| Fiscal Year:                                 | FY 2019  | Task Last Updated:                | FY 05/01/2020  |
|--|--|-----------------------------------|--|
| 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<br>Element/Subdiscipline: |  |                                   |  |
| Joint Agency Name:                           |  | TechPort:                         | No   |
| Human Research Program Elements:             | (1) <b>HHC</b> :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<br>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   | <b>Congressional District:</b>    | 8  |
| Comments:                                    |  |                                   |  |
| Project Type:                                | Flight   | Solicitation / Funding<br>Source: | 2013-14 HERO NNJ13ZSA002N-ILSRA.<br>International Life Sciences Research<br>Announcement |
| Start Date:                                  | 09/01/2015   | End Date:                         | 09/30/2021   |
| No. of Post Docs:                            | 0  | No. of PhD Degrees:               | 0  |
| No. of PhD Candidates:                       | 0  | No. of Master' Degrees:           | 3  |
| No. of Master's Candidates:                  | 5  | No. of Bachelor's Degrees:        |  |
| No. of Bachelor's Candidates:                | 9  | Monitoring Center:                | NASA JSC   |
| Contact Monitor:                             | Douglas, Grace   | <b>Contact Phone:</b>             |  |
| Contact Email:                               | grace.l.douglas@nasa.gov   |                                   |  |
| Flight Program:                              | ISS  |                                   |  |
| Flight Assignment:                           | NOTE: End date changed to 9/30/2021 per PI (Ed., 5/4/2020)<br>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:           | Fall 2019 report: Co-investigator (Co-I) Sandra Whitmire departed the project in Fall 2019, and Pete Roma was added as a Co-investigator for Behavioral Health and Performance (BHP). Diana Arias was added as a participant and test coordinator to support the BHP work. Jess Bunchek joined the project as a student intern in 2018 and was subsequently hired as a NASA contractor to continue working with this project. She was added as a Co-I. |                                   |  |

| COI Name (Institution):             | Douglas, Grace Ph.D. ( NASA Johnson Space Center )<br>Hummerick, Mary M.S. ( Amentum, Kennedy Space Center )<br>Mitchell, Cary Ph.D. ( Purdue Universitygrant NNX15AN78G )<br>Morrow, Robert Ph.D. ( Orbital Technologies Corporation )<br>Wheeler, Raymond Ph.D. ( NASA Kennedy Space Center )<br>Young, Millennia Ph.D. ( NASA Johnson Space Center )<br>Spencer, LaShelle M.S. ( Amentum, Kennedy Space Center )<br>Romeyn, Matt M.S. ( NASA Kennedy Space Center )<br>Roma, Peter Ph.D. ( KBR/NASA Johnson Space Center )<br>Buncheck, Jess M.S. ( Southeastern Universities Research Association, Kennedy 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<br>health promoting, bioavailable nutrients, enhance the dietary experience, and reduce launch mass as we move toward<br>longer-duration missions. However, studies of edible produce during spaceflight have been limited, leaving a significant<br>knowledge gap in the methods required to grow safe, acceptable, nutritious crops for consumption in microgravity. The<br>"Veggie" vegetable-production system on the International Space Station (ISS) offers an opportunity to develop a<br>"pick-and-eat" fresh vegetable component to the ISS food system as a first step to bioregenerative supplemental food<br>production. We propose growing salad plants in the Veggie unit during spaceflight, focusing on the impact of light<br>quality and fertilizer formulation on crop morphology, edible biomass yield, microbial food safety, organoleptic<br>acceptability, nutritional value, and behavioral health benefits of the fresh produce. Phase A of the project would<br>involve flight tests using leafy greens. Phase B would focus on dwarf tomato. Our work will help define light colors,<br>levels, and horticultural best practices to achieve high yields of safe, nutritious leafy greens and tomatoes to supplement<br>a space diet of prepackaged food. Our final deliverable will be the development of growth protocols for these crops in a<br>spaceflight vegetable production system.<br>Specific aim 1: Evaluate the effects of four light treatments and two different fertilizer compositions on the yield,<br>morphology, organoleptic acceptability, and nutritional attributes of leafy greens during flight-definition and flight<br>testing.<br>Specific aim 2: Perform cultivar selection and evaluate the effects of four different red: blue light treatments and two<br>different fertilizer compositions on the yield, morphology, organoleptic acceptability, and nutritional attributes of dwarf<br>tomato during ground and flight tests.<br>Specific aim 3: Perform hazard analysis, develop plans for minimi |
