Can Leaf Mold Substitute Peat as a Local, Renewable Seed Starting Medium?

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Abstract

High-quality substrate alternatives to peat in horticulture and agriculture are needed due to the increasing demand for peat, and the many environmental concerns associated with its use. Leaf mold, used alone or in combination with other substrates, can serve as a renewable alternative to peat in potting media in organic production systems. This study was conducted to evaluate the suitability of using leaf mold as an alternative to peat in growing media for organic transplant production. Twenty-four substrates were compared. The growth and yield of the transplants were measured by comparing leaf area, dried plant biomass, and root:shoot ratios. Transplants were grown for six weeks. Root:shoot ratios were not affected by soil medium, loosening agent, or crop. Total dry weight was not affected by soil medium, only by crop. Total dry weight for zinnias was significantly higher than for kale at the end of the experiment. Maximum leaf growth in transplants resulted from the substrate composed of equal amounts of leaf mold and compost, by volume, with vermiculite as the loosening agent. These results indicate that substrates containing leaf mold produce similar results and therefore are a viable alternative to peat for organic vegetable and flower production.

Keywords: leaf mold, peat, peat alternatives, peat substitutes, seedling production, growing media, sustainability, sustainable agriculture, sustainable food systems, regenerative agriculture, seed starting media

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Sphagnum peat moss is the most common ingredient used in soilless potting media in horticulture and agriculture transplant production in North America because of its widespread availability, low cost, structure, sterility, water holding capacity, and pore space (Kuepper and Everett 2004). Peat moss provides stable organic matter, structure and pore space to a potting media but contains few nutrients and requires liming (Kuepper and Everett 2004). Moreover, peat is a non-renewable resource; its harvesting disturbs threatened habitats and their biodiversity and contributes significantly to anthropogenic carbon dioxide emissions caused by aerobic peat decomposition (Muster et al. 2015).

1. Literature review

1.1 Concerns with peat use and ecology

Like other wetlands, peatlands help purify and store water and provide habitat for rare species (CC-GAP 2005). They are the single largest terrestrial store of carbon, storing 30% of the world's terrestrial carbon (Natural Resources Canada [NRC] 2016), equivalent to 75% of all carbon in the atmosphere, and twice the carbon stock in the global forest biomass, making them one of the best long-term stores (CC-GAP 2005; Joppa et al. 2016). Even though only 0.03%, or 73,500 acres, of Canadian peatlands, have been or are currently being harvested (Kuepper and Everett 2004), there are still many environmental concerns related to peat harvesting. When drained, peatlands become net sources of greenhouse gas (GHG) emissions (FAO 2019). According to the FAO, the oxidation of peatlands makes them the third largest emitter of GHGs after crop and livestock agriculture and net forest conversion (FAO 2019). In addition, draining peatlands changes vegetation cover, erodes biodiversity, lowers water quality, increases the frequency of fires, and causes land subsidence (FAO 2019). Moreover, the rate of re-growth of peat deposits after harvest is incredibly slow, with peat in Canadian peatlands forming at 0.5 to 1.0 mm per year (Kuepper and Everett 2004). Another alarming issue is the fact that peatlands are likely to become increasingly vulnerable to fire as climate change progresses (NRC, 2016). This is worrisome because in addition to releasing significant amounts of carbon dioxide and other greenhouse gases, peat fires release mercury into the atmosphere at a rate 15 times greater than upland forests, which is a serious human health concern (NRC, 2016). Consequently, the continued degradation of peat bogs plays a significant part in accelerating climate change (CC-GAP 2005). While the peat industry argues that peatlands can be managed at sustainable levels, it recognizes that alternatives to peat must be developed in order to meet environmental concerns of consumers and contend with increased regulation of peatland exploitation (McMahan 2006).

1.2 Growing media alternatives to peat

The search for alternative high-quality and low-cost substrates as growing media in horticulture and agriculture is a serious need due to the increasing demand for peat, as well as the many environmental concerns of its use (Bustamante et al. 2008). Renewable alternatives in growing media production are urgently required but slowly emerging. Some research experiments (Zhang, Sun, Tian, & Gong, 2013) have studied the feasibility of replacing peat with different percentages (0, 10, 30, 50, 70, 90, and 100%) of composted green waste as growth media. Others have explored specific waste materials as potting media substitutes for peat, such as bark, compost, sawdust, sewage sludge, coconut coir, biochar, or spent mushroom compost (Bustamante et al. 2008), but a study on the suitability of leaf mold as a seedling and potting medium is missing.

1.2.1. Coconut coir

In recent years, there has been an increase in the use of coconut coir as a ‘sustainable’ peat substitute. Coir originates from coconut husks and is a by-product of the coconut fibre industry (Kuepper and Everett 2004). Because the physical properties of coir are similar to sphagnum peat moss, it has been deemed an acceptable peat replacement in a potting mix recipe (Kuepper and Everett 2004). A study on coir as an alternative to peat in media for tomato transplant production found that transplants grown in coir-based media generated similar or greater tomato yields than those grown in peat-based media (Arenas et al. 2002). However, the same study found that media containing more than 50% coir resulted in reduced transplant growth and vigour, likely caused by nitrogen immobilization by microbes with the higher C:N ratio of coir (2002). This study also concluded that visual estimates of transplant growth were not closely associated with subsequent fruit yield (2002). Some consider coir to be more environmentally sustainable than peat because it is a by-product of industry rather than a product that is mined from a natural ecosystem (Kuepper and Everett 2004). However, one must consider that coir travels much farther in shipping, originating from Southeast Asian, African, and tropical American countries where coconuts are harvested commercially (McMahan 2006 & Abad et al. 2005).

1.2.2. Biochar

Interest and research into biochar as a potting mix amendment has been increasing. When substituted for peat in potting substrate mixtures in rates up to 15% (v/v), biochars (without acid treatment) produced from hardwood pellets and pelleted wheat straw were similar in performance to mixtures containing only peat moss (Vaughn et al. 2015). The high pH of biochar can also neutralize the acidity of peat and eliminate the need for lime (Kuepper and Everett 2004). A mix with 30% biochar and 70% peat moss had a pH and physical characteristics very similar to a commercial peat-perlite potting mix (Kuepper and Everett 2004).

1.2.3. Leaf mold

Leaf mold is an ingredient found in many older organic potting mix recipes in place of peat moss (Kuepper and Everett 2004). It is made from leaves that have been composted for six to twelve months and provides structure and water-holding capacity to a potting mix, along with adding beneficial microbes (Kuepper and Everett 2004). Unlike compost, leaf mold does not provide a significant source of fertility in the potting mix because it is made primarily from carbon-rich materials (Kuepper and Everett 2004). Though Heckman et al. (n.d.) would argue that after decomposition, leaves contain agronomically significant amounts of nutrients, including potassium, calcium, magnesium, sulfur, boron, iron, manganese, chloride, sodium, copper, and zinc. Leaf mold can be regarded as an environmentally-friendly alternative to both peat and coir, as it is locally-available and is considered a waste product in cities. There are also economic benefits as the use of peat moss and vermiculite is becoming increasingly expensive while the use of residues such as leaf mold means lower costs (Tsakaldimi & Ganatsas 2016).

