Fiscal Year:	FY 2023	**Task Last Updated:**	FY 09/29/2023
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		
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PI Organization Type:	NASA CENTER	**Phone:**	321-861-2938
Organization Name:	NASA Kennedy Space Center		
PI Address 1:	ISS Ground Processing and Research		
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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:	1	**No. of Master' Degrees:**	1
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:**	
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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:	Matthew Romeyn, Co-I, passed away in August, 2022.		

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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.
	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 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 data have all been analyzed, and the team is writing up the VEG-04 plant, chemistry, microbial, and organoleptic results for publication in a special edition of The Journal of Plant Interactions, Article Collection on “Plant Astrobiology”, with a submission deadline of August 31, 2023. The behavioral health data from VEG-04 A and B have been analyzed, but we are still compiling these data with data in HRF-VEG studies. 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 Human Research Program Control Board on Sept. 17, 2020. The Institutional Review 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.

All planned HRF VEG studies have been completed, and no additional tests have been added. Data are being compiled.

VEG-05 Experiment Summary The Veggie (Vegetable Production System) on the ISS offers an opportunity to develop a "pick-and-eat" fresh vegetable component for the ISS food system as the first step towards bioregenerative supplemental food production. In the Veggie unit, during spaceflight, salad plants will be grown, focusing on the impact of light quality and fertilizer formulation on crop morphology, edible biomass yield, microbial food safety, organoleptic acceptability, nutritional content, and behavioral health benefits of the fresh produce for the Solanum lycopersicum 'Red Robin' dwarf Tomato cultivar. The VEG-05 experiment will test different red to blue light ratios using the Veggie units on the ISS. Tomato plants will be grown in Veggie for ~100 days and astronaut crewmembers will provide plant care and pollination, and harvest fruit 3 times during and at the end of this growth period. Crewmembers will be asked to complete self-report surveys, including the Profile of Mood States - Short Form (POMS-SF) and a Veggie-specific questionnaire pre-flight, in-flight, and post-flight. And they will also be asked to perform an organoleptic evaluation of a portion of the fresh produce they consume at harvest.

Consumed tomato samples will have their mass measured on the ISS prior to crew consumption, and half of the produce will be frozen and returned to Earth for post-flight microbiological food safety and nutritional analysis. The goal of this experiment is to help define light colors and horticultural best practices to achieve high yields of safe, nutritious appealing tomato fruit to supplement a space diet of prepackaged food.

Experiment Verification Test (EVT) EVT began April 27, 2022, and ran for 99 days, with completion on August 4th, 2022. EVT consisted 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: 270 micromoles, Blue setting: 30 micromoles, Green setting: ON (30 micromoles)). We had initially planned to add water to the root mats at day 45, and to water plant pillows every other day at this point. Six days after water was added to the root mats, we observed wilting of all plants and the root mats were dry (Days after Initiation or "DAI" 51). Some leaves were lost during this wilting event and plants had some recovery after refilling the root mat. 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 monitored and added water to the root mats every 6 days, with watering to pillows every other day, and root mats continued to wick, and plants generally grew well. Leaking was observed after the first wilting event, and refill of the root mats and periodically after this when water was added to pillows, indicating that the plant pillows were back at capacity. A second wilting event occurred at 75 days after initiation, when plants again used all the water and emptied the root mat.

The first flower was observed in the red-rich treatment on DAI 32 with flowers open on DAI 34. The first flowers emerged in the blue-rich treatment on DAI 36 and opened on DAI 37. All plants had flowers by DAI 41. The first fruit was noted on DAI 47, and all plants had fruit visible by DAI 49. There were signs of intumescence on many leaves; intumescence is a physiological growth disorder often seen in tomatoes grown under narrow spectrum light sources, and this does not present a cause for concern. We did see slightly different rates of flowering, fruit formation, and fruit ripening between the two light treatments, with the red-rich light treatment generally being in advance of the blue-rich light treatment. We had ripe fruit detach prior to the first harvest, one each from plants 7 and 11 at 80 DAI, and one from plant 2 at 81 DAI, as well as an unripe fruit from plant 9 the same day. Three harvests were conducted over the course of the EVT, with the first harvest at 83 DAI, the second harvest at 90 DAI, and the final harvest of flowers and plant samples at 99 DAI. The mass measuring device that was planned to be used in the flight experiment was tested for the EVT harvests. EVT success criteria were analyzed.

Task Progress:

Microbiological analysis of tomato fruit from VEG-05 Experiment Verification Test (EVT)

The VEG-05 EVT consisted of an initial fruit harvest completed on 7/19/22, an interim harvest completed on 7/26/22, and a final harvest completed on 8/4/22. For the first two harvests, tomatoes were obtained from twelve plants, with the final harvest consisting of fruit from eleven plants. The team performed standard food safety testing on all samples. All samples were processed for aerobic plate counts (APC), yeast and mold counts, coliform/E. coli, S. aureus, and Salmonella sp. All three harvests yielded fungal counts that fell below the detection limit. Detection limits vary due to individual sample weights but are all below 70 (the highest detection limit) colony-forming units per gram fresh weight (CFU/gfw) for yeast and mold and 7 for Coliform/E. coli and S. aureus. No Salmonella were detected. The highest counts were on the fruit from the 2nd harvest and the colony type indicated one species of Bacillus. This identification has not been confirmed.

VEG-05 Flight Operations Flight Preparation VEG-05 plant pillows were prepared at the end of October 2023. All pillows were packed and sewn with all materials sanitized appropriately (pillows and wicks were treated with ethylene oxide and allowed to offgas, substrates were sieved, washed with deionized (DI) water to remove dust, autoclaved, and oven dried. Seeds were planted in a laminar flow hood. After allowing seeds to dry, pillows were packed in bags and sealed for flight. They were then turned over for flight packing and placement on the rocket.

