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Growth of Liverwort (*Marchantia polymorpha*) and Spotted Spurge (*Euphorbia maculata*) Decreases with Substrate Stratification and Strategic Fertilizer Placement

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Summary

Substrate stratification is a method of filling nursery containers with "layers" of substrates (e.g., pine bark) comprised of different physical properties to manipulate soil moisture dynamics, improve irrigation and fertilization efficiency. However, stratification could also potentially serve as a weed management tool. The objective of this research was to assess the effect of stratified substrates and strategic fertilizer placement on the germination and growth of spotted spurge (*Euphorbia maculata*) and liverwort (*Marchantia polymorpha*) establishment in nursery pots. Before experiment initiation, aged pine bark was screened to three different sizes that consisted of particles ranging from 0.3 to 0.6 cm, 0.6 to 1.3 cm, and 1.3 to 1.9 cm. Bark was also screened to pass through a 1.3 cm and included all fines (all particle sizes less than 1.3 cm). The stratified treatments consisted of either the 0.3 to 0.6 cm, 0.6 to 1.3 cm, or 1.3 to 1.9 cm pine bark applied at depths of either 2.5 or 5 cm on top of the < 1.3 cm substrate. An industry-standard treatment was also included in which the substrate was not stratified but consisted of only the < 1.3 cm bark used throughout the container. A controlled-release fertilizer (CRF) was used at same rate

(35 g per pot) in all the treatments; However, fertilizer was incorporated in only the bottom layer in all stratified treatments (no fertilizer in the top 2.5 or 5 cm of the container media) while the industry standard had fertilizer incorporated throughout. Results showed that in comparison with the industry (non-stratified) standard, substrate stratification decreased spotted spurge germination by 30% to 84%. Spotted spurge shoot dry weight was reduced by 45% to 55% in stratified treatments when the top layer was applied at a depth of 5 cm, while a decrease of 14% to 42% was observed when the top layer was applied at a depth of 2.5 cm. Liverwort coverage was substantially reduced by nearly 100% in all the stratified substrate treatments. Overall, results suggest substrate stratification could be implemented in container production as part of an integrated weed management strategy.

INTRODUCTION

Weed management in container nurseries is currently primarily performed using frequent PRE herbicide applications in conjunction with hand weeding. High reliance on PRE herbicides causes several negative consequences such as high chemical costs, or concerns with recycling irrigation water (Poudyal and Cregg, 2019; Wilson et al., 1995). Additionally, the nursery industry produces thousands of different taxa and there is no herbicide labelled for use on all species. Popular plants such as succulents, herbaceous annuals, perennials, ornamental grasses, and tropical plants can also be highly sensitive to herbicides. When herbicides cannot be used, hand weeding costs can be significant. Darden and Neal (1999) reported that $1367 was spent to hand weed '1000' 3L (0.66 gal.) pots in just four months. There is a clear and immediate need to develop new, integrated and sustainable weed management strategies to reduce the cost of hand weeding and the disadvantages associated with a herbicide-only management strategy.

One cultural practice that has received some attention is the placement of controlled-release fertilizers (CRF) in pots. CRF is added to a nursery potting substrate to supply nutrients as the substrate used is mostly made up of materials such as bark, peat, perlite, or sand that lack nutrients. This ability to control nutrients in a pot can be strategically utilized to manage weed growth. Strategic fertilizer placement reduces weed growth by limiting the access to nutrients and at the same time increases the desired crop's competitive ability by direct access to nutrients (Nkebiwe et al., 2016). A fertilization method called subdressing has been shown to decrease the growth of spotted spurge (*Euphorbia maculata*) and eclipta (*Eclipta prostrata*) by over 80% (Saha et al., 2019; Stewart et al., 2018). Subdressing is accomplished by adding a layer of fertilizer in a pot filled with 50% to 75% potting substrate and filling the remainder of the pot with the same substrate (Khamare et al., 2020; Saha et al., 2019). This creates easy access to nutrients for crop roots, without any nutrients available for weed seedlings on the surface of the pot. Several studies have also shown that subdressing, can limit weed growth and reduce nutrient leaching without causing injury to the ornamental crops (Bir and Zondag, 1986; Stewart et al., 2018).

