37.3 a	41.2 a	49.0 a	76.2 a	29.0 a
3/8 to 3/4:S:1	40.1 a	44.2 a	48.6 a	78.9 a	32.5 a
3/8 to 3/4:S:2	38.2 a	43.6 a	50.2 a	75.1 a	30.1 a

[i] Substrate consisted of 1/8 to 1/4-inch pine bark (PB) at 1 inch depth (1/8–1/4:S:1), 1/8 to 1/4-inch PB at 2 inches depth (1/8–1/4:S:2), 1/4 to 1/2-inch PB at 1 inch depth (1/4–1/2:S:1), 1/4 to 1/2-inch PB at 2 inches depth (1/4–1/2:S:2), 3/8 to 3/4-inch PB at 1-inch depth (3/8–3/4:S:1), 3/8 to 3/4-inch PB at 2 inches depth (3/8–3/4:S:2), ≤1/2-inch PB throughout (≤1/2:TO); 1 inch = 2.54 cm.

[ii] Growth index was determined by calculating [(height + width at widest point + perpendicular width) ÷ 3] from 0 to 24 weeks after planting (WAP); 1 cm = 0.3937 inch.

[iii] Shoot and root dry weight at 24 WAP; 1 g = 0.0353 oz.

[iv] Means followed by the same letter within a column are not significantly different according to Tukey's honestly significant difference test at $P < 0.05$.

Our previous study with stratified substrates showed an initial decrease in plant growth, likely due to water stress before full root development that resulted from the use of a coarser substrate in the upper portion of the container (Khamare et al., 2022). The initial growth reduction was transient, and at the conclusion of the study, no growth differences were observed. In the present study, a greater amount of total rainfall was received (52.9 vs. 42.5 inches in the 2021 experiment), and there was no reduction on plumbago growth, possibly because the experiment was initiated during the summer months and more frequent rainfall occurred (Fig. 1). Overall, plants in all the stratified treatments reached marketable sizes within the similar timeframe, but additional research may be needed to mitigate the reduced growth of small liners planted in a coarse substrate (that lacks incorporated fertilizer).

Effect of substrate stratification on emergence and shoot biomass of spotted spurge and bittercress. Spotted spurge emergence in all the stratified substrate treatments was lower than ≤1/2:TO with the exception of 1/4 to 1/2:S:1 at 4 WAP, with average spotted spurge counts ranging from 0.9 to 2.4 in comparison with an average of 5.6 in the standard ≤1/2:TO (Table 5, Fig. 2). At 9 WAP, spotted spurge emergence was higher in the ≤1.2:TO substrate compared with stratified treatments, with stratification resulting in a 30% to 84% decrease in spotted spurge counts (Table 5). In general, shoot dry weight followed a similar pattern with the exception of 1/4 to 1/2:S:1 and 3/8 to 3/4:S:1 that resulted in no significant reduction in shoot weight. Although the 1/8 to 1/4:S:1 resulted in a significant decrease in spotted spurge biomass, observing no reduction with the other stratified treatments applied at a 1-inch depth indicates that for spotted spurge, the top strata would likely need to be at least a 2-inch layer to have any meaningful effect. In all stratified treatments applied at a depth of 2 inches (1/8 to 1/4:S:2, 1/4 to 1/2:S:2, 3/8 to 3/4:S:2), lower spotted spurge biomass was recorded in comparison with the same substrate applied at a 1-inch depth with the exception of the 1/8 to 1/4-inch substrate. On average, shoot dry weight decreased by 45% to 55% compared with the ≤1/2:TO substrate, whereas shoot dry weight decreased by only 14% to 42% when the top strata were applied at only a 1-inch depth.

