Fiscal Year:	FY 2023	**Task Last Updated:**	FY 03/02/2023
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Project Title:	Spaceflight Effects on Plant-Microbe Interactions		
Division Name:	Space Biology		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	(1) Cell & Molecular Biology (2) Microbiology (3) Plant Biology		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Comments:	NOTE: PI formerly at Pennsylvania State University; moved to University of Wisconsin-Madison in 2007 (Info received 7/2009)		
Project Type:	Flight,Ground	**Solicitation / Funding Source:**	2018 Space Biology (ROSBio) NNH18ZTT001N-FG2. App D: Flight and Ground Space Biology Research
Start Date:	04/01/2021	**End Date:**	03/31/2024
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No. of PhD Candidates:	1	**No. of Master' Degrees:**	1
No. of Master's Candidates:	1	**No. of Bachelor's Degrees:**	
No. of Bachelor's Candidates:		**Monitoring Center:**	NASA KSC
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Flight Program:	ISS		
Flight Assignment:			
Key Personnel Changes/Previous PI:	NA		
COI Name (Institution):	Bakshi, Arkadipta Ph.D. (University of Wisconsin, Madison) Swanson, Sarah Ph.D. (University of Wisconsin, Madison) Barker, Richard Ph.D. (University of Wisconsin System) Hanson, David Ph.D. (University of New Mexico)		
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Task Description:	This proposal seeks to address: (1) how spaceflight modulates the interactions between plants and microbes and (2) how well microgravity analogs capture the events elicited by the spaceflight environment. Tomato plants will be grown on orbit in the NASA Vegetable Production System (Veggie) hardware on board the International Space Station (ISS) with and without the beneficial rhizosphere microbe Trichoderma hazianum. A third sample will be of this microbe growing under identical conditions on the ISS but without the plants. These samples will be compared to parallel ground controls at 1 x gravity as well as to samples growing on 1-axis and 3D clinostats. Assays will integrate RNAseq-based transcripomics and ionomics (nutrient uptake and distribution) alongside biochemical measures of photosynthesis and stress. These comparisons will provide measures of both how spaceflight affects the plant, the microbe, and the relationship between these organisms and additionally, how well microgravity analogs can reproduce these kinds of events on the ground. In addition, the omics-level data gathered from this study will be compared to the wealth of spaceflight-related omics data available through the GeneLab data repository. Using an approach of orthologous matrix mapping will allow identification of similar genes between diverse species and so allow for comparisons of, for example, the degree of similarity between patterns of gene expression to be compared between different species. Overall this research will help define how spaceflight may modulate plant, microbial, and plant-microbe responses and help understand whether defined beneficial microbes may provide a countermeasure to the deleterious effects of spaceflight on plants. The work will capitalize on the complementary expertise of two groups: the Gilroy lab team (plant spaceflight, transcriptomics) and the Hanson lab (biochemistry, photosynthesis).
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	This proposed research seeks to address how spaceflight modulates the interactions between plants and microbes using tomatoes and the beneficial soil microbe Trichoderma harzianum. The microbiome around the plant root is recognized as a crucial element in the productivity and hardiness of plants but the complex interactions and chemical signals that occur between plant and microbe have only recently begun to be dissected. Yet, for example, T. harzianum is used as a commercial biostimulant, being added to the soil to pormote plant growth and vigor. The research in this project will further define the molecular components of the interaction between plant root and this fungus and how spaceflight alters these events. Thus, the work will not only provide insight into how plant-microbe interactions are affected by spaceflight but address whether T. harzianum might be used as a biostimulant to counteract some of the stresses of spaceflight on plant growth. This research will also help provide molecular insight into how these interactions occur on Earth. Such a fuller understanding of plant-microbe interactions, especially of T. harzianum-plant root communications and response will be important steps towards optimizing these beneficial interactions and so increasing plant productivity in both space and on Earth.
