Preliminary Species and Media Selection for the Veggie Space Hardware

Gioia Massa[1], Gerard Newsham[2], Mary E. Hummerick[2], Janicce L. Caro[2], Gary W. Stutte[2], Robert C. Morrow[3], and Raymond M. Wheeler[4]

[1]ISS Ground Processing and Research, Mail Code UB-A-00, Kennedy Space Center, FL 32899; [2]ESC Team QNA, Mail Code ESC-54, Kennedy Space Center, FL 32899; [3]Orbital Technologies Corporation, 1212 Fourier Drive, Madison, WI 53717; [4]Surface Systems Division, Mail Code NE-S-1, Kennedy Space Center, FL 32899

ABSTRACT

Plants will be an important component of off-Earth life support systems for food production and atmosphere recycling. "Veggie" is a small vegetable production unit designed for space flight, with a passive water delivery system. Plants can be grown in Veggie using small bags with a wicking surface containing media and fertilizer, i.e., pillows. Pillows planted with seeds can be placed on the wicking surface of the Veggie reservoir and water will wick throughout the media. Multiple small salad and herb species were grown in Veggie analog conditions using both commercial peat-based media and arcillite. Biometric measurements and microbial loads were assessed. Some species grew better in a particular media, but no general trends were apparent. Lettuce plants grew best in the blends of the peat-based and arcillite media. Microbial counts were lower on plants grown in arcillite. Four media types (peat-based mix, arcillite, and blends of the two) were tested in the rooting pillows; tests included Chinese cabbage, Swiss chard, lettuce, snow pea, and radish. Most species grew best in blends of the commercial mix and arcillite. Edible biomass production varied from 3.5-8 grams dry mass/m^2/day with lettuce having the lowest biomass and Chinese cabbage highest. Radish plants showed an increasing percentage of partitioning to edible roots with increasing arcillite in the media. Pillows appear to offer a simple, effective strategy for containing rooting media and avoiding free water while growing plants in the Veggie hardware.

Key words: Crop Production; Food Production; Habitation; Herb; International Space Station; Life Support; Payload; Salad Machine; Spaceflight; Space Life Sciences; Vegetable

Correspondence to: Gioia D. Massa
NASA
ISS Ground Processing and Research
Mail Code UB-A-00
Kennedy Space Center, FL 32899
Phone: 321-861-2938
E-Mail: gioia.massa@nasa.gov

INTRODUCTION

As we approach long-term, off-Earth human exploration, mass and volume limitations will drive the need for sustainable closed-loop life-support systems. Plants will play a critical role in these systems, both for food production and atmosphere regeneration (Wheeler *et al.*, 2001). As a first step, a "salad machine" or vegetable production unit will serve as a technology demonstration with the potential to produce fresh food to augment a packaged diet, remove CO_2, and generate O_2 to supplement cabin air, as well as provide psychological benefits for the crew (Kliss and MacElroy, 1990).

The "Veggie" deployable salad-crop-production system, developed by the Orbital Technologies Corporation (ORBITEC, Madison, WI), is a modular, low mass, low energy unit with the versatility to grow a variety of crops in transit vehicles, space stations, or planetary habitat environments (Morrow *et al*., 2005; Morrow and Remiker, 2009; Stutte *et al*., 2011b). Salad crops recommended for space-life support scenarios that might be grown in a vegetable production unit such as Veggie include greens such as lettuce, spinach, chard, and mizuna, tomato, pepper, green onion, radish, herbs and strawberry (Wheeler, 2009). A Veggie unit consists of three main subsystems: an LED light cap, a bellows enclosure for the shoot environment, and a 0.14 m^2 root mat fed by a reservoir (Morrow *et al*., 2005 and design revisions since). Veggie is currently considered one of the most advanced salad-machine precursors available (Stutte *et al*., 2009) and has passed a NASA safety review for a planned delivery and test on the International Space Station (ISS) in early 2014.

For several years, researchers at ORBITEC (Morrow and Remiker, 2009) and Kennedy Space Center (Stutte *et al*., 2009; Stutte *et al*., 2011a, 2011b) have assessed the Veggie unit for baseline salad performance under 1 *g*. But a microgravity environment poses additional challenges for crop production, including maintenance of adequate nutrient and water delivery to the roots, without anoxia or salt-stress (Porterfield, 2002). A number of approaches for water/nutrient delivery have been tested for space, including porous membranes, either using constant pressure or ebb/flow delivery (Wright *et al*., 1988; Koontz *et al*., 1990; Berkovich *et al*., 2002), porous tubes (Morrow *et al*., 1992; Dreschel *et al*., 1994), contained, vacuum operated systems (Brown *et al*., 1992), natural and manufactured solid substrates with sub-irrigation (Bingham *et al*., 1996; Goins *et al*., 1997), and capillary wicking from a reservoir (Kliss *et al*., 2000; Morrow *et al*., 2005; Morrow and Remiker, 2009). Active nutrient delivery provides the capability for extended cultivation scenarios by continual replenishment of water and nutrients (Goins *et al*., 1997), but this increased control comes with increased energy and infrastructure requirements. Passive systems, like capillary wicking, have low energy requirements, but less control of delivery under variable environmental conditions, and can only function for limited durations until nutrients are exhausted. For salad crops growing for relatively short durations, however, passive nutrient delivery can be sufficient.

Our studies focused on testing of a passive nutrient and water delivery system using rooting pillows designed for direct contact with the Veggie root mat reservoir (Stutte *et al*., 2011a). Pillows are small packages of growth media mixed with time release fertilizer that have a wicking surface for passive water delivery from a water conducting surface. Solid media inside pillows provides structural support for plant roots and passively distributes water and oxygen in the root zone. Seeds can be planted directly in pillows when dry, and germination can be initiated by the addition of water on orbit. Pillows are initially designed to be single use, but might be capable of growing additional crops in future scenarios. Following use, the spent pillows, which are fabricated from plastics and wicking fibers, could be compacted using devices such as a plastic melt waste compactor (Johnson *et al*., 2012) and used as components of building materials or to generate radiation shield panels (Wilson *et. al*., 1997). Pillows and other disposable components can be made from polyethylene or similar materials, which are effective shields for hazardous galactic cosmic radiation (Guetersloh *et al*., 2006).

