

From Kennedy, to Beyond: Growing Plants in Space

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Astronauts cannot have their cake and eat it too, but what about growing a salad and eating it? As NASA continues to push the envelope on Space exploration and inhabitation the need for a fresh food source becomes more vital. The Life Support team at NASA is using a system developed by ORBITEC the VEGGIE, in which astronauts aboard the ISS, and potentially the Moon and Mars, will be capable of growing food. The introduction of plants not only gives astronauts a means of independently supplying food, but also recreation, oxygen replenishment and psychological benefits. The plants were grown in “pillows”, the system used for growing plants within the VEGGIE. This test included 4 types of media mixtures that are composed of a clay based media called Arcilite and Fafard #2, which is a peat moss-based media (<1 mm Arcilite, 1-2 mm of Arcilite, 1:1 <1mm & 1-2 mm mixture and 1:1 Arcilite & Fafard mixture). Currently, 3 lettuce cultivars are being grown in 4 mixtures of media. Tests were being conducted to see which form of media has the ratio of best growth and least amount of microbes that are harmful. That is essential because a person’s body becomes more susceptible to illness when they leave Earth. As a result, test must be conducted on the “pillow” system to assess the levels of microbial activity. The cultivars were tested at different stages during their growing process for microbes. Data show that the mix of Fafard and Arcilite had the best growth, but also the most microbes. This was due to the fact that Fafard is an organic substance so it contains material necessary for microbes to live. Data suggest that the <1mm Arcilite has an acceptable amount of growth and a lower level of microbes, because it is non-organic.

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Introduction

The aspirations of NASA to inhabit the moon, mars and other locations are dependent upon developing a source of sustenance that independent of food being transported to and from the site. Creation of self-sustaining practices, such as crop production, could initiate a system for being more independent. The growing of salad crops would have benefits outside of sustenance for the advancement of deep space ventures that include recreational activities for the crew to conduct that would have a positive physiological effect, and the plants themselves would help with the revitalization of the environment by reducing carbon dioxide and producing oxygen. Currently, the instrument used for experimentation of the previously stated idea is called VEGGIE. This instrument is constructed by ORBITEC, purchased by NASA and is used at the Kennedy Space Center in preparation for flight to the International Space Station. Scientists and engineers are trying to find ways to make this system meet the strict requirements for compatibility with space flight regulations. The goal is to have VEGGIE light enough, compact enough, and as microbial free as possible. The plants will not be grown hydroponically and will therefore need media to grow in space (Morrow & Remiker, 2009).

The media will be placed in a bag made of light weight, electrostatic material with surface of Nitex, a wicking material (figure 1). These bags are called pillows and the purpose of this wicking material is to draw water up from a capillary mat, which absorbs water from a reservoir. The media, for experimental purpose are a mix between different size particles of Arcillite, a clay-based media, and Fafard #2, a peat moss-based media. This particular experiment is focused on finding which type of media has the best balance between overall growth and microbial activity. General Knowledge tells us that the Fafard #2 would have the most microbe activity because it's organic, but maybe it can outgrow all other mixtures.



Figure 1. Pillow with wicking surface shown.

Materials and Methods

Four mixtures of media were tested with 3 lettuce (*Lactuca sativa*) cultivars in the experiment. The mixtures of media were Arcillite (Turface Proleague, Profile LLC, Buffalo Grove IL) and Fafard #2 (Conrad Fafard Inc., Agawam, MA) in the following combinations and sizes: <1 mm Arcilite, 1-2 mm Arcilite, 1:1 mixture of <1mm & 1-2 mm and 1:1 Arcilite (1-2mm) & Fafard #2mixture. The cultivars were 'Waldman's Green', 'Outredgeous' & 'Flandria'. The media was mixed in separate containers and Nutricote (18-6-8, type 180, Florikan, Sarasota, FL), a time release fertilizer, was added at a ratio of 7.5 grams of Nutricote per liter of dry media.

Seeds of the 3 cultivars were planted in each of the 4 media 5 times, meaning that 60 total pillows were planted. The pillows had tiny slits placed into the top (non-wicking) surface so that small inserts made of Nitex could be placed in them. These inserts act as wicks to draw water up through the media, much like the ones in the VEGGIE system and the ones placed in the tubs used for the experiment. The tubs contained a large foam sheet with a flexible material placed horizontally on top and these were both covered by a Nomex fabric wicking material that would draw water from the reservoirs. The pillows were placed in increments of 12 in 5 tubs equaling 60, the total number of pillows. The tubs were analog test for VEGGIE hardware that was much smaller.

