Fiscal Year:	FY 2022	**Task Last Updated:**	FY 07/14/2022
PI Name:	Massa, Gioia Ph.D.		
Project Title:	Pick-and-Eat Salad-Crop Productivity, Nutritional Value, and Acceptability to Supplement the ISS Food System		
Division Name:	Human Research, Space Biology		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	(1) **HHC**:Human Health Countermeasures		
Human Research Program Risks:	(1) **Food and Nutrition**:Risk of Performance Decrement and Crew Illness Due to Inadequate Food and Nutrition		
Space Biology Element:	(1) Plant Biology		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	(1) Bioregenerative Life Support		
PI Email:	gioia.massa@nasa.gov	**Fax:**	FY
PI Organization Type:	NASA CENTER	**Phone:**	321-861-2938
Organization Name:	NASA Kennedy Space Center		
PI Address 1:	ISS Ground Processing and Research		
PI Address 2:	Mail Code UB-A-00		
PI Web Page:			
City:	Kennedy Space Center	**State:**	FL
Zip Code:	32899-0001	**Congressional District:**	8
Comments:			
Project Type:	FLIGHT	**Solicitation / Funding Source:**	2013-14 HERO NNJ13ZSA002N-ILSRA. International Life Sciences Research Announcement
Start Date:	09/01/2015	**End Date:**	09/30/2025
No. of Post Docs:	0	**No. of PhD Degrees:**	0
No. of PhD Candidates:	0	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	0	**Monitoring Center:**	NASA JSC
Contact Monitor:	Douglas, Grace	**Contact Phone:**	
Contact Email:	grace.l.douglas@nasa.gov		
Flight Program:	ISS		
Flight Assignment:	NOTE: End date changed to 9/30/2025 per HRP HHC element management (Ed., 8/10/21) NOTE: End date changed to 9/30/2021 per PI (Ed., 5/4/2020) NOTE: End date changed to 8/31/2020 per PI (Ed., 8/17/18) NOTE: Element change to Human Health Countermeasures; previously Space Human Factors & Habitability (Ed., 1/18/17) NOTE: Period of performance changed to 9/01/2015-8/31/2018 (previously 7/1/15-6/30/18) per G. Douglas/HRP (Ed., 4/3/16)		
Key Personnel Changes/Previous PI:	Dr. Sara Whiting departed the project in April 2022, and Dr. Lauren Landon was added as a temporary Co-Investigator for Behavioral Health and Performance (BHP). Sydney Begerowski and Cara Spencer assumed Meredith Russell's position as participants to support the BHP work. No students worked directly on the project during this reporting period.		