Our goals were to compare the growth and performance of several species and media types in rooting pillows designed for use in the Veggie unit. Species and media trials were conducted in Veggie analog conditions that provided an environment (light level, temperature, relative humidity, and water delivery) similar to what would be expected in space. Plants included small species that could be grown rapidly within the constraints of a space habitat, and consumed fresh for salad or added to packaged diets to improve flavor. Plant growth conditions, nutrition and plant species can also influence the microbial population inhabiting plant surfaces (Lindow and Brandl, 2003). The microbial load on crops intended for consumption by astronauts must fall within an acceptable range according to NASA microbiological standards set for food. Thus, an additional aspect of these studies was to assess the microbial populations found on the plants grown using the pillow rooting system and capillary wicking.

MATERIALS AND METHODS

Pillow Construction

Pillows were constructed from 7.6 cm x 12.7 cm bags (3" x 5" re-closable static shielding bags, Uline, Pleasant Prairie, WI, external dimensions of 10 cm x 15 cm). On one side, a 5.3 cm x 10.5 cm window was cut, and a 7.5 cm x 11.5 cm piece of Nitex nylon mesh (Sefar Nytal PA-25-63, Sefar, Heiden, Switzerland) was heat welded to this surface to allow capillary wicking. Each pillow was filled with 100 mL of media and fertilizer (discussed below) and sealed using the re-closable zipper. In the non-wicking surface of the pillow, 1.5 cm slits were placed, and two 1.5 cm x 5.5 cm Nitex wicks were passed into each slit so that half of each wick protruded and the other halves were spread along the surface of the media in the pillow. Wicks were used to enclose seeds and promote early germination events by maintaining moisture around the seed.

Trial 1: Thirteen Cultivars and Five Types of Media

The first cultivar and media test examined 13 cultivars in eight species: lettuce (*Lactuca sativa* L.) cultivars 'Outredgeous' (Johnny's Select Seed, Winslow, ME), 'Flandria' (Rijk Zwaan USA, Salinas, CA), 'Sierra' (Veseys, York, PEI, Canada), and 'Oak leaf' (Terroir Seeds, Chino Valley, AZ), mizuna, (*Brassica rapa* cv. Nipposinica) (Seeds of Change, Santa Fe, NM), 'Tender leaf' vegetable amaranth (*Amaranthus viridis* cv. Tender leaf) (Evergreen YH Enterprises, Anaheim, CA), 'Bright lights' Swiss chard (*Beta vulgaris* var. cicla) (Terroir Seeds, Chino Valley, AZ), 'Tokyo Bekana' Chinese cabbage (*Brassica rapa*, Chinensis group) (Evergreen YH Enterprises, Anaheim, CA), 'Sugar Pod II ' snow pea (*Pisum sativum* cv. Sugar Pod II) (Evergreen YH Enterprises, Anaheim, CA), 'Spicy Globe' basil (*Ocimum basilicum* minimum 'Spicy Globe') (Ferry-Morse Seed Co., Fulton, KY), 'Genovese' basil (*Ocimum basilicum* 'Genovese') (Terroir Seeds, Chino Valley, AZ), common chives (*Allium schoenoprasum*) (Veseys, York, PEI, Canada), and Greek oregano (*Origanum heracleoticum*) (Terroir Seeds, Chino Valley, AZ).

All of the leafy greens selected can be grown using the cut-and-come-again strategy, essentially repeated harvests from the same plant, a harvest scenario that will potentially be employed in Veggie. Lettuce cultivars 'Flandria' and 'Outredgeous' have been previously studied for growth in off-Earth scenarios (Richards et al., 2006; Stutte et al., 2009; Stutte et al., 2011a, b). 'Sugar Pod II' snow pea was selected to test a small reproductive species in the pillows. The herbs were chosen to provide different growth habits and with the intent that they could easily be used to add interest and variety to a diet based on pre-packaged thermally stabilized foods.

These 13 cultivars were tested in both Fafard #2, a peat-based commercial potting mix also containing perlite and vermiculite, (Conrad Fafard Inc., Agawam, MA) and in arcillite (sifted 1-2mm, Turface Proleague, Profile LLC, Buffalo Grove IL) using the rooting pillows. The 1-2 mm arcillite medium has been well studied for use in microgravity (Jones et al., 2002; Norikane et al., 2005). Additionally, two cultivars, 'Outredgeous' and 'Sierra' lettuce were tested with five media types: Fafard # 2, arcillite (1-2mm), a 1:1 blend of the two media, a 7:3 blend (70% Fafard #2: 30% arcillite) and a 1:1 blend of coarse perlite and fine vermiculite (PVP Industries, North Bloomfield, OH). Media were autoclaved and mixed with Nutricote (18-6-8, type 180, Florikan, Sarasota, FL) slow release fertilizer at a rate of 7.5 g/L dry media. Pillows were weighed and then soaked in de-ionized (DI) H_2O for five minutes prior to planting.

Fresh media was soaked in DI H_2O for 70 hours to check pH levels. Starting pH of the media with fertilizer ranged from 5.76 for the Fafard #2 to 6.56 for the arcillite with the blends having intermediate values. The perlite: vermiculite blend was higher at 6.64. Two slit openings were planted in each pillow except for chives, where ten openings were planted per pillow. Two seeds were planted per opening and thinned to one plant per opening upon germination and emergence, except in the case of snow pea where one seed per opening was planted due to large seed sizes. Pillows were placed Nitex-side-down in Veggie reservoir analogs, which consisted of polypropylene sterilizing pans (14.2 L, Thermo Fisher Scientific, Rochester NY) with a sheet of soft foam (43.5 cm x 35.5 cm x 5 cm, Uline, Pleasant Prairie, WI), topped with a 420 mm x 340 mm x 2 mm sheet of PVC and wrapped with a 680 mm x 520 mm piece of Nomex wicking cloth (Aramid fabric, Insulsafe Textiles). PVC was placed over the foam to maintain uniform moisture and eliminate the impact of air bubbles under the cloth. Thirteen pillows were placed in each tub with cultivars randomized in three tubs (two with Fafard #2, one with arcillite). Thirteen pillows of 'Outredgeous' or 'Sierra' lettuce were placed in each of two other tubs with five media types randomized.