They were then placed in an environmental growth chamber that was configured to simulate the condition a VEGGIE unit placed on the International Space Station (figure 2). The days in the chamber were 16 hours long and the nights were 8 hours long. The temperature for the chamber was set for 26°C days and 24°C and 70% and 75% relative humidity, respectively. The Carbon Dioxide was set at 1200 ppm throughout the entire experiment. All of the settings were computed and monitored by a computer software application called OPTO. They were grown in these conditions for 28 days.



Figure 2. Pillows with plants grown in Environmental Growth Chamber with minimal to limit microbial levels

Microbial Testing

Samples for microbiological analysis were collected pre-planting and post-harvest from all four media used in this study. Before planting, two unused pillows were cut into individual components: Nitex wicking material, solid static shielding material, and the Nitex netting. Seeds of each cultivar were also analyzed. Post-harvest samples included plants, (see below), all four media types, pillow components from each type as well as root material retrieved from the soil. Weights were determined for samples of rooting media, root material and seeds and the surface

area was measured for the pillow material samples. Soil, root and pillow material samples were placed in 15 milliliters sterile DI water in 50 ml sterile centrifuge tube containing 5 milliliters sterile glass beads. The seeds were placed in smaller (15 milliliter) sterile tubes containing 2 milliliters sterile DI water with glass beads. Samples were shaken manually for two minutes to remove microbes from the surfaces.

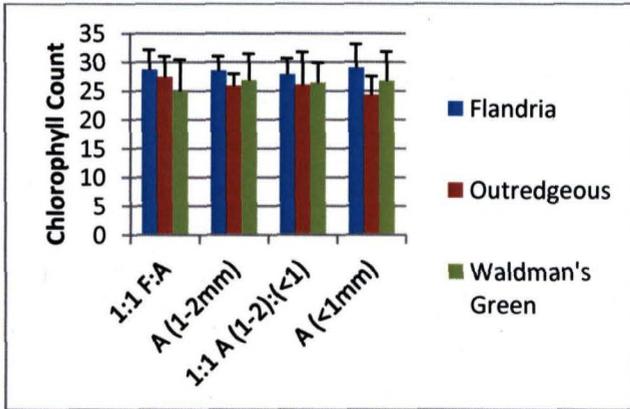
Plant samples for microbiological analysis were collected in triplicate. Portions of four plants of the 'Outredgeous' cultivar were cut using sterile scissors and forceps as follows: Samples from the top of the plant were comprised of the leaves cut approximately 5 cm from the plant /pillow interface, middle samples were the next 2.5 cm, leaving the bottom 2.5 cm sample. The leafy top samples were placed in sterile whirl-pack sample bags containing 60 milliliters sterile DI water with sterile glass beads. Smaller middle and bottom samples were placed in sterile 50 milliliter centrifuge tubes with 30 milliliters sterile water and approximately 5 milliliters sterile glass beads. All samples were weighed. Samples were shaken for two minutes to remove microbes from the plant surfaces. Sample solutions were serially diluted in sterile DI water and plated onto for heterotrophic plate counts. Inhibitory Mold was used for yeast and mold counts. R2A plates were incubated at 25^o C for up to 48 hours. IMA plates were incubated at room temperature for 5 days. Plates within the 25-250 colonies range, or lowest possible were counted to calculate colony forming units per gram of fresh plant weight.

Microbial enumeration data were transformed to log₁₀ values. A one way Anova was performed to determine significant spatial differences on harvested lettuce. A two way Anova was run to determine the effects of rooting media on microbial densities on plants and other surfaces.

Results

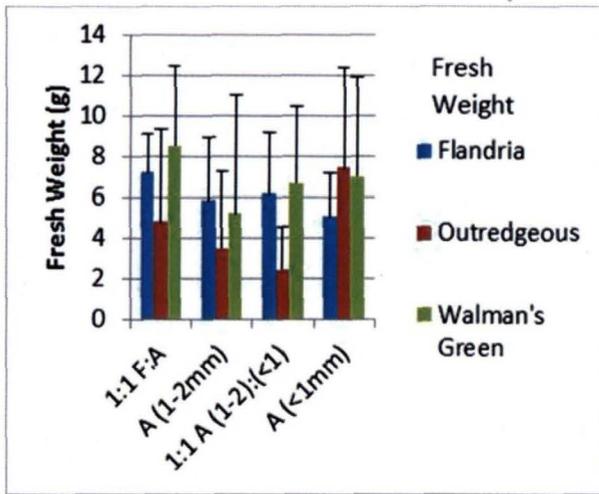
Plant Growth

The extensions on the graphs are the standard deviations and the units of the growth graphs are grams.



