| | | | |
|---|---|---|---|
| **Fiscal Year:** | FY 2019 | **Task Last Updated:** | FY 02/28/2019 |
| **PI Name:** | Lewis, Norman G Ph.D. | | |
| **Project Title:** | An Integrated Omics Guided Approach to Lignification and Gravitational Responses: The Final Frontier | | |
| **Division Name:** | Space Biology | | |
| **Program/Discipline:** | | | |
| **Program/Discipline--Element/Subdiscipline:** | SPACE BIOLOGY--Developmental biology | | |
| **Joint Agency Name:** | | **TechPort:** | No |
| **Human Research Program Elements:** | None | | |
| **Human Research Program Risks:** | None | | |
| **Space Biology Element:** | (1) Plant Biology | | |
| **Space Biology Cross-Element Discipline:** | (1) Reproductive Biology | | |
| **Space Biology Special Category:** | (1) Bioregenerative Life Support | | |
| **PI Email:** | lewisn@wsu.edu | **Fax:** | FY 509-335-8206 |
| **PI Organization Type:** | UNIVERSITY | **Phone:** | 509-335-2682 |
| **Organization Name:** | Washington State University | | |
| **PI Address 1:** | Institute of Biological Chemistry | | |
| **PI Address 2:** | 299 Clark Hall | | |
| **PI Web Page:** | http://ibc.wsu.edu/ | | |
| **City:** | Pullman | **State:** | WA |
| **Zip Code:** | 99164-6340 | **Congressional District:** | 5 |
| **Comments:** | | | |
| **Project Type:** | FLIGHT | **Solicitation / Funding Source:** | 2014 Space Biology Flight (ILSRA) NNH14ZTT002N |
| **Start Date:** | 05/01/2015 | **End Date:** | 04/30/2020 |
| **No. of Post Docs:** | 1 | **No. of PhD Degrees:** | 1 |
| **No. of PhD Candidates:** | 1 | **No. of Master' Degrees:** | |
| **No. of Master's Candidates:** | | **No. of Bachelor's Degrees:** | |
| **No. of Bachelor's Candidates:** | 1 | **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:** | ISS<br>NOTE: End date changed to 4/30/2020 per PI (Ed., 3/4/19)<br>NOTE: End date changed to 4/30/2019 per NSSC information (Ed., 8/16/18) | | |
| **Key Personnel Changes/Previous PI:** | | | |
| **COI Name (Institution):** | Davin, Laurence Ph.D. ( Washington State University )<br>Hanson, David Ph.D. ( University of New Mexico )<br>Lipton, Mary Ph.D. ( Battelle Memorial Institute )<br>Sayre, Richard Ph.D. ( New Mexico Consortium )<br>Starkenburg, Shawn Ph.D. ( Los Alamos National Security ) | | |
| **Grant/Contract No.:** | NNX15AG56G | | |
| **Performance Goal No.:** | | | |
| **Performance Goal Text:** | | | |
| | We propose a multi-omics study using the model plant Arabidopsis under both 1g and in microgravity conditions (spaceflight). Our approach spans comprehensive phenomics, metabolomics, transcriptomics, and proteomics strategies, and is incisively and uniquely melded via deployment of an integrated computational biology (ICB) approach we are pioneering. Our plant lines include wild type, various mutants we have generated with different lignin amounts through manipulation of the multigene family encoding arogenate dehydratases, and other lines enhanced in carbon assimilation capacity, and combinations thereof. We consider this places us in an unprecedented position to investigate how plants function in altered gravity environments.<br>We are very well positioned for incisive spaceflight and definition stage (1g) studies to investigate gene/metabolic network relationships and adaptations resulting from varying lignin and carbon assimilation levels, e.g., on photosynthesis; C allocation; water use efficiency (WUE); vascular plant growth/development; vasculature performance; auxin transport; and gravitational adaptations. Our overarching hypothesis is that a comprehensive interrogation (an integrative omics study) of our Arabidopsis lines with varying lignin levels and/or | | |

modulated carbon concentrating mechanisms (CCMs) or combination thereof will identify gene/metabolic networks, mechanisms and/or pathways that are differentially modulated at 1g and on exposure to microgravity, i.e., various omics (phenomics, transcriptomics, genomics, proteomics, metabolomics, and ICB) will allow us to study these in a truly unprecedented way.