Compost was included as an ingredient in some of the treatments in this experiment because of its high nitrogen content, since the carbon:nitrogen ratio of leaf mold is high and as such, it has the potential to tie up nitrogen in the soil. Compost often contains enough nitrogen, phosphorus, and potassium to meet a seedling's nutritional needs for the first two to three weeks of growth (Kuepper and Everett 2004). Generally, compost is added to a potting mix at a concentration of 20 to 50% by volume, depending on the crop and growing conditions (Grubinger 2012). Used in a potting mix, compost should be mature and screened to remove large particles (Kuepper and Everett 2004). It is a good practice to test compost by sending samples to a lab or conducting a germination test to ensure that it is fully mature before using it in a batch of potting mix (Kuepper and Everett 2004). For drainage, I chose builders' sand in place of perlite, as it is more locally-available. Coarse builder's sand was traditionally used to add porosity to a mix, before being replaced by perlite and vermiculite (Kuepper and Everett 2004). I also used vermiculite as an alternative drainage component and loosening agent. Research by Arenas et al. (2002) has suggested that the relationship between traditional attributes of transplant growth such as root dry weight and visual quality should be investigated further.

This study aimed to assess the suitability of using leaf mold as an alternative to peat in growing media for organic transplant production. This was accomplished by evaluating six growth substrates and studying the effects on growth and yield of two crop species commonly grown as transplants: kale (*Brassica oleracea* ‘Lacinato’) and zinnias (*Zinnia elegans* ‘Giant Blue Point’), by measuring leaf area, dried plant biomass, and root:shoot ratios.

My hypothesis is that transplants grown in leaf mold-based media will perform the same or better than transplants grown in peat-based media.

2. Materials and Methods

2.1. Materials & Growing Site

This experiment took place in Kwantlen Polytechnic University’s passive solar geodesic dome at the Research & Teaching Farm on the Garden City Lands, in Richmond, BC. All soil mix treatments were mixed, filled in their cells, and seeded at this location.

The leaf mold used in this experiment was a product made from local leaves harvested in Richmond and left to decompose in a black garbage bag for approximately one year. The compost was sourced from Net Zero Waste in Abbotsford and Hop Compost in Vancouver, BC. The sand was sourced from Lawnboy Enterprises in Vancouver, BC. The peat and vermiculite (medium A-2) were sourced from Sun Gro in Agawam, MA. Soil amendments were sourced from Kwantlen Polytechnic University’s Department of Sustainable Agriculture. All seeds were sourced from West Coast Seeds.

2.2. Methods

Before mixing the 36 soil treatments, I performed a compost maturity test on my first source of compost (Net Zero Waste) with fast-germinating radish seeds to determine if the level of phytotoxins in the compost was high enough to inhibit germination (Compost Info Guide 2019). I seeded 20 radish seeds each in a pot of peat-based soil mix and in a pot of Net Zero compost. Compost is said to be mature if the germination rate from the seeds grown in the compost is not significantly less than that of the control soil, after seven days (Compost Info Guide 2019). After 11 days, 85% of the seeds in the peat soil had germinated but none of the seeds in the pot of compost had germinated. The compost from Net Zero Waste also had a sour smell to it, so, for this reason, in addition to its low germination rate, I deemed this compost too immature to conduct my experiment and I selected another source of compost; Hop Compost. I performed a second compost maturity test with this new compost source, following the same methods as above. This time, after 11 days, the seeds in the pot of Hop compost had a germination rate of 45%. This result was not ideal but it was better than the performance of the compost from Net Zero Waste so I went ahead and used it in my soil blends.

Using the proportions of ingredients used in the 2015 KPU Soil Recipe (Table 1), I calculated the total amount of each ingredient needed to fill all four 18-cell plug trays, consisting of 72 27-in^3 cells. First, I measured out the nutrient amendments needed for the treatments with leaf mold and peat. I converted the amounts of nutrient amendments to ounces in Table 2. I decided to measure out all of the amendment ingredients as a batch, instead of measuring out each amendment in each individual cell.

KPU Soil Mix Recipe (2015)	
Ingredient	Amount
Peat	18 L
Loosening agent	18 L
Lime	100 g
Alfalfa Meal*	540 g
Kelp Meal	270 g
Bone Meal	140 g
Guano	70 g

Table 1. KPU soil mix recipe (2015) (36 L).
* Alfalfa meal was unavailable, therefore it was substituted for blood meal.

We did not have any more alfalfa meal on hand so I substituted it for blood meal at ⅙th of the amount by weight, to account for the nitrogen content of blood meal being around six times higher (13%) than that of alfalfa meal (2-3%). Each 27 in^3 cell had a volume of 442.45 mL, therefore I calculated a total soil mix batch of 31.86 L to fill 72 cells. I originally incorrectly attributed the 31.86 L of the total soil mix to the batch volume, and this led to incorrect amounts of amendments being used. The amendments only needed to fill 48 cells to add nutrients to the treatments containing leaf mold and/or peat, for a total volume of 21.2 L. The amounts of nutrient amendment were converted to ounces instead of grams, after I discovered that the scale in the soil lab only has two-decimal precision in ounces.

Nutrient Amendment Batch									
Nutrient Amendment Ingredient	Original Amount (36 L KPU recipe)	Proportion of Batch	Batch Amount Calculated	Actual Amount Needed for 21.2 L Batch		Amount Added/Cell	Actual Amount Needed/Cell	
Lime	100 g	2.78 g/L	88.57 g	58.94 g	2.08 oz	3.12 oz	1.23 g	0.04 oz
Blood meal	90 g	2.50 g/L	79.65 g	53.0 g	1.87 oz	2.80 oz	1.10 g	0.04 oz
Kelp meal	270 g	7.50 g/L	238.95 g	159.0 g	5.61 oz	8.43 oz	3.31 g	0.12 oz
Bone meal	140 g	3.89 g/L	123.94 g	82.47 g	2.91 oz	4.37 oz	1.72 g	0.06 oz
Guano	70 g	1.94 g/L	61.80 g	41.13 g	1.45 oz	2.18 oz	0.86 g	0.03 oz
Totals	670 g	18.61 g/L	592.91 g	394.54 g	13.92 oz	20.90 oz	8.22 g	0.29 oz

Table 2. Total amount of each nutrient amendment ingredient added to each cell for treatments composed of leaf mold and peat. Proportions are adjusted to reflect the smaller batch size required for this experiment and the ingredient replacement of blood meal substituting for alfalfa meal as a nitrogen source. Highlighted columns reflect how I should have calculated the amounts of amendments needed.

