International Life Sciences Research Announcement “Pick and Eat” Project Contributions

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Major: Botany
NIFS Intern, Spring Session
Date: 13 April 2018

The International Life Sciences Research Announcement (ILSRA) is a grant which focuses on "Pick and Eat" salad-crop productivity, nutritional value, and acceptability to supplement the International Space Station (ISS) food system. My contributions into this project were to 1) manage the Veggie chambers to maintain optimal plant growth and attend to any plant needs, 2) analyze data for the VEG-04 Science Verification Test to create a water delivery schedule for the astronauts aboard the ISS, and 3) assume the role as a VEG-04 Experiment Verification Test (EVT) "pseudonaut" to confirm that all schedules and flight procedures produce quality results. The VEG-04 EVT will continue up until the last day of my internship. Additionally, I'm currently working on two independent research projects, both of which provide insight to potential plant growth hardware options for lunar or Martian surfaces. One of which is a cable culture hydroponics system (favorable in areas with highly limited space), and the other are two aeroponic systems (a subset of hydroponics which uses *no media* and misting as its delivery for water and nutrients). All independent projects are still being tested and therefore results are not yet established. Lastly, I have been in charge of maintaining the AeroGarden®, a hydroponic-aeroponic hybrid system which is currently being investigated as a concept to the potential for minimizing human involvement in the process of growing plants. This has the potential to play a major role in future technological project designs for spaceflight hardware. This system is an ongoing project that will continue once my internship ends.

I. Introduction

As a space plant biologist at Kennedy Space Center, my main responsibility was to research optimal growth conditions for the advancement of agriculture in microgravity environments. I work in the Life Sciences and Utilization division (UB-A), and a grant called the International Life Sciences Research Announcement (ILSRA) was the focal point in my internship contributions, and the grant was awarded to the UB-A branch to primarily research the most productive and efficient elements for plant growth, specifically experimenting with lighting and nutrient supplementation. Within the ILSRA grant, I worked on a variety of tasks and projects. Additionally, I have created two independent research projects, reassessing previously-tested hydroponic equipment and exploring optimal growth responses in aeroponic systems.

II. ILSRA "Pick and Eat" Projects

The International Life Sciences Research Announcement (ILSRA) grant is a collaborative effort between the National Aeronautics and Space Administration (NASA), Sierra Nevada Corporation-ORBITEC, and Purdue University in determining crop productivity, nutritional value, and acceptability to supplement the ISS food system[1]. The goal of this research is to implement fresh food for astronauts aboard the International Space Station (ISS) along with finding the most optimal and efficient growth methods for various crops in microgravity environments[2]. The primary focus of this research was to experiment with different fertilizer and light color and ratio treatments to determine the most optimal combinations for exceptional plant growth in controlled environments. The ILSRA project has been titled "Pick and Eat" project, which is compiled of numerous projects and various stages in the projects. The equipment used to grow the crops for every experiment is called the "Veggie" vegetable production unit. Each Veggie unit contains six Passive Orbital Nutrient Delivery Systems (PONDS) which contain each individual growing crop. There are currently two Veggie units installed inside of the ISS, capable of staggered and simultaneous experiments to be conducted. For ground-based flight definition testing, analog systems called Biomass Production Systems for Education (BPSE, also developed by SNC-ORBITEC) and analog PONDS systems were used to mimic Veggie. For pre-flight verification, Veggie units are used in controlled environment chambers to mimic the ISS environmental conditions of temperature, relative humidity, and carbon dioxide.

A. VEG-04 SVT Data Analysis for Water Delivery

Each stage of the ILSRA "Pick and Eat" must go through a series of repetitions and tests before it's accepted as fit for a flight experiment. After verifying that the crop can withstand various environmental conditions similar to those of the ISS module, it is subject to two final tests. The first test is a Science Verification Test (SVT), which tests the science in the official hardware and is primarily used to answer a question specific to that crop—such as when and how much water to add to each plant, if liquid fertilizer is necessary, or how long it takes the seeds to germinate. Once the SVT is complete, data are analyzed, question answered, and a final procedure is created. The second test is an

Experiment Verification Test (EVT), which is a dress rehearsal replicate for the final flight experiment. This is performed precisely by following the procedures provided to astronauts aboard the ISS given to verify that all steps produce the expected results. After EVT is completed and produce successful results, the experiment is now ready for flight.

