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Influence of Substrate Stratification and Fertilizer Placement on Growth of Ligustrum (*Ligustrum japonicum*) and Germination and Biomass of Bittercress (*Cardamine flexuosa*) in Containers

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Summary

Substrate stratification is a method of filling nursery containers with pine bark (or other substrates) with different particle sizes in "layers" in order to improve soil moisture dynamics. Currently, substrate stratification, or layering, is being investigated by some researchers as a method to increase the efficiency of production inputs such as irrigation and fertilization. Itis typically performed using larger particle bark as the bottom substrate and finer particle bark as the top substrate to achieve more uniform moisture distribution within containers. The objective of this study was to evaluate the effect of stratified substrates and strategic fertilizer placement on the growth of common nursery weeds and ornamental crops. In contrast to typical methodology, this study evaluated use of coarse bark (screened to 1.3 or 1.9 cm) as the top substrate and finer bark (0.95 cm) as the bottom substrate with the goal of reducing water holding capacity in the top 5 to 7.5 cm of the substrate to reduce weed germination and growth. Results showed that substrate stratification significantly decreased the growth of bittercress (*Cardamine flexuosa*) by 85% to 90% in comparison with substrates

that were not stratified. While stratification initially reduced growth of ligustrum (*Ligustrum japonicum*), at 6-months after potting , there was no difference in ligustrum shoot or root weight in comparison with non-stratified industry standard substrates. The results indicate that substrate composition along with strategic fertilizer placement can be utilized as an effective weed management strategy.

INTRODUCTION

Nursery growers rely on frequent application of preemergence (PRE) herbicides along with supplemental hand weeding to manage weeds as there are no postemergence (POST) herbicides labeled to be safely applied on top of the ornamentals for broadleaf weed control. While PRE herbicides are effective, they are not labeled for use on all ornamental species as tropical plants, succulents, and herbaceous annuals and perennials are highly sensitive to herbicides. There are several negative consequences associated with frequent use of preemergence herbicides including high chemical costs, runoff and potential environmental effects, crop safety concerns, and inefficiency. For example, when applying herbicides to spaced containers, as much as 80% of the herbicide lands in between containers and is unavailable for weed control, resulting in sunk costs (Gilliam et al., 1992). Challenges associated with herbicide use often lead to the need for frequent hand weeding. However, hand weeding is often the costliest weed management strategy, costing up to \$9,000 ha^{-1} (Case et al., 2005; Stewart et al., 2017). Due to the cost of hand weeding and the challenges associated with PRE herbicides, more integrated weed management strategies are needed for nursery production.

Strategic fertilizer placement has been investigated as an integrated weed management method in recent years. Alternative, or "strategic" fertilizer placements, such as subdressing so that the crop has access to nutrients but weeds do not, has been shown to reduce growth of common nursery weeds such as spotted spurge (*Euphorbia maculata*) and eclipta *(Eclipta prostrata*) by over 80% (Saha et al., 2019; Stewart et al., 2019). An additional area that has recently been investigated as a method of improving moisture distribution in nursery containers is substrate stratification, or layering, in which different portions of a container are filled with substrates of varying particle sizes (Fields et al., 2020). As gravitation potential decreases from the top to the bottom of a container, there is a gradient of increasing substrate moisture from the top to the bottom of the container (Owen and Altland, 2008). With stratified substrates, substrates could be layered within a container so that larger particle materials with lower water holding capacity were at the bottom of the container and finer textured materials with higher water holding capacity were at the top. Consequently, a more constant moisture gradient would be created with the benefit of conserving both water and nutrients (Fields et al., 2020).

Depending upon the composition and layering, stratified substrates could also potentially reduce weed growth if substrates were stratified in an inverse manner to that described by Fields et al. (2020), using larger particle materials in the top portion of the container profile. This composition would lead to a substrate that dried quickly on the top surface, reducing weed growth, but held adequate moisture for crop growth. An essential prerequisite of seed germination is water uptake by seed (Harper and Benton, 1966). Previous research has shown that germination and growth of many common nursery weeds such as pearlwort (*Sagina procumbens*), northern willowherb (*Epilobium ciliatum*), and common groundsel (*Senecio vulgaris*) decrease as substrate particle size increased (Wada, 2005). Additionally, a container substrate surface with a larger particle size could cause weed seeds to be flushed deep into the substrate, reducing, or eliminating germination because many weeds require light in order to germinate (Keddy and Constabel, 1986). However, the effects of substrate stratification, used either alone or combined with alternative fertilizer placement on growth of nursery weeds and ornamental crops is unknown. The objective of these experiments was to evaluate the effect of stratified substrates and strategic fertilizer placement on growth of common nursery weeds and ornamental crops.

