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Project Title:	Effect of Spaceflight and Simulated Microgravity on Plant Defense Responses		
Division Name:	Space Biology		
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Space Biology Cross-Element Discipline:	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 PhD Candidates:	2	**No. of Master' Degrees:**	0
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Contact Monitor:	Massa, Gioia	**Contact Phone:**	321-861-2938
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Flight Program:			
Flight Assignment:	NOTE: End date changed to 08/31/2024 per NSSC information (Ed., 2/9/24). NOTE: End date changed to 08/31/2023 per NSSC information (Ed., 6/6/22).		
Key Personnel Changes/Previous PI:	June 2023 report: No changes to personnel. 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.

Task Progress:

Summary of research accomplishment 09/01/22 – 08/31/23
Specific Aim 1: Determine the effect of spaceflight on genome-wide transcriptomic and physiological defense responses in tomatoes.

i) Performed SVT tests in the NASA Advanced Plant Habitat (APH) with Red Wire team

This year (Year 4), work on Specific Aim 1 focused on conducting the Science Verification Test (SVT) experiments with Red Wire and finalizing protocols for the Experiment Verification Test (EVT) and spaceflight. In years 1 and 2 at Purdue, we optimized conditions (fertilizer, media) for tomato growth in the NASA science carrier. In year 3, we translated this work to NASA Kennedy Space Center (KSC) and conducted pre-SVT experiments with our NASA team. Starting in July 2022 (Year 4), we began working with Red Wire to conduct SVT experiments. In late Oct 2022, we initiated an SVT with Red Wire. However, this experiment was stopped 16 days after initiation (DAI) due to blue-green color developing on the wicks and unhealthy plants. After discussing the reasons for the problem, the team identified two primary possibilities: changes in fertilizer concentrations over time and windspeed strength. In Nov 2022 at Purdue, we tested three different combinations of fertilizer + substrate and identified a slightly different combination that appeared to promote better tomato growth in the science carrier.

We had an opportunity for SVT-1.5 in March 2023. The windspeed was reduced to 0.3 m/s for SVT-1.5. The goal of SVT-1.5 was to observe good germination and healthy, growing tomato plants. SVT-1.5 plants grew well.

Based on these results, we moved forward with SVT-2 in the Ground APH inside an International Space Station Environmental Simulator (ISSES) chamber in May 2023. All Moneymaker (MM) plants grew well during SVT-2, but only 5 NahG plants germinated and were healthy. Leaves were swabbed with salicylic acid (SA) or mock solution and harvested as expected. Frozen leaves were sent to Purdue, and we extracted total RNA. All Moneymaker plants had good quality RNA and had more than 250 µg/ul RNA. Only 2 of 5 NahG plants met the same metrics. Further examination of the data showed that plant size was related to RNA quality and amount. NahG plants germinated several days (typically 2 – 4) after the MM plants. The 2 NahG plants with good quality RNA were of comparable size to the MM plants. We concluded that promoting earlier germination of NahG would result in larger plants that would mitigate the issue of size and low quality/levels of RNA.

Based on our success criteria in our Experimental Research Design (ERD), all parts of SVT-2 met the minimum requirement for ‘acceptable’. However, there was a large difference between the two genotypes. If examining MM plants alone, many categories were ‘excellent’ while those for NahG would have been minimum acceptable or unacceptable.

Moving forward for EVT, we made several changes to promote increased NahG germination. We planted an entire quadrant of NahG (an increase of 8 plants to 9 plants), planted extra seeds to ensure better germination, and started the NahG watering (flood fill) three days before quadrants were planted with MM plants. We passed our EVT readiness review on June 16, and are currently conducting our EVT.

Importantly, our experimental design has built-in redundancy. The central question of our research is: "How does spaceflight impact plant immune responses?" We address this question using a chemical method (treating plants with SA) and a genetic method (NahG plants). Thus, even if a sufficient number of NahG plants do not grow, the experimental design still allows us to answer our question.

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 we could grow tomato cultivars to maturity under continuous clinorotation. In years 2 and 3, we successfully demonstrated the infection of Moneymaker tomato plants by Fusarium oxysporum in the enclosed rhizoboxes, and built a second clinostat to enable the simultaneous analysis of plants under clinorotation perpendicular and parallel to the gravity vector. We also optimized the growth conditions on the paired clinostat (clinorotated and upright) and established conditions where the plants were healthy for 15 days on the clinostat. In Year 4, we optimized F. oxysporum inoculations and inoculated two replicates. We are currently growing additional replicates and are cutting sections from roots and stems.

In an exciting new result for Year 4, we have identified changes in stem vascular anatomy due to clinorotation. These defects vary among the two cultivars tested: MM and Hawaii7996 (HA7996), and can be classified as two distinct changes:

1) The xylem protrusions into the pith, evident in the upright control stems, are mostly lost in both cultivars. 2) Xylem protrusions have either expanded into a complete band surrounding pith (in HA7996) or are partially absent (in MM).

These differences are not observed in sections from the root-stem junction, and we are currently processing samples from the root. These anatomical differences have significant implications for plant physiology in spaceflight and the potential of vascular pathogens to colonize the xylem. The anatomical differences we observed are not associated with stem diameter or height differences. However, MM clinorotated plants have a reduced shoot weight as compared to their upright counterparts.

In addition to the work in Specific Aims 1 and 2, in year 4 we worked with our NASA colleagues to develop a final version of the ERD.

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