Fiscal Year:	FY 2024	**Task Last Updated:**	FY 11/28/2023
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Project Title:	Dissecting Beneficial Plant-Microbe Interactions and Their Efficacy in the ISS Spaceflight Environment, a Model Study		
Division Name:	Space Biology		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	(1) Cell & Molecular Biology (2) Microbiology (3) Plant Biology		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Comments:			
Project Type:	Flight	**Solicitation / Funding Source:**	2018 Space Biology (ROSBio) NNH18ZTT001N-FG. App B: Flight and Ground Space Biology Research
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No. of PhD Candidates:		**No. of Master' Degrees:**	
No. of Master's Candidates:		**No. of Bachelor's Degrees:**	1
No. of Bachelor's Candidates:	1	**Monitoring Center:**	NASA KSC
Contact Monitor:	Mickens, Matthew	**Contact Phone:**	
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Flight Program:			
Flight Assignment:	NOTE: End date changed to 12/31/2024 per NSSC information (Ed, 3/12/24) NOTE: End date changed to 12/31/2023 per NSSC information (Ed, 1/15/23)		
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Davin, Laurence Ph.D. (Washington State University, Pullman) Kahn, Michael Ph.D. (Washington State University, Pullman)		
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Task Description:	Significance of objectives to NASA and this Solicitation: Deep space exploration or extraterrestrial colonization (e.g., Moon or Mars) will require the ability to sustainably produce plants for human/animal consumption, as well as providing aesthetic benefits of plant life to future crews and personnel in extra-terrestrial colonies. One key challenge in spaceflight/microgravity is in overcoming long-standing difficulties in efficaciously providing water and nutrients to germinating and maturing plants. Another important research challenge that has seen little attention is in productively exploiting beneficial plant-microbe interactions in spaceflight/microgravity, particularly for nitrogen (N) fixation. When both challenges are resolved for optimal, productive, and efficacious plant growth in space, this will provide the exciting opportunity to recycle organically bound carbon (C) and N that was sequestered in these plants. Through subsequent recycling of those organics (e.g., derived from human and animal consumption waste and from unused plant parts), this will help enable sustainable plant growth over multiple generations. Another benefit of studying beneficial plant microbe interactions is at the fundamental science level, i.e., by gaining much improved understanding of how the spaceflight/microgravity environment affects this important physiological process. Central objectives of proposed research: Complementary purposes of our 2 Specific Aims are to initially dissect, understand, and optimize plant growth/development in spaceflight/microgravity via exploiting beneficial plant-microbe interactions. Then to ultimately recycle organic C and N from them suitable for subsequent multiple plant generations. To do this, we will use model Medicago plant species (e.g., alfalfa), and its beneficial bacterial symbiont, which together can potentially displace the need for N-containing fertilizer in spaceflight/microgravity. Specific Aims: 1. Comprehensively compare and contrast efficacy of beneficial symbiotic plant-microbe interactions between Medicago and Sinorhizobium growing on the International Space Station (ISS) and on Earth (NASA Kennedy Space Center/KSC), including understanding changes occurring at the molecular level; 2. Compare and contrast ease of biodegradation of the ISS and Earth grown Medicago plant material, in order to assess whether there are any differences in the ability to recycle C, N, etc. for multiple generations of plant growth/development. Justification for this work is threefold: The first is to demonstrate that beneficial plant microbe interactions during N-fixation can be efficaciously achieved in spaceflight/microgravity. The second is to gain a fundamental understanding of spaceflight/microgravity environment effects on these beneficial plant microbe interactions, and their potential usage for deep space exploration and colonization. The third is to demonstrate that organic C and N can be facilely recycled to support multiple generations of plant growth in space. Methods/techniques: During growth, FluorPen and plant size measurements will be carried out to assess comparative N-fixation efficacy for each condition, both on the ISS and on Earth. Tissues (leaf, stem, and root) from the ISS and Earth control will be collected after ca. 6-8 weeks growth, frozen (-160°C). They will be subjected to transcriptomic and metabolomic (including amino acid) analyses; the microbiomes present in aerial/underground tissues will be determined. The multi-omics approaches employed are as for our Arabidopsis study. Medicago plant material, from the ISS and ground control, will also be subjected to biodegradation to establish whether there are any differences in N-mineralization (for recycling) in spaceflight/microgravity or ground control tissues.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Among the benefits on Earth envisaged: improving our knowledge of N-fixing process and the symbiosis between Medicago and Sinorhizobium, and determining optimal lignin contents for space and Earth will be very instructive, as will the recycling C/N capabilities for both wild type and genetically modified plant lines. Demonstrating this in space is also a very effective means of demonstrating to aspiring young scientists (including Middle and High School students) and others of the importance of plant life, of N-fixation, and of C/N recycling in a sustainable manner.
Task Progress:	1. Progress on Ground Verification (GVT) Test Preparations and Execution The GVT used 2 NASA Vegetable Production System (Veggie) units, each housing twelve APEX Growth Chambers (AGCs). Twelve had an alfalfa reduced-lignin line and its corresponding control (VEGGIE 1; lignin experiment); whereas the other twelve had M. sativa v. Ladak (VEGGIE 2; symbiosis experiment). For VEGGIE #1 (lignin experiment), the overall goal is to compare and contrast the ease of biodegradation of alfalfa grown on the International Space Station (ISS) and Earth, including low-lignin alfalfa plants. This is to assess whether there are differences in the ability to recycle C, N, etc., for multiple generations of plant growth/development. Alfalfa was grown with added nitrogen. For VEGGIE #2 (symbiosis experiment), the hypothesis is that rhizobia will be able to generate good plant growth and root nodulation in plant growth medium lacking an added nitrogen source. There are 3 experimental configurations to test this hypothesis: • Rhizobia (Sinorhizobium meliloti) added: This configuration should generate good plant growth and root nodulation in a plant growth medium lacking added nitrogen. • No rhizobia or added nitrogen source in plant growth medium: In this configuration, the plants should be stunted because they lack access to a usable nitrogen source. • No rhizobia, but added nitrogen source. This configuration is to test the growth of the plants when supplied with nitrogen-containing fertilizer. 1.1. GVT-1 For the first GVT, the Principal Investigator (PI) team used the Plant Growth Systems (PGSs), previously named Apex Growth Chambers (AGCs). Members of the PI team travelled to NASA Kennedy Space Center (KSC) on May 20, 2023 and 24 PGSs were assembled. A Magenta box was next placed on the top of each PGS. The GVT was initiated May 23, 2023, by addition of nutrient solution (125 ml) to each PGS. Each PGS was weighed before and after addition of nutrient solution. This was done at this stage in order to estimate nutrient solution loss (due to plant growth and evaporation) over time.