MATERIALS AND METHODS

All experiments were conducted at the Mid-Florida Research and Education Center in Apopka, FL in November 2019 and repeated in February 2020. Pine bark was purchased from a local supplier and then screened through standard soil sieves to yield three different particle sizes including 0.95, 1.3, and 1.9 cm pine bark. Each resulting particle size included all bark particles that were equal to or smaller than each sieve size. The first three substrate treatments were constructed using one of the three particle sizes (0.95, 1.3, or 1.9 cm bark) throughout the container and had controlled release fertilizer (CRF) (Osmocote® 17-5-11, 8 to 9 mo.) incorporated at 35 g pot^{-1} throughout the substrate profile (TO) (0.95:TO, 1.3:TO, and 1.9:TO, respectively) following standard industry practices. Stratified substrate treatments were constructed by having either the 1.3 or 1.9 cm pine bark as the top substrate with the bottom substrate comprised of 0.95 cm bark. The top substrate (1.3 or 1.9 cm pine bark) was applied at depths of either 5 or 7.5 cm, resulting in four stratified (S) substrate treatments (1.3:S:5, 1.3:S:7.5, 1.9:S:5, and 1.9:S:7.5). In all stratified treatments, the CRF and rate mentioned previously was incorporated at the same rate per pot in all cases, but was only incorporated into the bottom 0.95 cm substrate. An additional treatment consisted of removing all fines from the 1.9 cm bark and using it as the top substrate applied at a 5-cm depth (1.9:S:5: N/F) as described previously.

This resulted in a total of eight substrate treatments with the 0.95:TO, 1.3:TO, and 1.9:TO considered industry standard substrates in that they were comprised of particle sizes often selected for use by growers and contained CRF incorporated throughout the substrate profile.

Uniform liners of ligustrum (*Ligustrum japonicum*) grown in 5-cm plug trays were used to assess the response of a common ornamental to the stratified substrate treatments. During transplanting, liners were planted using standard planting methods in 3.8 L nursery containers, and the top substrates were not applied as a mulch would be applied. That is, the roots of the ligustrum were planted into the top portion of all substrates. After ligustrum were transplanted into the above-mentioned substrates, all plants were placed on a full sun nursery pad, irrigated 1.3 cm per day via overhead irrigation, and were evaluated for 6 months after transplanting (MAT). Data collected included plant growth index [(height + width at widest point + perpendicular width) ÷ 3] measured every 2 months, in addition to root and shoot dry weights at study conclusion. The first experimental run was initiated on Nov. 20, 2019 and the second on Feb. 2, 2020.

To assess weed growth, 25 seeds of bittercress (*Cardamine flexuosa*) were surface sown into a separate set of 3.8 L nursery containers that were filled and fertilized as mentioned previously, placed inside a shade house (60% ambient light), and were irrigated 1-cm per day via overhead irrigation. Data collection included counts of emerged bittercress after 4 and 10 weeks after potting (WAP) and shoot dry weight were recorded at trail conclusion (10 WAP).

All data were analyzed using mixed model analysis of variance with statistical software (JMP® Pro ver. 14, SAS Institute, Cary, NC) with replication as a random factor and all other factors as fixed. Data were inspected to ensure the assumptions of ANOVA were met and then post hoc means separation was performed using Tukey's Honest Significant Differences test at a 0.05 significance level.