Initially, 2 L of DI H_2O were placed in each tub. Water use was monitored and the level was adjusted daily with DI H_2O to maintain adequate water in the tubs. Tubs were covered with plastic wrap to increase internal humidity until ¾ of the seeds had germinated. Plants were grown under 16-h photoperiods with initially 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ triphosphor fluorescent light (Sylvania FP541/841/HO). Light levels were increased to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 9 days after planting (DAP). Seeds that failed to germinate were replanted within 5 DAP. Plants were grown at 24°C for the first week and then the temperature was ramped to 28°C day / 24°C night by 9 DAP. Relative humidity was adjusted to 70% day / 75% night and CO_2 was set to 1200 $\mu mol\ mol^{-1}$ so that environmental conditions were relevant to those measured in the Veggie hardware with bellows closed and lights cycling in a high CO_2 environment like the ISS. All plants were harvested at 36 DAP and root-filled pillows were weighed and characterized at 37 DAP.

Upon harvest, roughly one third of the plants were reserved for microbial load analysis. Plants were photographed and biometric analysis included measurements of chlorophyll, leaf area, fresh and dry mass. Chlorophyll measurements were obtained by averaging the SPAD values of three non-senescent leaves per plant (SPAD-502, Konica Minolta, Osaka, Japan), and average values per pillow were calculated from the two plants. Leaf area was only measured on plants not used for microbial analysis. Shoot tissues were bagged and dried in an oven at 70°C for greater than 72 h. Oven dry tissue was allowed to equilibrate to room temperature and RH for an hour prior to weighing.

Qualitative measurements of root status were obtained by observing pillow fill with roots through the translucent Nitex surface using the following criteria: Pillows filled to excess with roots bulging and solid mats of roots were considered root bound. Pillows ¾ full to full but without bulging were considered full. Pillows ½ to ¾ full were considered half full. Pillows less than ½ full were considered low. Pillows were also weighed and dried, and dry mass was obtained, however pillow-to-pillow media variability and the relative mass of roots to media made it difficult to estimate root dry mass. Arcillite is significantly heavier than dried root tissue, while peat, perlite and vermiculite are similar in mass to dried root tissue.

Microbial Load Analysis

Plants used for microbial analysis were from one set of all cultivars grown in Fafard #2 and one set each of 'Outredgeous' and 'Sierra' lettuce grown in three types of rooting media: Fafard #2, 1:1 blend of Perlite and Vermiculite, and arcillite (1-2 mm), described above. The mixes of Fafard #2 and arcillite were not analyzed for microbial load. The two plants grown in one pillow were pooled samples. Shoot tissue was cut from the plants and weighed using aseptic technique and placed into sterile blender bags (BagLight, Interscience Labs, Weymouth, MA). Sterile DI H_2O was added to each sample in a 1:10 weight/volume ratio. Bags containing sample and diluent were placed in a bag mixer (Interscience Labs, Weymouth, MA) and blended for 2 minutes to remove microbes from plant surfaces. The sample extracts were serially diluted in sterile DI H_2O and plated in duplicate onto Difco R2A agar (BD, Franklin lakes, NJ). Plates were incubated at 28°C for 48 hours before enumeration of colonies to determine colony-forming units (CFU) per gram of tissue. Microbial sampling and analysis methods were modified from Pouch Downs and Ito (2001).

Trial 2: Five Cultivars in Four Types of Media

Five cultivars from four plant families were tested in the second trial. Cultivars were 'Cherry

Bomb II' radish (*Raphanus sativus* L. cv. Cherry Bomb II, family Brassicaceae) (W. Atlee Burpee & Co., Warminster, PA), 'Sugar Pod II' snow pea (*Pisum sativum* cv. Sugar Pod II, family Fabaceae) (Evergreen YH Enterprises, Anaheim, CA), 'Tokyo Bekana' Chinese cabbage (*Brassica rapa*, Chinensis group, family Brassicaceae) (Evergreen YH Enterprises, Anaheim, CA), 'Bright Lights' Swiss chard (*Beta vulgaris* var. cicla, family Chenopodiaceae) (Terroir Seeds, Chino Valley, AZ) and 'Outredgeous' lettuce (*Lactuca sativa* cv. Outredgeous, family Asteraceae) (Johnny's Select Seed, Winslow, ME).

Four previously used media were tested: Fafard #2, arcillite (1-2 mm), and 7:3 and 1:1 mixtures of the two of these. Pillows and planting were as described for Trial 1. Sixty pillows were grown in five tubs, therefore there were twelve pillows of each cultivar with three pillows of each media type per cultivar. The sixty pillows were completely randomized within the five tubs, giving twelve pillows per tub arranged in alternating orientation within the tubs to provide maximum spacing for each plant and reduce shading.

Planting and experimental design followed the procedures described above, except that temperature was ramped up to 28°C by 7 DAP and light was maintained at 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the duration of the experiment. Plants were harvested at 28 DAP and root-filled pillows were characterized and weighed at 29 DAP. Harvest measurements were similar to those described above except that microbial analyses were not performed. Also, plant height and qualitative status were noted but leaf area measurements were not performed. Photographs and measurements of chlorophyll, fresh mass, dry mass, pillow status, and pillow mass were all taken as previously described.

Statistical Analysis

Data are presented on a per pillow basis. Data were analyzed using GraphPad Prism® software with reference to Motulsky, 2003. Analysis of variance (ANOVA) was performed on data for chlorophyll, leaf area, height, fresh mass, dry mass, and biomass partitioning (radish). Data are presented as true means. Mean separation was performed by Bonferroni post-tests for two-way ANOVA.

RESULTS AND DISCUSSION

Two plants, (except for chives), were allowed to grow in each pillow as shown in Figure 1, however seeds did not always germinate simultaneously, and light interception often gave the earlier plant a competitive advantage. For this reason, data are discussed on a per-pillow basis rather than on a per-plant basis. Chlorophyll and height measurements are averages of both plants in a pillow, while leaf area, fresh mass, and dry mass are totaled per pillow.