COI Name (Institution):	Douglas, Grace Ph.D. (NASA Johnson Space Center) Hummerick, Mary M.S. (Amentum, Kennedy Space Center) Mitchell, Cary Ph.D. (Purdue University--grant NNX15AN78G) Morrow, Robert Ph.D. (Orbital Technologies Corporation) Wheeler, Raymond Ph.D. (NASA Kennedy Space Center) Young, Millennia Ph.D. (NASA Johnson Space Center) Spencer, LaShelle M.S. (Amentum, Kennedy Space Center) Romeyn, Matt M.S. (NASA Kennedy Space Center) Buncheck, Jess M.S. (Southeastern Universities Research Association, Kennedy Space Center) Bell, Suzanne Ph.D. (NASA Johnson Space Center) Whiting, Sara Ph.D. (KBR/Johnson Space Center) Landon, Lauren (Wyle, Johnson Space Center)
Grant/Contract No.:	Internal Project
Performance Goal No.:	
Performance Goal Text:	
Task Description:	The capability to grow nutritious, palatable food for crew consumption during spaceflight has the potential to provide health promoting, bioavailable nutrients, enhance the dietary experience, and reduce launch mass as we move toward longer-duration missions. However, studies of edible produce during spaceflight have been limited, leaving a significant knowledge gap in the methods required to grow safe, acceptable, nutritious crops for consumption in microgravity. The “Veggie” vegetable-production system on the International Space Station (ISS) offers an opportunity to develop a “pick-and-eat” fresh vegetable component to the ISS food system as a first step to bioregenerative supplemental food production. We propose growing salad plants in the Veggie unit during spaceflight, focusing on the impact of light quality and fertilizer formulation on crop morphology, edible biomass yield, microbial food safety, organoleptic acceptability, nutritional value, and behavioral health benefits of the fresh produce. Phase A of the project would involve flight tests using leafy greens. Phase B would focus on dwarf tomato. Our work will help define light colors, levels, and horticultural best practices to achieve high yields of safe, nutritious leafy greens and tomatoes to supplement a space diet of prepackaged food. Our final deliverable will be the development of growth protocols for these crops in a spaceflight vegetable production system. Specific aim 1: Evaluate the effects of four light treatments and two different fertilizer compositions on the yield, morphology, organoleptic acceptability, and nutritional attributes of leafy greens during flight-definition and flight testing. Specific aim 2: Perform cultivar selection and evaluate the effects of four different red: blue light treatments and two different fertilizer compositions on the yield, morphology, organoleptic acceptability, and nutritional attributes of dwarf tomato during ground and flight tests. Specific aim 3: Perform hazard analysis, develop plans for minimizing microbial hazards, and screen flight-grown produce for potential pathogens.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Research Project: Our work on “Pick-and-Eat Salad-Crop Productivity, Nutritional Value, and Acceptability to Supplement the ISS Food System” focuses on developing a fresh food production capability on the International Space Station (ISS). We are using the Veggie hardware to develop light and fertilizer combinations that generate nutritious and appealing leafy green vegetables and dwarf tomatoes that astronauts can safely consume. The results of this research will directly translate to Earth-based controlled environment production of these and similar crops in vertical farms and urban plant factories. The capability to grow nutritious, palatable food for crew consumption during spaceflight can potentially provide health-promoting, bioavailable nutrients, enhance the dietary experience, and reduce launch mass as we move toward longer-duration exploration missions. However, studies of edible produce during spaceflight have been limited, leaving a significant knowledge gap in the methods required to grow safe, acceptable, nutritious crops for consumption in microgravity. The Veggie vegetable-production system on the ISS offers an opportunity to develop a “pick-and-eat” fresh vegetable component to the ISS food system as a first step to bioregenerative supplemental food production. Our goal is to grow salad crops in the Veggie unit during spaceflight and assess the impact of light quality and fertilizer formulation on crop morphology, edible biomass yield, microbial food safety, organoleptic acceptability, nutritional value, and behavioral health benefits. Our work will help define light color ratios, fertilizer composition, and horticultural best practices to achieve high yields of safe, nutritious leafy greens and tomatoes to supplement a space diet of prepackaged food. Our final deliverable will be to develop growth protocols for these crops in a spaceflight vegetable-production system. This will reduce the risk and close the gap of inadequate nutrition by helping us advance bioregenerative food production to supplement the packaged diet for future space exploration.
	Pick-and-Eat Salad-Crop Productivity, Nutritional Value, and Acceptability to Supplement the ISS Food System (VEG-04A, VEG-04B, and VEG-05) is a set of hybrid experiments of plant research with human organoleptic and behavioral research. These experiments are sponsored by the NASA Human Research Program but are implemented in partnership with the NASA Space Biology Program. The VEG-04 flight experiments were conducted in 2019, and the VEG-05 experiment has been delayed due to hardware development and implementation, but it is planned for launch to the International Space Station (ISS) in fall 2022. Because of this delay, an additional component was added to this experiment, known as HRF VEG (which stands for Human Research Facility – Veggie). The HRF VEG experiment consists of collecting data-of-opportunity from other space biology investigations with plants that are being conducted on the ISS between the VEG-04 and VEG-05 tests. Data collected are specific to the human research foci of this project and include only behavioral health and performance data as part of the Veggie Questionnaire and Profile of Mood States data collected, and Organoleptic data on the crops. VEG-04A and B VEG-04A was conducted during Increment 57-58 and ran from June 4, 2019-July 9, 2019. VEG-04B was conducted during Increment 61-62, and ran from October 1, 2019-November 28, 2019, and both had ground controls run ~48 hours later. Science samples were returned from the

Task Progress:

ISS and were processed for microbiological food safety and chemical analyses.
Some chemical analyses were delayed due to COVID-19 and equipment failures, but these now have been completed. These final data are being analyzed and the team is writing up the VEG-04 plant, chemistry, microbial, and organoleptic results for publication in a special edition of Frontiers in Plant Science, “Higher Plants, Algae and Cyanobacteria in Space Environments, Volume II” for fall of 2022. The behavioral health data from VEG-04 A and B have been analyzed but we are considering if we want to publish these with the data from other studies or as a stand-alone component at this point.

HRF VEG Plans for HRF VEG, essentially the collection of human data-of-opportunity on plant growth tests including VEG-03 I, J, K, and L and PH-04, were approved by the NASA Human Research Program Control Board on Sept. 17, 2020. The Institutional Revew Board (IRB) and crew informed consent briefings were modified to include these additional experiments; all crew members for the possible missions received Informed Consent Briefings; and organoleptic and veggie questionnaires were modified to allow the additional crops (‘Outredgeous’ lettuce, ‘Dragoon’ lettuce, ‘Wasabi’ mustard. ‘Red Russian’ Kale, ‘Extra Dwarf’ Pak Choi, ‘Amara’ mustard, and ‘Española improved’ Hatch Chile peppers) and additional hardware (Advanced Plant Habitat) to be evaluated by participating subjects. Collection of plant data was not a part of the HRF VEG studies.