RESULTS AND DISCUSSION

Effect of substrate composition on growth of ligustrum. At 2 months after transplanting, growth index measurements showed plants were smaller in stratified substrates (1.3:S:7.5, 1.9:S:5, and 1.9:S:7.5) compared to the incorporated CRF substrates of 0.95:TO and 1.3:TO (Table 1). By 4 MAT, some treatment differences were observed, but all stratified treatments had growth indices similar to the 0.95:TO treatment. At 6 MAT, all stratified substrates had growth indices similar to standard incorporated treatments, indicating that while growth was initially reduced in stratified substrates, likely due to the unfertilized layer in the top 5 to 7.5 cm, the reduced growth index was only transient (Fig. 1). However, dry wt. data collected at trial conclusion revealed that while all stratified substrates had similar root and shoot biomass compared with the 0.95:TO treatment, 1.9:S:5 and 1.9:S:7.5 had lower shoot and root wt. compared with 1:3:TO.

Table 1. Effect of substrate composition on growth index and biomass of container-grown ligustrum (*Ligustrum japonicum*).

	Growth index (cm)[a]			Biomass[b]	
Substrate[c]	2MAT	4MAT	6MAT	Shoot wt (g)	Root wt (g)
0.95:TO	16.7 a	27.6 abc	45.8 ab	58.7 abc	25.0 abcd
1.3:S:5	17.5 a	28.8 ab	45.0 ab	61.3 ab	28.2 a
1.3:S:7.5	13.7 b	24.1 bc	42.8 ab	47.7 bc	20.3 bcd
1.9:S:5	13.8 b	22.8 c	40.8 b	42.4 c	19.2 d
1.9:S:7.5	13.6 b	23.8 bc	43.1 ab	44.6 bc	20.0 cd
1.3:TO	17.3 a	30.1 a	49.2 ab	60.8 ab	26.8 ab
1.9:TO	16.1 ab	28.7 abc	45.6 ab	58.6 abc	26.0 abc
1.9:S:5: N/F	17.8 a	28.3 abc	50.8 a	68.8 a	27.8 a

[a]Growth index was determined by calculating [(height+ width at widest point + perpendicular width) ÷ 3] from 2 to 6 months after transplanting (MAT). First experimental run was initiated on (Nov. 20, 2019) and second on (Feb. 2, 2020).

[b]Shoot and root dry wt. taken at trial conclusion at 6 months after transplanting (MAT).

[c]Substrate consisted of incorporated substrates with 0.95, 1.3 or 1.9 cm pine bark (PB) throughout the container with controlled release fertilizer (Osmocote® 17-5-11, 8 to 9 mo.) incorporated at 35 g pot-1 throughout (TO) (0.95:TO, 1.3:TO, and 1.9:TO) and stratified substrate treatments consisted of having the top substrate (1.3 or 1.9 cm pine bark) applied at depths of 5 or 7.5 cm, resulting in four stratified (S) substrate treatments (1.3:S:5, 1.3:S:7.5, 1.9:S:5, and 1.9:S:7.5) with the bottom substrate consisting of 0.95 cm bark with CRF incorporated. 1.9:S:5: N/F consisted of 1.9 cm bark with fines removed, applied at a 5 cm depth with 0.95 cm bark as the bottom substrate with CRF incorporated.

