1 Introduction to Veggie

The Veggie vegetable production system is a small plant growth chamber designed and built by ORBITEC (Madison, WI) to grow vegetable crops in space (Morrow et al., 2005; Morrow and Remiker, 2009). Veggie was designed to be a low mass, low power, and low crew time-requiring simple, expandable, food crop production system. After several iterations of design, development, and testing, Veggie was launched to the International Space Station (ISS) in April, 2014 in the SpaceX Dragon capsule as part of the SpaceX CRS-3 mission. Veggie was installed in the ISS Columbus module in May of 2014 into an Expedite-the-Processing-of-Experiments-to-Space-Station (ExPRESS) rack providing approximately 70 W of power to the hardware lighting and fans as well as cooling air to the lighting array. The LED lighting can be run in manual or automatic modes and is discussed in a recent publication by Massa et al., (2016). The body of the hardware is a flexible, extensible, transparent bellows which attaches via magnets to the lighting array. The baseplate of the bellows also attaches to the lighting array via flexible support arms, and the bellows and arms allow the baseplate height below the lighting array to be fully adjustable. This enables height modification of the hardware for differing crops or for different phases of growth. The Veggie baseplate is 29.2 cm wide by 36.8 cm deep with a maximum available shoot height in an empty chamber of 47 cm. Figure 1 shows the Veggie hardware during ground testing.

The baseline Veggie watering system is a passive wicking design consisting of a reservoir that wicks to substrate filled pouches or plant pillows. The reservoir is designed to hold up to 2 L of water. This reservoir interfaces with Veggie plant pillows secured to the wicking surface with elastic bands. Plant pillows are sewn Teflon™ coated Kevlar® and Nomex® pouches that are prepared for flight
by 1) cutting and inserting polypropylene germination wicks (cut from KIMTECH PURE* W4 Dry Wipers, Kimberly-Clark Professional, Roswell, GA) 2) filling with substrate and fertilizer mix, and 3) sewing the open end closed. The pouches have an external quick disconnect connected to a water tube passing into the pouch and terminating in an internal irrigation ring. Pillows contain solid porous ceramic arcillite substrate (sieved, washed Turface Proleague, Profile Products, LLC) and controlled release polymer fertilizer (Nutricote 18-6-8, type 180, Florikan, Sarasota, FL), and they were designed from data collected by Stutte et al., (2011). For VEG-01, half the pillows in each set were filled with substrate sieved to 600 µm-1 mm and the other half were filled with a 50:50 blend of 600 µm-1 mm and 1-2 mm. Figure 2 shows a map of the pillow location inside Veggie indicating the position of the different substrates. Procedures for substrate preparation and pillow filling are thoroughly outlined in Massa et al., (2017). Two surface sanitized seeds are glued with guar in each plant pillow in between paired germination wicks. Planted pillows are sealed in gas impermeable bags,
packaged in foam, and launched to the ISS or used in ground controls.

At the initiation of an experiment, the light intensity, duration, and fan speed are programmed. Plant pillows are removed from their packages and attached to the reservoir via elastic silicone bands. Pillows are hydrated with ISS potable water via a quick disconnect attachment leading to a drip ring inside each pillow. The Veggie bellows are closed and the reservoir is filled with water via an external port. Plant care operations include separating the germination wicks at three days after initiation to allow seedlings to emerge, thinning seedlings to one plant per pillow at seven days after initiation, and periodic watering and photographing. Plants or plant material is harvested in a crop-specific manner. Ground control experiments are conducted at Kennedy Space Center in Veggie hardware located in ISS environmental simulator (ISSES) chambers controlling temperature, relative humidity, and CO₂ levels based on data downlinked from the ISS.

As a method to assess Veggie hardware functionality and operations, three sets of plant pillows were sent with the first Veggie unit to ISS. These experiments were classified as the VEG-01 series. VEG-01A and B both grew for 33 days, from May 8–June 10, 2014 and July 8–Aug. 10, 2015, respectively. VEG-01C grew 90 days from Nov. 16, 2015-Feb. 14, 2016.

