Fiscal Year:	FY 2022	**Task Last Updated:**	FY 07/01/2022
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
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No. of Post Docs:	0	**No. of PhD Degrees:**	0
No. of PhD Candidates:	2	**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:	NOTE: End date changed to 08/31/2023 per NSSC information (Ed., 6/6/22).		
Key Personnel Changes/Previous PI:	June 2022 report: No changes to personnel. June 2021 report: No changes to personnel. June 2020 report: No changes to personnel.		
COI Name (Institution):	Sparks, Erin Ph.D. (University of Delaware)		
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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.
	Scientific Goals and Objectives of the Proposed Research (2019 – 2022; extension until 2023 because flight has not launched yet) The central hypothesis of this proposal is: ‘Spaceflight and simulated microgravity increase tomato susceptibility to pathogens through altered transcriptional defense responses and increased pathogen colonization.’ The goals of this proposal are twofold: 1) gain fundamental insights into how the activated plant immune system responds in space in a horticultural crop, and 2) gain knowledge of the impact of simulated microgravity on plant colonization and disease development by a pathogenic fungus. To accomplish these goals, we have two Specific Aims: Specific Aim 1: Determine the effect of spaceflight on genome-wide transcriptomic and physiological defense responses in tomatoes. Aim 1 Hypothesis: In response to a defense elicitor, spaceflight-grown wild type (WT) plants will have delayed transcriptional defense responses compared to ground controls. Spaceflight grown immune-deficient plants will have altered transcriptional responses and slower plant growth rates compared to ground treated and untreated plants. To test our hypothesis, we will use RNA-seq analysis to investigate genome-wide defense responses before and after treatment with a defense elicitor in wild type and immune-deficient tomatoes during spaceflight. Differential gene expression will be examined in wild-type tomatoes +/- the defense elicitor and in immune-deficient tomatoes compared to wild-type. We will also monitor growth rate of tomatoes using cameras in the APH before and after treatment with the defense elicitor. Specific Aim 2: Investigate how simulated microgravity affects pathogen colonization of tomato plants. Aim 2 Hypothesis: Simulated microgravity will cause earlier and increased colonization of tomato by the plant fungal pathogen Fusarium oxysporum. To test our hypothesis, tomato plants will be grown in simulated microgravity conditions using a 2D clinostat at the University of Delaware, the Co-I’s institution. As a control, tomatoes will be grown parallel to the gravity vector while rotating clockwise. Tomato plants at the 3-leaf stage will be inoculated with Fusarium oxysporum or water as a control. At 10, 14, and 18 days post inoculation, plants will be removed from the clinostat and a 2 cm section below the root shoot junction will be harvested. Samples will be sent to Purdue for processing and microscopy to observe F. oxysporum colonization within the root tissues. Summary of research accomplishment 09/01/21 – 08/31/22, year 3 Specific Aim 1: Determine the effect of spaceflight on genome-wide transcriptomic and physiological defense responses in tomatoes. i) Optimized conditions for tomato growth in the NASA Science Carrier This year (year 3), work on Specific Aim 1 focused on finalizing protocols necessary for tomato growth in the APH during spaceflight. In years 1 and 2, we optimized conditions (fertilizer, media) for tomato growth, using similar substrates as in the Advanced Plant Habitat /APH (year 1), and in the NASA science carrier (year 2). In year 3, we identified proper light conditions using a MARS hydro system and the NASA science carrier. We, and the NASA team, subsequently used these conditions to conduct pre-Science Verification Test (SVT) tests at NASA to test our salicylic acid (SA) treatment and leaf harvest protocol. The first test was conducted in December of 2021. Plants grew well and RNA integrity (RIN) values of the extracted RNA were mostly acceptable, and there were challenges with SA application and leaf harvest. We therefore conducted a second pre-SVT test in March 2022 with changes in leaf harvesting and RNA extraction procedures. SA application and leaf harvest was improved in this second pre-test. RNA RIN values improved compared to the first pre-SVT test, but were not excellent. For these tests, we harvested leaf tissue using -80°C cold blocks. Currently, only one question remains – whether harvesting leaf tissue with -160°C blocks would further improve RNA RIN values. Since pre-SVT

	tests are resource-intensive, at Purdue we are testing whether tissue that has been immediately frozen in liquid N2 (as a proxy for -160°C) will yield improved RIN values. These data should be finished by the end of July 2022, and then we will be ready for the SVT.
Task Progress:	For both pre-SVT tests, photographs were taken of plants to test whether Purdue could accurately locate which leaf to swab. In Year 2 we tested whether seeds would germinate if they were watered several weeks and months after planting, and found 100% germination rates even 4 months after planting. We also found the best configuration for our experiments (three seeds per row). In Year 3, we tested seed sterilization methods and found that using NASA's hydrochloric acid (HCL) sterilization method provided good germination. 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. In Years 1 and 2, 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 addition, we initiated experiments to test whether the chemical will elicit defense responses in tomatoes grown in the APH conditions. At the end of year 1, we found that swabbing tomato leaves with 5 mM salicylic acid (SA) using a Q-tip enabled 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. In year 2, we optimized 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 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. In year 3, we harvested RNA from leaves grown in our optimized conditions and treated with 7.5mM SA and found that the RIN score was acceptable for all samples. In year 1, we found that storing tissue for one month did not alter the RNA quality. In year 2, we tested storing tissue up to four months in storage and found no change in RNA quality. In year 3 we tested whether SA that has been stored for 3 and 6 months would still able to elicit defense responses. Results were positive. Together, these experiments support our ability to grow tomato plants in the APH during spaceflight and perform our experiment. 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 US Department of Agriculture (USDA) permits for Fusarium oxysporum. In year 2, we successfully demonstrated the infection of Moneymaker tomato plants by Fusarium oxysporum in the enclosed rhizoboxes, and build a second clinostat to enable the simultaneous analysis of plants under clinorotation perpendicular and parallel to the gravity vector. In year 3, we have optimized the growth conditions on the paired clinostat (clinorotated and upright). We have successfully demonstrated that tomato roots respond to simulated microgravity as predicted after 3 weeks of stimuli. These responses include the agravitropic growth of roots and larger shoot biomass (as previously shown for rice plants – Jagtap et al. 2011; data not shown). We are now inoculating plants growing in the clinostat with Fusarium oxysporum. In addition to the work in Specific Aims 1 and 2, in year 3 we worked with our NASA colleagues to develop a near-final version of the Experiment Requirements Document. This document will be finalized in July 2022. Reference: Jagtap SS, Dhumal KN, Vidyasagar PB (2011) Effects of slow clinorotation on growth and yield in field grown rice. Gravit Space Biol 25(1):48-50.
Bibliography Type:	Description: (Last Updated: 06/23/2025)