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

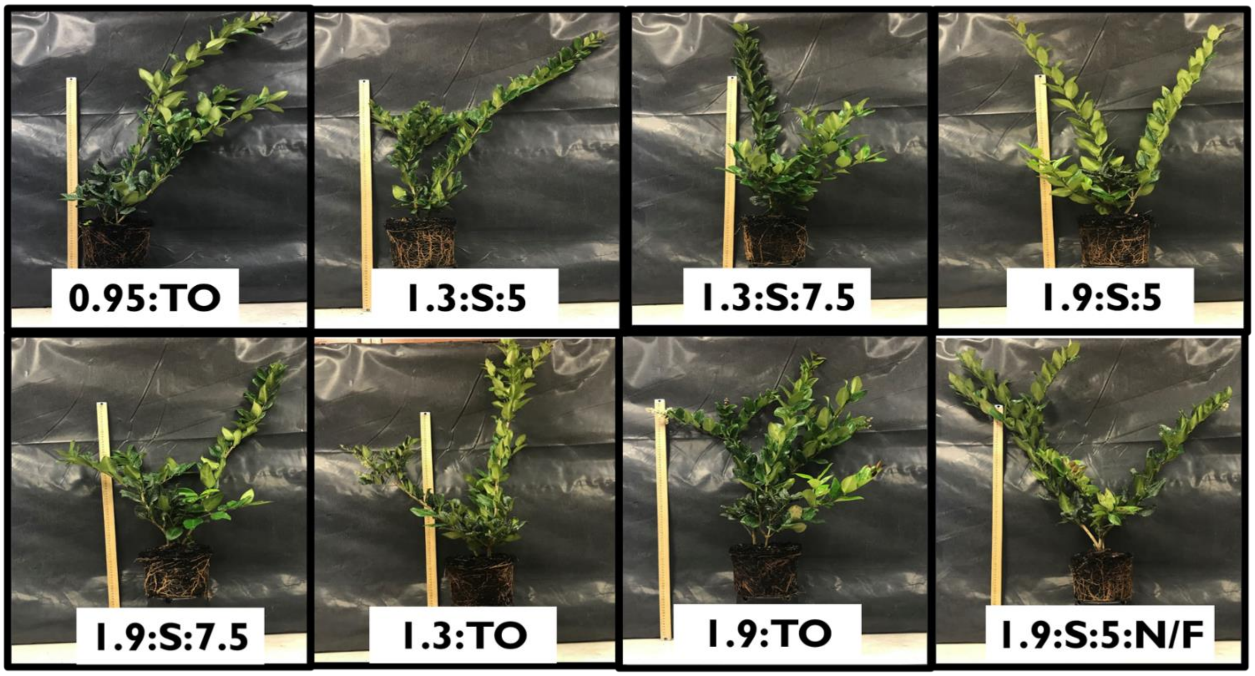

Figure 1. Growth of ligustrum (*Ligustrum japonicum*) at 6 months after transplanting. 0.95:TO = 0.95 cm pine bark (PB) incorporated with CRF throughout the container, 1.3:S:5 = 1.3 cm PB as top substrate applied at a 5 cm depth with 0.95 cm bark as the bottom substrate with CRF incorporated, 1.3:S:7.5 =1.3 cm PB as top substrate applied at a 7.5 cm depth with 0.95 cm bark as the bottom substrate with CRF incorporated, 1.9:S:5 =1.9 cm PB as top substrate applied at a 5 cm depth with 0.95 cm bark as the bottom substrate with CRF incorporated, 1.9:S:7.5 = 1.9 cm PB as top substrate applied at a 7.5 cm depth with 0.95 cm bark as the bottom substrate with CRF incorporated, 1.3:TO = 1.3 cm pine bark (PB) incorporated with CRF throughout the container, 1.9:TO = 1.9 cm pine bark (PB) incorporated with CRF throughout the container, 1.9:S:5:N/F = 1.9 cm bark with fines removed, applied at a 5 cm depth with 0.95 cm bark as the bottom substrate with CRF incorporated.

Interestingly, the 1.9:S:5:N/F treatment which had the lowest water holding capacity in the top 5 cm (data not shown) had shoot and root dry wt. similar to or greater than all incorporated treatments, even though no fertilizer was incorporated into the top 5 cm of the substrate.

Effect of substrate composition on bittercress (Cardamine flexuosa) germination and biomass. At 4 WAP, bittercress germination was highest in the 0.95:TO treatment followed by the 1.9:TO treatment which was similar while germination was lowest in the stratified substrate treatments (Table 2). The lowest germination was observed in the stratified substrate of 1.9:S:5: N/F (Table 2). By 8 WAP, fewer differences were observed in germination, but germination was significantly reduced in stratified treatments including 1.3:S:5, 1.9:S:5, and 1.9:S:5: N/F compared with 0.95:TO (Fig. 2).

Table 2. Effect of substrate composition on bittercress (*Cardamine flexuosa*) germination and biomass.

