Fiscal Year:	FY 2019	**Task Last Updated:**	FY 09/18/2019
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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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:**	09/11/2020
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:	15	**Monitoring Center:**	NASA KSC
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Flight Program:	ISS		
Flight Assignment:	ISS NOTE: End date changed to 9/11/2020 per NSSC information (Ed., 9/18/19) 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:	Overview: APEX05 successfully launched on SpaceX-13 on December 2017 and after conducting the successful RNAseq element of the APEX05, samples were returned January 2018. The experimental plants grew for 8 days and showed the expected levels of vigorous development, comparable to the parallel ground controls. All flight success criteria were in the excellent range. Analysis has been completed of the on-orbit plant growth images. The returned samples have also been imaged using confocal microscopy to follow the dynamics of the RBOHDpromoter::GFP and Ubiquitin10promoter::mCherry signals and the imaging data is under analysis. RNAseq analyses of the flight vs ground samples has also been completed. For these analyses, all success criteria also met the excellent range. Insights from RNAseq analyses of APEX05 samples: Comparison of gene expression patterns in flight vs ground samples support the main hypotheses driving the experimental design of APEX05 that spaceflight induces both hypoxic and oxidative stress in plants. Thus, the wild type control plants show patterns of gene expression consistent with both hypoxia and oxidative stress. The hypoxia resistant cax2 mutants show less up-regulation of hypoxia-related transcripts, consistent with the idea that in these lines hypoxia responses are constitutively induced, allowing the plants to survive better once hypoxic conditions are established. The rbohD mutant also shows an altered pattern of oxidative stress-induced transcripts and some unique patterns of gene expression. For example, there was a significant increase in the differential expression of a cluster of loci involved in, e.g., defense signaling and ion transport. Insights from GFP imaging: The imaging of the oxidative stress-responsive promoter:GFP construct from both flight and ground samples suggests rbohD plants show altered reactive oxygen species production in the roots on both the ground and during spaceflight. The imaging of this reporter system on-orbit in the LMM (light microscopy module) further suggests that there is no obvious change over the 24h of the imaging window in the development of signal from either the ROS- and stress- responsive promoter-reporter lines. These observations imply the oxidative stress related to flight has already built up over the time period of growth prior to imaging. These results further suggest that the RBOHD enzyme may be a critical element of spaceflight-induced oxidative stress responses. To further define whether this is indeed true, test are underway using hypoxic chambers and clinostat responses in these GFP bioreporter plants to see how closely these conditions mimic spaceflight response in wild type and how well the rbohD mutants resist these stresses. Ground-based Analyses: In parallel to the APEX05 flight experiment, ground-based analyses have been pursued to complement the flight data. The critical experiments have been focused on delivering hypoxic stress to plants undergoing clinorotation to try and mimic the spaceflight environment as closely as possible. Patterns of growth in 2-d and 3-d clinorotated plants have therefore been followed with the 3-d clinostat most closely mimicking the growth patterns seen in spaceflight. High-quality, 3-d clinostats compatible with use in custom hypoxic chambers have been developed and to allow experiments where these stresses are combined to now be performed. Studies of ground-based stress signaling systems in plants has also been pursued to place the spaceflight data in context. A previously undescribed long-range signaling system that plants use to communicate throughout the plant body has been uncovered by this research. The system is based around rapid, long-range, propagating changes in calcium levels within cells that carry stress information from one part of the plant to other organs that themselves are not experiencing the stress. The system is driven by the action of the Ca2+ channels of the glutamate-like receptor family. Characterization of this effect in response to a wide range of stresses from wounding to cold stress, hypoxia, and pathogen attack is underway. Presentations and Outreach/Education: During 2018-2019, the APEX-05 project was presented at the 2019 MidWest Plant Cell Dynamics Meeting, the annual meeting of the American Society for Gravitational and Space Research, the ISS (International Space Station) Research and Development Conference and at venues such as the Academia Sinica in Taiwan. APEX-05 has also been presented at outreach-oriented events ranging from the University of Wisconsin sponsored outreach days (e.g., University of Wisconsin's Science Expeditions) to presentations for high school students and undergraduates and middle school and K-12 teachers (such as at the Biotechnology Institute's summer teacher

training program).

The laboratory maintains 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 2018-2019 approximately 15 students have been mentored on various plant molecular and engineering projects related to our general space biology program and APEX05, with a large number working on bioinformatics-based projects driven by the APEX05 RNAseq datasets. This work also helps maintain a close collaboration with Dr. Andrea Henle at Carthage College, a 4 year liberal arts college in Wisconsin, where APEX05 analyses are an integral component of her space biology class.

This last year has also been notable with providing support with interviews and lab visits to about 20 groups of middle/high school students who are part of the First Lego League Championship. This year this league focused on challenges related to sustaining astronauts during long-term manned spaceflight. Many of the groups chose to design solutions for plant growth in space, hence their interest in projects such as BRIC-based studies and especially Veggie and experiments such as APEX05.

The lab has also been lucky to be able to share space/stress science with the local, national and international media through, e.g., interviews ranging from the local Badger Herald and The Sun Prairie Star newspapers through to the New York Times and National Geographic. We have also been fortunate to do a range of radio interviews with local radio stations such as Madison’s WORT and Wisconsin Public radio through to national distributors such as such as NPR (e.g., Science Friday and Big Picture Science).

Bibliography Type:	Description: (Last Updated: 02/22/2025)
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