Another cultural method that could have potential for weed management is engineered substrates or substrate stratification. This is a new area of research that has the potential to decrease weed growth, water use, nutrient leaching that can result in reduced production time. Substrate stratification involves layering different substrates or the use of same substrate with different textures in a single pot (Fields et al., 2020). Fields et al (2021) reported that by using substrates with a high level of moisture and nutrient retention placed on top of a coarse, freely draining substrate, fertilizer rates could be reduced by 20% with no negative effects on the growth or quality of Red Drift roses (*Rosa* 'Meigalpio' PP17877) compared with an industry-standard substrate.

Theoretically, stratification of the top layer with freely draining, larger particle substrate without any fertilizer and the bottom layer with fine-textured, high moisture-retentive substrate could be used as a weed management tool. The top coarse-textured layer would hold less moisture and no fertilizer where weed seeds are introduced. Whereas the bottom layer would hold enough moisture and nutrients for the crop roots to access because as substrate particle size decreases, water holding capacity typically increases (Gruda and Schnitzler, 2004; Puustjarvi and Robertson, 1975; Richard and Beardsell, 1986). In this scenario, weed germination could be potentially reduced because weed seeds are introduced on the surface on the container substrate and require moisture for germination (Harper and Benton, 1996; Wada, 2005). Thus, it is possible that the top layer of substrate with less water holding capacity could result in reduced weed seed germination. Additionally, because the size of the most common container weed seeds is small, a top layer with a larger particle size could cause weed seeds to be flushed deep into the substrate, decreasing their chances of germination because many weed species require light to germinate (Keddy and Constabel, 1986).

Stratification could also potentially eliminate the disadvantages associated with mulching. First, the current industry practice is to fill the container with the same substrate with a space of 2 to 7 cm gap or more for mulch application (Altland et al., 2016; Bartley et al., 2017; Marble et al., 2019; Richardson et al., 2008) which reduces substrate volume and potential root growth. Mulching can also be costly, prone to blowing out of pots with high winds, or can be lost when pots are blown over. With substrate stratification, the extra step of mulching is eliminated as the top layer of stratified substrate will cover the pot surface and will be part of the growing substrate itself. As the plant liner is planted into this coarse bark layer, stratification would increase potential root volume compared with typical mulching practices. This would reduce the cost required for labor, mulching materials, and because crop roots would grow in this top stratified layer, less substrate would be lost due to wind or pot blow over. In theory, substrate stratification combines two of the most successful nonchemical weed management practices: strategic fertilizer placement and a 'mulch' like top layer that holds less moisture and no nutrients, but research is needed to verify these assumptions. The objective of this study was to evaluate the effect of substrate stratification on the growth of liverwort and spotted spurge.

MATERIALS AND METHODS

Experiments were conducted at the Mid-Florida Research and Education Center in Apopka, FL in 2020. Aged pine bark was purchased from a local supplier and further thoroughly screened to three different sizes that consisted of particles ranging from 0.3 to 0.6 cm, 0.6 to 1.3 cm, and 1.3 to 1.9 cm. An additional bark was also screened to pass through a 1.3 cm screen and included all fines (all particle sizes less than 1.3 cm). The stratified treatments were constructed by having either the 0.3 to 0.6 cm, 0.6 to 1.3 cm, or 1.3 to 1.9 cm bark as the top substrate with the bottom substrate consisting of ≤ 1.3 cm bark. The top substrate was applied at a depth of either 2.5 or 5 cm, resulting in six stratified substrate treatments (abbreviated as *top substrate size: screen size:" **S**" for stratification: top depth* in cm or 0.3-0.6:**S**:2.5, 0.3-0.6:**S**:5, 0.6-1.3:**S**:2.5, 0.6-1.3:**S**:5, 1.3-1.9:**S**:2.5 and 1.3 -1.9:**S**:5). An industry-standard treatment was also included in which the substrate was not stratified but consisted of only the ≤ 1.3 cm bark used throughout the container. A controlled-release fertilizer (CRF) (Osmocote® Blend 17-5-11 N-P-K [8 to 9 mo], ICL Specialty Fertilizers, Dublin, OH) at 35 g pot^{-1} was used at the same rate in all the treatments; However, fertilizer was incorporated in only the bottom layer in all stratified treatments (no fertilizer in the top 2.5 or 5 cm of the container media) while the industry standard treatment had fertilizer incorporated throughout. All the treatments consisted of pine bark and CRF without the addition of any other amendments such as peat moss or sand.