| 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 the development of a fresh food production capability on the International Space Station. Using the Veggie hardware we have been testing light and fertilizer combinations that will generate nutritious and appealing leafy green vegetables and dwarf tomatoes that astronauts can consume in a safe manner. The results of this research will be directly translatable 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 has the potential to 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 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. Our goal is to grow salad plants 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 the development of growth protocols for these crops in a spaceflight vegetable-production system. This will help reduce the risk and close the gap of inade  |
|                                     | Reporting Period: 08/31/2018-08/31/2019<br>Crop Testing   |
|                                     | Mizuna: After the VEG-04 experiment with mizuna was transitioned to become a plant pillow experiment in Veggie, ground testing was required to optimize fertilizer formulation and growth duration for this different growing system. It was decided to split the VEG-04 mizuna testing for flight into two tests, VEG-04A, lasting 28 days, and VEG-04B, a 56 day test with repeated harvests. Three ground tests were conducted the first a 28 day test to quickly narrow down fertilizer for VEG-04A preflight verification testing. The second and third tests were 56 day tests which further narrowed down the fertilizer and also the timing of harvests. The VEG-04A verification testing for flight proceeded in parallel with these tests. VEG-04A testing revealed issues of differential thermal heating through absorbance of visible radiation and re-radiation of infrared energy. The two light treatments selected for flight – 90% red: 10% blue plus green resulted in differential heating and water use in the plant pillows. This was mitigated by the addition of reflective plant pillow shades. Following fertilizer testing a change was made in the levels of fertilizer for fight. VEG-04A launched in Dec. 2018, and plants for that test were grown on ISS (with ground controls on a 52-hour delay) in June-July of 2019.  |

|                | Several preflight verification tests had to be conducted for VEG-04B, and it was found that previous ground tests in analog systems did not act as good analogs for Veggie and plants in Veggie showed much worse growth than they did in ground analogs under the same conditions. This required changes in levels of fertilizer, spacing from lights, horticultural procedures, and water applications when compared to planned treatments. Ground verification testing and a water use test helped to clarify methods changes and the VEG-04B plant pillows launched in July of 2019 for planned growth in September.  |
|----------------|---|
|                | During all preflight testing crew procedures and surveys were finalized, crew were consented, and preparations were made for the human subject aspects of the research. Additionally, ground samples were grown from the VEG-04A selected fertilizer, and organoleptic analyses of plants grown under different light settings were conducted. Analyses indicated that produce was acceptable to tasters, and there were no important changes in overall appeal over time, or between light treatments.   |
|                | Dwarf tomato: Tomato testing was generally put on hold as the PONDS (Passive Orbital Nutrient Delivery System) hardware was redesigned for better functionality in microgravity. One test was conducted to grow tomatoes in plant pillows of either 250 mL or 500 mL substrate capacity in Veggie. While tomato plants grew in both pillow sizes, only 50% of the tomato plants survived. Fruit were produced on remaining plants and roughly the same amounts of fruit were produced in both.  |
|                | HACCP plan development: A hazard analysis critical control point (HACCP) plan has been developed, based on baseline microbiological data and a risk assessment for crops grown in the Veggie. The HACCP plan consists of clarification of process step control points, identification of food safety hazards at this point, and determination of methods to reduce the hazard. The following seven points have been identified:   |