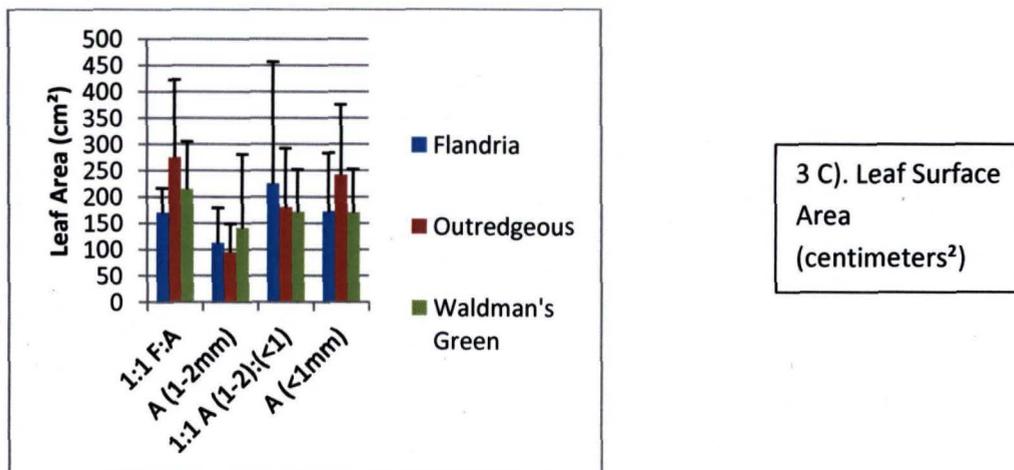
3 A). Chlorophyll Content (SPAD)

As previously stated, the SPAD is a measurement of the chlorophyll within the leaves of a plant. This is an important measurement because it gives an idea of how much chlorophyll the plant is making. 'Flandria' had the highest SPAD in all four types of media with 'Outredgeous' having the second highest and 'Waldman's Green' lowest. The amount of chlorophyll in a plant has little value by itself but in correlation with fresh weight it can tell you valuable information.



3 B). Fresh Weight (Grams)

The fresh weight is the mass, in grams, of the plant directly after it is detached from the pillow. Fresh weight would be the premier way of gathering data on how much growth a plant has had. The weight is related to the area of the leaves and the chlorophyll content. 'Waldman's green' had the highest fresh weight on average, based on its SPAD count this information tells you that it doesn't need as much chlorophyll to grow bigger than the other cultivars.



The leaf area is a representation of the surface area of each plant. These numbers were gathered and averaged to get a scope on how large each plant physically grows. 'Outredgeous' had the largest leaf area and 'Waldman's Green' had the least. Noticeably, all three plants were the greatest in different media.

The result of this test indicated useful characteristics of all three cultivars in general. As previously stated, 'Waldman's Green' had a high fresh weight and low chlorophyll count meaning it doesn't need much to chlorophyll to grow. 'Flandria' had a high chlorophyll count and both slightly lower leaf area and mass, which indicates that it had dark, dense, and thinner leaves than 'Waldman's Green'. 'Outredgeous' had a high leaf area and low fresh weight, which means it had dark and very thin leaves.



Figure 4. The three cultivars. A) Outredgeous. B) Flandria. C) Waldman's Green

The media effect on the plants is the most significant in the experiment. Based on growth alone the 1:1 Fafard and Arcilite mix and the < than 1 mm Arcilite were the best. The media that grew plants with the highest SPAD was A (<1mm), but it was followed very closely by 1:1 F: A. The same is the case between the two for the fresh weight. The difference was in leaf area. The leaf area of 1: 1 F: A was significantly greater than any other media and A (<1mm) was close to third behind the <1mm & 1-2 mm mixture. A deciding factor between the mixtures was the microbe activity.

