Fiscal Year:	FY 2024	**Task Last Updated:**	FY 10/16/2023
PI Name:	Schuerger, Andrew Ph.D.		
Project Title:	Microgravity Can Down-Regulate Host Resistance and thus May Up-Regulate Plant Disease Development in Space		
Division Name:	Space Biology		
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Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
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Space Biology Cross-Element Discipline:	None		
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Zip Code:	321-261-3774	**Congressional District:**	8
Comments:			
Project Type:	FLIGHT	**Solicitation / Funding Source:**	2020 Space Biology NNH20ZDA001N-SB E.12. Flight/Ground Research
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No. of Post Docs:	1	**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 KSC
Contact Monitor:	Massa, Gioia	**Contact Phone:**	321-861-2938
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Flight Program:			
Flight Assignment:			
Key Personnel Changes/Previous PI:	Dr. Natasha Haveman, University of Florida (UF), has left the project and joined a NASA Kennedy Space Center (KSC) Space Biology Team in 2023. The funds for Dr. Haveman were transferred to Dr. Rob Ferl at UF. Dr. Vicken Aknadibossian (at UF) was selected to replace Dr. Haveman. In addition, a new Biological Scientist 1 (Ms. Kylee Soltez) was hired in Schuerger's lab to assist the project.		
COI Name (Institution):	Ferl, Robert Ph.D. (University of Florida, Gainesville) Paul, Anna-Lisa Ph.D. (University of Florida, Gainesville) Reed, David M.S. (Techshot, Inc.) Aknadibossian, Vicken (Dept. of Horticulture)		
Grant/Contract No.:	80NSSC22K0209		
Performance Goal No.:			
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Task Description:	Space-faring nations are utilizing small plant-growth payloads in microgravity (micro-g) to develop the knowledge and technology infrastructures to advance the development of food production systems on other planetary bodies. As the use of small plant-growth payloads in micro-g continues, plant disease outbreaks will increase over time, once the systems are integrated into the open-air microbiomes of spacecraft. This situation presents an opportunity to address directly Section 2.3.2.B of NASA Solicitation 2020 Space Biology NNH20ZDA001N-SB E.12. Flight/Ground Research – the combined effects of various space-relevant stressors – in a manner that further enables exploration. A solid literature base exists that indicates that plant host resistance is down-regulated in micro-g and includes studies that describe decreased cell wall rigidity, cell wall thickness, cellulose and matrix polysaccharides, lignin, and altered host-resistance gene pathways in micro-g. An equally solid literature base indicates that microbial virulence may be up-regulated in microgravity and includes up-regulation of virulence in microbe/microbe, microbe/insect, and pathogen/plant interactions. However, no data exists on the interactions of a foliar phytopathogen on a plant host with concomitant host-resistance transcriptomics data. The alternative hypothesis (Ha) for the International Space Station (ISS)-flight experiment is: Microgravity Can Down-Regulate Host Resistance and thus May Up-Regulate Plant Disease Development in Space. Results will fill key knowledge gaps into how plant diseases and host resistance are affected by micro-g. Proposed here is a novel flight experiment that will study the development of a foliar plant pathogen (i.e., phytopathogen) on the well-studied, Arabidopsis thaliana (At) host. The phytopathogen – Golovinomyces cichoracearum (Gc), a powdery mildew fungus - on A. thaliana is a well-studied pathosystem. The Gc/At pathosystem is chosen here because (i) both Gc and At are sequenced and annotated, (ii) extensive literature is available on host-resistance in At, (iii) diverse At cultivars with differential expression of easily measured host resistance mechanisms are available, (iv) most stages of the Gc life-cycle are on external surfaces of leaves and can be easily observed, (v) the expected ease of sanitizing flight hardware, and (vi) maximized crew safety on the ISS because Gc has no established interaction with humans. We will use the Multi-Use Variable-Gravity Platform (MVP) facilities built by Techshot, Inc. (2 units are in orbit on the ISS) to investigate the development of Gc on leaves of At. [Ed. Note: Techshot, Inc. was acquired by Redwire Corporation in November, 2021.] Each MVP has two independently controlled centrifuge rotors fitted with up to 4 Phytofuge plant-growth modules that will be rotated at 1g or left stationary in micro-g. Each