2 Overview of VEG-01 validation tests

2.1 VEG-01A – ‘Outredgeous’ red romaine lettuce

The first ISS test of Veggie utilized red romaine lettuce selected through analog ground testing (Massa et al., 2013). The goals of this initial test were to assess Veggie hardware functionality and crew procedures, and to collect data necessary to understand the food safety of lettuce grown in Veggie. Seeds of red romaine lettuce (Lactuca sativa cv. ‘Outredgeous’) were initiated by Astronaut Steve Swanson following the installation of the Veggie hardware, with Steve adding between 100 and 150 mL of water to each plant pillow and 1020 mL of water to the reservoir. 10 mL were withdrawn and frozen for later comparison with a post-growth water sample. LED lights were set to red and blue on high and green on, with the bellows baseplate positioned 30.5-31.75 cm below the light array to provide approximately 200 μmol·m⁻²·s⁻¹ of photosynthetically active radiation at the surface of the pillows in the center of the Veggie unit at a ratio of 12 red (630 nm): 3 Blue (455 nm): 1 green (530 nm). A photoperiod was set to 16 h light / 8 h dark. A temperature and relative humidity data logger programmed to collect data every 15 min (HOBO® data logger U12, Onset, Bourne, MA) was inserted into the bellows behind the plant pillows at the back left corner of the hardware. The VEG-01A ground control pillows were initiated with a 26 h delay from flight pillows. Table 1 shows the ISS environmental data during this experiment.

At initiation, one of the flight pillows, pillow A, could not be hydrated. This was thought to be the result of a blocked or damaged pillow quick disconnect or internal tube. This pillow was swapped with one of the other set of red romaine lettuce plant pillows. Following initiation, germination was noted in all pillows except pillow B. Plants were thinned so that each pillow (except pillow B) had one seedling per pillow on day 7. Additional water (420 mL) was added to the reservoir. The HOBO data logger was also moved to the back right side of the hardware to assess any thermal gradients (Table 2). Photos were taken weekly, and photos at 14 days after initiation showed that three of the five plants were stunted and did not appear to be growing. The Veggie team suspected that the reservoir was not providing sufficient water to the plant pillows, and the astronauts were sent directions to manually add water to the plant pillows to save the plants. One of the three recovered from the low water event, but two plants died. The three remaining plants were originally intended to grow for 28 days, but due to the growth limiting water conditions, plants were allowed to grow for 33 days. After 33 days the experiment was completed (Fig. 3), and plants, pillows, and veggie bellows were swabbed packaged in foam, and launched to the ISS or used in ground controls.

Table 1. Durations and ISS environmental data for VEG-01A, B, and C. Data points are averages ± standard deviations for all days of experimentation from initiation until harvest. VEG-01A data were downlinked from sensors in the US Laboratory Module, while for VEG-01B and C, data were downlinked from Columbus module sensors. VEG-0-1A and C data are averages of data collected every minute, while VEG-01B data points are averages of data at 5 minute intervals.

<table>
<thead>
<tr>
<th></th>
<th>Duration (days)</th>
<th>Temperature (°C)</th>
<th>Relative Humidity (%)</th>
<th>CO₂ Level (μmol mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEG-01A</td>
<td>33</td>
<td>22.2 ± 0.2</td>
<td>43.9 ± 3.7</td>
<td>2798.9 ± 677.7</td>
</tr>
<tr>
<td>VEG-01B</td>
<td>33</td>
<td>23.3 ± 0.4</td>
<td>38.3 ± 4.5</td>
<td>2559.5 ± 660.3</td>
</tr>
<tr>
<td>VEG-01C</td>
<td>90</td>
<td>23.1 ± 0.3</td>
<td>40.3 ± 2.1</td>
<td>3156.2 ± 451.2</td>
</tr>
</tbody>
</table>
Table 2. Environmental data collected from within the Veggie hardware during VEG-01A. Data points are averages ± standard deviations for days of experimentation from initiation until harvest. Data were collected every 15 minute using a HOBO data logger. Day-to-night transitions were instantaneous, but for calculation simplicity to avoid the photoperiod transition period, night data are averages of the hours between midnight and 05:45 AM and day data are averaged between 10:00 AM and 15:45 PM to give 24 data points for each period. On day 13 following initiation the data logger was shifted from the back left corner to the back right corner of the hardware. Average data from each side are displayed with the day of shifting omitted from calculations.