To create the different soil media treatments, I measured and mixed the ingredients for each treatment in a large sealable plastic bag. Treatments with leaf mold and peat were pre-mixed with nutrient amendments to achieve an equivalent comparison between these two media with the compost (see Table 2). The compost factor did not receive these amendments as it already contained nutrients that would serve as a slow-release fertilizer. The amendment batch was measured by weight with a scale and the substrate and loosening agent ingredients were measured with a graduated cylinder beaker. Leaf mold was sifted as needed with a compost sifter.

After all the cells were filled with their respective soil mixes, the three crops, head lettuce (*Lactuca sativa* ‘Aerostar’), kale (*Brassica oleracea* ‘Lacinato’), and zinnia (*Zinnia* *elegans* ‘Giant Blue Point’), were seeded at a rate of 2-3 seeds per cell, on June 21, 2019. Each cell was later thinned to the strongest plant.

2.3. Experimental design

The experiment was conducted in a completely randomized full factorial design with two replicates of 36 treatments with three factors, in four 18-cell seedling trays, resulting in 72 experimental units.

Each treatment was a different combination of a growing medium, a loosening agent, and a crop. Treatments were made up of different combinations of the following three experimental factors:

1) Growth medium (Six levels):
    a) Peat
    b) Leaf mold
    c) Compost
    d) Leaf mold/Compost (1:1)
    e) Peat/Compost (1:1)
    f) Peat/Leaf mold (1:1)
2) Loosening Agent (Two levels):
    a) Sand
    b) Vermiculite
3) Crop (Three levels):
    a) Kale
    b) Lettuce
    c) Zinnia

The dependent factors were growth and yield measured as leaf area, plant biomass and root:shoot ratio.

Each cell was given a separate tag with a unique identifying code. Seedlings were overhead-irrigated every other day. None of the lettuce plants germinated, therefore, the experiment was analyzed with 24 treatments. The kale and zinnia transplants were grown for six weeks.

2.4. Data collection methods

Leaf area was estimated weekly by randomly selecting and measuring the length and width of four leaves from each plant. The means of the estimated leaf area values for each plant were multiplied by 75% to better represent the shape of the leaves.

After six weeks, kale and zinnia transplants were harvested, rinsed and dried, and their fresh weights recorded, on July 29, 2019. Plants were then oven-dried at 60 °C in an air-forced oven for 72 hours. Dry weights were then recorded and root:shoot ratios were calculated.

2.5. Statistical analysis

All data were tested for normality using the Shapiro-Wilk Test. Dried biomass and root:shoot ratio data were analyzed by ANOVA. Leaf area data were analyzed using a Linear Mixed Model test with week, medium, and loosener as fixed effects and crop as a random effect. All analyses were conducted using the jamovi (v. 1.0.8.0) interface for R statistical software. Tukey's Honestly Significant Difference test and the Bonferroni test were used to compare differences between treatments ($\alpha = 0.05$).

3. Results

3.1. Root:shoot ratio

Soil medium (*p*=0.264), loosening agent (*p*=0.142), and crop (*p*=0.060) had no significant effect on root:shoot ratios of kale and zinnia plants (Table 3). There was no interaction between crop and medium (p=0.372), crop and loosening agent (p=0.424), or medium and loosening agent (p=0.274).

	Sum of Squares	df	Mean Square	F	p
Crop	0.0760	1	0.0760	3.846	0.060
Medium	0.1355	5	0.0271	1.372	0.264
Loosening Agent	0.0450	1	0.0450	2.278	0.142
Crop ✻ Medium	0.1106	5	0.0221	1.119	0.372
Crop ✻ Loosening Agent	0.0130	1	0.0130	0.658	0.424
Medium ✻ Loosening Agent	0.1327	5	0.0265	1.343	0.274
Residuals	0.5731	29	0.0198		

Table 3. Results for the ANOVA test on the effect of crop, loosening agent, soil medium, and their interactions, on root:shoot ratios (p > 0.05).

3.2. Total dry weight

Total dry weight (biomass) showed no significant difference by soil medium (p=0.499). However, a significant difference in total dry weight was found between crops (p=0.003) (Table 4). Total dry weight for zinnias was significantly higher than the total dry weight for kale at the end of the experiment (Figure 1).

	Sum of Squares	df	Mean Square	F	p
Crop	9.20	1	9.196	10.679	0.003
Medium	3.84	5	0.769	0.892	0.499
Loosening Agent	1.16	1	1.156	1.343	0.256
Crop ✻ Medium	4.07	5	0.814	0.945	0.467
Crop ✻ Loosening Agent	1.34	1	1.337	1.552	0.223
Medium ✻ Loosening Agent	4.07	5	0.813	0.944	0.467
Residuals	24.97	29	0.861		

Table 4. Results for the ANOVA test on the effect of crop, loosening agent, soil medium, and their interactions, on total dry weight (p > 0.05).

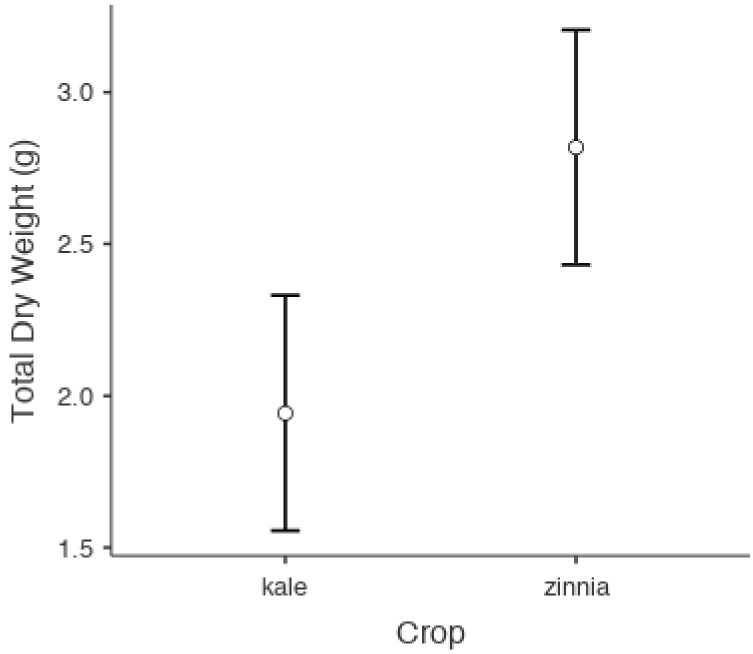

Figure 1. Total dry weight (g) of plant biomass for kale and zinnia (*n*=24 for each crop).