The question addressed in the VEG-04 SVT was how frequent the plants needed to be watered, and how much water needed to be added to each plant. My duty was to compile the data from the completed SVT for the VEG-04 experiment and create a watering schedule for the astronauts. I sourced my data primarily from Fig. 1, which represents the Day After Planting (DAP)s in hierarchical order of all averaged PONDS units which received the most amount of water deliveries. My other primary data were from the percentage of water remaining in each PONDS per each DAP, by comparing all the PONDS units (Fig. 2). This helped in determining any outliers. Fig. 1 provided data on which days would most likely be implemented as watering days based on the majority, while Fig. 2 provided individual PONDS data to identify if any plants used more water than the others and thus may require more frequent waterings. By comparing these data with pictures taken throughout the entire SVT experiment (e.g. pictures like Fig. 3), I was able to correlate any excessive or reduced water intake with the size of plant in the case that plant size was influential of the amount of water absorbed by the plant. It was concluded that plant size had negligible impacts on water intake, but all data was taken into account before results were finalized.

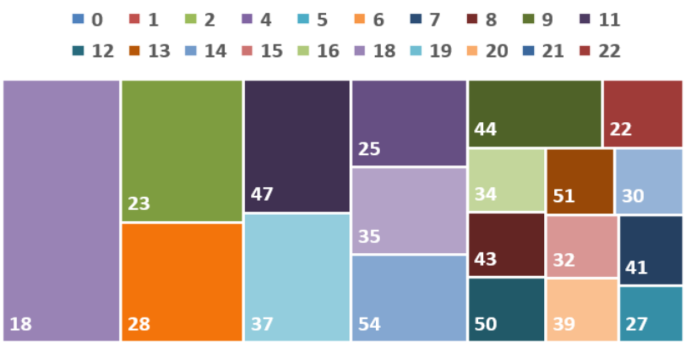

Figure 1. Hierarchical depiction of the water deliveries on each Day After Planting (DAP), averaging all six Passive Orbital Nutrient Delivery System (PONDS) units.

My finished analysis of VEG-04 SVT watering delivery schedule determined that each plant, regardless of size, was to be watered following the schedule of:

1st water: 18 DAP
2nd water: 22-23 DAP
3rd water: 25-26 DAP
4th water: 28-29 DAP
5th water: 34-35 DAP
6th water: 39-40 DAP
7th water: 46-48 DAP
8th water: 53-54 DAP

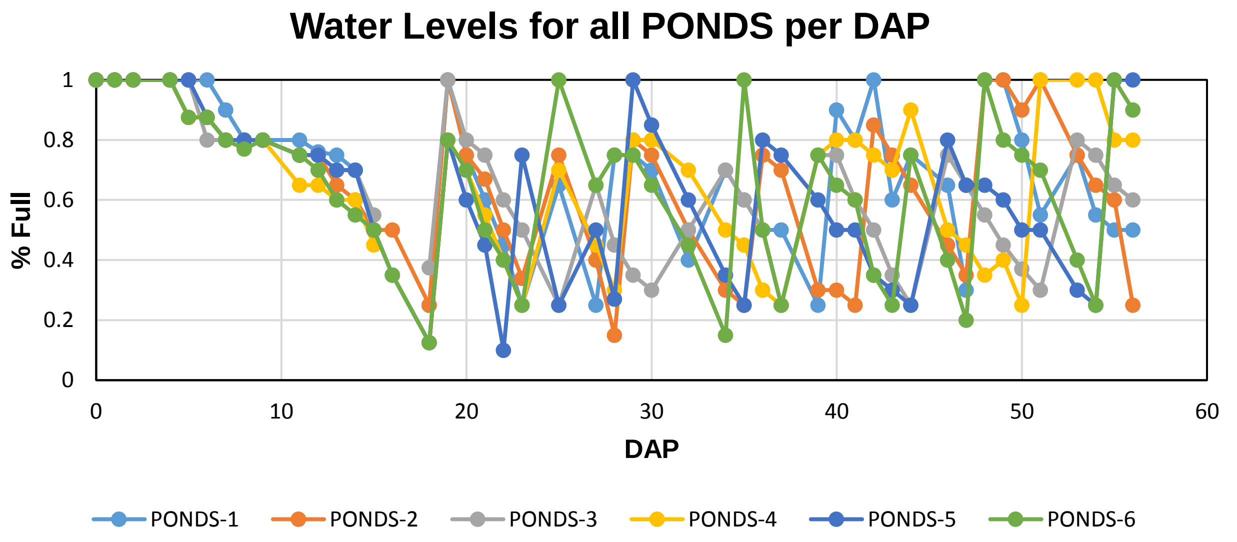

Figure 2. Water levels related to each Day After Planting (DAP). *Water levels are shown as a percentage from full (100% = 1). Watering deliveries instructed to occur whenever a water level reads below 0.25. Each Passive Orbital Nutrient Delivery System (PONDS) is represented by a different color.*

Figure 3. Top view of VEG-04 SVT Mizuna plants at Day After Planting (DAP) 18. *Each Passive Orbital Nutrient Delivery System (PONDS) is numbered 1-6 to distinguish plants. Photo taken 17 December 2017.*

This water delivery schedule is made specifically for the mustard crop *Brassica juncea var. japonica* (common name, Mizuna) for the duration of 56 days. Therefore, it cannot guarantee success to any other crop species chosen, or for a duration longer than 56 days (a duration shorter than 56 days for Mizuna may still be appropriate). This schedule is being applied to the current VEG-04 EVT, which was initiated 10 April 2018.