Similar to the results observed with spotted spurge, bittercress emergence at 4 WAP was lower in all stratified treatments, with mean counts

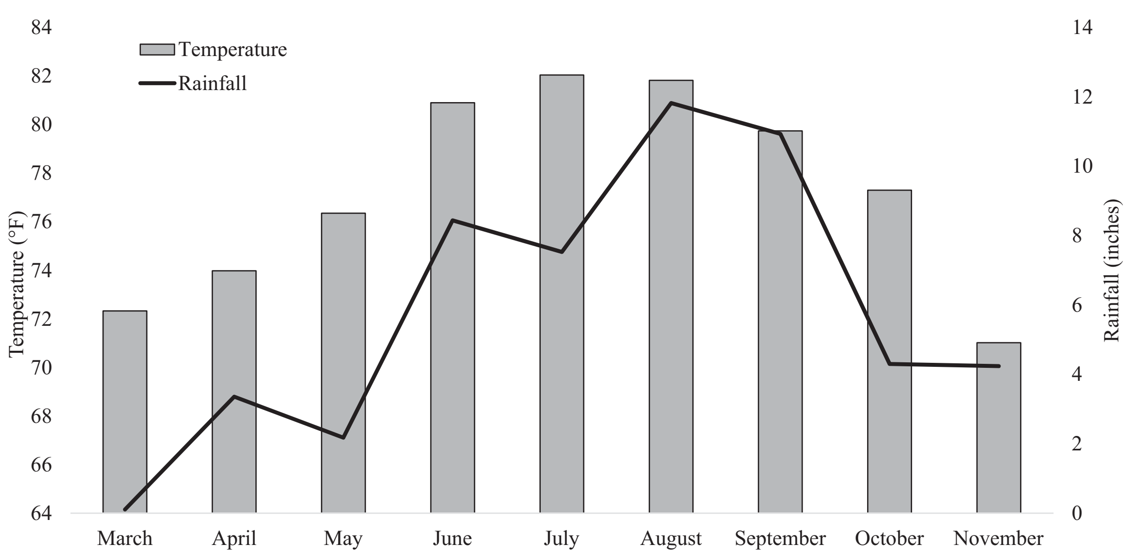

Fig. 1. Average monthly temperature and cumulative rainfall over two experimental runs [Mar to Nov 2020 (Florida Automated Weather Network 2022)]. The first experimental run was initiated on 11 Mar 2020, and the second run on 12 May 2020, with both experiments being harvested at 24 weeks after planting; (°F – 32) ÷ 1.8 = °C, 1 inch = 2.54 cm.

Table 5. Spotted spurge and bittercress emergence and biomass in stratified and non-stratified pine bark substrates.

	Spotted spurge			Bittercress		
	Weeds (no.)[ii]		Biomass[iii]	Weeds (no.)		Biomass
Substrate[i]	4 WAP	9 WAP	Shoot wt (g)	4 WAP	9 WAP	Shoot wt (g)
≤1/2:TO	5.6 a[iv]	11.4 a	22.4 a	9.6 a	11.6 a	5.9 a
1/8 to 1/4:S:1	1.6 c	5.1 bc	13.0 bc	2.5 bc	4.0 b	1.6 bc
1/8 to 1/4:S:2	0.9 c	3.5 c	10.0 c	1.9 c	3.8 b	0.8 bc
1/4 to 1/2:S:1	3.9 ab	7.1 b	19.2 a	1.5 c	4.0 b	1.3 bc
1/4 to 1/2:S:2	1.9 c	6.3 bc	12.4 c	0.6 c	2.4 b	0.4 c
3/8 to 3/4:S:1	2.4 bc	7.3 b	17.7 ab	4.1 b	4.7 b	1.7 b
3/8 to 3/4:S:2	0.9 c	4.6 bc	11.2 c	1.4 c	2.6 b	0.4 c

[i] Substrate consisted of 1/8 to 1/4-inch pine bark (PB) at 1 inch depth (1/8–1/4:S:1), 1/8 to 1/4-inch PB at 2 inches depth (1/8–1/4:S:2), 1/4 to 1/2-inch PB at 1 inch depth (1/4–1/2:S:1), 1/4 to 1/2-inch PB at 2 inches depth (1/4–1/2:S:2), 3/8 to 3/4-inch PB at 1-inch depth (3/8–3/4:S:1), 3/8 to 3/4-inch PB at 2 inches depth (3/8–3/4:S:2), ≤1/2-inch PB throughout (≤1/2:TO); 1 inch = 2.54 cm.

[ii] Weed count was assessed by surface sowing 25 seeds of spotted spurge and bittercress to each container separately and counting established seedlings at 4 and 9 weeks after planting (WAP).

[iii] Shoot dry weight at trial conclusion at 10 WAP; 1 g = 0.0353 oz.