3.3. Leaf area

There was a significant effect of medium (*p*=0.013), week (time) (*p*<0.01), and loosening agent (*p*=0.037) on leaf area (Table 5). There was also a significant interaction between medium and loosening agent on leaf area (*p*=0.041) (Table 5).

Leaf area was significantly greater during each week for the leaf mold/compost (Figure 5) and peat/compost (Figure 7) substrates compared to the pure compost medium (Figure 4). There was an interaction between growing medium and loosening agent. Plants grown in the leaf mold/compost substrate with vermiculite as the loosening agent showed greater leaf growth than the same substrate with sand (Figure 5), pure leaf mold with vermiculite (Figure 2), or pure compost with either loosening agent (Figure 4). All other soil mix treatments showed similar levels of growth. The substrates composed of only leaf mold were the only substrates to show better growth when combined with sand as the loosening agent, but this difference was not significant (Figure 2).

Fixed Effect Omnibus tests				
	F	Num df	Den df	p
Medium	3.034	5	118	0.013
Week	94.242	2	118	<.001
Loosening Agent	4.428	1	118	0.037
Medium ✻ Week	0.483	10	118	0.899
Medium ✻ Loosening Agent	2.402	5	118	0.041
Week ✻ Loosening Agent	0.605	2	118	0.548

Table 5. Results for the Linear Mixed Model test on the effect of medium, week, loosening agent, and their interactions, on leaf area (*n*=24 for each crop) ($p > 0.05$).

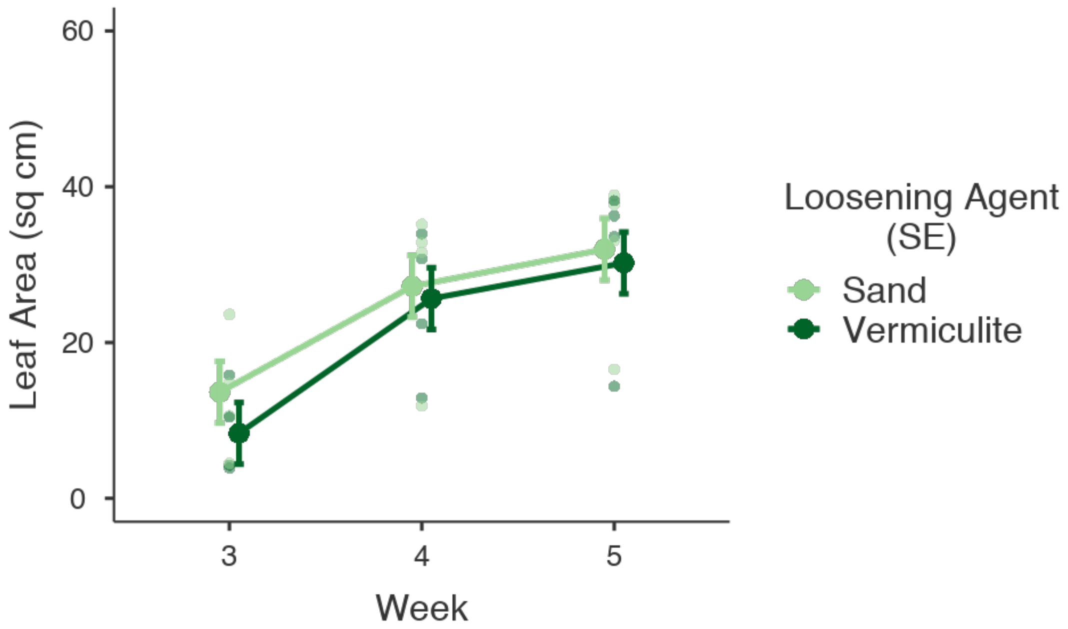

Figure 2. Leaf area (cm^2) increase with time in leaf mold medium with two different loosening agents (*n*=24 for each crop).

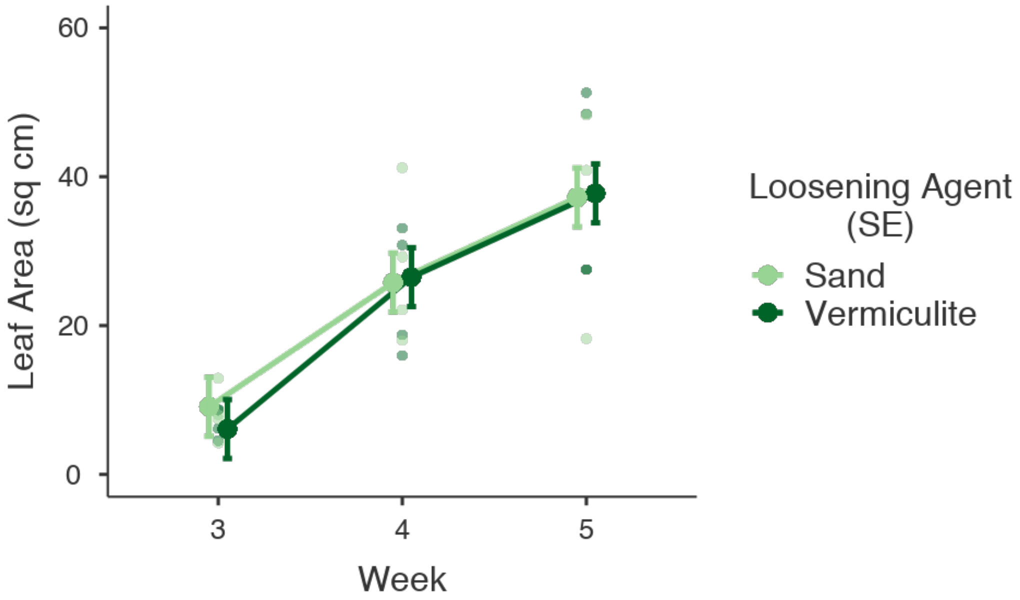

Figure 3. Leaf area (cm^2) increase with time in peat medium with two different loosening agents (*n*=24 for each crop).