Veggie Light Mapping SpX-CRS-26 launched the VEG-05 payload on Nov. 26, 2022, and it docked on Nov. 27. The first VEG-05-related activity was the light meter activity. Here an astronaut used the light meter with a Photosynthetically Active Radiation (PAR) sensor to measure both the external ambient light in the Veggie units when the Veggie lights were off, as well as the light output for red, blue, and green lights at different settings at a 10 cm height and 5 positions on the baseplate in both Veggie units. From the data, steps were conducted to determine the appropriate light setpoints for the flight Veggie lights to get the same PAR output with the different light ratios of 90: 10 Red: Blue and 50: 50 Red: Blue. Using similar calculations and confirming measurements, ground settings for Veggie units were also determined. Using this approach, we were able to get an average PAR per unit of approximately 277-281 µmol/m2/s, which is lower than the planned intensity of 330 µmol/m2/s, but it was the highest level that could be obtained given the limitations of the flight hardware.

Flight and Ground Control Initiation Initiation of VEG-05 was started on 12/9/2022 but there were challenges. On 12/14/2022 all pillows were watered, and photos were taken, and the height was set, and the fans were turned on low. The lights were turned on at setpoints later that day. Lights were started later to have lights on during the night cycle running from 16:00-8:00 to keep the Veggie lights from interfering with other activities in Columbus. The ground control experiment was initiated on 12/16/2022. Plant pillows dried out much earlier than expected, and this drying was at a critical period during seed germination, when plants are most vulnerable. Emergency water was provided to try and rescue germination. This was partially successful in flight due to the behavior of water, and ultimately all but 3 flight pillows (9 of 12) had plants in them, though germination was delayed for most and few had more than 1 plant. Even though water was added more quickly on the ground, only 3 of 12 ground pillows ended up with plants and all were in the blue-rich treatment. The root cause of this premature drying was a low relative humidity event on the ISS starting at the end of DAI 2, which caused increased evaporation from the pillows and drying much earlier than anticipated. Due to the poor germination on the ground, after it was obvious that no additional ground plants would germinate, a decision was made to terminate the original ground control and re-run this asynchronously. Because in flight an extra 100 mL of water was added on day 9 to try and rescue the dry pillows, and due to the fluid physics differences between ground and flight, the restarted ground control was run at the same environmental conditions, but the additional water was split into multiple additions and dosed daily from DAI 3 until DAI 10 to get the pillows through the drying events. Following this action, we had plants germinate successfully in all plant pillows. The asynchronous ground control was restarted at the beginning of Feb. 2023 and ran through May, with successful growth of all 12 plants.

Both flight and ground control ran 100 days, with harvests of fruit at day 83, day 90, and day 100. Flight plants had uneven growth, and following the early drying events, excess water was frequently observed, which led to a variety of plant stress responses, including uneven plant growth, excess adventitious root formation, flower and fruit abortion, and visible microbial growth. This was likely due to overperformance and uncontrolled wicking from the root mat reservoir. While flight plants received approximately half the water that ground plants received, the uneven growth of these and conditions of the flight environment caused significant overwater stress to occur with flight plants. While ground control plants required water to both the root mat and plant pillows throughout their life after the initial root mat fill at DAI 45, flight plants required very little water added to pillows and primarily the root mat was used after DAI 45.

The root mat appeared to wick water to excess. Judging wetness was often difficult; and due to the length of the experiment, adding water daily to plant pillows was not practical, so the root mats continued to be used. In total, from the five-surviving red-rich lighted plants, only 5 ripe fruit were produced, and from the four-surviving blue-rich lighted plants, 10 fruit were produced, with only 6 of these ripe by day 100. Pillows were also collected and returned for analysis of roots, wicks, and substrates. Swab samples and pre- and post-growth water samples from the root mat were collected.

Because of the small fruit number and the unsatisfactory growth, crewmembers were not allowed to consume the tomatoes, and all fruit, as well as large branches with leaves, samples of the adventitious roots, two plant rooting pillows from each treatment, microbial sampling swabs, and some water samples were returned for analysis.

The restarted ground control had significantly better plant growth, primarily due to the ability of excess water to drain out of the plant pillows. Plants showed normal growth and fewer adventitious roots. All harvest dates had excellent fruit production. Interestingly, when compared to the yields from EVT, blue-rich plants produced a similar average fruit mass, but red-rich plants produced a much lower mass of ripe fruit.

Because of the small sample sizes and factors affecting growth on the ISS, objectives of assessing light quality effect (red: blue light treatments) will not be achieved. Revised objectives of this study include to compare stressed flight plants with normal ground plants to determine the impact of plant overwatering stress in space on food safety and the plant microbial community, to determine nutrient content changes in fruit and leaves from stressed plants, and to evaluate stress metabolism changes in returned tissue by transcriptomic analysis. Postflight analysis is underway. While not generating the desired information on spaceflight growth responses of healthy crops, our team is hopeful that these analyses will shed light on tomato responses to stress in this environment, as plant overwatering stress is a mission-relevant condition that could occur in future space crop growth systems.

Bibliography Type:	Description: (Last Updated: 10/26/2023)
Articles in Peer-reviewed Journals	Morsi AH, Massa GD, Morrow RC, Wheeler RM, Elsysy MA, Mitchell CA. "Leaf yield and mineral content of mizuna in response to cut-and-come-again harvest, substrate particle size, and fertilizer formulation in a simulated spaceflight environment." Life Sci Space Res. 2023 Sep 20. Online ahead of print. https://doi.org/10.1016/j.lssr.2023.09.005 , Sep-2023