[iv] Means followed by the same letter within a column are not significantly different according to Tukey's honestly significant difference test at $P < 0.05$.

ranging from 0.6 to 4.1 compared with 9.6 in the industry-standard substrate of ≤1/2:TO. Similar results were observed at 9 WAP with stratification resulting in bittercress counts 57% to 94% lower than the ≤1/2:TO. In addition to lower bittercress counts, shoot dry weight was substantially reduced in all the stratified substrates, ranging from 71% to 93% in comparison with the ≤1/2:TO substrate.

Although the depth of the top substrate had a clear effect on spotted spurge growth, there were no differences in substrates screened to the same size regardless of application depth, with the exception of the 3/8 to 3/4-inch substrate where the 2 inches depth provided a greater reduction in bittercress growth. This result was similar to the response observed for spotted spurge.

The aforementioned results are similar to the previous study with stratified substrates, where 80% to 97% reduction in bittercress growth has been reported (Khamare et al., 2022). The stratification technique performed in this study resulted in the top strata containing no fertilizer whereas the bottom strata had fertilizer incorporated. Consequently, this served as a form of strategic fertilizer placement, such as dibbling or subdressing which has been shown to reduce germination of large crabgrass (*Digitaria sanguinalis*), eclipta (*Eclipta prostrata*), and spotted spurge by 22%, 60%, 43% and shoot biomass by 84%, 90%, and 89% respectively (Khamare et al., 2020; Saha et al., 2019; Stewart et al., 2018). Another cultural practice that substrate stratification mimics is mulching, as the coarse bark top strata hold less moisture, acts as a barrier for weed seeds to germinate, and additionally maintains moisture and temperature in the bottom strata. Mulching has been studied extensively as a weed management tool in container nurseries (Altland et al., 2016; Bartley et al., 2017; Marble et al., 2019; Richardson et al., 2008); whereas stratification provides additional benefits. The top strata act as a mulch from a weed management perspective, and the top strata

