Fiscal Year:	FY 2020	**Task Last Updated:**	FY 12/23/2020
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 have quantified 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 has been made using fluorescence microscopy. Comparison to plants grown on the ground help define 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 has addressed how spaceflight may induce stresses related to reduced oxygen availability in plants. The work has targeted the role of Ca2+ signaling and reactive oxygen species as components of this response 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:	APEX-05 mission and sample analyses: APEX05 successfully launched on SpaceX-13. Plants were grown on orbit in the Veggie hardware, were returned frozen, and gene expression analysis using RNAseq also successfully completed. The experimental plants grew for 8 days on orbit and showed the expected levels of vigorous development, comparable to the parallel ground controls. All flight success criteria and subsequent sample analyses were in the excellent range. The overarching hypothesis for the APEX05 experimental design is that spaceflight imposes hypoxic and oxidative stress that will be disrupted in the cax2 and atrbohd mutants and that this will be reflected in the patterns of gene expression from plants growing on orbit. This idea is supported by direct analysis of various Arabidopsis ecotypes grown on the ISS and by comparison of the transcriptional responses from multiple plant spaceflight experiments analyzed using the Test Of Arabidopsis Space Transcriptome (TOAST) database, which aggregates much of the available data in this area. Comparison of gene expression patterns in flight vs ground samples for APEX-05 indicate these ideas are likely correct. The wild type control plants showed patterns of gene expression consistent with both hypoxia and oxidative stress as well as reduction in both root and shoot growth when compared to ground controls. APEX-05 cax2 responses: The hypoxia resistant cax2 mutants showed reduced induction of hypoxia-related transcripts in spaceflight. Consistent with these observations, the cax2 mutants also showed high basal expression of several molecular markers of hypoxic response, suggesting they were constitutively adapted to hypoxia, even when O2 levels were normal. Further, in ground-based controls on Earth showed these mutants were resistant to hypoxia and to flooding stress (another low oxygen environment), again consistent with a constant hypoxia response pre-adapting them to this stress. CAX2 is a Ca2+ pump that should attenuate the Ca2+ signaling triggered by low oxygen levels and the cax2 mutants showed elevated basal Ca2+ levels and significantly higher hypoxia-induced Ca2+ signals, providing a potential mechanism for their constitutive and enhanced low O2 stress resilience. APEX-05 rbohd responses: The wild-type plants in space also showed evidence of the induction of genes responsive to oxidative stress, such as a suite of molecular chaperones of the heat shock protein family. These genes were not induced in the rbohD mutant, which maintained growth in spaceflight above levels of the wild type control plants. In ground controls, the rbohd mutants also showed resistance to hypoxic and flooding stresses, suggesting hypoxia and ROS signaling may be intimately interrelated in spaceflight responses. Ground-based analyses: As part of these studies a series of ground-based analyses have been conducted to help either develop assays for the spaceflight experiments, or to better define the Ca2+ and ROS-based signaling in plants likely triggered by spaceflight. An improved red-light based system to impose dormancy on Arabidopsis seeds to aid in their transfer to orbit for APEX-05 has been developed and a collaboration with the Townsend group at the University of Wisconsin-Madison has led to production of a hyperspectral imaging system to investigate how this technology might be applied to monitoring plant stress in growth chamber environments such as the Veggie hardware used for APEX-05. During analyses of signaling events, a new plant-wide ROS and Ca2+ signaling system based on propagating waves of Ca2+ has been revealed. Additionally, a new transcriptional reporter for monitoring the dynamics of ROS-imposed stress throughout the plant has been developed. Presentations and Outreach/Education: The APEX-05 project has been presented at numerous national and international meetings including regularly at the Plant Cell Dynamics Meeting, the ISS Research and Development Conference, the annual meetings of the American Society for Gravitational and Space Research, and the American Society of Plant Biology, and at university venues ranging from South Dakota State to the Academia Sinica in Taiwan. APEX-05 has also been central to a series of outreach activities, being regularly presented at events ranging from University of Wisconsin sponsored outreach days (e.g., University of Wisconsin’s Science Expeditions) to presentations for high school students, undergraduates, and middle school and K-12 teachers (such as regularly at the BioPharmaceutical Technology Center Institute summer training program for teachers). APEX-05 has also been featured in some unique mentoring opportunities such as to multiple groups participating in the plant space biology themed First Lego League Championship over 2018-2019. Multiple cohorts of undergraduates have also participated in designing and fabricating

	hardware such as the red-light dormancy inducing system used in the APEX-05 flight or clinostats and low O2 chambers used in the ground-based controls for the APEX-05 experimentation.
Bibliography Type:	Description: (Last Updated: 04/23/2024)
Articles in Peer-reviewed Journals	Barker RJ, Lombardino J, Rasmussen K, Gilroy S. "Test of Arabidopsis Space Transcriptome: A discovery environment to explore multiple plant biology spaceflight experiments." Front Plant Sci. 2020 Mar 4;11:147. https://doi.org/10.3389/fpls.2020.00147 ; PMID: 32265943 ; PMCID: PMC7076552 , Mar-2020
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