Fiscal Year:	FY 2015	**Task Last Updated:**	FY 07/14/2015
PI Name:	Gilroy, Simon Ph.D.		
Project Title:	Spaceflight-Induced Hypoxic/ROS Signaling		
Division Name:	Space Biology		
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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		
PI Email:	sgilroy@wisc.edu	**Fax:**	FY
PI Organization Type:	UNIVERSITY	**Phone:**	608-262-4009
Organization Name:	University of Wisconsin-Madison		
PI Address 1:	Department of Botany		
PI Address 2:	430 Lincoln Dr.		
PI Web Page:			
City:	Madison	**State:**	WI
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
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No. of PhD Candidates:	0	**No. of Master' Degrees:**	0
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No. of Bachelor's Candidates:	2	**Monitoring Center:**	NASA KSC
Contact Monitor:	Levine, Howard	**Contact Phone:**	321-861-3502
Contact Email:	howard.g.levine@nasa.gov		
Flight Program:	ISS		
Flight Assignment:			
Key Personnel Changes/Previous PI:	None		
COI Name (Institution):	Swanson, Sarah Ph.D. (University of Wisconsin, Madison)		
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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,		

Task Description:	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 research is capitalizing on the VEGGIE hardware to address how spaceflight affects plant growth and gene expression related to hypoxic response in Arabidopsis thaliana. Hypoxia is thought to develop in spaceflight as the lack of buoyancy-driven convection caused by the microgravity environment reduces the gas exchange that normally occurs around and within organisms. Metabolic consumption then yields a reduction in available oxygen levels that are thought to lead to the development of oxygen-limiting conditions with subsequent reduced plant vigor. Previous research from our BRIC17 experiment and mining of data from other spaceflight researchers suggests that some of the hallmark molecular markers of hypoxic stress are upregulated in spaceflight. In addition, transcriptional profiling has highlighted molecular fingerprints of reactive oxygen species (ROS). ROS-related stress is a well-defined element of hypoxic response on Earth, and so the spaceflight data on ROS response is also consistent with the development of hypoxic stress in spaceflight. For this APEX-05 experiment, we plan to analyze mutants in a gene linked to hypoxic signaling (cax2) and in a major ROS producing enzyme (AtRBOHD). We plan to use a fixation/imaging protocol for green fluorescent protein (GFP) that will allow us follow the dynamics of this ROS response with high spatial resolution in the spaceflight materials. The major goal of the initial period of this work has therefore been to develop the genetic tools to support this last goal of being able to image GFP-expressing reporter lines. Making Transgenic Reporter Plants: In order to visualize ROS response throughout the plant in space-flown plants, we will use plants transformed with a GFP reporter system driven by a ROS-inducible promoter. The ROS-responsive promoter is that from the AtRBOHD gene. In order to make this a quantitative assay, we also plan to use a constitutive promoter (from Ubiquitin 10, UBQ10) driving a different GFP (mCherry, a red fluorescent version). The ratio of signal between GFP and mCherry will then provide a measure of ROS response independent of any variation between plants or individual organs in their general levels of protein production. Over the reporting period, we have cloned the requisite genes/promoters and are currently generating transgenic reporter lines in the model plant Arabidopsis expressing these constructs. Supressing Germination: This experiment requires Arabidopsis seeds to be planted at Kennedy Space Center but to germinate on orbit in the VEGGIE aboard the ISS. To delay germination of plants until they are in the VEGGIE hardware we have been testing the far-red light germination suppression system developed in the Blancaflor lab for APEX-03. This involves far-red light irradiation of seeds planted in Petri dishes using a 730 nm LED and then storage of the irradiated seed in darkness. Germination is subsequently induced by moving the Petri plates to white light, such as provided by the VEGGIE. Testing indicates this system can robustly delay germination for at least 2 weeks and our current analysis is testing the reliability of germination delays well beyond this period. At a practical level, testing is showing that manually wrapping the Petri dishes in foil and then placing these in a plasticized-foil bag provides a robust method of maintaining the plates in darkness after irradiation with a level of protection compatible with the handling needed for spaceflight. To increase throughput for the far-red irradiation step, we have also partnered with the Mechanical Engineering Department at UW-Madison and as part of their introductory engineering program (InterEGR 160), we have had 2 groups of undergraduate students design automated irradiation systems that will handle 6 Petri dishes at a time and automatically load them into the foil bags at the end of the treatment. They have delivered 2 prototypes for the equipment and these are currently under testing. Mutant analyses: We have also targeted ~20 genes from analysis of our transcriptomics data from previous spaceflights as highly likely to be related to low oxygen and/or ROS-related signaling in spaceflight. These include a range of heat shock-related proteins and ROS-responsive genes such as AtRBOHD. We are isolating homozygous knockout mutants in these genes and are currently assaying these mutants and wild-type controls for their response to different levels of O2 using growth and assaying the level of genes known to be changed in spaceflight as our measures of response. Presentations and outreach: During 2015, we have presented the spaceflight hypoxia project at the MidWest Plant Cell Dynamics Meeting in Madison, the International Space Station Meeting in Boston, the Plant Oxidative Group meeting in Verona, the Plant Stress meeting in Heidelberg, and the Plant Pathology student and postdoc seminar series at UW Madison. We plan to present at the meeting of the American Society of Plant Biologists in Minnesota in July and at the ASGSR meeting in November. As part of our space science outreach we have held events at the Westside Christian High School, Sigma

	Alpha Iota music society and the UW Health Center. We have also established a Facebook page: < https:// > in preparation for beginning the active flight component of this grant.
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