<table>
<thead>
<tr>
<th>Sensor Position</th>
<th>Duration (days)</th>
<th>Temperature (°C)</th>
<th>Relative Humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data Logger-Day</td>
<td>Both</td>
<td>33</td>
<td>24.77 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>12</td>
<td>24.36 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>20</td>
<td>24.97 ± 0.10</td>
</tr>
<tr>
<td>Data Logger-Night</td>
<td>Both</td>
<td>33</td>
<td>21.70 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>12</td>
<td>21.66 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>20</td>
<td>21.72 ± 0.18</td>
</tr>
</tbody>
</table>

Figure 3. Astronaut Steve Swanson harvesting thirty three day-old Veggie-grown lettuce on the ISS on June 10, 2014.

with microbial sampling swabs. At this time the three surviving plants were harvested, wrapped in aluminum foil, and frozen at -80°C in the Minus Eighty Laboratory Freezer aboard ISS (MELFI). Two plant pillows containing roots were also frozen, along with the swabs and a 10 mL water sample from the reservoir. All samples and the HOBO data logger were returned to Earth and analyzed beginning in Oct. 2014. Table 2 highlights the HOBO data logger data.

HOBO data indicate that there was a slight difference in average temperature between the back left and back right corners of the Veggie unit, primarily during the day period (Table 2). This temperature variation also impacted internal relative humidity. Causes of this temperature variation remain unknown.

Sample analysis included the following: fresh mass of frozen plant samples, culturable microbial assessment of plants, water samples, and plant pillow components, identification of cultured microbes, RNA sequencing and identification of the total microbial population, quantification of anthocyanin, antioxidant capacity, and total phenolics, elemental analysis of plants and water samples, and X ray tomography of plant pillows to image root distribution. These data are being compiled for publication with similar analyses from subsequent flight samples.
Table 3 shows the average frozen fresh mass of the plants.

<table>
<thead>
<tr>
<th></th>
<th>Flight</th>
<th>Ground</th>
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<tbody>
<tr>
<td>Sample Number</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Average Fresh Mass</td>
<td>20.61 g</td>
<td>15.29 g</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>11.66 g</td>
<td>9.60 g</td>
</tr>
<tr>
<td>Maximum</td>
<td>31.51 g</td>
<td>26.11 g</td>
</tr>
<tr>
<td>Minimum</td>
<td>8.31 g</td>
<td>2.81 g</td>
</tr>
</tbody>
</table>

Table 3. Average fresh mass of flight and ground lettuce plants from VEG-01A. Plants were harvested, frozen at -80°C, returned to Earth, and weighed while still frozen. Standard deviations and maximum and minimum values are presented to highlight variability.

Our primary goal was to examine the fresh produce for food safety analysis. Plants were examined for the specific pathogens *E. coli*, *S. aureus*, and *Salmonella* sp., and these were not found. Although there are currently no set standards for space-grown produce, the microbial load on crops intended for consumption by astronauts presumably should approximate other NASA microbiological standards for food. Currently, the limit for aerobic bacteria on a non-thermostabilized food item is < 20,000 colony-forming units (CFU) per gram (Perchonok et al., 2012); however, these standards apply to prepackaged food sent from Earth. Aerobic plate counts detected from Veggie-grown lettuce were less than this limit for non-thermostabilized food on all flight plants and all but one ground plant. The similar limit for total yeasts and molds on food is <1000 CFU/g and for our samples total yeasts and molds were all below the limit except on one flight plant (plant C, the largest, which had levels of 1120 CFU/g). Bacterial and fungal species isolated from flight samples appeared to be typical station microbes. After presentation of these data to flight medicine and safety boards, the Veggie team received approval for the crew to grow and consume the second set of ‘Outredgeous’ lettuce with a precautionary produce sanitizing step.