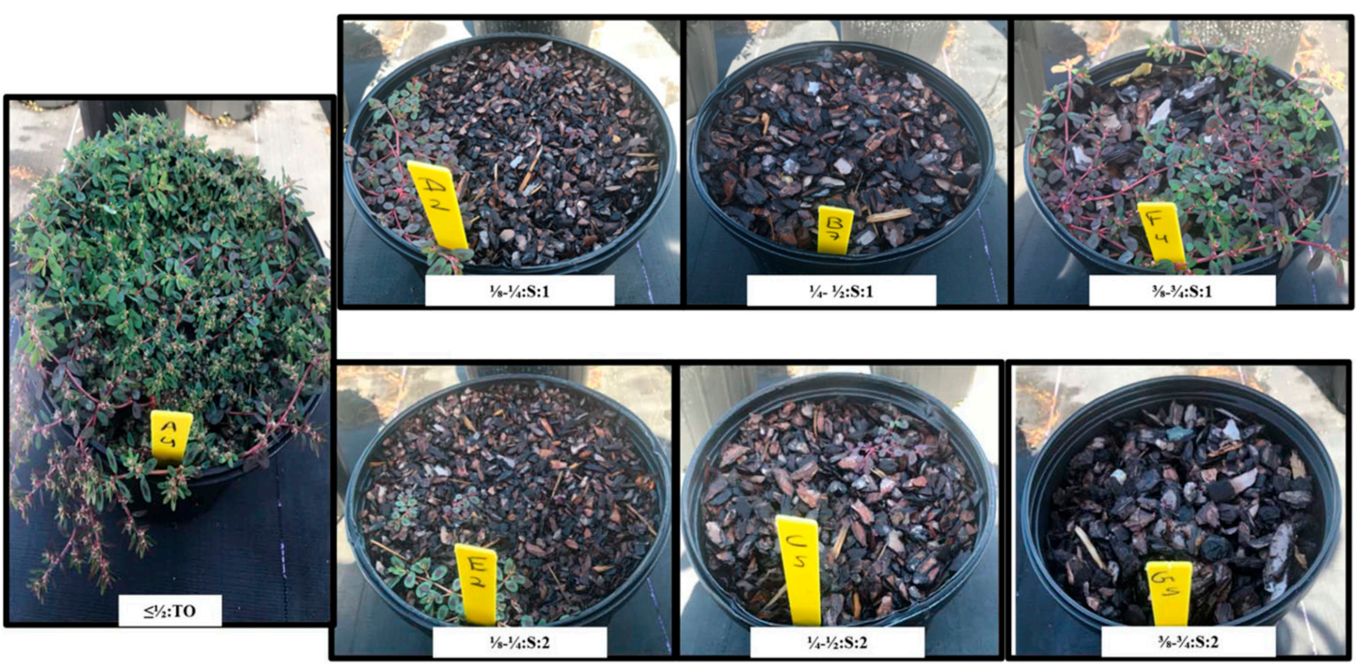

Fig. 2. Established seedlings of spotted spurge at 4 weeks after planting. Substrate consisted of 1/8 to 1/4-inch pine bark (PB) at 1 inch depth (1/8–1/4:S:1), 1/8 to 1/– inch PB at 2 inches depth (1/8–1/4:S:2), 1/4 to 1/2-inch PB at 1 inch depth (1/4–1/2:S:1), 1/4 to 1/2-inch PB at 2 inches depth (1/4–1/2:S:2), 3/8 to 3/4-inch PB at 1-inch depth (3/8–3/4:S:1), 3/8 to 3/4-inch PB at 2 inches depth (3/8–3/4:S:2), ≤1.2-inch PB throughout (≤1/2:TO); 1 inch = 2.54 cm.

Fig. 3. Liverwort coverage at 16 weeks after planting. Substrate consisted of 1/8 to 1/4-pine bark (PB) at 1 inch depth (1/8–1/4:S:1), 1/8 to 1/4-inch PB at 2 inches depth (1/8–1/4:S:2), 1/4 to 1/2-inch PB at 1 inch depth (1/4–1/2:S:1), 1/4 to 1/2-inch PB at 2-inch depth (1/4–1/2:S:2), 3/8 to 3/4-inch PB at 1 inch depth (3/8–3/4:S:1), 3/8 to 3/4-inch PB at 2 inches depth (3/8–3/4:S:2), ≤1/2-inch PB throughout (≤1/2:TO); 1 inch = 2.54 cm.

is the part of the growing media that results in greater rooting volume and could reduce some of the time and cost typically associated with a mulch application (Khamare et al., 2022).

EFFECT OF SUBSTRATE STRATIFICATION ON THE ESTABLISHMENT OF LIVERWORT. Liverwort growth was highest in the industry-standard ≤1/2:TO substrate with an average coverage of 77% (Table 6) at 16 WAP. In all other treatments, liverwort coverage was negligible at less than 1% (Fig. 3). Similar results were reported in the previous study where the growth of liverwort was <1% in all stratified substrates (Khamare et al., 2022). Liverwort is known to be sensitive to cultural conditions, such as moisture levels and high fertility [i.e., nitrogen (Newby et al., 2007)], and research has shown that mulch and alternative fertilization methods, such as dibbling or subdressing can suppress liverwort growth and spread (Altland and Krause 2014; Svenson, 1998). As stratified substrates consisted of 1 to 2 inches of top substrate strata with low water-holding capacity and without any fertilizer, stratification would be an ideal strategy to help manage liverwort growth in nurseries because it eliminates two major factors that contribute toward its spread.

Conclusions

Results from these experiments indicate that substrate stratification has promise as a weed management tool in container nurseries, but because some negative growth impacts were observed with ligustrum, additional research is warranted to optimize stratification techniques that balance crop needs with improvements in weed management. The previous study by Khamare et al. (2022) was conducted in late fall with lower temperatures and low rainfall, whereas the current experiments were conducted in spring and summer with higher and more frequent rainfall. Overall, similar results were observed in both the current study and previous work (Khamare et al., 2022) where weed growth was reduced with minimal impact on the ornamentals evaluated. Given that the initial liners used in this experiment were 2 inches in height, the top unfertilized strata of bark might have minimal impact on the growth of the ornamental species evaluated because the root ball was in contact with the fertilized bottom portion of the substrate, seemingly providing sufficient nutrition and water holding capacity for the ornamental crop to establish. As stated previously by Khamare et al. (2022), this substrate stratification technique could potentially reduce losses associated with mulching (from container blow-over and a reduction in economic costs) and allow for greater root volume within a container while still providing a weed-control benefit. Further research is needed to determine

Table 6. Establishment of liverwort in stratified and nonstratified pine bark substrates.

