Fiscal Year:	FY 2016	**Task Last Updated:**	FY 07/14/2016
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
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No. of Post Docs:	1	**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:**	0
No. of Bachelor's Candidates:	4	**Monitoring Center:**	NASA KSC
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Flight Program:	ISS		
Flight Assignment:			
Key Personnel Changes/Previous PI:	None		
COI Name (Institution):	Swanson, Sarah Ph.D. (University of Wisconsin, Madison)		
Grant/Contract No.:	NNX14AT25G		
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Performance Goal Text:			

Task Description:	This research will capitalize 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 experiment is consistent with the plants grown in space having experienced long-term hypoxic stress. These plants also showed hallmarks of using 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 propose to use the plant growth capabilities of the VEGGIE to significantly extend our insights into hypoxic stress. Wild-type, rbohD, and cax2 mutant seedlings will be grown on orbit. After 2 weeks, samples will 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:	This experiment (Advanced plant Experiment 05; APEX-05) requires Arabidopsis seeds to be planted at Kennedy Space Center but to germinate on orbit in the Veggie hardware on board the ISS. To delay germination of plants till they are in the Veggie hardware the far-red light germination suppression system developed in the Blancaflor lab for the APEX-03 experiment has been adopted. Arabidopsis seeds are normally induced to germinate by red light and this germination is inhibited by far-red light (~730 nm). Therefore, dormancy can be induced by far-red light irradiation of seeds planted in Petri dishes, even though those seeds are imbibed and ready to germinate. Subsequent storage of the far-red irradiated seed in darkness maintains the dormancy. Germination is induced by unwrapping the seeds and irradiating them with the LEDs (light emitting diodes) of the Veggie hardware. The far-red irradiation procedure has been optimized by using a custom light rack that allows 12 Petri dishes to be simultaneously treated. The light rack is run in a custom made bench top darkroom and the irradiated dishes are wrapped in foil and then bundled in sets of 4 into plasticized-foil bags to allow for ease of handling and transport with maximal protection to the foil wrapping. The darkroom allows these manipulations to be made without exposing the seeds to room lights that could trigger germination. The 4 packs are then removed from the darkroom and maintained at 4°C through launch and berthing until they are opened by the astronauts for installation into the Veggie. Testing indicates far-red treatment of as little as 1 minute in this apparatus reliably delays germination for >2 weeks, with extended testing suggesting >10 weeks storage without germination is routinely obtained. The experiment will use 4 different genotypes of Arabidopsis: wild type, the cax2-2 and cax2-3 mutants, and a mutant in the gene AtRBOHD. CAX2 is an ion transporter that previous analysis has shown likely plays a critical role in signaling of hypoxic stress. RBOHD is an enzyme that produces reactive oxygen species in response to a wide range of environmental cues. The mutant plant lines for these studies have been generated and the project has moved to testing and validation in preparation for a flight opportunity. Both the Science and Experiment Verification Tests (SVT and EVT) have been successfully completed during 2015-2016 in order to troubleshoot and test the experimental design and procedures. The SVT was performed February-March 2016 and results met the “excellent” success criteria for all elements of the experiment, indicating the experimental approaches and procedures were likely sufficient to support a flight. Therefore, an EVT was conducted May-July 2016. The results from EVT again met the “excellent” level for all success criteria. The far-red irradiation effectively delayed germination in all samples and once triggered to germinate by the lights of the Veggie hardware, seedling growth was efficiently initiated in all samples. At the end of the experiment, samples were chemically fixed and frozen and subsequently RNA was isolated in sufficient quantities and with sufficient purity to perform the RNAseq gene expression analysis that is one major focus of this research. The lines used for these experiments have also been engineered with green fluorescent protein (GFP)-based reporter for monitoring ROS response. Therefore, prior to isolation and analysis of RNA, GFP imaging will be performed on flight and ground samples. GFP fluorescence can be maintained despite fixation of the samples in RNAlater and freezing at -80 degrees and so all EVT samples were imaged to assess GFP signal after fixation and freezing. All samples showed preservation of fluorescence signal to the levels required for analysis of cellular distribution. In parallel to preparing for flight, research has been progressing on defining the ROS-based signaling system in Arabidopsis and analyzing the effects of genes targeted as being related to hypoxia and/or ROS-related signaling from analysis of previous spaceflight transcriptomics data. These genes include Ca2+-signaling–related genes, molecular chaperones, and oxidative stress-related genes. The knockout mutants in these genes have been verified and are now being tested for alterations in responses linked to spaceflight, such as changes in gravitropic growth and sensitivity to hypoxia and ROS (H2O2) treatment. In collaboration with Dr. Richard Morris at the John Innes Centre, Norwich, UK, work has also been conducted on modeling how ROS and Ca2+ interact to propagate stress responses in plants. Using a combination of in vivo imaging of Ca2+ and ROS dynamics and analysis of responses in the AtRBOHD mutant, the role of this enzyme in ROS-assisted Ca2+-dependent stress signaling has been dissected. This quantitative analysis allowed

	Richard Morris to develop a math-based model to describe this phenomenon. The model predicts a role for AtRBOHD-dependent extracellular ROS production in cell-to-cell movement of stress information within the plant. Using measurements of responses to inhibitors of AtRBOHD, mutants, and quantitative imaging, the predictions of the model have been tested and the model validated. APEX-05 research is also being used as the core of a suite of outreach efforts ranging from participation in local University sponsored outreach days (e.g., UW's Science Saturdays and Science Expeditions) to presentations at Botany and Astronomy clubs and hands-on programs in adult education (e.g., the DIY Science program). Educational experiences for both local high school and college students (including those who may not have been initially attracted to biological sciences) are also being offered. Thus, UW-Madison's introductory engineering class has been mentored over the course of the 2015/2016 academic year to develop practical solutions to projects related to space science and the phenotyping needed to understand the results from APEX-05. For example, the students were tasked with the development of 3-D clinostats and slow speed plant growth centrifuges. Partnering with Washington University in St Louis is now being explored to extend this program to involve design and architecture students and so broaden the multidisciplinary nature of this opportunity. This interdisciplinary approach has also allowed the establishment of the Astro-Botanical Engineering Society (ABES) based at the Wisconsin Institute of Discovery Center as part of the Wisconsin Outreach Collaborative (WOC). The group mentors approximately 20 regularly participating students, ranging from engineers, computer scientists, and mathematicians to botanists. These students also focus on generating solutions for the large scale phenotyping and measurement systems to support APEX-05-related analyses.
Bibliography Type:	Description: (Last Updated: 02/22/2025)
Articles in Peer-reviewed Journals	Evans MJ, Choi WG, Gilroy S, Morris RJ. "A ROS-assisted calcium wave dependent on the AtRBOHD NADPH oxidase and TPC1 cation channel propagates the systemic response to salt stress." Plant Physiol. 2016 Jul;171(3):1771-84. http://dx.doi.org/10.1104/pp.16.00215 ; PubMed PMID: 27261066; PubMed Central PMCID: PMC4936552 , Jul-2016
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Articles in Peer-reviewed Journals	Choi WG, Hilleary R, Swanson SJ, Kim SH, Gilroy S. "Rapid, long-distance electrical and calcium signaling in plants." Annu Rev Plant Biol. 2016 Apr 29;67:287-307. http://dx.doi.org/10.1146/annurev-arplant-043015-112130 ; PubMed PMID: 27023742 , Apr-2016