|                | 1. Ground processing of pillows/PONDS, where introduction of microbes via handling and materials could occur and a plan to sterilize components and aseptic technique while assembling will help mitigate this hazard.  |
|                | 2. Ground processing of seeds, where introduction of microbes via handling and indigenous microbes present on seeds could present a hazard and this can be mitigated by disinfection, certification of pathogen free seed, and use of sanitary handling practices.  |
|                | 3. Integration with the Veggie hardware, where introduction of microbes via handling could occur and use of sanitary handling will help mitigate this risk.   |
| Task Progress: | 4. Watering, where introduction of microbes via water supply or unsanitary handling is possible and can be mitigated by ensuring that water is potable quality and treated with biocide.  |
|                | 5. Growth of plants, where potential contamination from air and human presence, and an increase in indigenous flora due to availability of nutrients are possible risks, and use of sanitary handling and minimizing handling of plants before harvest will help mitigate this risk.  |
|                | 6. Harvest of crops, where introduction of microbes due to harvest procedures/human handling presents a risk, and sanitized instruments should be used, and gloves worn to mitigate it.   |
|                | 7. Post-harvest handling, where microbial presence established during plant growth may be introduced via handling, and crops should be sanitized before consumption following procedures to mitigate this. As well the Veggie facility should be thoroughly sanitized.  |
|                | Packing and transport of plant pillows and PONDS are not considered control points and no additional mitigation is needed for these steps. Data from verification and flight tests continue to be taken to validate these HACCP points and mitigation steps.  |
|                | Purdue University Research: During the present reporting period, our team members from Purdue University worked with Mizuna and 'Outredgeous' lettuce, two candidate salad-crop species. A ground-based Mizuna study was conducted with two main objectives: to investigate the effect of a cut-and-come-again procedure effect on biomass yield and mineral content of Mizuna over time, and to evaluate controlled-release fertilizer treatments for growing Mizuna under ISS conditions.   |
|                | During the study, two Nutricote fertilizer treatments were evaluated. Mizuna plants grown under the mix with T180, the slower release fertilizer, had higher yield during the first harvest. However, plants grown under the mix with T100 had a higher increase in yield during the second harvest and less decrease for the third harvest. So, the T100 mix ended up with higher total yield for the three harvests. The T100 mix gave an increase in micro-nutrients but decrease in macro-nutrients from harvest to harvest. The T180 mix gave an increase in P and Mg content, but a decrease in N, K, Na, Ca, and S, and an increase in micro-nutrients from harvest to harvest to harvest to harvest and the T180 mix-grown plants showed a higher level of Mn.  |
|                | A similar test was performed with 'Outredgeous' lettuce. Lettuce plants grown with the T100 mix had higher fresh weight than plants grown under the T180 treatment. We concluded that both Mizuna and lettuce grew better with a fertilizer mix with T100. Further studies are needed to understand the response of Mizuna to T180 fertilizer mix. At the end of each experiment, leachate samples were collected from each substrate for electrical conductivity (EC) measurements. Despite the higher yield for T100 fertilizer treatment, leachate samples indicated high EC for both T100 and T180. This led to an examination of the root environment to determine if it may limit fertilizer uptake. Substrate physical-properties including container capacity, air space, total porosity, and bulk density for different substrate combinations were measured. Analysis indicated that standard substrate physical properties can be met with the mix of 60%Turface: 40% Profile. |
|                | SNC ORBITEC Research: The SNC ORBITEC testing assessed a range of wick materials, wick configurations, wick processing steps, and seed-placement position to determine effectiveness for germinating seeds in Veggie plant pillows. Since a large number of plant pillows were not available for this assessment, a cup system mimicking a plant pillow was developed. The substrate formulation used inside the cups was the same as that used for the VEG-04B flight experiment.  |

Tested wicks were cut from a variety of materials, passed through a foam gasket and lid, and positioned in the cup while the substrate is filled and packed around each of the paired wicks (depending on the configuration used). 'Outredgeous'