B. VEG-04 EVT "Pseudonaut"

Because EVT experiments are to be completed by strictly following the procedures, not familiarity, the executioner is chosen based on lack of thorough knowledge of the flight hardware logistics. The person following the procedures written for astronauts is termed as a "pseudonaut", and I was chosen for the role of VEG-04 EVT pseudonaut. Assuming this role requires strict following of written astronaut procedures for Veggie hardware initiation, maintenance, and harvesting of the crops. I am filmed for all aforementioned activities to create an instructional tutorial for astronauts, as a reference before they perform their duties on VEG-04 experiments executed on-orbit on the ISS.

Additional duties include reading over procedures to prepare before performing them, recording data in a logbook, taking pictures of the plants for visual record, and following a strict schedule for various maintenance activities (e.g. wick opening, plug removal, plant thinning, germination cap removals, and watering at particular intervals).

C. Veggie Chamber Management

One of my daily routines was to maintain the various crops planted in multiple BPSE units. Located in one of the growth chambers in the SSFP, it was my duty to record chamber conditions (e.g. temperature, relative humidity, and CO_2 levels), water measurements (amount and frequency), pH and electrical conductivity, and change the height of the light canopy when appropriate. Weekly checks included inspections of light concentrations per BPSE unit to make sure ratios were maintained, as well as taking water samples for chemical analysis. It was also my job to record any abnormal occurrences—this might have been seeds that never germinated and therefore I needed to replant new seeds, or it could have been that plants began showing signs of stress and I needed to figure out why it was occurring. Daily checks are imperative in the case that circuits would trip in the chambers, causing lights and ventilation to stop. In these instances, immediate action was crucial.

Figure 4. Picture of current chamber contents. *Biomass Production Systems for Education (BPSE) units vary in color because of current experiments investigating different light ratio affects on crops. From left to right: BPSE 1, BPSE 3, BPSE 3, BPSE 4, and BPSE 6. Photo taken 26 March 2018.*

III. Independent Research Projects

My mentor was very encouraging of me to initiate personal research projects, so I chose to undertake projects which I could develop in my free time. These projects were collaborative efforts between Ruqayah Bhuiyan (NIFS intern) and myself, and Oscar Monje (LSSO employee) assisted us when the required work was beyond our skillsets (e.g. electrical and mechanical work). These projects are intended for potential plant growth hardware to be applied in Lunar or Martian surface environments. Although these systems have been previously investigated, we are aiming to advance knowledge of optimal performance and results from these systems.

A. Cable Culture Hydroponic System

Cable culture is a hydroponic system initially developed for expandable Lunar and Martian greenhouse modules, created by the University of Arizona and Sadler Machine Co. [3]. Kennedy Space Center's long-standing relationship with Phil Sadler and Gene Giacomelli has led to the possession of the cable culture system. It presents the basic function of nutrient film technique, allowing for water to passively continually flow past the roots while the roots absorb only what they need, but the cable culture is assembled so it can expand and compact when necessary. The majority of its growth is vertical (demonstrated in Fig. 5) and a structure such as this possesses great potential for compact environments such as those on the moon or Mars, where space is very limited. My main objective in this project was to start it up and running for the first time at Kennedy Space Center. I also experimented with the "wicking" for the plants, which is essentially a substrate which gives roots a place to grow and access to water (and oxygen in lower gravity environments). R. Bhuiyan and I are currently testing two different wick structures.

Figure 5. Cable culture hydroponics. *Water enters though black tubes at top left and slowly drains out of the bottom right tubes, which collect in a single reservoir for recycled use. Two crops, extra dwarf Pak Choy and Dragoon lettuce, planted alternately. Wicks assembled in clumps of two: top right two and bottom left two are the single wide strips, top right and bottom left are the three narrow strip wick shapes.*

R. Bhuiyan and I chose two different plants to grow in our cable culture, *Brassica rapa var. chinensis* and *Lactuca sativa var. longifolia*, commonly known as extra dwarf Pak Choy and Dragoon lettuce, respectively. These are placed at two different heights to test potential effects from differing light intensities (height differences were fixed because of the initial infrastructure of the cable culture). We created two different wick shapes; one wick had a single wide strip to the bottom of the water reservoir, while the other had three narrow strips to the reservoir. We chose to test two different wick structures to determine if roots have a preference of wick configuration. I hypothesize that the three narrow strips may show high plant productivity because they potentially allow for easier root growth and more opportunity for root aeration. Experiment is still in progress; no results thus far.