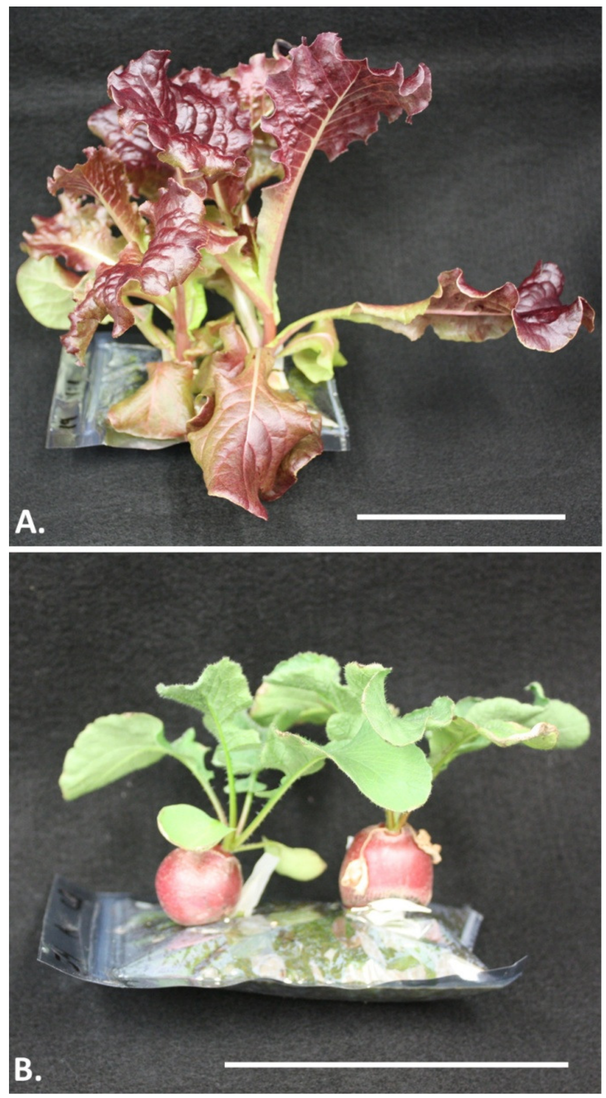

Figure 1. Examples of salad crops growing in pillows. A. 'Outredgeous' lettuce in 1:1 Fafard #2: arcillite and B. 'Cherry Bomb II' radish in arcillite at harvest of trial 2 (28 DAP). Scale bars are 10 cm.

Trial 1A: Thirteen Cultivars in Two Types of Media

For the broad survey of thirteen species in two types of media, media was not found to be a significant factor in any of the parameters measured across species. Across media, differences between species were found to account for large percentages of the variation in chlorophyll content (59%), leaf area (78%), fresh mass (48%) and dry mass (80%). Greek oregano seeds failed to germinate in pillows with either of the media used, so only twelve species were analyzed, and due to the diversity of chlorophyll levels, size, and growth rate of these species, no general response to media effects can be assessed. Media response was observed in some species, with, for example, delayed growth in a particular type of media, however small sample sizes limit conclusions. Figure 2 shows the dry mass per pillow of the twelve species tested in two media types.

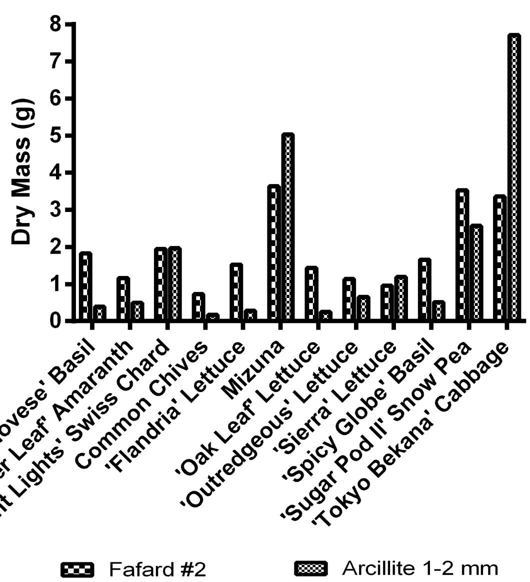

Figure 2. Shoot dry mass of twelve species grown in pillows in two different media. Data are per pillow and a pillow contains two plants (except chives which had 10 plants per pillow). Mass is the sum of the shoot dry mass of all plants in a pillow. Each bar represents one pillow.

Cultivars with favorable growth in pillows include 'Tokyo Bekana' Chinese cabbage, 'Sugar Pod II' snow pea, mizuna, 'Bright Lights' Swiss chard, 'Spicy Globe' basil and the lettuce varieties 'Outredgeous' and 'Sierra', based on biomass accumulation and observations of plant health.

Trial 1B: Two Species in Five Types of Media

Two cultivars of lettuce, 'Outredgeous' and 'Sierra', were tested for growth in five types of media. Both grew well in pillows and showed no significant variation in leaf area due to different media types. 'Sierra' lettuce showed a chlorophyll response to media, with plants growing in perlite: vermiculite having significantly higher SPAD readings than plants growing in arcillite ($P<0.05$), but no chlorophyll response was observed in 'Outredgeous'. Fresh and dry mass were significantly different depending on media type. Plants growing in mixtures of Fafard #2 and arcillite had greater mass than those grown in other media, as shown in Figure 3, and in both cultivars, plants grown in 7:3 and 1:1 mixtures of Fafard #2: arcillite showed significantly greater fresh (not shown) and dry mass accumulation than those grown in the 1:1 mixture of perlite: vermiculite. Trends for the observed percentage of root fill in the pillows mirrored those for dry mass (data not shown), with percentages varying from half to full in Fafard #2 and perlite: vermiculite, full in arcillite, and the Fafard: arcillite blends being mostly root-bound.

