Fiscal Year:	FY 2018	**Task Last Updated:**	FY 07/11/2018
PI Name:	Gilroy, Simon Ph.D.		
Project Title:	Spaceflight-Induced Hypoxic/ROS Signaling		
Division Name:	Space Biology		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:	SPACE BIOLOGY--Cellular and molecular biology		
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	(1) Cell & Molecular Biology (2) Plant Biology		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	(1) Bioregenerative Life Support		
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Zip Code:	53706-1313	**Congressional District:**	2
Comments:	NOTE: PI formerly at Pennsylvania State University; moved to University of Wisconsin-Madison in 2007 (Info received 7/2009)		
Project Type:	FLIGHT	**Solicitation / Funding Source:**	2014 Space Biology Flight NNH14ZTT001N
Start Date:	09/12/2014	**End Date:**	09/11/2019
No. of Post Docs:	2	**No. of PhD Degrees:**	0
No. of PhD Candidates:	0	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	3
No. of Bachelor's Candidates:	10	**Monitoring Center:**	NASA KSC
Contact Monitor:	Levine, Howard	**Contact Phone:**	321-861-3502
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Flight Program:	ISS		
Flight Assignment:	ISS NOTE: End date changed to 9/11/2019 per NSSC information (Ed., 9/14/18) NOTE: End date changed to 9/11/2018 per NSSC information (Ed., 12/13/17)		
Key Personnel Changes/Previous PI:	None		
COI Name (Institution):	Swanson, Sarah Ph.D. (University of Wisconsin, Madison)		
Grant/Contract No.:	NNX14AT25G		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	This research has capitalized on the capabilities of the VEGGIE hardware to address how spaceflight affects plant gene expression and growth related to low oxygen stress (hypoxia). Hypoxia is thought to develop in spaceflight as weightlessness nullifies the buoyancy-driven convection that usually aids in mixing and supplying gas (oxygen) around organisms. Our analysis of Arabidopsis grown on the International Space Station (ISS) as part of the BRIC17 (Biological Research in Canisters) experiment is consistent with the plants grown in space having experienced long-term hypoxic stress. These plants also showed hallmarks of up-regulating Ca2+- and reactive oxygen species- (ROS-) pathways (such as those supported by the enzyme RBOHD). Further, we have identified a Ca2+ transporter named CAX2 as playing a critical role in this hypoxic signaling system. We therefore have used the plant growth capabilities of the VEGGIE to significantly extend our insights into hypoxic stress. Wild-type, rbohD, and cax2 mutant seedlings were grown on orbit. After 8 days, samples were photographed, fixed in RNAlater using Kennedy Fixation Tubes, and frozen for subsequent post-flight analysis. For analysis, we will quantify patterns of growth and gene expression using the techniques of RNAseq and qPCR. In addition, analysis of a ROS reporter gene tagged with green fluorescent protein will be made using fluorescence microscopy. Comparison to plants grown on the ground will be used to ask how much of the responses seen on orbit can be explained by the development of long-term hypoxia linked to the microgravity environment. Results from this analysis are expected to advance our understanding of hypoxic response in plants grown in both space and on Earth in addition to testing whether the hypoxic Ca2+ signaling system provides targets for genetically engineering potential countermeasures to low oxygen stress.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	This research is addressing how spaceflight may induce stresses related to reduced oxygen availability in plants. The work targets the role of Ca2+ signaling and reactive oxygen species as components of this response system to define molecular components of the system. The results from this work will both provide insight into a potentially important element of spaceflight-related stress and also help to define elements of the low oxygen response system that operates on Earth. Plants on Earth experience such conditions during flooding of the soil, when there is a large microbial population in the soil consuming available oxygen and even when the metabolic activities within the plant's own tissues are intense enough to consume available oxygen. These natural low oxygen events are sensed by plants and can lead to either changes in growth and development to accommodate or escape them, or in extreme cases they can lead to significant losses in productivity and even death. These spaceflight experiments on low oxygen sensing mechanisms will therefore help provide molecular targets for potential manipulation to help make plants more tolerant of low oxygen and so contribute to agronomically important traits such as flooding tolerance in crop plants.
Task Progress:	APEX05 successfully launched on SpaceX 13 on December 15th, 2017 and the APEX05 experiment was installed in the Veggie hardware December 20th, 2017. This phase of the experimentation consisted of 26 Petri dishes with 10 seeds/dish plated on to solid Phytagel media. To prevent germination, the plates were irradiated with far red light prior to launch. Far red light inhibits germination in Arabidopsis and this treatment was 100% effective for the flight samples (and their parallel ground controls) with no germination of seeds prior to mounting in the Veggie. In addition, there was no detectable contamination of the plates in both the flight samples and the ground controls throughout the entire experiment. The plants grew for 8 days and grew vigorously as expected from the ground controls and the previous results from the Science and Experiment Verification tests. Insertion of 26 plates into the Veggie and subsequent growth were nominal. Astronaut Scott Tingle took daily photographs of the 4 genotypes (wild-type plants and the cax2-2, cax2-3, and rbohD mutants) from plates mounted in the corners of the Veggie and at 4 and 8 days for the entire 26 plate set of the experiment. At day 8, the plates were photographed, opened, and the seedlings harvested into RNAlater containing Kennedy Fixation Tubes (KFTs). All samples were successfully harvested and the KFTs stored in the MELFI at -80°C prior to return. Samples were returned successfully with a splashdown on January 13th, 2018. After successful de-integration of both the flight and parallel ground control materials, samples were shipped on dry ice to the university of Wisconsin-Madison for post-flight analysis by the PI team. Post flight analysis consists of: 1. Analysis of the on-orbit plant growth images. This analysis has been completed. 2. Imaging the samples using the confocal microscope to follow the dynamics of the RBOHDpromoter::GFP and Ubiquitin10promoter::mCherry signals. All the samples have now been successfully imaged and the imaging data is under analysis. 3. Isolation of RNA from samples dissected into root and shoots and analysis of gene expression patterns in flight vs ground samples using RNAseq is currently underway Ground-based research: In parallel to the APEX05 flight experiment, we have continued to pursue ground-based analyses to complement the flight data. We have developed a series of hypoxic chambers where we can regulate O2 levels around plants. We have analyzed the response of wild type, cax2-2, cax2-3, and rbohD mutants to lowered O2 levels at the levels of both growth and qPCR analysis of marker gene expression. We have also been analyzing ~20 genes targeted from an analysis of spaceflight transcriptomics data as being both up- or down-regulated in spaceflight and related to hypoxia and/or ROS-related signaling in ground-based research. This work is now showing an unexpected relationship between spaceflight response genes and effects on gravity signaling on Earth, suggesting that some of the spaceflight transcriptome changes may reflect disruption of a gravity sensing and response network of elements not predicted from classic gravitropism analysis on Earth. Presentations and Outreach/Education: During 2017-2018, we have presented the APEX-05 project at the 2018 MidWest Plant Cell Dynamics Meeting, the annual meeting of the American Society for Gravitational and Space Research, and the American Society of Plant Biologists and at universities, such as Carthage College. We have also used APEX-05 as a base for our outreach efforts, where we have presented it at events ranging from University of Wisconsin sponsored outreach days (e.g., University of Wisconsin’s Science Expeditions) to presentations for high school students and undergraduates (e.g., BioHouse and summer undergraduate research programs) and middle school and K-12 teachers (Biotechnology Institute summer training program) and to Madison’s Boys and Girls club. We are also working closely with Madison West High School’s Rocketry Society. These students have designed, built, and flown Arabidopsis experiments on their rocket flights, including now multiple NASA-sponsored launches at Marshall Space Flight Center. We have then been able to train them in plants science analysis such as phenotyping and