B. Aeroponics Systems

Aeroponics is a type of hydroponics system—the sole difference is that aeroponics uses no growing medium at all, and roots are in primary contact with air. The roots are only temporarily exposed to water and nutrients when scheduled, most often using timed misting intervals to supply water and nutrients to the roots. We chose to explore aeroponics systems because previous interns had done experiments with aeroponics and we wanted to continue gathering information on optimal aeroponic performance, but also because O. Monje had previously flown an aeroponic system on parabolic flights, therefore its importance to crop technological development had already been established.

The system tested on parabolic flights was one of the systems we used, while we had another created that was a rectangular shape made of a black opaque material to inhibit any light from entering the interior and fostering algae growth in the water reservoir. We decided that both systems would use only one reservoir for draining water, and recycling to reuse for waterings. The single reservoir system made it easier for us to determine how much water and nutrients the plants were absorbing, while also making access to check pH and electrical conductivity easier. O. Monje assisted us in creating a data logger, which incorporated coding that instructed the system to mist on intervals, for a certain amount of seconds per mist. We also created and altered electrical circuits to connect and activate the data logger, spray nozzle, and air compressor.

R. Bhuiyan and I chose to test the same crops as the cable culture, the *Brassica rapa var. chinensis*, common name extra dwarf Pak Choy and *Lactuca sativa var. longifolia* , common name Dragoon lettuce. We chose to test the length of the wick to determine whether or not the roots had a preference to long or short wicks, and if their growth patterns affected their overall productivity performance. We used two different aeroponics units, one a square shape and one rectangle, to see if the plants' proximity to the spray nozzle will affect water distribution, and therefore affect the root growth and plant productivity. I hypothesize that wick length will not affect the overall productive growth of the plant, but I do predict that closer plant proximity to the spray nozzle will promote higher plant productivity. This experiment is currently in progress; no results yet.

IV. AeroGarden® Management

The Human Research Program wanted UB-A to investigate the potential that Miracle-Gro's AeroGarden® could provide for plant growth hardware research in a human-space analog. The system resembles that of a hybrid between hydroponics and aeroponics because it uses water from a recycling water reservoir, yet it has an aeration pump which cyclically runs to oxygenate the plant's root systems (this is why it is considered "aeroponics"). Because the AeroGarden® is made originally for household use, it is meant to be incredibly user-friendly and grow plants successfully for people who are not gardening-savvy. The unit includes a screen that provides the current water levels and time left until nutrients need to be added, so the user knows when the plants need water or nutrients. The AeroGarden® is a gateway to experimentation with the idea to minimize human involvement in growing plants, a concept which may provide insight for potential future technological project designs[2].

Figure 6. Miracle-Gro's AeroGarden®. *Left side: contains herbal plants which are removed/harvested regularly and replanted with various crops when necessary. Left side: contains four tomato plants which have remained since their initial planting, clearly producing large amounts of fruit.*

My responsibilities to the Aerogarden® are to maintain water and nutrient levels, height of light canopy, and to check overall plant health. Over the course of my time with it, I have been able to plant a variety of crops, and in doing so, understand how to grow crops in order to produce optimal results from the system. Herbs, leafy greens, and flowering crops all react differently to the system:

1) Herbs performed overall very well in the system. The Genovese basil flourished immediately and continued to remain healthy for months. The dill and chives remained consistently healthy throughout their lifespan.

2) Leafy greens did not respond well to the system overall. The high heat from the light canopy caused the leaves to mildly wilt, and the lack of air flow caused tip burn to rot a few young inner leaves (rendering them inedible). The stems also became excessively elongated and a few of the plants began bolting, both signs of stress from the plant. Consequently, stress can manifest as a bitter taste in plant leaves, thus causing them to be undesirable to eat.

3) Tomatoes required more frequent nutrient supplementation than the other plants tested, but produced large amounts of fruit. One tomato plant exhibited extremely low leaf growth and fruit never ripened, but I suspect it's a genetic mutation in the plant, not a technological issue since all other tomato plants are healthy and all are equally exposed to resources.

From the time these initial crops were planted and some harvested or removed, I have since put in a pepper plant, a radish, and mustard greens. None of the new crops are far enough along to determine the AeroGarden®'s success in their productivity. This unit is an ongoing experiment, testing various crop types and will continue to be explored after my internship ends.

Acknowledgments

I would like to thank Matthew Mickens, Gioia Massa, Matthew Romeyn, and the UB-A branch for taking me as NIFS intern. I'd also like to thank the Kennedy Space Center's Office of Education and the Intern Coordinators Gwen Gamble, Grace Johnson, and Priscilla Moore. Lastly, I would like to thank the Oregon Space Grant Consortium for funding this opportunity.

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