Trial 1: Microbial Load Analysis

Currently the limit for aerobic bacteria on a non-thermostabilized food item is $\leq 2 \times 10^4$ colony forming units (CFU) per gram (Perchonok and Douglas, 2012). Studies done to determine the microbial numbers in field and retail samples of a variety of leafy green produce items report total aerobic bacteria levels in the range of 10^4 to 10^7 CFU/gram (Johnston et al., 2005; Ruiz et al., 1987). Our analysis of twelve cultivars of leafy greens and herbs indicates microbial densities (2.2×10^3 to 1.8×10^6) consistent with these studies with the exception of basils, Chinese cabbage, and lettuces grown in arcillite, in which bacterial counts were less than 10^4/gram. Higher numbers of aerobic bacteria on produce are not necessarily correlated with decreased shelf life, quality or an indication of pathogen contamination (Johnston et al., 2005).

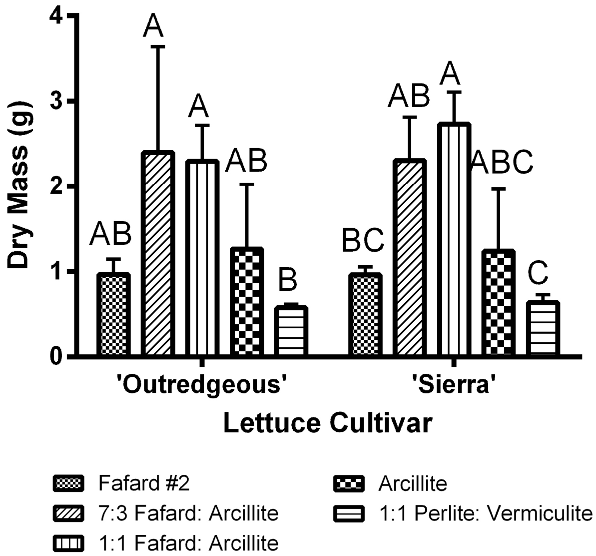

Figure 3. Shoot dry mass of lettuce grown in five different media types. Data are means of two pillows per media type for each cultivar, with each pillow containing two plants. Error bars indicate standard deviations. Means within a cultivar with different letters are significantly different at *P*<0.05.

For the cultivars grown in Fafard #2, counts ranged from 1.9 x 10^3 on snow pea to the highest bacterial count, 1.8 X 10^6, on mizuna. Microbial analysis was also performed on one pillow of each 'Sierra' and 'Outredgeous' lettuce grown on three media types: Fafard #2, arcillite, and 1:1 perlite: vermiculite. Lettuce plants grown in arcillite exhibited aerobic plate counts approximately 1-2 orders of magnitude lower than those grown in perlite: vermiculite and Fafard #2. This information is important in the selection of potential food crops and growth media when considering microbiological standards, however further analysis with greater numbers of replicates needs to be performed, and the more-productive media blends were not tested. Additionally, seeds were not sterilized prior to these tests, and sterilization might lead to reduced microbial levels on produce. Many of the microorganisms detected on the leaves are likely brought in through air flow and human tending, however, so produce sanitation using a sanitizing agent safe for spaceflight is one option to reduce microbial loads in crops and conditions where they exceed allowances.

Trial 2: Five Species in Four Types of Media

Several of the highly productive species observed in trial 1 were selected for further study in trial 2. Radish, a crop well-studied for use in space (Goins et al., 2003; Richards et al., 2006) was added for comparison. Due to the media response seen among lettuce cultivars in trial 1, the perlite: vermiculite blend was dropped from consideration and only four media types were examined: Fafard #2, arcillite (1-2 mm), and 7:3 and 1:1 mixtures of the two media. Of the parameters measured, average plant height showed no media dependency (data not shown). Plants grown in pillows reached the same average height at harvest regardless of growth media, indicating that height in these species may be predetermined and not responsive to root zone environments that affect other aspects of plant growth.

Interestingly, qualitative observations of root fill in the pillows also were species-dependent with only slight media differences (data not shown). Snow pea pillows were root bound in all media types, while radish pillows were less than half-full of fibrous roots in all media. Other species were intermediate, with Swiss chard pillows full to root bound, and Chinese cabbage and lettuce pillows half to full. These three species generally showed an increase in roots with increasing levels of arcillite (data not shown).

Chlorophyll content was affected by the type of species and to a lesser extent the type of media. Figure 4 shows that the SPAD readings of radish, snow pea, and chard were not different from one another and averaged in the 40-50 range. Chinese cabbage and lettuce both had lower chlorophyll contents (SPAD~15-30) and were not different from each other regardless of media (Fig. 4). Some media effects were observed, but generally chlorophyll contents (SPAD data) were much more species dependent than media dependent, with species accounting for 80% of the observed variation and media only accounting for 3%. This response is consistent with sufficient macro/micronutrients being available from the slow release fertilizer in the media.

In contrast to the slight impact of media type on chlorophyll levels, fresh mass was dependent on both media type and species, with species accounting for 33% of the observed variation and media accounting for 28%. Interaction between species and media was not significant. Radish had the greatest fresh mass of all species tested, as seen in Figure 5. For media, Fafard#2 consistently produced plants of all species with the lowest fresh mass, and the Fafard #2: arcillite mixtures tended to produce larger plants, likely related to the balance between moisture holding capacity and aeration of the media.

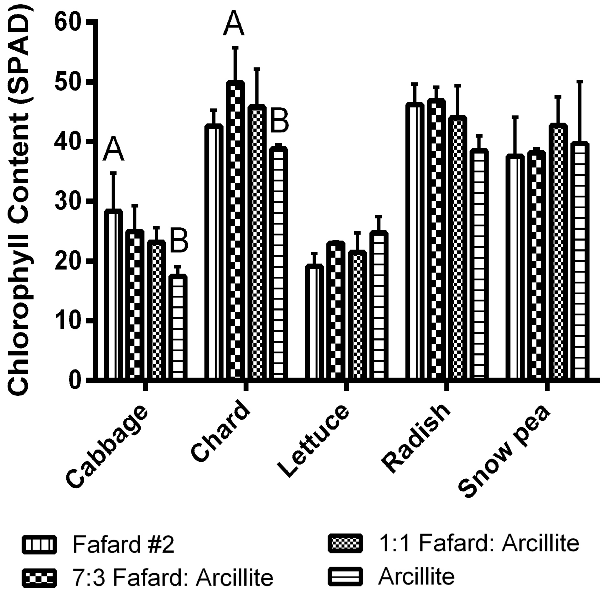

Figure 4. Average chlorophyll content for five species grown in four types of media. Data are averages for three pillows, with pillow values the average of two plants, and plant values averaging three leaves on a plant. Means within plant species between media types with different letters are significantly different at *P*<0.05. Where letters are absent there is no significant difference within a species. Error bars indicate standard deviations.

