Fiscal Year:	FY 2022	**Task Last Updated:**	FY 06/01/2022
PI Name:	Gilroy, Simon Ph.D.		
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
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No. of Post Docs:	2	**No. of PhD Degrees:**	
No. of PhD Candidates:		**No. of Master' Degrees:**	
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)		
Grant/Contract No.:	80NSSC21K0577		
Performance Goal No.:			
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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. DEFINING FLIGHT PROTOCOLS The major focus for work in the reporting period has been to optimize and define flight procedures and protocols. Extensive testing revealed that 12 cm square Petri dishes offered the required balance between plant and fungal growth. Harvesting of both tomato and fungal samples has also been optimized. The plants/fungus are grown on the surface of a nutrient gel. However, removing the plant and associated fungal hyphal mat intact was initially challenging, as the fungus adhered to the growth matrix. A series of surface substrates was therefore tested before settling on 1 mm pore size cotton fiber mesh, which allowed both normal plant growth and the lifting of the whole plant and fungal samples from the gel surface intact. The large pore size of this mesh likely provides enough area of direct contact between the plant roots and the Phytagel growth medium below the cloth to sustain "normal" plant growth. The mesh was laid on the nutrient gel (1/2 strength LS medium with 1% Phytagel) surface and then seeds were placed on this layer and the plants grown along the surface. This approach allowed the whole plant, with Trichoderma attached, to be harvested by peeling the cotton substrate off of the surface of the gel. These samples were then snap frozen by placing the entire sample in a foil bag and rapidly freezing using aluminum blocks conditioned to -160°C. Delaying germination: Cold temperatures were optimized to delay germination of both the tomato seeds and the Trichoderma spores before insertion into the NASA Vegetable Production System (Veggie) in orbit. Germination of both the tomato and Trichoderma is delayed by storage in the dark at 4°C for 2 weeks. At this time point, the Trichoderma (but not the tomato seeds) begins to germinate and grow, setting the limit on the timing of storage prior to initializing the experiment in the Veggie. Confirmation of fungal inoculant: Sequencing data from samples of the fungal isolates to be used from 2 diagnostic genes (ITS1/2 and TEF1) was BLASTed against both the National Institutes of Health (NIH) sequence databases and using the species identification tools at Trichokey.com used to confirm the fungal inoculant as T. harzianum. RNA quality and quantity: Initial testing indicates a single 3-week-old tomato seedling from each plate will provide > 1µg of RNA of sufficient quality to analyze the required separate root and shoot samples by both RNA-seq and quantitative (polymerase chain reaction) PCR tests. II. GROUND-BASED CHARACTERIZATION In parallel with the efforts to define flight procedures, the interaction between T. harzianum and both tomato and Arabidopsis plants has been further characterized. A key observation is that, as proposed by several groups, the growth promoting factor of the interaction between plant and fungus seems to be delivered by volatiles from the fungus. Thus, increased growth and stress resilience in the plants have been evident when the Trichoderma is confined away from the plant using a spit plate system (i.e., plates with a divider separating fungal growth media from the plants). Unexpectedly, growth of the plant roots has been seen towards the fungus and fungus towards the plants, suggesting that the plant root may also be releasing volatiles that are detected by the fungus. Defining the potential mechanism of both of these directional growth responses is a key goal for the coming year as, at a practical level, it may be possible to grow the fungus remotely from direct contact with the plant and/or just deliver the fungal volatiles but still gain the beneficial effects on plant growth. III. PRESENTATIONS AND OUTREACH These spaceflight-related projects have been presented at multiple outreach events at venues -- ranging from colleges and universities such as Elizabethtown College, Oregon State University, and the Instituto de Biologia Experimental e Tecnologica in Oeiras, Lisbon, Portugal -- to outreach to the general public at events such as the University of Wisconsin’s Science Expeditions, the Friends of Allen Centennial Garden and Madison Master Gardeners, and the Sidmouth Science Festival in the UK, as well as to school students as part of "Space Camp" at the Deke Slayton

	Memorial Space and Bike Museum in Sparta, WI.
Bibliography Type:	Description: (Last Updated: 04/23/2024)
Articles in Peer-reviewed Journals	Bakshi A, Gilroy S. "Moving Magnesium." Mol Plant. 2022 May 2;15(5):796-98. http://doi.org/10.1016/j.molp.2022.04.005 ; PMID: 35422405 , May-2022
Books/Book Chapters	Barker R, Johns S, Trane R, Gilroy S. "Analysis of Plant Root Gravitropism." in "Environmental Responses in Plants. Methods in Molecular Biology series." Ed. P. Duque, D. Szakonyi. New York, NY: Humana, 2022. https://doi.org/10.1007/978-1-0716-2297-1_1 , Apr-2022