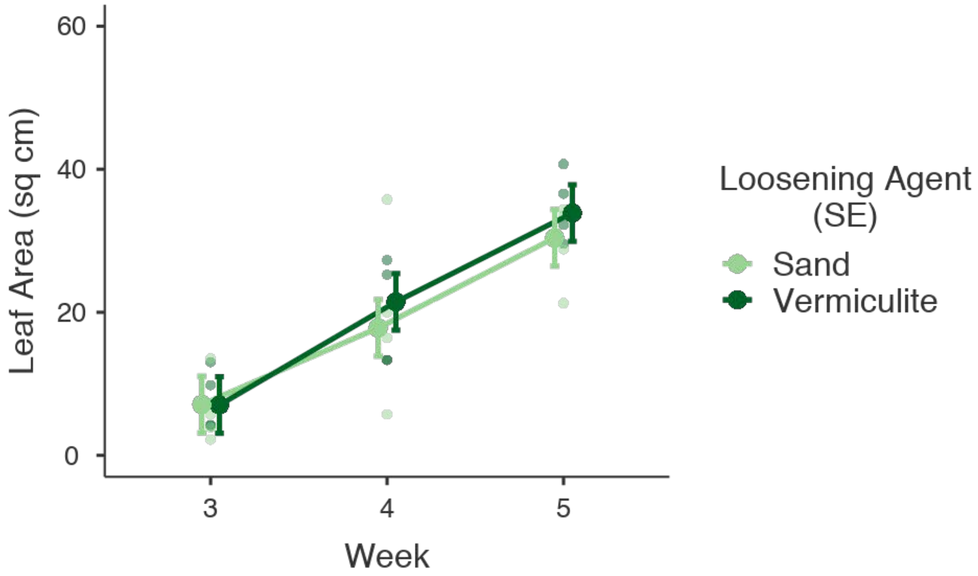

Figure 4. Leaf area (cm^2) increase with time in compost medium with two different loosening agents (*n*=24 for each crop).

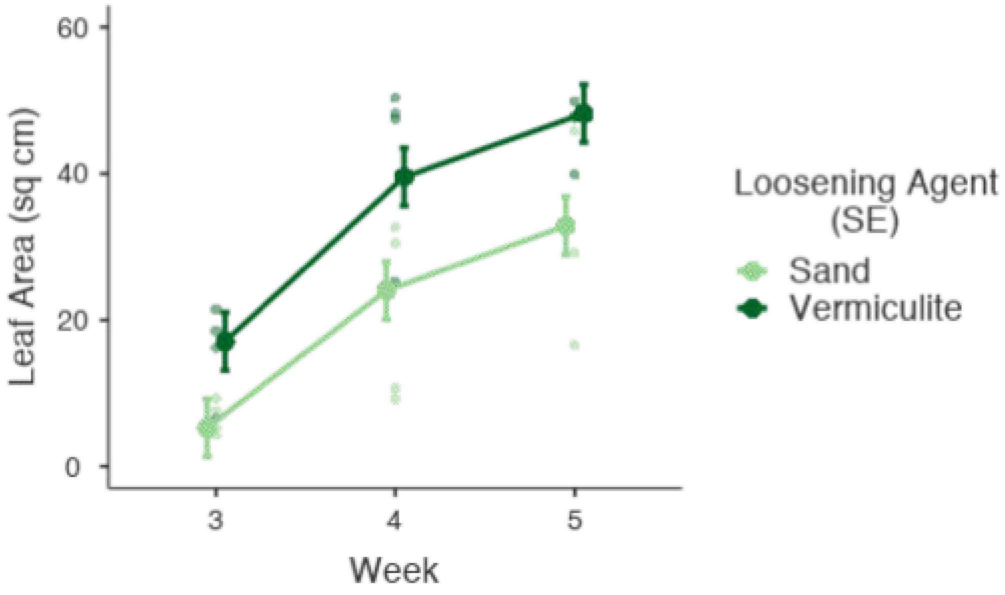

Figure 5. Leaf area (cm^2) increase with time in leaf mold/compost medium with two different loosening agents (*n*=24 for each crop).

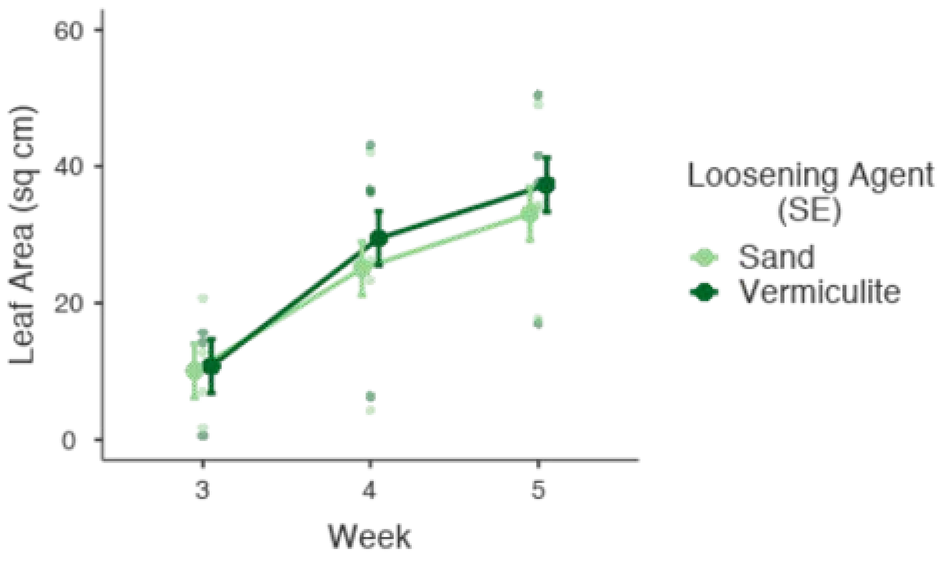

Figure 6. Leaf area (cm^2) increase with time in peat/leaf mold medium with two different loosening agents (*n*=24 for each crop).

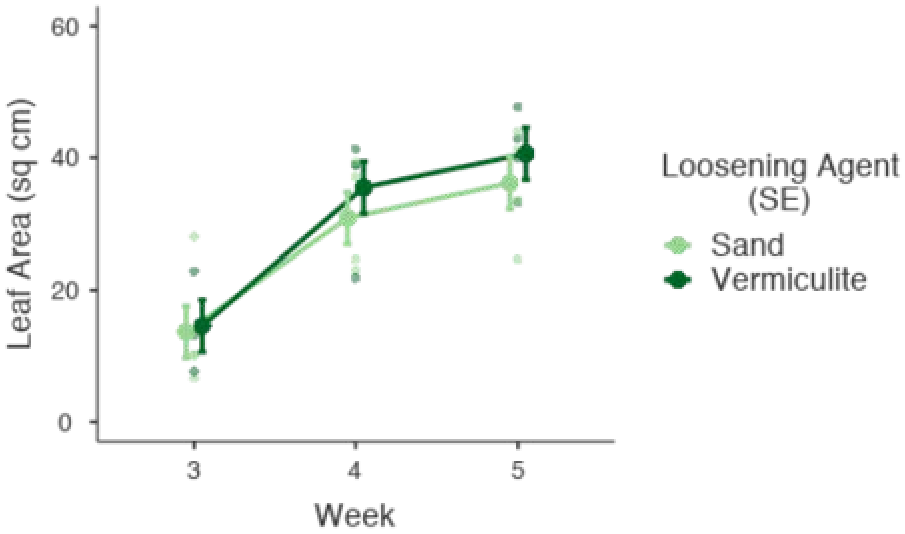

Figure 7. Leaf area (cm^2) increase with time in peat/compost medium with two different loosening agents (*n*=24 for each crop).