Dry mass data displayed an interaction between species and media that was not observed in fresh mass or other parameters, documented in Table 1. Swiss chard, lettuce and snow pea showed no significant response to media and produced the same dry mass as each other. Chinese cabbage plants however, produced a significantly greater dry mass in 1:1 Fafard #2: arcillite than in any of the media tested (P<0.05). The dry mass of Chinese cabbage produced in 1:1 Fafard #2: arcillite was significantly greater than any other species in the same media (P<0.01). Also, Chinese cabbage produced significantly more dry mass than lettuce in every media tested (P<0.05), however there were no differences between the two in fresh mass (see Fig. 5) suggesting a high quantity of water in lettuce compared to the cabbage. Radish plants produced significantly more dry mass in 7:3 Fafard #2: arcillite than in any of the other media types (P<0.05), and since radish showed excellent growth in the 7:3 mixture, a significantly greater dry mass was produced in this media by radish than by either Swiss chard, lettuce or snow pea (P<0.01), and a slightly greater dry mass than Chinese cabbage (Table 1).

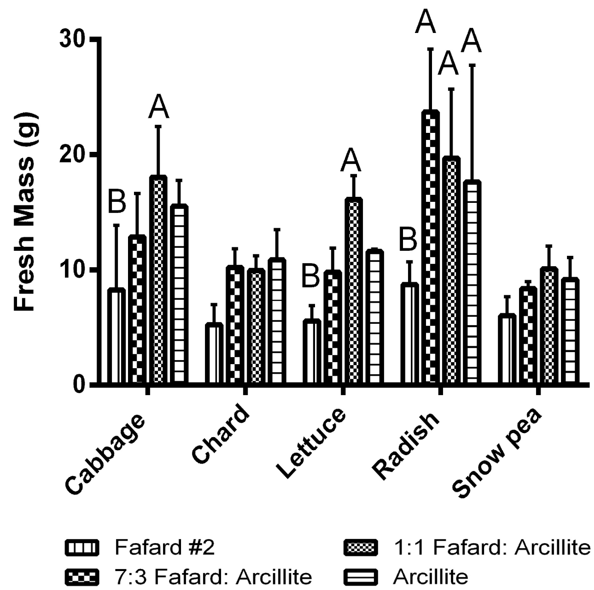

Figure 5. Shoot fresh mass of five species grown in four types of media. Data are averages for three pillows, with pillow values the sum of the shoot (shoot and tap root for radish) fresh mass of two plants. Means within plant species between media types with different letters are significantly different at *P*<0.05. Where letters are absent there is no significant difference within a species. Error bars indicate standard deviations.

General trends indicate that Chinese cabbage and radish dry mass accumulation responds to media more than other species. Although similar in fresh mass, Chinese cabbage generally produces the greatest dry mass, while lettuce the least of any of the crops tested. Radish produced significantly greater dry mass than most other species in the 7:3 mixture, however, these data are total biomass. When only the tap root is examined this value falls to 1.95 g dry mass on average. Table 2 provides an estimate of the maximum growth rate of each of these species. These edible growth rates are comparable to those for lettuce and soybean observed by Wheeler et al., 2003, though they are lower than for other staples such as potato.

Table 1. Average shoot dry mass (g) (shoot and tap root for radish) per pillow of five species grown in four media types. Data are averages for three pillows, with pillow values the sum of the dry mass of two plants. Means between plant species within a media type with different letters are significantly different at P<0.05. Asterisks indicate means within a species that are significantly different between media types (*P*<0.05)

Plant	MEDIA TYPE			
	Fafard #2	7:3 Fafard: Arcillite	1:1 Fafard: Arcillite	Arcillite
Cabbage	2.21 A	2.29 AB	3.36 A*	2.21 A
Chard	0.92 B	1.86 BC	1.78 B	1.55 AB
Lettuce	0.78 B	1.24 C	1.46 B	1.07 B
Radish	1.63 AB	3.10 A*	1.93 B	1.61 AB
Snow pea	1.25 B	1.53 BC	1.74 B	1.84 AB

Table 2. Calculated maximum edible growth rates of tested species in highest yielding media (highest yielding for dry mass). Calculations use a 28 day growth period and a pillow with external area of 0.015 m^2. Mass is an average of three pillows with two plants per pillow. Radish data are for tap root mass while other data are for shoot mass.

Plant	Edible Growth Rate		Media
	(g FM/m^2/day)	(g DM/m^2/day)	
Cabbage	42.9	8.0	1:1 Fafard#2: arcillite
Chard	24.3	4.4	7:3 Fafard#2: arcillite
Lettuce	38.3	3.5	1:1 Fafard#2: arcillite
Radish	44.5	4.6	7:3 Fafard#2: arcillite
Snow pea	21.8	4.4	arcillite

Interestingly, although total fresh mass of radish demonstrated a significant influence of media, individually, there was no significant difference between shoots grown in different media types or roots grown in different media types. Figure 6 shows that dry mass, however, was significantly different, with plants grown in 7:3 Fafard: arcillite having significantly greater shoot mass than arcillite- or 1:1-grown plants and significantly greater root mass than Fafard #2-grown plants.

When the percentage of biomass in the root and shoot are calculated we observe increasing partitioning into the edible root with increasing concentration of arcillite and decreasing percentage of Fafard #2 in the media, as shown in

Figure 7, and the interaction between media and the location of partitioning is highly significant (P<0.0001).