	Liverwort coverage (%)[i]
Substrate[ii]	16 WAP
≤1/2:TO	77.2 a[iii]
1/8 to 1/4:S:1	0.4 b
1/8 to 1/4:S:2	0.3 b
1/4 to 1/2:S:1	0.2 b
1/4 to 1/2:S:2	0.02 b
3/8 to 3/4:S:1	0.02 b
3/8 to 3/4:S:2	0 b

[i] Liverwort % coverage was measured by capturing photos at a height of 3 ft (0.9 m) above the container and analyzed using the software program (ImageJ; U.S. National Institutes of Health, Bethesda, MD) at 16 weeks after planting (WAP).

[ii] Substrate consisted of 1/8 to 1/4-inch pine bark (PB) at 1 inch depth (1/8–1/4:S:1), 1/8 to 1/4-inch PB at 2 inches depth (1/8–1/4:S:2), 1/4 to 1/2-inch PB at 1 inch depth (1/4–1/2:S:1), 1/4 to 1/2-inch PB at 2 inches depth (1/4–1/2:S:2), 3/8 to 3/4-inch PB at 1-inch depth (3/8–3/4:S:1), 3/8 to 3/4-inch PB at 2 inches depth (3/8–3/4:S:2), ≤1/2-inch PB throughout (≤1/2:TO); 1 inch = 2.54 cm.

[iii] Means followed by the same letter within a column are not significantly different according to Tukey's honestly significant difference test at $P < 0.05$.

the suitability of this method on additional weeds and ornamental species, as well as to determine the effect on water use and nutrient leaching during container ornamental production.

References

Abramoff, M.D., P.J. Magalhães, and S.J. Ram. 2004. Image processing with ImageJ. Biophoton. Int. 11:36–42.

Altland, J.E., J.K. Boldt, and C.C. Krause. 2016. Rice hull mulch affects germination of bittercress and creeping woodsorrel in container plant culture. Am. J. Plant Sci. 7:2359–2375, https://doi.org/10.4236/ajps.2016.716207.

Altland, J.E. and C.C. Krause. 2014. Parboiled rice hull mulch in containers reduces liverwort and flexuous bittercress growth. J. Environ. Hortic. 32:59–63, https://doi.org/10.24266/0738-2898.32.2.59.

Altland, J.E., J.S. Owen, and M.Z. Gabriel. 2011. Influence of pumice and plant roots on substrate physical properties over time. HortTechnology 21:554–557, https://doi.org/10.21273/HORTTECH.21.5.554.

Altland, J.E., J.S. Owen, B.E. Jackson, Jr., and J.S. Fields. 2018. Physical and hydraulic properties of commercial pine-bark substrate products used in production of containerized crops. HortScience 53:1883–1890, https://doi.org/10.21273/HORTSCI13497-18.

Bartley, P.C., G.R. Wehtje, A.M. Murphy, G.W. Foshee, and C.H. Gilliam. 2017. Mulch type and depth influences control of three major weed species in nursery container production. HortTechnology 27:465–471, https://doi.org/10.21273/HORTTECH03511-16.

Berchielli-Robertson, D.L., C.H. Gilliam, and D.C. Fare. 1990. Competitive effects of weeds on the growth of container grown plants. HortScience 25:77–79, https://doi.org/10.21273/HORTSCI.25.1.77.

Bilderback, T., C. Boyer, M. Chappell, G. Fain, D. Fare, C. Gilliam, B. Jackson, J. Lea-Cox, A. LeBude, and A. Niemiera. 2013. Best management practices guide for producing nursery crops. South. Nurs. Assoc., Ackworth, GA.

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

Fain, G.B., P.R. Knight, C.H. Gilliam, and J.W. Olive. 2003. Effect of fertilizer placement on prostrate spurge growth in container production. J. Environ. Hortic. 21:177–180, https://doi.org/10.24266/0738-2898-21.4.177.

Fields, J.S., J.S. Owen, and J.E. Altland. 2020. Stratified substrates: A media management strategy for increased resource efficiency (abstr). HortScience 55:399–S400.