As the nutrient solution was dispensed, the PGSs were next placed in both Veggie systems in the ISS Environmental Simulator (ISSES) chamber. Light intensity and photoperiod were set. Temperature, relative humidity (RH), and CO2 level settings used in the ISSES chamber were those of “ISS average”.

Temperature, RH, and CO2 levels were recorded daily by KSC personnel, with averages of each being 22.6 °C, 39%, and 2,610 ppm, respectively.

In addition, two HOBO Data Loggers (one in each Veggie system) were programmed to measure and record temperature, RH, and light at 15-minute intervals during plant growth. Pictures were taken on days 6, 8, 10, 13 and every 3 days thereafter until harvest.

Eight days after initiation (DAI) of the experiment, the top Magenta box on each PGS was removed. Additional water was provided at day 10, and every 3 days thereafter until harvest, with weights again taken before and after adding water. Germination rate (10 DAI) and number of plants that grew in each PGS were recorded.

GVT-1 did not fully meet our success criteria, due to germination issues.

1.2. GVT-2

GVT-2 employed fresh seed stocks and was initiated October 5, 2023 and carried out as described above. From a biomass production perspective, GVT-2 appeared to be fully successful in meeting all Success Criteria. Plant tissues are currently under analyses.

2. Lignin-reduced Alfalfa Lines

As described in the previous Progress Report, application of CRISPR/Cas9 was carried out to generate lignin-reduced alfalfa lines. The CRISPR/Cas9 gene-editing approach was selected to disable arogenate dehydratase (ADT) genes, potentially allowing for multiple gene knock-out targets in a single experiment. To date, over 100 CRISPR/Cas9 transgenic plants have been generated.

Bibliography Type:	Description: (Last Updated: 01/22/2025)
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