4. Discussion

Previous studies have evaluated various alternative substrates such as biochar, coconut coir, bark, compost, sawdust, sewage sludge, and manure, but studies on the suitability of leaf mold as a seed starting and potting medium are lacking. In this experiment, plant growth and yield kale and zinnia transplants were measured as the increase in leaf area over time in peat, leaf mold, and compost media, and final dry weights and root:shoot ratios were compared. The plants grew very well and without any added fertilizers for the six week duration of this experiment.

Early in the study, the germination failure of the lettuce plants may have been due to the warm growing conditions inside the passive solar growing dome. Lettuce grows best under cooler temperatures and has difficulty germinating when temperatures are above 22°C, as they were in June (MacDonald 2014). They might have germinated had it been possible to shade them without shading the other crops.

As expected, leaf area increased over time in both kale and zinnia transplants. The greatest leaf growth was found in the plants grown in the substrate composed of leaf mold and compost with vermiculite as the loosening agent. An explanation for this combination outperforming the others could be that the carbon to nitrogen ratio was balanced between the high nitrogen of the compost and the high carbon of the leaf mold, in addition to being a high nutrient substrate, compared to peat or leaf mold on its own. I suspect that the sand as a loosening agent could have performed better if it had been more uniform in size. There was high variability in sand particle size, ranging from fine sand (125–250 µm) to very fine gravel (2–4 mm), though I intended to study coarse builders' sand (0.5 - 1 mm) (classification based on the Krumbein phi scale) (Krumbein 1934).

Root:shoot ratios were compared to determine if there were any environmental factors causing competition between plants for a certain element such as light quality, water availability, root space, or nutrient availability. The root:shoot ratios for all transplants were in the same range and therefore the plants do not appear to have suffered any environmental stress.

Figure 1 shows that total dry weights of the plants' biomass were not driven by soil media, but by crop difference. Seeing higher biomass in zinnia than in kale plants was expected since the zinnia plants were larger than the kale plants at the time of harvest. From these findings, it appears that soil medium did not influence biomass but it certainly influenced leaf growth.

Despite the benefits to plant growth, there are several limitations for the use of leaf mold. One limitation of these methods is that leaf mold takes six months to one year to produce. The leaves need to be composted until they break down sufficiently to produce a fluffy substrate. Another concern is the ecological impact of harvesting leaves that provide organic matter in sensitive areas. Leaf litter serves as habitat and overwinter shelter for many invertebrates (Baird n.d.). It also recycles important nutrients back into the soil, prevents erosion, and conserves moisture. In considering this, we should avoid collecting leaves from natural spaces. This may also present a barrier to large-scale production of leaf mold. In cities, however, leaves are abundant and largely considered a waste product. They are generally raked up and sent away to be composted with other yard waste by the City. Composting municipal leaves on their own would be much more beneficial than mixing them with other waste as the resulting leaf mold has a valuable application as a substrate.

5. Conclusion

The pressure to find sustainable alternatives to peat has been increasing in recent years due to ecological concerns and this pressure will only rise with the advance of climate change. It is imperative that we reduce our dependence on peat as a substrate and transition to using more renewable soil media for transplant production. The goal of this study was to determine if transplants grown in leaf mold-based media will perform similarly or better than transplants grown in peat-based media. Maximum leaf growth occurred in transplants grown in the substrate composed of equal parts leaf mold and compost, with vermiculite as the loosening agent. Leaf mold on its own cannot totally replace peat as a substrate, but, in combination with compost, it can serve as a substitute for peat in seed starting media and produce similar crop growth. From these results, it is clear that leaf mold is a valuable substrate that supports vegetable and flower growth and its use should be explored further. The results obtained from this study will contribute to existing knowledge on alternative substrates to peat for transplant production and benefit KPU’s Science and Horticulture programs, small-scale farmers, and backyard gardeners, by providing evidence that leaf mold can be used as a locally-available, renewable, and low-cost substrate.

Further experiments should compare the use of different proportions of compost, loosening agents, and amendments combined with leaf mold on a wider selection of crops to determine optimal mixes for transplant growth. It is worth noting that different batches and different types of both compost and leaf mold will produce different results. The combination of leaf mold and compost also has the potential to reduce the use of fertilizers in transplant production, since these transplants received no added nutrients during their six weeks of growth. It would also be interesting to conduct an experiment that examines the effect of leaf mold as a seed starting medium on the yield of crops after they've been transplanted in the field. This kind of research is valuable in advancing regenerative methods of food production. Most of our crop production starts with planting a seed and no food system can be truly regenerative if it relies on non-renewable resources.

6. Acknowledgments

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Addendum: Data analysis and presentation in R and jamovi

A data table called 'LeafMold' was constructed in R.

	Code	Cell	Rep	Treat
1	A1	1	A	Treat1
2	A2	2	A	Treat2
3	A3	3	A	Treat3
4	A4	4	A	Treat4
5	A5	5	A	Treat5
6	A6	6	A	Treat6
7	A7	7	A	Treat7
8	A8	8	A	Treat8
9	A9	9	A	Treat9
10	A10	10	A	Treat10
11	A11	11	A	Treat11
12	A12	12	A	Treat12
13	A13	13	A	Treat13
14	A14	14	A	Treat14
15	A15	15	A	Treat15
16	A16	16	A	Treat16
17	A17	17	A	Treat17
18	A18	18	A	Treat18
19	A19	19	A	Treat19
20	A20	20	A	Treat20
21	A21	21	A	Treat21
22	A22	22	A	Treat22
23	A23	23	A	Treat23
24	A24	24	A	Treat24
25	A25	25	A	Treat25
26	A26	26	A	Treat26

This data table was used to create the experimental design layout by generating a matrix in R:

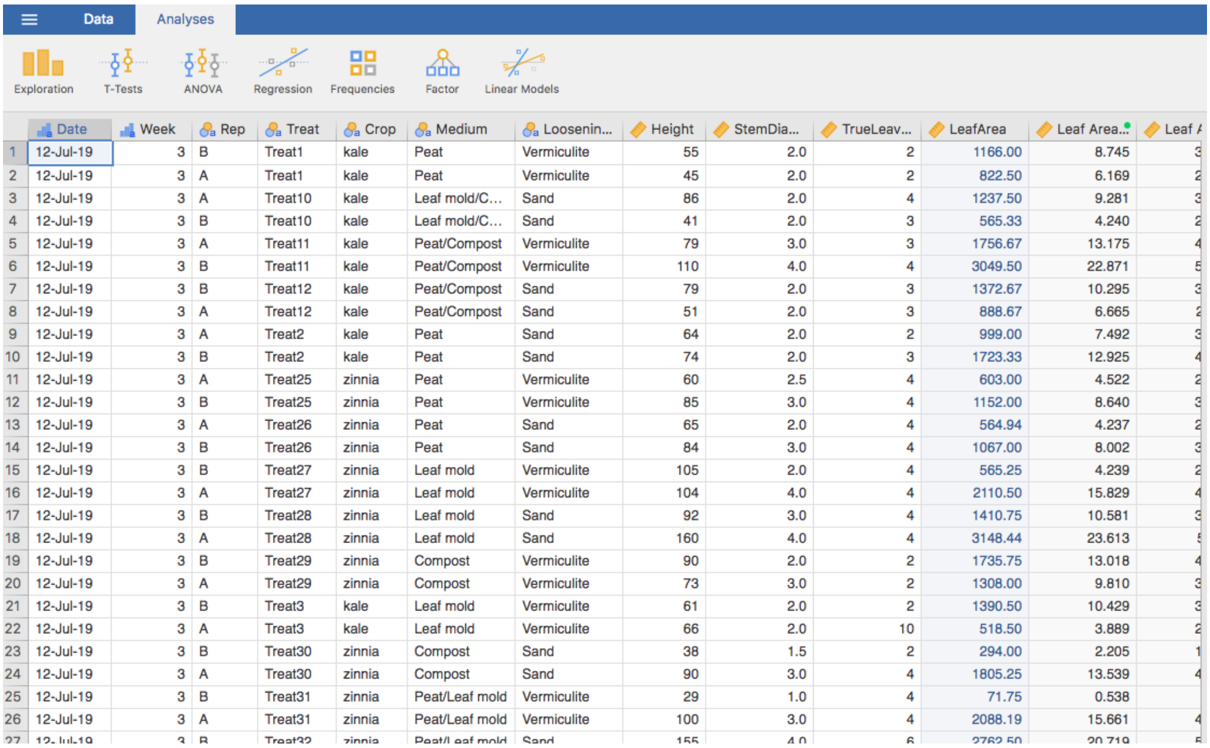
```
> Code <- LeafMold$Code

> matrix(sample(Code), nrow=6)

     [,1]  [,2]  [,3]  [,4]  [,5]  [,6]  [,7]  [,8]  [,9]  [,10] [,11] [,12]
[1,] "B18" "A35" "A24" "B19" "B4"  "A21" "B12" "A2"  "A26" "B30" "B34" "A25"
[2,] "A23" "B29" "A28" "B24" "A3"  "A15" "B1"  "A30" "A14" "B6"  "B15" "B5"
[3,] "B9"  "A29" "A1"  "B23" "B11" "B10" "B21" "B25" "A6"  "B27" "B14" "A10"
[4,] "A5"  "B13" "A9"  "A17" "B36" "A13" "A4"  "A33" "A22" "A34" "A8"  "B16"
[5,] "B7"  "A7"  "B26" "B8"  "B2"  "B33" "B3"  "B22" "A11" "B20" "B28" "A19"
[6,] "B35" "A36" "A20" "A12" "A18" "B17" "A27" "A32" "A16" "B32" "B31" "A31"
```

This matrix was used to create a table to divide experimental units into four trays:

CRD	Tray 1			Tray 2			Tray 3			Tray 4		
	[,1]	[,2]	[,3]	[,4]	[,5]	[,6]	[,7]	[,8]	[,9]	[,10]	[,11]	[,12]
[1,]	"B18"	"A35"	"A24"	"B19"	"B4"	"A21"	"B12"	"A2"	"A26"	"B30"	"B34"	"A25"
[2,]	"A23"	"B29"	"A28"	"B24"	"A3"	"A15"	"B1"	"A30"	"A14"	"B6"	"B15"	"B5"
[3,]	"B9"	"A29"	"A1"	"B23"	"B11"	"B10"	"B21"	"B25"	"A6"	"B27"	"B14"	"A10"
[4,]	"A5"	"B13"	"A9"	"A17"	"B36"	"A13"	"A4"	"A33"	"A22"	"A34"	"A8"	"B16"
[5,]	"B7"	"A7"	"B26"	"B8"	"B2"	"B33"	"B3"	"B22"	"A11"	"B20"	"B28"	"A19"
[6,]	"B35"	"A36"	"A20"	"A12"	"A18"	"B17"	"A27"	"A32"	"A16"	"B32"	"B31"	"A31"

Table I. Completely randomized experimental design layout with 72 experimental units in four 18-cell plant trays. ‘As’ and ‘Bs’ represent replicates.

After data collection, a flat data table was imported from Excel into jamovi (v. 1.0.8.0) for statistical analysis:



	Date	Week	Rep	Treat	Crop	Medium	Loosenin...	Height	StemDia...	TrueLeav...	LeafArea	Leaf Area...	Leaf A
1	12-Jul-19	3	B	Treat1	kale	Peat	Vermiculite	55	2.0	2	1166.00	8.745	3
2	12-Jul-19	3	A	Treat1	kale	Peat	Vermiculite	45	2.0	2	822.50	6.169	2
3	12-Jul-19	3	A	Treat10	kale	Leaf mold/C...	Sand	86	2.0	4	1237.50	9.281	3
4	12-Jul-19	3	B	Treat10	kale	Leaf mold/C...	Sand	41	2.0	3	565.33	4.240	2
5	12-Jul-19	3	A	Treat11	kale	Peat/Compost	Vermiculite	79	3.0	3	1756.67	13.175	4
6	12-Jul-19	3	B	Treat11	kale	Peat/Compost	Vermiculite	110	4.0	4	3049.50	22.871	5
7	12-Jul-19	3	B	Treat12	kale	Peat/Compost	Sand	79	2.0	3	1372.67	10.295	3
8	12-Jul-19	3	A	Treat12	kale	Peat/Compost	Sand	51	2.0	3	888.67	6.665	2
9	12-Jul-19	3	A	Treat2	kale	Peat	Sand	64	2.0	2	999.00	7.492	3
10	12-Jul-19	3	B	Treat2	kale	Peat	Sand	74	2.0	3	1723.33	12.925	4
11	12-Jul-19	3	A	Treat25	zinnia	Peat	Vermiculite	60	2.5	4	603.00	4.522	2
12	12-Jul-19	3	B	Treat25	zinnia	Peat	Vermiculite	85	3.0	4	1152.00	8.640	3
13	12-Jul-19	3	A	Treat26	zinnia	Peat	Sand	65	2.0	4	564.94	4.237	2
14	12-Jul-19	3	B	Treat26	zinnia	Peat	Sand	84	3.0	4	1067.00	8.002	3
15	12-Jul-19	3	B	Treat27	zinnia	Leaf mold	Vermiculite	105	2.0	4	565.25	4.239	2
16	12-Jul-19	3	A	Treat27	zinnia	Leaf mold	Vermiculite	104	4.0	4	2110.50	15.829	4
17	12-Jul-19	3	B	Treat28	zinnia	Leaf mold	Sand	92	3.0	4	1410.75	10.581	3
18	12-Jul-19	3	A	Treat28	zinnia	Leaf mold	Sand	160	4.0	4	3148.44	23.613	5
19	12-Jul-19	3	B	Treat29	zinnia	Compost	Vermiculite	90	2.0	2	1735.75	13.018	4
20	12-Jul-19	3	A	Treat29	zinnia	Compost	Vermiculite	73	3.0	2	1308.00	9.810	3
21	12-Jul-19	3	B	Treat3	kale	Leaf mold	Vermiculite	61	2.0	2	1390.50	10.429	3
22	12-Jul-19	3	A	Treat3	kale	Leaf mold	Vermiculite	66	2.0	10	518.50	3.889	2
23	12-Jul-19	3	B	Treat30	zinnia	Compost	Sand	38	1.5	2	294.00	2.205	1
24	12-Jul-19	3	A	Treat30	zinnia	Compost	Sand	90	3.0	4	1805.25	13.539	4
25	12-Jul-19	3	B	Treat31	zinnia	Peat/Leaf mold	Vermiculite	29	1.0	4	71.75	0.538	
26	12-Jul-19	3	A	Treat31	zinnia	Peat/Leaf mold	Vermiculite	100	3.0	4	2088.19	15.661	4
27	12-Jul-19	3	B	Treat32	zinnia	Peat/Leaf mold	Sand	155	4.0	6	2762.50	20.719	5