	Bittercress		
	Germination count pot^{-1a}		
Substrate[c]	4 WAP	9 WAP	Biomass[b]
0.95:TO	13.2 a[d]	14.0 a	7.6 a
1.3:S:5	6.9 cd	8.7 bc	1.2 b
1.3:S:7.5	6.7 cd	10.1 abc	0.5 b
1.9:S:5	6.9 cd	8.1 bc	1.1 b
1.9:S:7.5	7.9 bc	10.4 ab	0.5 b
1.3:TO	8.6 bc	11.9 ab	5.9 a
1.9:TO	11.3 ab	11.8 ab	7.7 a
1.9:S:5: N/F	3.4 d	6.2 c	0.2 b

[a]Germination count was assessed by surface sowing 25 seeds of bittercress (*Cardamine flexuosa*) to each pot and counting germinated seedling at 4 weeks and 9 weeks after potting (WAP). First experimental run was initiated on (Nov. 20, 2019) and second on (Feb. 2, 2020).

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

[c]Substrate consisted of incorporated substrates with 0.95, 1.3 or 1.9 cm pine bark (PB) throughout the container with controlled release fertilizer (Osmocote® 17-5-11, 8 to 9 mo.) incorporated at 35 g pot-1 throughout (TO) (0.95:TO, 1.3:TO, and 1.9:TO) and stratified substrate treatments consisted of having the top substrate (1.3 or 1.9 cm pine bark) applied at depths of 5 or 7.5 cm, resulting in four stratified (S) substrate treatments (1.3:S:5, 1.3:S:7.5, 1.9:S:5, and 1.9:S:7.5) with the bottom substrate consisting of 0.95 cm bark with CRF incorporated. 1.9:S:5: N/F consisted of 1.9 cm bark with fines removed, applied at a 5 cm depth with 0.95 cm bark as the bottom substrate with CRF incorporated.

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

Bittercress dry wt. taken at trial conclusion revealed that all stratified treatments had significant lower dry wt. compared with incorporated treatments, with stratification and strategic fertilizer placement resulting in decreases ranging from 80 to 97% in bittercress biomass. The top layer of stratified treatments had no fertilizer in top 5 or 7.5 cm of the substrate, hence the top layer lacked nutrients for bittercress growth. Similar results were reported by Saha et.al (2019), where 90% reduction in eclipta growth was observed when fertilizer was subdressed resulting in a similar effect where no nutrients were contained on the surface of the substrate.

Figure 2. Bittercress (*Cardamine flexuosa*) at 10 weeks after potting. 0.95:TO = 0.95 cm pine bark (PB) incorporated with CRF throughout the container, 1.3:S:5 = 1.3 cm PB as top substrate applied at a 5 cm depth with 0.95 cm bark as the bottom substrate with CRF incorporated, 1.3:S:7.5 =1.3 cm PB as top substrate applied at a 7.5 cm depth with 0.95 cm bark as the bottom substrate with CRF incorporated, 1.9:S:5 =1.9 cm PB as top substrate applied at a 5 cm depth with 0.95 cm bark as the bottom substrate with CRF incorporated, 1.9:S:7.5 = 1.9 cm PB as top substrate applied at a 7.5 cm depth with 0.95 cm bark as the bottom substrate with CRF incorporated, 1.3:TO = 1.3 cm pine bark (PB) incorporated with CRF throughout the container, 1.9:TO = 1.9 cm pine bark (PB) incorporated with CRF throughout the container, 1.9:S:5:N/F = 1.9 cm bark with fines removed, applied at a 5 cm depth with 0.95 cm bark as the bottom substrate with CRF incorporated.

Stewart et al (2017) also showed that depths of 2.5 cm are efficient to control spotted spurge, large crabgrass (*Digitaria sanguinalis*), bittercress and liverwort (*Marchantia polymorpha*) growth. While the growth of bittercress decreased in stratified substrates, it could be due to the strategic placement of fertilizer and more research is needed to quantify the effect of stratification on weed germination and growth.

Results from this study showed that substrate stratification and strategic fertilizer placement may result in early growth reductions to ligustrum, but no differences were observed between stratified substrates and an industry standard 0.95:TO substrate by the time plants reached marketable size or were ready to be transplanted into larger containers. The growth of bittercress substantially decreased in the stratified substrates indicating that stratified substrate could potentially be used as part of an overall weed management strategy. As these methods of substrate stratification and strategic fertilizer placement have not been evaluated widely, additional research is currently being conducted to determine the effects of these strategies on the growth of other common ornamental species and problematic weeds in container nurseries, as well as the effects of these methods on irrigation and fertilization efficiency.

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