Fiscal Year:	FY 2021	**Task Last Updated:**	FY 06/22/2021
PI Name:	Iyer-Pascuzzi, Anjali Ph.D.		
Project Title:	Effect of Spaceflight and Simulated Microgravity on Plant Defense Responses		
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) Microbiology (2) Plant Biology		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Comments:			
Project Type:	FLIGHT,GROUND,New Investigation	**Solicitation / Funding Source:**	2018 Space Biology (ROSBio) NNH18ZTT001N-FG. App B: Flight and Ground Space Biology Research
Start Date:	09/01/2019	**End Date:**	08/31/2022
No. of Post Docs:	0	**No. of PhD Degrees:**	0
No. of PhD Candidates:	1	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	1	**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:	June 2021 report: No changes to personnel. June 2020 report: No changes to personnel.		
COI Name (Institution):	Sparks, Erin Ph.D. (University of Delaware)		
Grant/Contract No.:	80NSSC19K1495		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	As on Earth, in space plants must defend themselves against microbial and fungal pathogens. For example, plants grown on the International Space Station (ISS) recently became diseased from the fungal pathogen Fusarium oxysporum. Although previous studies have demonstrated that plant defense pathways are altered by spaceflight when plants were grown in sterile conditions, fundamental knowledge of how the plant immune system responds to microbes or defense elicitors during spaceflight is lacking. This knowledge is critical to growing plants that can withstand the rigors of long duration spaceflight, and is particularly important in a horticultural crop that will provide nutrition to the space crew. In this proposal, we will perform the following objectives: 1) Investigate physiological and whole genome transcriptional responses to defense activation in wild-type and immune-deficient tomatoes during spaceflight. Tomatoes will be grown in the Advanced Plant Habitat (APH). We will activate defense responses with a chemical elicitor. At 24 and 48 hours after defense activation, we will harvest tissue and subsequently perform next-generation sequencing to identify genome-wide transcriptional defense responses. In addition, we will use next-generation sequencing to examine the transcriptional response to spaceflight in immune-deficient tomatoes. All plants will be imaged daily to understand the impact of spaceflight on growth rates of immune-activated and immune-deficient tomatoes. All experiments will be performed in parallel on the ground. 2) Determine whether colonization of tomato by the fungal plant pathogen Fusarium oxysporum is impacted by simulated microgravity. We will grow plants in a 2D-ground-based microgravity simulator and inoculate them with Fusarium oxysporum. We will assess tomato plant colonization using histological techniques. This work will generate key fundamental knowledge of plant-microbe interactions that is important for understanding plant production in space. It is consistent with the goal of the Plant Biology Element in the Space Biology Science Plan 2016-2025.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	On Earth, plant pathogens cause upwards of 20–40% global crop loss from disease. Microbes, including plant pathogens, have been isolated from the International Space Station (ISS), and thus disease loss also threatens crop production in space. Spaceflight studies are important to life on Earth because these studies have the potential to uncover new interactions between gravity and plant responses to pathogens. For example, the plant hormone auxin is known for its critical roles in plant development and defense. Recent experiments on the ISS revealed novel interactions between gravity and auxin. This new information will be informative for developing new plant disease resistance strategies on Earth and will enhance our ability to grow crops on Earth.