|                                    | lettuce was used as a test species. Each cup was bottom watered in a controlled environment room under similar temperature and light to the ISS with slightly higher humidity and Earth-ambient CO2.  |
|------------------------------------|---|
|                                    | Wick material, wick configuration, and seed placement were evaluated. Five wick materials that should be safe for ISS were tested: Shamtastic (85% rayon and 15% olefin), Crew wipe (polypropylene), Synthetic gauze (polyester-rayon), Nomex (aramid polymer), and Capmat 2 (non-woven polyester). Most of these wicks, aside from the crew wipes, have an open fibrous structure. They are also thicker than the crew wipe wicks currently used in Veggie pillows. Wick configurations tested included 1) wicks cut flush to the foam gasket, 2) wicks cut so they protrude 2 cm above wick gasket, and with the two wick pieces spread at the base, and 3) wicks cut so they protrude 2 cm above the foam gasket, with the two wick pieces together at base. Seed position treatments included 1) placing the seeds at the midpoint of the gasket thickness, and 2) placing the seeds just below the gasket. Seedlings were thinned, and the germination rate was recorded. At 21 days, plants were assessed for any damage, measured to obtain height, and then harvested. Fresh and dry weights were collected from harvested plants. Wicks were assessed for salt accumulation and contamination. |
|                                    | In general, the crew wipe and synthetic gauze materials had the highest germination rates. Germination did not appear<br>to be impacted by wick configuration or by seed location. Plants grew best in the Crew Wipe wicks, closely followed by<br>Synthetic gauze wicks, and then by the Shamtastic wicks. The Nomex and CapMat 2 wicks showed consistently poor<br>growth. Plant growth did not appear to significantly differ due to wick configuration or seed placement. So far, the crew<br>wipe and synthetic gauze materials appear to perform better than the other wick materials tested. A long wick opening<br>away from the plant stem may be slightly better than a wick cut flush to the gasket. Salt deposits seemed to be more<br>apparent on the crew wipes, though this might be due to either the tight fiber configuration causing more salt deposition,<br>or this configuration just making salt deposition more visible. The Shamtastic material appeared particularly susceptible<br>to mold development and degradation than the other wick materials. For this reason we will not evaluate this wick<br>material in more detail.   |
|                                    | Upcoming assessments will include testing of additional wick materials. In addition, some seeds will be grown without wicks for comparison. Other wick treatments will include additional wick lengths and autoclaved wicks vs non autoclaved wicks to determine if this step helps reduce mold and algae growth.   |
| Bibliography Type:                 | Description: (Last Updated: 07/26/2024)   |
| Articles in Peer-reviewed Journals | Mickens MA, Torralba M, Robinson SA, Spencer LE, Romeyn MW, Massa GD, Wheeler RM. "Growth of red pak choi under red and blue, supplemented white, and artificial sunlight provided by LEDs." Sci Hortic (Amsterdam). 2019 Feb;245:200-9. Epub 2018 Oct 22. <u>https://doi.org/10.1016/j.scienta.2018.10.023</u> , Feb-2019  |
| Articles in Peer-reviewed Journals | Mickens MA, Skoog EJ, Reese LE, Barnwell PL, Spencer LE, Massa GD, Wheeler RM. "A strategic approach for investigating light recipes for 'Outredgeous' red romaine lettuce using white and monochromatic LEDs." Life Sci Space Res (Amst). 2018 Nov;19:53-62. Epub 2018 Sep 18. <u>https://doi.org/10.1016/j.lssr.2018.09.003</u> ; PubMed <u>PMID: 30482283</u> , Nov-2018   |
| Papers from Meeting Proceedings    | <ul> <li>Burgner SE, Mitchell C, Massa G, Romeyn MW, Wheeler RM, Morrow R. "Troubleshooting Performance Failures of<br/>Chinese Cabbage for Veggie on the ISS." 49th International Conference on Environmental Systems, Boston, MA, July<br/>7-11, 2019.</li> <li>49th International Conference on Environmental Systems, Boston, MA, July 7-11, 2019. ICES paper ICES-2019-328. ,<br/>Jul-2019</li> </ul>  |
| Papers from Meeting Proceedings    | Romeyn MW, Spencer LE, Massa GD, Wheeler RM. "Crop Readiness Level (CRL): A Scale to Track Progression of Crop Testing for Space." 49th International Conference on Environmental Systems, Boston, MA, July 7-11, 2019. 49th International Conference on Environmental Systems, Boston, MA, July 7-11, 2019. ICES paper ICES-2019-342. , Jul-2019   |