Fields, J.S., J.S. Owen, and J.E. Altland. 2021. Substrate stratification: Layering unique substrates within a container increases resource efficiency without impacting growth of shrub rose. Agronomy (Basel) 11:1454, https://doi.org/10.3390/agronomy11081454.

Florida Automated Weather Network. 2022. Apopka weather summary Mar to Nov 2020. UF/IFAS Extension. http://fawn.ifas.ufl.edu/. [accessed 22 May 2022].

Fonteno, W.C. and C.T. Harden. 2010. North Carolina State University horticultural substrates lab manual. North Carolina State Univ., Raleigh, NC.

Fretz, T.A. 1972. Weed competition in container grown Japanese holly. HortScience 7:485–486.

Goodwin, D. 1980. Bark—A valuable resource for horticulture. N. Z. J. Agric. 141:17.

Ingram, D.L., C.R. Hall, and J. Knight. 2016. Carbon footprint and variable costs of production components for a container-grown evergreen shrub using life cycle assessment: An east coast U.S. model. HortScience 51:989–994, https://doi.org/10.21273/HORTSCI.51.8.989.

Ingram, D.L., C.R. Hall, and J. Knight. 2017. Comparison of three production scenarios for *Buxus microphylla* var. japonica 'Green Beauty' marketed in a No. 3 container on the west coast using life cycle assessment. HortScience 52:357–365, https://doi.org/10.21273/HORTSCI11596-16.

Keddy, P.A. and P. Constabel. 1986. Germination of ten shoreline plants in relation to seed size, soil particle size and water level: An experimental study. J. Ecol. 74:133–141, https://doi.org/10.2307/2260354.

Khamare, Y., S.C. Marble, J.E. Altland, B.J. Pearson, J. Chen, and P. Devkota. 2022. Effect of substrate stratification on growth of common nursery weed species and container-grown ornamental species. HortTechnology 32:74–83, https://doi.org/10.21273/HORTTECH04965-21.

Khamare, Y., S.C. Marble, and A. Chandler. 2020. Fertilizer placement effects on eclipta (*Eclipta prostrata*) growth and competition with container-grown ornamentals. Weed Sci. 68:496–502, https://doi.org/10.1017/wsc.2020.44.

Lu, W., J.L. Sibley, C.H. Gilliam, J.S. Bannon, and Y. Zhang. 2006. Estimation of US bark generation and implications for horticultural industries. J. Environ. Hortic. 24:29–34, https://doi.org/10.24266/0738-2898-24.1.29.

Marble, S.C., S.T. Steed, D. Saha, and Y. Khamare. 2019. On-farm evaluations of wood-derived, waste paper, and plastic mulch materials for weed control in Florida container nurseries. HortTechnology 29:866–873, https://doi.org/10.21273/HORTTECH04437-19.

Newby, A., J.E. Altland, C.H. Gilliam, and G. Wehtje. 2007. Pre-emergence liverwort control in nursery containers. HortTechnology 17:496–500, https://doi.org/10.21273/HORTTECH.17.4.496.

Owen, J.S. and J.E. Altland. 2008. Container height and Douglas fir bark texture affect substrate physical properties. HortScience 43:505–508, https://doi.org/10.21273/HORTSCI.43.2.505.

Richardson, B., C.H. Gilliam, G. Fain, and G. Wehtje. 2008. Nursery container weed control with pinebark mininuggets. J. Environ. Hortic. 26:144–148, https://doi.org/10.24266/0738-2898-26.3.144.

Saha, D., S.C. Marble, N. Torres, and A. Chandler. 2019. Fertilizer placement affects growth and reproduction of three common weed species in pine bark-based soilless nursery substrates. Weed Sci. 67:682–688, https://doi.org/10.1017/wsc.2019.49.

Stewart, C., S.C. Marble, B.E. Jackson, B.J. Pearson, and P.C. Wilson. 2018. Effects of three fertilization methods on weed growth and herbicide performance in soilless nursery substrates. J. Environ. Hortic. 36:133–139, https://doi.org/10.24266/0738-2898-36.4.133.

Svenson, S. 1998. Suppression of liverwort growth in containers using irrigation, mulches, fertilizers, and herbicides. Hortscience 33:485.

Yu, P. and S.C. Marble. 2022. Practice in nursery weed control—Review and meta-analysis. Front. Plant Sci. https://doi.org/10.3389/fpls.2021.807736.