To assess weed growth, on Apr. 2020 and May. 2020, twenty-five seeds of spotted spurge were seeded in each pot to evaluate its growth and germination. Nursery pots (3.8 L) were filled and fertilized by the method mentioned above and seeds were surface sown. The pots were placed on a full sun nursery pad, irrigated 1.3 cm per day via overhead irrigation (Xcel® wobblers, Senninger Irrigation, Clermont, FL) via two irrigation cycles. Data collection included counts of emerged spotted spurge (mature and cotyledon) at 4 weeks after potting (WAP) and mature spotted spurge at 10 WAP. Shoot dry weight was collected at the trial conclusion (10 WAP). The experiment was a completely randomized design with eight single pot replication per treatment and repeated.

A separate set of nursery pots were used to evaluate liverwort (*Marchantia polymorpha*) growth on stratified substrates in Dec. 2020. Ten weeks before initiating the experiment and filling pots, 4 to 5 pieces of liverwort were transplanted onto the surface of 1.7 L nursery pots that had been previously filled with a pine bark: peat substrate (80:20 v: v) amended with the CRF via incorporation as described above. The pot was placed inside a shade house (60% ambient light) and was irrigated 1-cm per day via overhead irrigation. Pots remained in the shadehouse until the surface of the pots was filled with liverwort (no visible substrate upon visual inspection). At this time (approximately 10 wks after planting), these pots were used as inoculum to naturally sporulate the treatments as liverwort can spread asexually through the splashing of gemmae or sexually via airborne spores (Newby et al., 2007). Square 1.7 L nursery pots were filled and fertilized with the stratified and industry-standard treatments mentioned previously and placed inside the same shadehouse. To initiate the experiment, the inoculum pots were placed around each substrate treatment replication at a distance of 0.5 cm so that the experimental pots had an inoculum pot on all four sides. Liverwort surface coverage was assessed at 16 WAP by taking digital photos of each treatment using an iPhone (iPhone 8 Plus, Apple, Cupertino, CA) from a height of 0.9 m. Images were cropped using

Microsoft Paint (Microsoft Corp., Redmond, WA) 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 in ImageJ software (Abramoff et al., 2004). In all cases, data were subjected to analysis of variance using statistical software (JMP® Pro ver. 14, SAS Institute, Cary, NC). Prior to analysis, all data were inspected to ensure the assumptions of ANOVA were met. When appropriate, post hoc means comparisons were performed using Tukey's Honest Significant Differences test at a 0.05 significance level.

RESULTS AND DISCUSSION

Effect of substrate stratification on germination and growth of spotted spurge

At 4 WAP, spotted spurge germination was lower in most of the stratified substrate treatments in comparison with the 1.3:TO treatment (Table 1). The only exception was the 0.6-1.3:S:2.5 treatment which had similar germination in comparison with the 1.3:TO treatment. At 9 WAP, germination was still highest in the 1.3:TO treatment with reduced germination in all the stratified treatments. Overall, substrate stratification decreased spotted spurge germination by 30% to 84% in comparison with the industry-standard treatment of 1.3:TO (Table 1).

Shoot dry weight analysis showed that while germination was reduced, stratified treatments including 0.6-1.3:S:2.5 and 1.3-1.9:S:2.5 had shoot weight similar to the industry-standard treatment of 1.3:TO treatment. In the remaining stratified substrate treatments with a top layer of 5 cm (0.3-0.6:S:5, 0.6-1.3:S:5, 1.3-1.9:S:5), shoot weight decreased by 45% to 55% in comparison with the 1.3:TO treatment whereas shoot weight only decreased by 14% to 42% when the top layer was applied at a 2.5 cm depth.