### 2.2 VEG-01B – ‘Outredgeous’ red romaine lettuce

The goals of VEG-01B were to grow lettuce to feed to crew, to see if increasing the initial priming water volume allowed the plant pillows to better establish a wicking water column, and to identify any changes in hardware function or plant/microbial growth with hardware age. The VEG-01B test was initiated by ISS astronaut Scott Kelly in July, 2015, with the same lighting and photoperiod settings as VEG-01A. The original non-functional plant pillow A had been saved from VEG-01A, and the quick disconnect water attachment had been swapped after harvest with one that was known to function. Kelly attempted to prime this plant pillow in VEG-01B but again water was unable to enter, indicating failure in a component other than the quick disconnect. This pillow was left unprimed in the hardware. After it was returned to Earth, a failure analysis noted that the internal fill tube was clogged, likely during manufacture. All other pillows were primed with 165 mL of water, an increased volume designed to better establish an initial water column. The reservoir was filled similarly to VEG-01A. Wick opening and thinning procedures and watering were also conducted similarly to VEG-01A but daily photos were taken of plants following thinning. Ground controls were run at a 24 h delay from flight. At 15 days after initiation plant growth appeared to cease. Plant pillows and the reservoir were watered at 16 days and water was directly added to plant pillows on every scheduled watering event after that. The plants recovered and were harvested after 33 days of growth (Fig. 4A). Half of the produce harvested was consumed after a precautionary sanitizing step using Pro-San® (Microcide, Inc., Sterling Heights, MI) wipes (Fig. 4B). Leaves were placed between layers of wipes in a re-closable bag and pressed for 30 seconds before being removed and consumed. The remainder of the produce was harvested and frozen similarly to VEG-01A for return to Earth for analysis. Samples were returned on May 18, 2016. Table 1 shows environmental data during this experiment.

Returned lettuce samples were found to contain no *E. coli*, *S. aureus*, or *Salmonella* sp. Flight lettuce microbial levels were higher than ground levels, however the use of Pro-San® wipes on consumed produce would have reduced any risk to the astronauts.

### 2.3 VEG-01C – ‘Profusion’ zinnia

The goals of VEG-01C were to monitor the growth of a longer duration crop in Veggie and to assess any hardware limitations on the process of flowering. Plant pillows containing seeds of a five-color mix of zinnia (*Zinnia hybrida* cv. ‘Profusion’) were initiated on November 16, 2015 by Kjell Lindgren and subsequently cared for by Kjell and Scott Kelly. Zinnias were initiated and grown similarly to lettuce except that the photoperiod of this short day plant was set to 10 h light /14 h dark. As in previous tests, one plant pillow failed to hydrate,
Figure 4. Thirty three day-old VEG-01B lettuce plants A in veggie prior to harvest and, B when Kjell Lindgren and Scott Kelly are sampling the Veggie-grown lettuce.
however in this case there was successful germination of zinnia plants in that malfunctioning pillow, likely due to excess water wicking into the pillow from the reservoir and the wicking surfaces of other plant pillows. Photo frequency was reduced to save crew time, and water was directly added to plant pillows with only initial water added to the reservoir. Early growth of zinnias looked abnormal, with significant leaf curling (Fig. 5A). Additionally, photos showed symptoms of excessive moisture around plants, and condensation on bellows. Plants also exhibited guttation (Fig. 5A). Several of the plants were stunted, exhibited chlorosis, and later died.

Two issues were identified as potentially responsible for this excess moisture: 1) the addition of water to pillows at levels that exceeded the plant demands and 2) inhibited air flow through the hardware. Water volumes for zinnia were based on previous volumes used for lettuce, but the growth rate of zinnia was slower than lettuce. Additionally the fan, when switched to high, dried out the chamber rapidly, but at low speed water remained in the chamber for much longer than in previous tests, indicating that the fan may have been malfunctioning at low speed, or that fan screens or cabin air intake screens in the bellows baseplate were occluded. At 36

![Image A](image1.png)