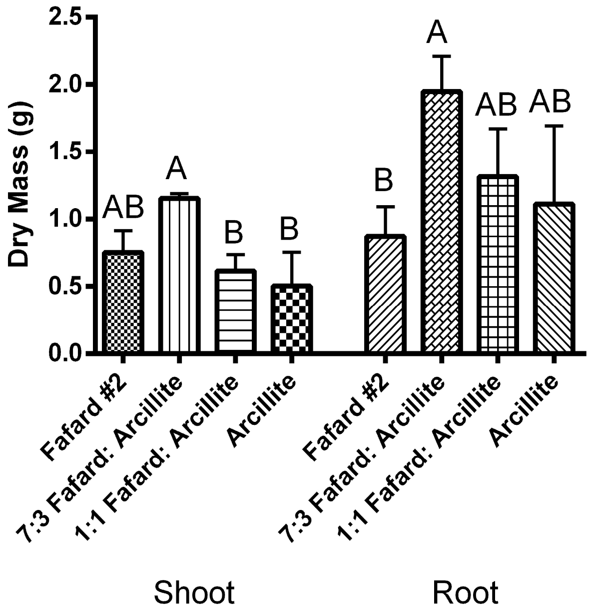

Figure 6. Dry mass of radish shoots and edible tap roots when grown in four types of media. Data are averages for three pillows, with pillow values the sum of two plants. Error bars indicate standard deviations. Means with different letters are significantly different at P<0.05.

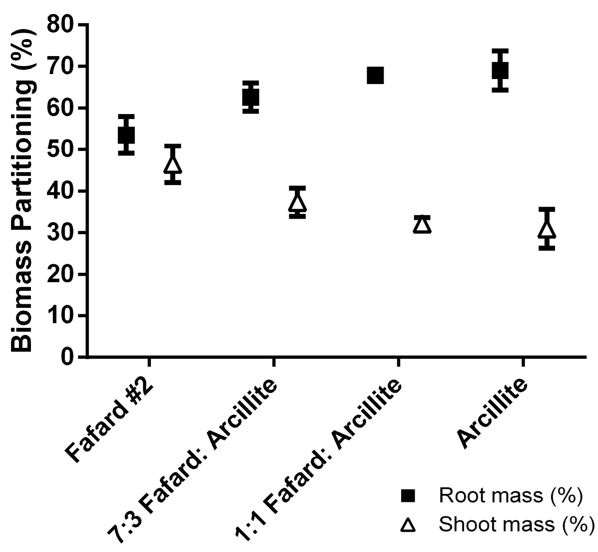

Figure 7. Biomass partitioning between the storage root and shoot calculated from dry mass. Data are means from three pillows (six plants) of each media types and error bars indicate standard deviations.

The observed differences in response to media may be due to a variety of media characteristics including moisture, aeration, and cation and anion exchange capacity, however since water is not leaching through the pillows it seems likely that water and aeration may be playing the largest role. The arcillite, a porous clay, has a lower water holding capacity (~50% by weight) than the peat-based Fafard #2 medium which expands when wet and can hold ~60% moisture by weight. The physical and chemical parameters of the media can lead to differences in root growth and nutrient availability, and several of these parameters are gravity-dependent. Future testing will further assess these challenges; however initial results using the pillow concept indicate that this technique may be a good approach to growth of salad and herb crops for the Veggie hardware.

CONCLUSIONS

The Veggie space hardware is designed to grow small salad crops using passive water distribution. Pillows, small packets of media and time-release fertilizer, were designed to interface with this hardware to supply water, nutrients, and structural support for crop growth. Species varied in their growth in pillows, however most salad greens and small herbs tested grew acceptably in these confined volumes. Some species had media-dependent growth, but certain parameters, such as plant height and chlorophyll level, did not vary much with media type. Shoot microbial loads were generally lower in plants grown in arcillite than in other types of media tested. A mixture of Fafard #2 and arcillite produced larger lettuce plants than these media separately, while lettuce did not grow well in a perlite: vermiculite combination. Chinese cabbage and radish had a greater dry mass response to media than other species tested, and both did best in blends of Fafard #2: arcillite though not at the same ratios. Radish plants showed increased carbon partitioning to the root zone and decreased partitioning to the shoot with increasing levels of arcillite in the media. Although a number of media characteristics may influence the growth of these species, it seems likely that water and aeration may be the most important parameters for these small pillows, and the impact of microgravity on these characteristics will need to be determined. Small, single-use pillows appear to provide acceptable growth of a variety of salad and herb species for the Veggie spaceflight hardware.

REFERENCES

Berkovich, Y.A., Tynes, G.K., Norikane, J.H., and Levine, H.G. 2002. Evaluation of an ebb and flow nutrient delivery technique applicable to growing plants in microgravity. *Society for Automotive Engineers (SAE) Technical Paper* 2002-1-2383.

Bingham, G.E., Jones, S.B., Pololsky, I.G., and Yendler, B.S. 1996. Porous substrate water relations observed during the Greenhouse-2 flight experiment. *Society for Automotive Engineers (SAE) Technical Paper* 961547.

Brown, C.S., Cox, W.M., Dreschel, T.D., and Chetirkin, P.V. 1992. The vacuum-operated nutrient-delivery system: Hydroponics for microgravity. *HortScience.* 27: 1183-1185.

Dreschel, T.W., Brown, C.S., Piastuch, W.C., Hinkle, C.R., and Knott, W.M. 1994. Porous tube plant nutrient delivery system development: a device for nutrient delivery in microgravity. *Advances in Space Research.* 14: 47-51.

Goins, G.D., Carr, J.D., Levine, H.G., Wheeler, R.M., Mackowiak, C.L., Ming, D.W. 1997. Comparison studies of candidate nutrient delivery systems for plant cultivation in space. *Society for Automotive Engineers (SAE) Technical Paper* 972304.

Goins, G.D., Yorio, N.C., Stutte, G.W., Wheeler, R.M., and Sager, J.C. 2003. Baseline environmental testing of candidate salad crops with horticultural approaches and constraints typical of spaceflight. *Society for Automotive Engineers (SAE) Technical Paper* 2003-01-2481.