Phytofuge unit has three separate petri dishes with light-emitting diode (LED) illumination and an internal camera. Seed of three cultivars of At will be (1) sown onto growth media in independent petri dishes, (2) held dormant for up to 30 days, and (3) once in orbit, one-half of the petri dishes will be inoculated with the powdery mildew phytopathogen Gc. The aerial mycelia, conidiophores, and spores of Gc will be allowed to develop for 5-7 days and then leaves harvested for two separate research pipelines. First, half of the healthy and half of the infected At plants will be fixed in glutaraldehyde and stored at 4C until processed on the ground for fluorescent staining, Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM) studies into the process of host infection. Second, the remaining healthy and infected plants will be frozen at -80C and later processed for transcriptomics of host-resistance genes. Results will inform future horticulturists, space engineers, and technologists of the risks of maintaining plant-host resistance in space when challenged by an airborne phytopathogen. The results will also assist in the design of future plant-growth modules for crewed missions to the Moon and Mars.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Plant disease development in space has profound impact on the future of human exploration of the Solar System. Currently the assumption is that plants grown in space-based bioregenerative life support systems (BLSS) modules will develop normal plant-resistance mechanisms to exposure of biological agents (e.g., bacteria, fungi, viruses). If disease resistance is "normal" in space-based BLSS modules, the use of crops for food, oxygen, and water recycling will be a viable option for crewed habitats on the Moon and Mars. In contrast, if plant diseases develop more quickly in space than on Earth, new and unique plant production protocols may have to be developed. The research outlined in this project seeks to identify if "plant resistance" against a fungal phytopathogen in microgravity progresses normally in the mustard plant, Arabidopsis thaliana. The fungal pathogen has the general name of "powdery mildew", but the species name is Golovinomyces cichoracearum. Powdery mildew phytopathogens have no proven disease risk to humans, and thus, there is no health risk to the astronauts on the International Space Station (ISS) during the flight experiment. The hypothesis being tested here is: Microgravity can down-regulate plant host resistance, and thus, may up-regulate plant disease development in space. The primary benefit to Earth-based agriculture will be to identify how disease resistance mechanisms operate under the unusual conditions of microgravity. Results may identify how to improve disease resistance in field crops on Earth.
Task Progress:	The plant pathosystem to be tested in the International Space Station (ISS) flight experiment is the fungal powdery mildew (PM) phytopathogen Golovinomyces cichoracearum (Gc) infecting leaves of Arabidopsis thaliana (At). The Gc/At Team has successfully caught up with several lagging lines of protocol development (see below), and we believe we are in a good position to schedule the Science Verification Test (SVT) and Experiment Verification Test (EVT) in the 1st and 2nd quarters of 2024. The three criticality-1 activities that must be successfully completed before developing an Experiment Requirements Document (ERD) document for the SVT are: 1) Complete a successful grow out of Col-0 and pmr4 At lines in the Phytofuge modules. As of this writing, the Phytofuge #1 test was started on 11-Oct-2023. 2) Develop a spore applicator that successfully delivers adequate conidia to the leaves of both At lines within the Phytofuge petri dishes. 3) Develop a 4°C storage protocol for Gc conidia that will retain high viability for up to 28 days for maximum flexibility in conducting the ISS flight experiment. The Gc/At Team is in a good position to complete a series of ongoing protocol development experiments that will permit

us to create the ERD no later than 31-Jan-2024. We have two working flight-rated Phytofuge units in the University of Florida Space Life Science Lab (SLSL) that will be used to verify that the At lines of choice – Col-0 (susceptible) and pmr4 (resistant) – can grow nominally in the flight hardware and allow nominal development of the Gc phytopathogen on inoculated leaves. The Phytofuge tests [ongoing] and other protocol development assays will be completed before 15-Dec-2023.

We are on track to move forward with SVT planning no later than 02-Jan-2024.

Bibliography Type:	Description: (Last Updated:)