molecular analyses (qPCR) of their rocket flown samples. This is a continuing program where we are developing a pipeline of talented and engaged students who are going on to College in STEM (Science, Technology, Engineering, and Math) areas.

We maintain a "Collaboratory" where biology and engineering undergraduates and high school students come together to develop high throughput phenotyping equipment and other hardware relevant to our spaceflight-related work. Over 2017-2018 we have mentored approximately 10 independent study students working on various plant molecular and engineering projects related to space biology and APEX05. We also run a space biology related practical lab course called AstroBotanical Engineering. Here a pool of plant biology and engineering students collaborate to develop space-related hardware for ground-based testing of plant science space-related projects. We have ~15 students per semester. We are also working with Dr. Andrea Henle at Carthage College, a 4 year liberal arts college in Wisconsin to help use APEX05 and space plant biology as an integral component of her new space biology class.

We have also been fortunate to have interest in this research from several media organizations and so we have participated in a series of interviews with groups ranging from local newspapers such as the Badger Herald and local and national radio (Wisconsin Public Radio, WORT radio, and Greensense radio), to podcasts (e.g., EdgeEffects and Founders Fund) to outreach venues such as Madison's Space Place, Soundwaves, and Science on Tap.

Bibliography Type:	Description: (Last Updated: 04/08/2024)
Articles in Other Journals or Periodicals	Barker RJ, Gilroy S. "Life in space isn't easy, even if you are green." The Biochemist. 2017 Dec 39(6):10-3. http://www.biochemist.org/bio/03906/0010/039060010.pdf , Dec-2017
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