For weeks 3-5, each row contained the following data points for each transplant:

- Date: Date of observation
- Week: Week of observation since crops seeded
- Rep: One of two replicates; labeled A or B
- Treat: One of 36 treatments; labeled Treat1 through Treat36
- Crop: One of three crops; kale, zinnia, or lettuce
- Medium: One of six growing media combinations; leaf mold, peat, compost, peat/compost, leaf mold/compost, or peat/leaf mold

- Loosening Agent: One of two loosening agents; sand or vermiculite
- StemDiameter: The stem diameter at the base of each transplant, in mm
- TrueLeaves: The number of true leaves per transplant, not including cotyledons
- Leaf Area: Estimated leaf area, calculated by multiplying the mean length and width of four leaves per transplant by 75%, in cm^2
- DryShootWt: Weight of shoot biomass, in grams
- DryRootWt: Weight of root biomass, in grams
- Total Dry Weight: Total weight of dried biomass, in grams (DryShootWt+DryRootWt)
- Root:shoot Ratio: Ratio of dried root biomass to dried shoot biomass (DryRootWt/DryShootWt)

The null hypothesis that growing media had no effect on root:shoot ratios was tested by ANOVA. We failed to reject the null hypothesis.

	Sum of Squares	df	Mean Square	F	p
Crop	0.0760	1	0.0760	3.846	0.060
Medium	0.1355	5	0.0271	1.372	0.264
Loosening Agent	0.0450	1	0.0450	2.278	0.142
Crop ✻ Medium	0.1106	5	0.0221	1.119	0.372
Crop ✻ Loosening Agent	0.0130	1	0.0130	0.658	0.424
Medium ✻ Loosening Agent	0.1327	5	0.0265	1.343	0.274
Residuals	0.5731	29	0.0198		

The null hypothesis that growing media had no effect on total dry weights was tested by ANOVA. The null hypothesis was rejected since the ANOVA found significant differences between crops. Since there were only two crops compared, we did not need to run a Tukey HSD test. We know that the difference in dry weight is between the kale and zinnia transplants.

	Sum of Squares	df	Mean Square	F	p
Crop	9.20	1	9.196	10.679	0.003
Medium	3.84	5	0.769	0.892	0.499
Loosening Agent	1.16	1	1.156	1.343	0.256
Crop ✻ Medium	4.07	5	0.814	0.945	0.467
Crop ✻ Loosening Agent	1.34	1	1.337	1.552	0.223
Medium ✻ Loosening Agent	4.07	5	0.813	0.944	0.467
Residuals	24.97	29	0.861		

The null hypothesis that growing media had no effect on leaf area was tested by a Linear Mixed Model test. The null hypothesis was rejected, as significant differences were found between media, week, and loosening agent. A significant interaction between medium and loosening agent was also found.

Fixed Effect Omnibus tests

	F	Num df	Den df	p
Medium	3.034	5	118	0.013
Week	94.242	2	118	<.001
Loosening Agent	4.428	1	118	0.037
Medium ✻ Week	0.483	10	118	0.899
Medium ✻ Loosening Agent	2.402	5	118	0.041
Week ✻ Loosening Agent	0.605	2	118	0.548

Means were separated by the Bonferroni test, which found significant differences between leaf mold/compost and compost media. Leaf area was also significantly different between weeks.

Post Hoc Comparisons - Medium

Comparison							
Medium		Medium	Difference	SE	t	df	$p_{bonferroni}$
Compost	-	Leaf mold	−3.220	2.69	−1.198	117	1.000
Compost	-	Leaf mold/Compost	−8.217	2.69	−3.058	117	0.041
Compost	-	Peat	−4.127	2.69	−1.536	117	1.000
Compost	-	Peat/Compost	−8.937	2.69	−3.326	117	0.018
Compost	-	Peat/Leaf mold	−4.680	2.69	−1.742	117	1.000
Leaf mold	-	Leaf mold/Compost	−4.996	2.69	−1.859	117	0.982
Leaf mold	-	Peat	−0.907	2.69	−0.337	117	1.000
Leaf mold	-	Peat/Compost	−5.717	2.69	−2.127	117	0.532
Leaf mold	-	Peat/Leaf mold	−1.460	2.69	−0.543	117	1.000
Leaf mold/Compost	-	Peat	4.090	2.69	1.522	117	1.000
Leaf mold/Compost	-	Peat/Compost	−0.720	2.69	−0.268	117	1.000
Leaf mold/Compost	-	Peat/Leaf mold	3.537	2.69	1.316	117	1.000
Peat	-	Peat/Compost	−4.810	2.69	−1.790	117	1.000
Peat	-	Peat/Leaf mold	−0.553	2.69	−0.206	117	1.000
Peat/Compost	-	Peat/Leaf mold	4.257	2.69	1.584	117	1.000

Post Hoc Comparisons - Week

Comparison							
Week		Week	Difference	SE	t	df	$p_{bonferroni}$
3	-	4	−17.18	1.90	−9.04	117	< .001
3	-	5	−25.59	1.90	−13.47	117	< .001
4	-	5	−8.41	1.90	−4.43	117	< .001