Task Progress:	I. OVERVIEW This research seeks to address: (1) how spaceflight modulates the interactions between plants and microbes and (2) how well microgravity analogs capture the events elicited by the spaceflight environment. Tomato plants will be grown on orbit in the NASA Vegetable Production System (Veggie) hardware on board the International Space Station with and without the beneficial rhizosphere microbe Trichoderma harzianum. These samples will be compared to parallel ground controls at 1 x gravity as well as to samples growing on 1-axis and 3D clinostats and on random positioning machines. Planned assays integrate RNAseq-based transcriptomics alongside biochemical measures of photosynthesis and stress. These comparisons will help define how spaceflight affects the plant, the microbe, and the relationship between these organisms, and additionally, how well microgravity analogs can reproduce these kinds of events on the ground. II. PROGRESS: Science and Experiment Verification Tests. The major focus for work in the reporting period has been to define protocols and analyses through a Science Verification Test (SVT) and Experiment Verification Test (EVT). The SVT was completed in June of 2022 and EVT in October 2022. Both tests were successful with a rating of excellent across all of the experiment's success criteria. Overview of procedures: The combination of SVT and EVT have allowed definition of experiment procedures for the flight experiment. Briefly, seeds, Trichoderma spores, or seeds inoculated with Trichoderma spores will be planted on 12 cm square Petri dishes containing gel growth medium supplemented with ½ strength Linsmaier and Skoog salts. A polyester mesh (1 mm mesh size) supported by a custom rim 3d printed in polyethylene tetraphthalate will be laid on the gel surface prior to planting. This aids in subsequent harvest of plants and fungal hyphae as the crew can lift off the mesh with all samples attached and intact for further processing. After planting, germination will be delayed for pre-flight operations by holding the plates at 4 °C. The samples will then be installed in the Veggie growth chamber and grown for 14 days with photography every other day. At 14 days, the tomato seedling roots are close to the bottom of the plate, and this growth feature defines when harvest will occur. The plates are then opened and mesh with samples attached is removed, wrapped in foil, and snap frozen between aluminum bricks conditioned to -160 °C. These samples are then stored at -80 °C until analysis. Current work is aimed to modify the harvest procedures to allow them to operate inside the Life Science Glovebox. This approach is to provide an extra level of containment on orbit. It is important to delay germination of both the tomato seeds and the Trichoderma spores before insertion into the Veggie on orbit. Testing has revealed that germination of both the tomato and Trichoderma is delayed by storage in the dark at 4 °C. This approach proves to be effective for 2 weeks, at which point the Trichoderma spores begin to germinate and grow. Once installed in the Veggie at room temperature, the seeds and Trichoderma geminate and grow until the plants fill the 12 cm Petri plate growth space after 14 days. At this point, the plants are well developed with true leaves and highly branched root systems and the Trichoderma colonies have grown to cover ~ 50% of the growth area. Analysis of the images through the growth time course shows that inoculation with Trichoderma alters both growth of the primary root and branching patterns, as predicted from previous research on this interaction. Biochemical Assays: In addition to growth analysis from the imaging described above, the frozen samples have been successfully assayed for chlorophyll and carotenoid content (as a measure of photosynthetic capacity), accumulation of anthocyanin (as a general stress marker) and levels of malondialdehyde, (as a biochemical marker for oxidative stress). Testing of all of these assays has been highly successful in both the SVT and EVT. RNA quality and quantity: One major aim of this research is to perform transcriptional profiling of the samples using RNA-seq. To test how well the samples perform for these kinds of assays, quantitative polymerase chain reaction

	(qPCR) has been used as a proxy for full RNA-seq analyses. Frozen samples provided material for isolation of RNA of sufficient quantity and quality (average A260/280 of 2.00) to separate root and shoot samples (average of 3.6 µg RNA/root or shoot sample/plate) and analyze them for quantitative PCR. Three representative genes were assessed in SVT and EVT as proof-of-concept that the nucleic acid will be suitable for broad-scale molecular analyses: PRP1b is a pathogenesis-related protein that is known to be induced in response to successful Trichoderma infection of the plant roots, HSP22 is a heat shock protein that is a marker for oxidative stress responses, and NT is a nitrate transporter that should be unresponsive to Trichoderma infection. The reference gene for the qPCR analysis was Elongation Factor 1-alpha. These genes were successfully analyzed and preliminary analyses show significant elevation of PRP1b and HSP22 in plants treated with Trichoderma, whereas NT was unaffected. Stable C-isotope analysis: In order to monitor photosynthetic parameters such as water use efficiency, stable isotope analysis is also planned to be used. To test the sampling method for these analyses, 4 gas samples were taken of the ambient atmosphere in the vicinity of the Veggie using Silonite MiniCans, with 2 samples at the start (day 0) of the experiment and 2 at the end (day 14). These samples were shipped to the University of New Mexico for analysis by the Hanson lab. All 4 samples were successfully analyzed, showing an average 12C:13C CO2 isotopic ratio of 25.6 +/- 4.6 per mil. These provide the baseline atmospheric measurements for use in modeling from tissue stable isotope content.
Bibliography Type:	Description: (Last Updated: 02/22/2025)
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