Microbial Activity

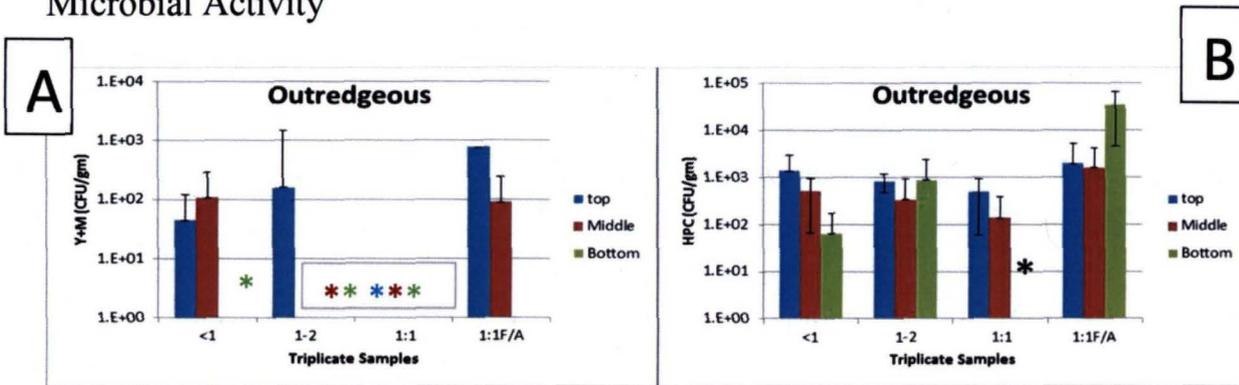
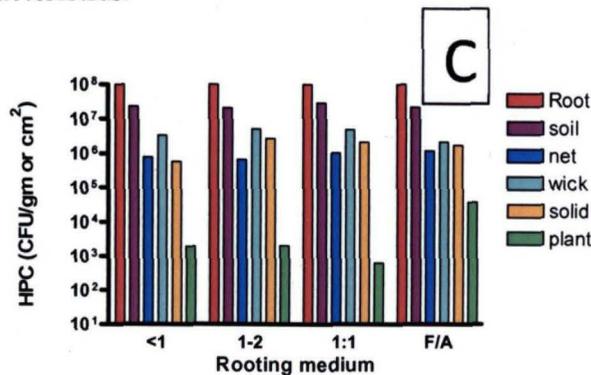


Figure 4. Results of microbial test that display the relation between media and cultivar
 A) Yeast and Mold count. B) Heterotrophic Plate counts of Outredgeous lettuce. C)
 Heterotrophic Plate counts for post-harvest pillow content.

There were very noteworthy differences in the results of the microbial test. Y+M indicate the yeast and mold count and HPC are heterotrophic plate counts. The asterisks indicate counts that were below the detection limit. The extensions indicate the standard deviations. The units are log based 10 colony forming units (CFU) per gram of tissue.

The “Triplicate Sample” graphs (Fig. 5 A and B) indicate the counts at the bottom, middle and top portions of the plants. The Y+M graph (Fig 5 A) indicates that the top and middle portions of the plants had the highest counts of activity, but this could have been influenced by human interaction. The bottom portion of the plants had relatively little amounts of activity, which is not as hypothesized because it is the portion closest to the growing media. The F: A mixture had more activity than the other media while the 1:1 Arcillite had very little.

HPC designates the count of distinguishable microbial colonies within the R2A plates from the plant samples (Fig. 4 B). Similarly to the Y+M, the HPC of plants grown in 1:1 Arcillite was low and the same correlation is evident with all other samples. As expected, the mixture with Fafard had the highest count by far due to the organic material in the media. The <math><1</math>mm and 1-2 mm Arcillite media were relatively close with <math><1</math> having the least amount of activity. Speculation can be made that the 1:1 Arcillite has low counts due to the fact that it is a mixture of <math><1</math> and 1-2 , both have low counts in different areas and could cause the mixture of both to have shared characteristics.



After all the plant section microbe test and growth test were concluded, a microbial test was conducted on the contents of the pillow for each media type. After the data were collected this graph (FIG 4 C) was constructed to show the bacterial activity count in each tested part of the pillow (root, soil, net, wick, solid and plant). The level of HPC did not substantially vary from media to media, with the only significant variation being the count for F: A plant.

The microbial test showed that the 1:1 Arcillite mix had the least amount of activity overall while the Fafard had the most. <1 Arcilite had the least amount of activity in the test on the contents of the pillow. This information justifies the hypothesis that the organic components of Fafard would make it more susceptible to high numbers of microbes.

Conclusion

Much of the media in the experiments had similar results in the test. F: A had the best results when it came to overall growth of the plants and 1:1 had the least amount of microbial activity, but neither would be the media that had the most viable conditions growing plants in space. In conclusion, <1 mm Arcillite would be the best media type to use for the VEGGIE system because It has the best ratio between growth and having the least amount of microbial activity, producing plants that displayed great growth and also having an acceptable level or microbial activity to correlate

Even though it did not have the best growth or least microbes it had consistently positive results throughout the course of the project. Being inorganic makes it less susceptible to microbial inhabitation and it also is easily remoistened after being dried, which is convenient in space.

References

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