

<b>Fiscal Year:</b>	FY 2020	<b>Task Last Updated:</b>	FY 06/30/2020
<b>PI Name:</b>	Iyer-Pascuzzi, Anjali Ph.D.		
<b>Project Title:</b>	Effect of Spaceflight and Simulated Microgravity on Plant Defense Responses		
<b>Division Name:</b>	Space Biology		
<b>Program/Discipline:</b>			
<b>Program/Discipline-- Element/Subdiscipline:</b>			
<b>Joint Agency Name:</b>		<b>TechPort:</b>	No
<b>Human Research Program Elements:</b>	None		
<b>Human Research Program Risks:</b>	None		
<b>Space Biology Element:</b>	(1) Microbiology (2) Plant Biology		
<b>Space Biology Cross-Element Discipline:</b>	None		
<b>Space Biology Special Category:</b>	None		
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<b>Comments:</b>			
<b>Project Type:</b>	FLIGHT,GROUND,New Investigation	<b>Solicitation / Funding Source:</b>	2018 Space Biology (ROSBio) NNH18ZTT001N-FG. App B: Flight and Ground Space Biology Research
<b>Start Date:</b>	09/01/2019	<b>End Date:</b>	08/31/2022
<b>No. of Post Docs:</b>	0	<b>No. of PhD Degrees:</b>	0
<b>No. of PhD Candidates:</b>	1	<b>No. of Master' Degrees:</b>	0
<b>No. of Master's Candidates:</b>	0	<b>No. of Bachelor's Degrees:</b>	0
<b>No. of Bachelor's Candidates:</b>	0	<b>Monitoring Center:</b>	NASA KSC
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<b>Flight Program:</b>			
<b>Flight Assignment:</b>			
<b>Key Personnel Changes/Previous PI:</b>	June 2020 report: No changes to personnel.		
<b>COI Name (Institution):</b>	Sparks, Erin Ph.D. ( University of Delaware )		
<b>Grant/Contract No.:</b>	80NSSC19K1495		
<b>Performance Goal No.:</b>			
<b>Performance Goal Text:</b>			

Task Description:	<p>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 <i>Fusarium oxysporum</i>. 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:</p> <ol style="list-style-type: none"> <li>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.</li> <li>2) Determine whether colonization of tomato by the fungal plant pathogen <i>Fusarium oxysporum</i> is impacted by simulated microgravity. We will grow plants in a 2D-ground-based microgravity simulator and inoculate them with <i>Fusarium oxysporum</i>. We will assess tomato plant colonization using histological techniques.</li> </ol> <p>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.</p>
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	<p>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.</p>
Task Progress:	<p>Specific Aim 1: Determine the effect of spaceflight on genome-wide transcriptomic and physiological defense responses in tomatoes.</p> <p>This year, work on Specific Aim 1 focused on optimizing protocols and documents necessary for tomato growth in the APH during spaceflight. The following was accomplished:</p> <ol style="list-style-type: none"> <li>i) Optimized conditions for tomato growth using similar substrates as in the Advanced Plant Habitat (APH). We first aimed to identify optimal tomato growth conditions using the same substrate and fertilizer as used by NASA in the APH. NASA suggested two different combinations of soil and fertilizer and we identified a combination that was best suited for tomato growth. We will continue going forward with this version of fertilizer. We used the same tomato genotypes that will be grown during spaceflight: Moneymaker and NahG. We used LED (light-emitting diode) lights that were on a 16 hour/8 hour day/night schedule, with a temperature of 25°C day and night. The substrate was kept very wet during germination. A humidity dome was placed on top of the substrate to prevent evaporation. We had 100% germination and will continue future experiments with these conditions. These experiments support our ability to grow tomato plants in the APH during spaceflight.</li> <li>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 and will examine transcriptomic responses to spaceflight in wild-type and immune deficient tomatoes. This year, we tested 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).</li> </ol> <p>We first tested the chemical elicitors BTH (a chemical analog of salicylic acid) and salicylic acid (SA; a plant defense hormone). Because BTH is more toxic than SA, we switched to SA for subsequent experiments. In the first experiment we used cotton balls to swab the top and bottom of the leaf of 4-week-old plants. The cotton balls were messy and required several dips in solution to swab both the top and bottom of the leaf. The plants were large and we decided to use smaller plants so that less solution is required.</p> <p>For the next experiments we only used SA. We treated the leaves of 3-week-old plants with a Q-tip to swab the top and bottom of the leaves. This was much less messy and we were able to wet the leaf more quickly. We extracted RNA from these leaves and were about to test gene expression for SA-marker genes just before Purdue shut down due to COVID-19. In the next steps, we will generate cDNA from the RNA and test for SA-dependent defense gene expression. In addition, we will test different concentrations of SA.</p> <ol style="list-style-type: none"> <li>iii) Testing whether leaves stored at -20° or -80°C freezer would yield similar quality RNA. Tissue for RNA extraction is typically stored in -80°C conditions if it is not used immediately. However, because -80°C space is limited on the ISS, we are testing whether tissue stored at -20°C will yield as high quality RNA as that stored in a -80°C freezer. To do this, we placed 15 tomato leaves in a -80C freezer and 15 in a -20°C freezer. We removed tissues one month later and extracted RNA. At this timepoint, no difference was observed between -80° and -20°C and RNA extracted from fresh leaves. We are continuing to test this monthly during 2020-2021.</li> </ol> <p>Specific Aim 2: Investigate how simulated microgravity affects fungal colonization of tomato plants.</p> <p>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 have redesigned the 2D clinostat to accommodate eight plants as opposed to the original four plant design. In the first round of experiments, tomato</p>

cultivars Moneymaker and NahG were grown to the 5-leaf stage under continuous clinorotation. Plants germinated and grew well. At the end of the experiments, root systems were examined and found to have agravitropic behavior.

One major outcome of this experiment was the need for a quick-release system for the rhizoboxes to ensure rapid sample acquisition. During future experiments, root systems will be harvested at specific time points after infection with a fungal pathogen. Rapid sample acquisition is needed to ensure results are due to the effects of clinorotation. We modified the rhizobox attachment to the frame to reduce the time to access samples.

A second experiment grew Moneymaker plants to maturity. Plants were successfully grown for three months under continuous clinorotation before experiments were halted due to the COVID-19 pandemic. Consistent with growth in simulated microgravity, shoots biomass was 4X reduced compared to plants grown under standard conditions. These preliminary results support the ability to complete ground-based experiments in Years 2 and 3.

<b>Bibliography Type:</b>	Description: (Last Updated: 06/22/2021)
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