Effect of substrate stratification on the establishment of liverwort

Liverwort growth was highest in the industry-standard treatment of 1.3:TO with an average coverage of 77% (Table 1). In all other treatments, liverwort coverage was negligible and less than 1% (Fig. 1). Liverwort thrives in an environment that has high moisture, high humidity, high fertility, and low ultraviolet light levels (Newby et al, 2007). As stratified substrates consist of a 2.5 to 5 cm of layer on top with low water holding capacity without any fertilizer, liverwort was unable to establish on the surface of the stratified treatments.

Overall, the growth of spotted spurge and liverwort was significantly reduced in the stratified substrates. Although not reported here for sake of brevity, additional experiments have been conducted with the same stratification technique described here with no adverse effects on some common ornamental species such as Japanese ligustrum (*Ligustrum japonicum*) and blue plumbago (*Plumbago auriculata*). Overall, current data suggest stratified substrates could be used as part of an overall integrated weed management program for container nurseries. Further research is ongoing to determine the impact of this method of substrate stratification on other weed and ornamental species.

Table 1. Effect of substrate composition on spotted spurge (*Euphorbia maculata*) germination and biomass and liverwort (*Marchantia polymorpha*) establishment.

	Spotted spurge			Liverwort
	Germination count[a]		Biomass[b]	% Coverage[d]
Substrate[c]	4WAP	9WAP	Shoot wt (g)	16WAP
1.3:TO	5.6 a[e]	11.4 a	22.4 a	77.2 a
0.3-0.6:S:2.5	1.6 c	5.1 bc	13.0 bc	0.4 b
0.3-0.6:S:5	0.9 c	3.5 c	10.0 c	0.3 b
0.6-1.3:S:2.5	3.9 ab	7.1 b	19.2 a	0.2 b
0.6-1.3:S:5	1.9 c	6.3 bc	12.4 c	0.02 b
1.3-1.9:S:2.5	2.4 bc	7.3 b	17.7 ab	0.02 b
1.3-1.9:S:5	0.9 c	4.6 bc	11.2 c	0 b

[a]Germination count was assessed by surface sowing 25 seeds of spotted spurge (*Euphorbia maculata*) to each pot and counting germinated seedlings at 4 weeks and 9 weeks after potting (WAP)

[b]Shoot dry wt was taken at trial conclusion at 10 weeks after seeding

[c]Substrate consisted of either the 0.3 to 0.6 cm, 0.6 to 1.3 cm, or 1.3 to 1.9 cm bark as the top substrate with the bottom substrate consisting of ≤ 1.3 cm bark and controlled release fertilizer (CRF) (Osmocote® Blend 17-5-11 N-P-K [8 to 9 mo]. The top substrate was applied at a depth of either 2.5 or 5 cm, resulting in six stratified substrate treatments (abbreviated as *top substrate size: screen size:" S" for stratification: top depth in cm* or 0.3-0.6:S:2.5, 0.3-0.6:S:5, 0.6-1.3:S:2.5, 0.6-1.3:S:5, 1.3-1.9:S:2.5 and 1.3 -1.9:S:5). An industry-standard treatment was also included in which the substrate was not stratified but consisted of only the ≤ 1.3 cm bark and CRF used throughout the pot

[d]Liverwort % coverage was measured by capturing photos at a height of 0.6 m above the pots and analyzed using the ImageJ software program at 16 WAP (week after potting) (5/22/2020)

[e]Means followed by the same letter within a column are not significantly different according to Tukey's HSD test α = 0.05.