Task Progress:	Task Progress 09/01/20 – 08/31/21 Specific Aim 1: Determine the effect of spaceflight on genome-wide transcriptomic and physiological defense responses in tomatoes. This year, work on Specific Aim 1 continued to focus on optimizing protocols and documents necessary for tomato growth in the APH during spaceflight. The following was accomplished: i) Optimized conditions for tomato growth in the NASA Science Carrier. Last year, we optimized conditions (fertilizer, media) for tomato growth, using similar substrates as in the APH. This year, we used the NASA science carrier to ask the following questions: a. Can we grow healthy tomato plants in the science carrier with NASA substrates? We tested these conditions using a science carrier from NASA and two genotypes of tomato, Moneymaker and NahG, that will be used in spaceflight. We used NASA protocols for seed sterilization and planting. Results were good and we obtained 100% germination for the Moneymaker tomato variety, and nearly 100% for the NahG. These experiments support our ability to grow tomato plants in the APH during spaceflight. Tomatoes are grown at 16/8 hour day length, with 25ºC day/night. Currently, we are optimizing the LED (light-emitting diode) lighting conditions for germination. b. Will seeds still germinate if they are watered several weeks and months after planting? Because the experiment may need to wait for several weeks or months once at the ISS, we tested whether our seeds would continue to germinate if watered months after planting. We planted four quadrants in the science carrier, and watered just one for each of four months. We had nearly 100% germination rates even 4 months after planting. c. What is the best growth configuration for the experiments? We found that planting three tomato seeds in each row of each quadrant gave us sufficient experimental replicates, and allowed sufficient leaf growth while not overcrowding neighbor plants. ii) Developing a safe spaceflight protocol for defense elicitation in tomato leaves. The goal of specific aim 1 is to investigate the impact of spaceflight on defense responses during spaceflight. To address this, we will elicit defense responses in space using a chemical elicitor. Last year, we started to test methods of treating tomato leaves with an elicitor. The method needs to work well in space and be easy to perform in a timely manner. In addition, we initiated experiments to test whether the chemical will elicit defense responses in tomatoes grown in the conditions in (i). At the end of last year, we found that swabbing tomato leaves with 5 mM salicylic acid (SA) using a Q-tip was able to elicit expression of a SA response gene. However, the response was not as robust as we had hoped. We hypothesized that this could be due to the side of the leaf that had been swabbed, or the amount of SA applied from the Q-tip. This year, we aimed to optimize this. We searched for a better SA application method; we tested applying SA to different sides of tomato leaves, we tested different leaf ages, and we tested higher concentrations of SA. We identified a swab from Fisher that worked well. We found that applying 7.5 mM SA to top and bottom of leaf #4 consistently activated the SA marker gene PR1-a. In contrast to 10 mM SA, 7.5 mM SA was not toxic to leaves. iii) Testing whether leaves stored at -20º or -80ºC freezer would yield similar quality RNA. Tissue for RNA extraction is typically stored in -80ºC conditions. However, because -80ºC space is limited on the ISS, last year we initiated testing whether tissue stored at -20ºC would yield as high quality RNA as that stored in a -80ºC freezer. Last year, we found that storing tissue for one month did not alter the RNA quality. This year, we tested this up to four months in storage and found no change in RNA quality.

	iv) Test whether SA that has been stored at RT for several months still elicits defense gene expression. Once in space, the experiment may not be initiated for several weeks or months. We tested whether SA stored at RT for one month was still able to elicit PR-1a gene expression. Results were positive, and we are continuing this year to test SA that has been stored for 3 and 6 months. Specific Aim 2: Investigate how simulated microgravity affects fungal colonization of tomato plants. In Aim 2, we proposed a series of ground-based experiments to investigate the impact of simulated microgravity on the ability of fungal pathogens to infect tomato roots. In the first year of this project, we redesigned the 2D clinostat to accommodate eight plants as opposed to the original four plant design. Using our redesigned system, we showed that we could grow tomato cultivars to maturity under continuous clinorotation. In Year 1, we also acquired the USDA (U.S. Department of Agriculture) permits for Fusarium oxysporum. For Year 2, we have successfully demonstrated the infection of Moneymaker tomato plants by Fusarium oxysporum in the enclosed rhizoboxes. We are currently testing different inoculation strategies to ensure reproducible disease progression under clinorotation. In addition, we built a second clinostat to enable the simultaneous analysis of plants under clinorotation perpendicular and parallel to the gravity vector. A methods paper is preparation describing the clinostat design and build based on this process.
Bibliography Type:	Description: (Last Updated: 06/22/2021)
Abstracts for Journals and Proceedings	Insley N, Iyer-Pascuzzi AS, Sparks EE. "The Effects of Simulated Microgravity on Plant Defense Responses." Presented at the 15th Delaware Space Grant Symposium, April 12, 2021. Delaware Space Grant Symposium, April 12, 2021. , Apr-2021