At this point all planned HRF VEG studies have been completed, and no additional tests have been added. The final HRF VEG test was the PH-04 test growing ‘Española improved’ Hatch Chile peppers in the Advanced Plant Habitat which grew July 12th to Nov. 26th, 2021 (137 days). Behavioral health and organoleptic data were collected.

VEG-05 Work to prepare VEG-05 for flight was completed in parallel with testing on the ISS of the Passive Orbital Nutrient Delivery System (PONDS) hardware. Because the PONDS hardware had yet to be successfully tested in flight, it was decided to test tomato plant growth both in PONDS units and in Veggie plant pillows.

Prior to this, in late 2018-early 2019 we grew two ‘Red Robin’ dwarf tomato plants in #6 plant pillows and 2 plants in #3 plant pillows – the only time tomato growth had been tested in actual flight pillows. Of these, only 1 plant in each pillow type survived, which we believe was caused by early overwatering and excessive fertilizer salt buildup. Additionally, both plants were severely epinastic (leaves curled or bent), though the reasons for this are unknown. ‘Red Robin’ is prone to epinasty when stressed. In this early pretest, both surviving plants produced roughly the same amount of fruit (21 in the #3 pillow and 24 in the #6 pillow). Other harvest data were lost due to the government shutdown in December through January 2019.

Pre-SVT Fertilizer Testing: Given the challenges with salt stress and the limited knowledge of tomato growth in both PONDS and pillows, the team was given an opportunity to do a pre-Science Verification Test (pre-SVT) definition test under flight-like conditions using Veggie hardware. For the pillows, a method to lengthen the wicks and pin them back away from the plants was tested to keep salt from burning the plant stems. Also, the Calcium Nitrate used in previous tests had been shown to release very quickly and contribute to burning. An alternate form of calcium, Calcium Carbonate, was tested in this pre-SVT test. After considerable assessment of the results of previous tests with tomatoes, and team discussions, two different low fertilizer formulations totaling not more than 10 g/L were selected for testing. There were 3 pillows and 3 PONDS of each of two treatments.

The Pre-SVT fertilizer test was initially designed to run 45 days to the onset of flowering; however, the opportunity arose to continue this test longer to start to assess fruit formation, so the test was run for 53 days. Two Veggie facilities were used, both with the Red-rich light treatment (330 micromoles, Red setting: 270 micromoles, Blue setting: 30 micromoles, Green setting: ON (30 micromoles)). The photoperiod was 16 h on/ 8 h off and the fan setting was supposed to be low, but fans were initially accidently set to high and then reset after starting. For pillows, a new way to pin the wicks back was devised as “beltloops” sewn through the pillows. The wicks extended over the gasket and thus were considerably longer than previous Veggie wicks. Also, the pillow shades had to be trimmed to account for the wicks, and the combination of longer wicks plus wicks touching shades led to an unforeseen issue where the pillows evaporated more water than anticipated. The pillow shades also absorbed and wicked water, providing a large evaporative surface, and because of this (and possibly the high fan setting early on) the pillows dried out from their initial priming in 4 days, prior to additional water being added or any anticipation that water would be needed. The team decided to replant the pillows and to shorten the wicks and pin them to the gasket instead of the pillow surface to reduce excess evaporation. The pillows were restarted 1 week later and operations for pillows were offset from the PONDS plants by one week.

Plants in both pillows and PONDS units grew well and appeared healthy. At harvest we assessed the height of each plant; the diameter in two dimensions; the number of flowers, leaves, and fruit; the leaf area and chlorophyll content (Soil Plant Analysis Development/SPAD); the leaf fresh mass; and the fruit fresh mass. Although both sets of plants had fruit, none were ripe.

In addition to the plant data, water from the PONDS cylinders was analyzed at the completion of the growth cycle. Data indicated few nutrients present in the water, with Electrical Conductivity (EC) between 77-228 µS/cm (for reference tap water often exceeds 600 µS/cm). In both PONDS and pillows, the higher fertilizer (Treatment 2) showed the best growth (fruit) or growth potential (flowers) and leaves, as well as in pillows the highest chlorophyll concentration. Since the amount of fertilizer in the PONDS water at the completion of the pre-SVT test was so low (at only 53 days when the planned growth period is 104 days), there was concern that even the higher level of treatment 2 was insufficient to maintain flowering and fruiting. Because of these factors, the team decided to select the highest treatment to move forward, but also to add an additional even higher treatment for comparison in SVT and again to split the plants with 3 pillows or PONDS per treatment.