Guetersloh, S., Zeitlin, C,; Heilbronn, L., Miller, J., Komiyama, T., Fukumura, A., Iwata, Y., Murakami, T., and Bhattacharya, M. 2006. Polyethylene as a radiation shielding standard in simulated cosmic-ray environments. *Nuclear Instruments and Methods in Physics Research Section B*: *Beam Interactions with Materials and Atoms*. 252: 319-332.

Johnson, J., Marten, A., and Tellez, G. 2012. Design of a High Efficiency, High Output Plastic Melt Waste Compactor. *AIAA Technical Paper No.* 2012-3544.

Johnston, L.M., Jaykus, L., Moll, D., Martinez, M.C., Anciso, J., Mora, B., and Moe, C.L. 2005. A field study of the microbiological quality of fresh produce. *Journal of Food Protection.* 68: 1840-1847.

Jones, S.B., Or, D., Bingham, G.E., and Morrow, R.C. 2002. ORZS: optimization of root zone substrates for microgravity. *Society for Automotive Engineers (SAE) Technical Paper* 2002-01-2380.

Kliss, M., Heyenga, A.G., Hoehn, A., and Stodieck, L.S. 2000. Recent advances in technologies required for a "Salad Machine". *Advances in Space Research.* 26(2): 263-269.

Kliss, M. and MacElroy, R.D. 1990. Salad machine: A vegetable production unit for long duration space missions. *Society for Automotive Engineers (SAE) Technical Paper* 901280.

Koontz, H.V., Prince, R.P., and Berry, W.L. 1990. A porous stainless steel membrane system for extraterrestrial crop production. *HortScience.* 25: 707.

Lindow, S.E. and Brandl, M.T. 2003. Microbiology of the phyllosphere. *Applied and Environmental Microbiology*. 69: 1875-1883.

Morrow, R.C., Bula, R.J., Tibbitts, T.W., and Dinauer, W.R. 1992. A matrix-based porous tube water and nutrient delivery system. *Society for Automotive Engineers (SAE) Technical Paper* 921390.

Morrow, R.C. and Remiker, R.W. 2009. A deployable salad crop production system for lunar habitats. *Society for Automotive Engineers (SAE) Technical Paper* 2009-01-2382.

Morrow, R.C., Remiker, R.W., Mischnick, M.J., Tuominen, L.K., Lee, M.C., and Crabb, T.M. 2005. A low equivalent system mass plant growth unit for space exploration. *Society for*

Automotive Engineers (SAE) Technical Paper 2005-01-2843.

Motulsky, H.J. 2003. *Prism®4 Statistics Guide-Statistical analyses for laboratory and clinical researchers*. GraphPad Software Inc., San Diego CA.

Norikane, J.H., Jones, S.B., Steinberg, S.L., Levine, H.G., and Or, D. 2005. Porous media matric potential and water content measurements during parabolic flight. *Habitation.* 10(2): 117-126.

Perchonok M. and Douglas, G. 2012. Risk factor of inadequate food system. Human health and performance risks of space exploration missions. http://humanresearchroadmap.nasa.gov/evidence/reports/food.pdf , accessed 05/08/2013.

Porterfield, D.M. 2002. The biophysical limitation in physiological transport and exchange in plants grown in microgravity. *Journal of Plant Growth Regulation*. 21: 177-190.

Pouch Downs, F. and Ito, K. 2001. *Compendium of methods for the microbiological examination of foods*, 4th ed. Washington D.C. : American Public health Association.

Richards, J.T., Edney, S.L., Yorio, N.C., Stutte, G.W., and Wheeler, R.M. 2006. Yields of salad crops grown under potential lunar or Mars habitat environments: effect of temperature and lighting intensities. *Society for Automotive Engineers (SAE) Technical Paper* 2006-01-2029.

Ruiz, B.G., Vargas, R.G., and Garcia-Villanova, R. 1987. Contamination on fresh vegetables during cultivation and marketing. *International Journal of Food Microbiology*. 4: 285-291.

Stutte, G.W., Monje, O., Yorio, N.C., Edney, S.L., Newsham, G., Connole, L., and Wheeler, R.M. 2009. Sustained salad crop production requirements for lunar surface. *Society for Automotive Engineers (SAE) Technical Paper* 2009-01-2381.

Stutte, G.W., Newsham, G., Morrow, R.C., and Wheeler, R.M. 2011a. Concept for sustained plant production on ISS using VEGGIE capillary mat rooting system. *AIAA Technical Paper No.* 2011-5263.

Stutte, G.W., Newsham, G., Morrow, R.C., and Wheeler, R.M. 2011b. Operational evaluation of VEGGIE food production system in the habitat demonstration unit. *AIAA Technical Paper No.* 2011-5262.

Wheeler, R.M. 2009. *Roadmaps and strategies for crop research for bioregenerative life support systems: a compilation of findings from NASA's advanced life support meetings*. NASA Technical Memorandum 2009-214768.

Wheeler, R.M., Mackowiak, C.L., Stutte, G.S., Yorio, N.C., Ruffe, L.M., Sager, J.C., Prince, R.P., Peterson, B.V., Goins, G.D., Berry, W.L., Hinkle, C.R., and Knott, W.M. 2003. *Crop production for advanced life support systems. Observations from the Kennedy Space Center Breadboard Project.* NASA Technical Memorandum 2003-211184.

Wheeler, R.M., Stutte, G.W., Subbarao, G.V., and Yorio, N.C. 2001. Plant growth and human life support for space travel. *In*: M. Pessarakli (ed.), *2nd Edition. Handbook of Plant and Crop Physiology*. New York : Marcel Dekker Inc., pp. 925-941.

Wilson, J.W., Miller, J., Konradi, A., and Cucinotta, F.A. (eds). 1997. *Shielding Strategies for Human Space Exploration.* NASA Conference Publication 3360.

Wright, B.D., Bausch, W.C., and Knott, W.M. 1988. A hydroponic system for microgravity plant experiments. *Transactions of the ASAE* (American Society of Agricultural Engineers). 31: 440-446.