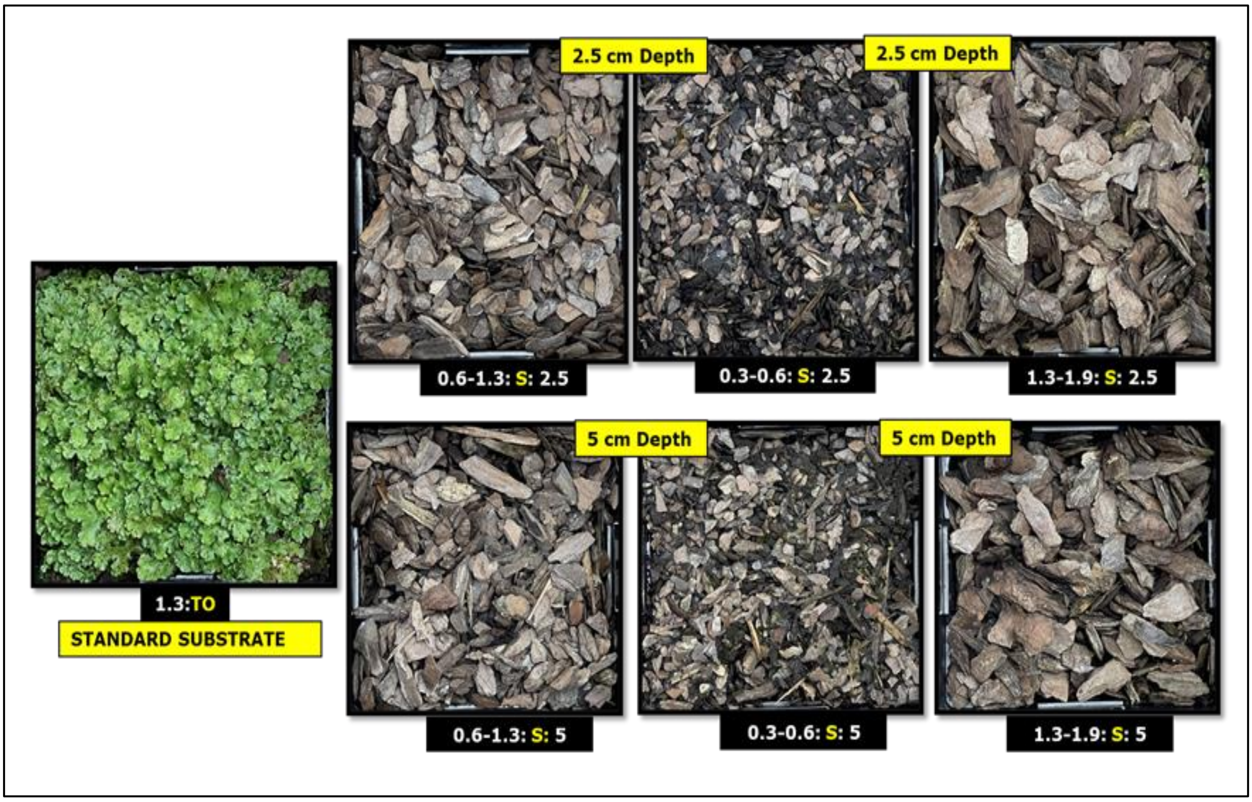

Figure 1. Liverwort (*Marchantia polymorpha*) coverage at 16 weeks after potting. Substrate consisted of either the 0.3 to 0.6 cm, 0.6 to 1.3 cm, or 1.3 to 1.9 cm bark as the top substrate with the bottom substrate consisting of ≤ 1.3 cm bark and controlled release fertilizer (CRF) (Osmocote® Blend 17-5-11 N-P-K [8 to 9 mo]. The top substrate was applied at a depth of either 2.5 or 5 cm, resulting in six stratified substrate treatments (abbreviated as top substrate size: screen size:" S" for stratification: top depth in cm or 0.3-0.6:S:2.5, 0.3-0.6:S:5, 0.6-1.3:S:2.5, 0.6-1.3:S:5, 1.3-1.9:S:2.5 and 1.3 -1.9:S:5). An industry-standard treatment was also included in which the substrate was not stratified but consisted of only the ≤ 1.3 cm bark and CRF used throughout the pot.

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