Science Verification Test (SVT): SVT was initiated on January 4, 2022, with the two fertilizer treatments in both plant pillows and PONDS units. Each pillow and PONDS unit contained three Red Robin Tomato seeds. Two Veggie facilities were used, both with the Blue-rich light treatment (330 micromoles, Red setting: 150 micromoles, Blue setting: 150 micromoles, Green setting: ON (30 micromoles)). The photoperiod was 16 h on/ 8 h off and the fan setting was low. The primary difference between the settings for this test and the pre-SVT test was that the Red-rich light treatment was used in Pre-SVT, while the Blue-rich treatment was used in SVT.

All plants showed excellent flowering and fruit production except the plant in pillow 5 which had abnormal growth. Although this plant flowered, flowering was very delayed, and all flowers aborted, leading to no fruit production. This abnormality was never previously seen with Red Robin tomato.

In general from SVT, fruit ripened later than anticipated (original harvest plans for DAI 80, 90, 104 were modified to DAI 90, 97, 104). The number of fruit produced and the size of the fruit was greatest in the first harvest and decreased over time. Fertilizer Treatment 2 (a slightly higher concentration) produced a slightly greater fruit mass on average than Treatment 1. Some unripe fruit and flowers remained at the final harvest. While growth could have continued longer, it is anticipated that fruit yield would continue to decline. Photos taken when Veggie lights are off are easier to use to detect flowering and fruiting, judge plant health, and determine ripeness level of fruit. Plant data will not be taken inflight, so these were not collected during SVT. Three mature leaves from each plant will be collected in flight and returned for analyses, similar to plant sampling that was performed in PH-04. Some fungus was observed on several stems and one leaf, likely due to excess water. After this was observed, water volumes were reduced, the leaf with fungus was removed, and stems were cleaned with ProSan® wipes. Fungal growth did not reoccur.

Microbiological analysis of tomato fruit from VEG-05 SVT: The VEG-05 SVT consisted of an initial fruit harvest completed on 4/4/22, an interim harvest completed on 4/11/22, and a final harvest completed on 4/18/22. At each harvest, tomatoes were obtained from five plants and standard food safety testing was performed on both sanitized and non-sanitized samples. Fruit samples were sanitized by wiping with wipes containing 1% ProSan solution for 30 seconds. All samples were processed for analysis of aerobic plate counts (APC), total yeasts and molds (Y & M), coliform/E. coli, S. aureus, and Salmonella sp. All three harvests yielded microbial and fungal counts that fell below the detection limits. Detection limits vary due to individual sample weights but are all below 70 (the highest detection limit) CFU/gfw for APC and Y & M and 7 for Coliform/E. coli, and S. aureus. No Salmonella was detected.

Experiment Verification Test (EVT): After completion of SVT, results were presented to NASA Biological and Physical Sciences (PBS) management in an EVT Readiness Review on April 25, 2022, and the team received approval to proceed with EVT. EVT began April 27, 2022, and is scheduled to run for 104 days with completion on August 9, 2022. EVT consists of 12 plant pillows with pillows 1-6 in the blue-rich light treatment ((330 micromoles, Red setting: 150 micromoles, Blue setting: 150 micromoles, Green setting: ON (30 micromoles)), and pillows 7-12 in the red-rich light treatment ((330 micromoles, Red setting: 270150 micromoles, Blue setting: 30 micromoles, Green setting: ON (30 micromoles)). SVT fertilizer treatment 2 will be used for all pillows. We had initially planned to add water to the root mats at day 45, and to water plant pillows every other day. This was successful in EVT, but 6 days after water was added to the root mats, we observed wilting of all plants and the root mats were dry. Some leaves were lost during this wilting event. To mitigate wilting, water was added to the pillows in excess of the planned volume, and the root mats were filled for a second time. This seemed to remedy the issue, but the plants again used up all the water within 6 days (though not to the point of wilting). The team has been monitoring and adding water every 6 days, and root mats continue to wick, and plants are growing well. Leaking was observed after the first wilting event and refill of the root mats, indicating that the plant pillows were back at capacity at that time.

EVT continues to progress. At this point, all plants appear to be flowering and setting fruit. The method of pollination was changed from tapping individual plants to instead knocking on the Veggie baseplate. This approach was successfully performed in analog testing, and it minimized damage to plants and potential loss of leaves, flowers, and fruit. It is working very well for EVT, and it also significantly reduces the time needed to pollinate all plants. Plans are for fruit to be harvested at day 90, 97, and 104. The mass measuring device will be used in the flight experiment and the ground unit will be tested for the EVT harvests.

Bibliography Type:	Description: (Last Updated: 10/26/2023)
Articles in Peer-reviewed Journals	Morsi A, Massa GD, Morrow RC, Wheeler RM, Mitchell CA. "Comparison of two controlled-release fertilizer formulations for cut-and-come-again harvest yield and mineral content of Lactuca sativa L. cv. Outredgeous grown under International Space Station environmental conditions." Life Sci Space Res. 2022 Feb;32:71-78. https://doi.org/10.1016/j.lssr.2021.12.001 ; PMID: 35065764 , Feb-2022
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