Fiscal Year:	FY 2017	**Task Last Updated:**	FY 07/20/2017
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:**	09/11/2018
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:**	2
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:	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 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 (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 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:	Optimizing Far-Red Light-Induced Dormancy: For APEX-05, Arabidopsis seeds will be planted at Kennedy Space Center and germinated on orbit in the Veggie aboard the ISS. To delay germination we are using a far-red light germination suppression system originally developed in the Blancaflor lab for APEX-03. Arabidopsis seed germination is inhibited by far-red light (~730 nm) and we have optimized the far-red irradiation procedure with a custom light rack that allows 12 Petri dishes to be simultaneously treated. The irradiated dishes are wrapped in foil, bundled in sets of 4 in plasticized-foil bags, loaded into Nomex bags, and maintained at 4°C until they are opened by the astronauts for installation into the Veggie. Inhibition of germination lasts >10 weeks under these conditions. We have also extended our analysis of the utility of far-red irradiation as a means of delaying seed germination to screening a broad spectrum of target crops/cultivars that may potentially be used with the Veggie. Seeds are currently being assayed over 6 months for efficiency and duration of germination suppression. Flight Readiness: Both the Science and Experiment Verification Tests have been successfully completed during 2015 and 2016, meeting the “excellent” level for all success criteria. The experiment has passed Flight Readiness Review and is scheduled for launch on SpaceX13. Ground-based Analyses: In parallel to preparing for flight, we have developed a series of hypoxic chambers where we can regulate O2 levels around plants. We have been testing the response of Arabidopsis to lowered O2 levels to develop a database to compare the APEX-05 flight materials to. QPCR analysis of marker gene expression suggests that some of the transcriptional fingerprints of genes upregulated in spaceflight can be mimicked at 10% O2 or lower. For example, induction of HSP101 and HSP70 occurs as seen in spaceflight samples at 6% O2. However, hypoxic challenge on Earth does not mimic all aspects of spaceflight. For example, spaceflight repressed genes such as the peroxidase superfamily are not repressed by hypoxia. Transcriptional fingerprints from spaceflight data also strongly suggest reactive oxygen species stress is part of the plant responses to spaceflight. Treatment of Arabidopsis with short-term/acute oxidative stress from addition of H2O2 does not mimic spaceflight responses as assessed by QPCR analysis of marker genes. However, we postulate that the spaceflight environment is more likely delivering long-term (many days), low levels of oxidative stress. Long-duration H2O2 application is complex as the H2O2 degrades rapidly when added to plant samples. We have addressed the long-term treatment challenge in two ways. We have added H2O2 to plants at 2 day intervals during their growth and also developed a system to apply continuous H2O2 application to plants growing on Petri dishes. This 3-d printed ‘ROS-flow’ system provides a laminar flow of H2O2 solution over the face of a Petri dish where the plants are growing and is allowing us to monitor response to plants experiencing constant but low level H2O2 application. Mutant Analyses: We are 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. These include a range of heat shock-related, Ca2+ signaling and ROS-related genes such as AtRBOHC, D and F. We have isolated homozygous knockout mutants in these genes and are phenotyping them for alterations in ROS, hypoxic, touch, and gravitropic response. These analyses are revealing previously unknown links between spaceflight responsive transcriptome and signaling pathways thought to be altered in the microgravity environment. For example, we have found that mutants in HSP family members that are upregulated in spaceflight but classically linked to molecular stress responses, have significant defects in gravitropic response in the root. Thus, this analysis targeting spaceflight transcriptomics is now also helping increase the molecular details of the gravitropic response machinery. Presentations and Outreach/Education: During 2016-2017, we have presented the APEX-05 project at the 2017 MidWest Plant Cell Dynamics Meeting, the annual meeting of the American Society for Gravitational and Space Research, and the American Society of Plant Biologists. 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., UW’s Science Saturdays and Science Expeditions) to presentations for high school students and undergraduates (e.g.,

BioHouse and summer undergraduate research programs) and K-12 teachers (Biotechnology Institute summer training program). We have also used large attendance opportunities such as the Madison Garden Expo to perform space biology-related outreach to the general public. We have also had the opportunity to talk about APEX-05 and space biology internationally, e.g., through Skype interviews with high school students in the UK and will be presenting at Sir Isaac Newton College in the UK this summer. Dr. Barker, a scientist in the lab working on APEX-05 also presented the APEX-05 work at the Chinese Academy of Science/National Academy of Science Forum for New Leaders in Space Science

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 NASA-sponsored launches at Marshall Space 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 areas.

We are also blending our biology-based plant space science with educational experiences for both local high school and college students (including those who may not have been initially attracted to biological sciences). Thus, we have mentored the UW-Madison introductory engineering class over the course of the 2016/2017 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, this Spring semester the students were tasked with the development of 3-d clinostats and acoustic levitators to grow plants in an environment lacking physical contact with a growth medium. Many of these students continue with these projects by working in the lab for credit as independent study students or enrolling in the AstroBotanical Engineering class (see below).

We also 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. We mentor approximately 10 independent study students working on various plant molecular and engineering projects related to space biology. This interdisciplinary approach has also allowed us to establish 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 15-20 students.

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, to space-oriented podcasts (e.g., L9).

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