**Figure 5.** A Zinnia plant exhibiting guttation and leaf curling, likely due to excess water at twenty nine days after initiation. A stunted, chlorotic plant is also visible. B ‘Profusion’ zinnias with numerous flowers at 87 days after initiation on the ISS.
days after initiation, prior to switching the fan to high speed, fungal growth was observed on plants in Veggie. Scott Kelly removed visibly damaged plant tissue, froze the tissue for later analysis, and cleaned the inside of the hardware and the plants using sanitizing wipes. He also switched the fan to high. He asked to take responsibility for watering and overall plant care. A one-page general guide was developed to help him implement autonomous gardening. Scott repeated removal of dead tissue during later checks, and 53 days into the experiment, additional fungal growth was noted and two of the remaining four plants (and part of a third) were removed. At 61 days after initiation, both remaining plants flowered under Kelly’s care. Plants continued to flower and thrive from this point, producing more than 27 flowers (Fig. 5B). Plants and flowers were harvested after 90 days of growth. The crew were allowed to retain approximately half of the flowers as souvenirs. The remainder of the flowers were individually wrapped in dry wipes and left in an open bag to promote desiccation. Plants were harvested and frozen along with swabs, plant pillows, and water samples. All science samples were returned on May 18, 2016. Table 1 shows environmental data during this experiment.

Several fungi were recovered from symptomatic tissues including Aspergillus sp., Penicillium sp., and Fusarium sp. Plant pathogenicity tests are underway to identify the true plant pathogen and determine if it was opportunistic or aggressive. Human pathogens were not detected in any samples. From the 12 flight flowers returned in dry wipes, 111 seeds have been recovered, however viability of these has not been established and flowers and seeds showed evidence of post-harvest fungal growth.

3 Future Directions

The VEG-03 payload launched in February, 2016. Similarly to VEG-01, this payload consists of three sets of plant pillows, with one set of ‘Outredgeous’ lettuce, and two sets of ‘Tokyo Bekana’, a small Chinese cabbage (Brassica rapa var. chinensis). This cultivar was selected from leafy greens crop testing to assess horticultural performance, nutritional values, and organoleptic appeal (Massa et al., 2015). Crops grown for VEG-03 will be tested using a cut-and-come-again repetitive harvest technique to test a more sustainable, longer duration growth cycle for improvement of food yield and any changes in microbial composition. One crop of ‘Outredgeous’ lettuce was initiated Oct. 25, 2016 and repeatedly harvested until a final harvest on Dec. 28, 2016, with both crew consumption and science samples taken from the plants.

Following VEG-03, an enhanced passive water delivery system will be developed and flown. Given the challenges with the original reservoir water delivery system, a different approach designed to better provide water and maintain continuous moisture has been selected. VEG-01 demonstrated evidence of both insufficient and excess water in the plant root zone environment. Historically space flight experiments have had challenges delivering fluid and oxygen to plant root zones (e.g. Porterfield, 2002 and references therein). Plant hypoxic stress responses associated with a reduction of buoyancy-driven thermal convection of oxygen have been observed (Liao et al., 2004). One solution to the challenges of plant irrigation in space is to use powered approaches with pumps, solenoids, and water sensors of some type to control moisture levels in the root zone. The Plant Habitat hardware that will launch to the ISS in 2017 will use that approach (Massa et al., 2016). Oxygen levels can also be monitored and controlled with active aeration. Veggie took an unpowered approach to plant irrigation, and the first attempts with this approach were insufficient for plant needs leading to a higher need for crew involvement as well as sub-optimal plant growth. It is expected that the enhanced Veggie watering system will reduce crew operations, enhance plant growth, and provide better crop uniformity.

In addition, a second Veggie flight unit will be launched and installed on the ISS in close proximity to the current unit. This will enable side-by-side testing of different crops and different lighting conditions. VEG-04 and VEG-05 are planned experiments examining the impact of light quality and fertilizer formulation on yield, nutrition, and organoleptic characteristics of a leafy green crop and a dwarf tomato, respectively. Each crop will be tested simultaneously on ISS in two Veggie units having different red: blue light ratios to assess if differences in light quality have the same consequences under microgravity conditions as they do in a 1g environment. In addition to these and other future VEG-series experiments, the Veggie hardware will be used for the Advanced Plant EXperiment (APEX) series of experiments where smaller plants are grown in petri dishes or other sample containers. Other spaceflight investigations may also take advantage of the lighting environment provided by Veggie.

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