**Task Description:**

Overall objectives:

1. Establish multi 'omics' effects of modulating lignin and CCM levels i) at 1g and ii) in spaceflight.

2. Compare/contrast data, using an ICB approach, to better define and understand gravity sensing and responses, and if threshold/induction parameters are modified or changed, when lignin and CCM levels are varied.

More specifically, we address distinct hypotheses for our various teams, and integrate, dissect, and incisively analyze them holistically in a manner hitherto not possible. These 5 hypotheses include that: modulating lignin and CCM levels differentially affect carbon assimilation/re-allocation, photosynthesis, and WUE (Team 1); modulating lignin and CCM levels differentially affect secondary and primary metabolite levels (metabolomics) (Team 2); system-wide modification in the transcriptome occurs through a common transcriptional regulatory mechanism, and transcriptome/proteome 'discrepancies' result from over-simplification of transcript analyses (Team 3); differential alterations in lignin and CCM levels can often be attributed to overall distinct changes in protein expression and phosphorylation patterns in a defined set of proteins (Team 4); an integrated omics analysis will provide urgently needed new insights into global effects on plant biological processes at both 1g and in microgravity (Teams 1-4). Each hypothesis draws upon the most advanced technologies available for study. We consider that our ICB approach will transform omics analysis through our advanced instrumentation and analytical tools. We will utilize (or design) computational tools/mathematical algorithms for integration and correlation of high resolution phenotype measurements (phenomics) with 'low' resolution global subcellular system measurements (transcriptomics, etc.) through 'nth' dimensional analysis.

Our study aligns with Research Emphasis 1 and 3, and decadal survey elements in Cell, Microbial, and Molecular Biology (CMM-3, CMM-5), Organismal and Comparative Biology (OCB 2-5), Developmental Biology (DEV-4), and Plant and Microbial Biology, chapter 4 (P2). Our data generation will also be seamlessly integrated with various web-based platforms to handle, disseminate, and inter-actively utilize through iPlant and OpenMSI, and thus are made available to NASA as well as being a community resource.

**Rationale for HRP Directed Research:**

**Research Impact/Earth Benefits:**

1). This research will provide NASA and science in general with the first “big “omics” data” analysis, integration, and assessment – at the gene, protein, and metabolic outcome levels – as to how microgravity alters the basic responses of plants when the influence of gravity is removed/minimized. This will serve as the foundation “omics” analyses in subsequent spaceflight and colonization experiments in space, as well as shedding new insights into the manifold effects of gravity during plant growth and development.
2). We have partnered with Ms. Kathy Lucchesi (K-7/8 teacher), at McCaffrey Middle School in Galt, California, and their largely Hispanic students. Supplemental funding was provided to the school by NASA and the California Space Grant Consortium so that these middle school students can safely follow and utilize many of the plant growth and development protocols developed for the International Space Station (ISS) experiments. One purpose here is that the students grow plants under similar conditions and obtain information and insights on how the research impacts or benefits life on Earth and beyond (in the future).

Written materials on, and seeds for, the experiments at hand are also routinely provided. The additional aim here is in helping teach and inspire these young students about the joys and fun of the scientific method in experimental plant biology. Periodically, the middle school students present results to Dr. Lewis over where such work is routinely evaluated.

Astronaut Sunita Williams, Dr. Lewis (Washington State University), and Dr. Sato (NASA Ames) visited the school on April 13, 2018. Astronaut Williams gave presentations on her time on International Space Station and answered questions from students. The Galt Herald ( http://www.galtheraldonline.com/ ) and the Lodi News-Sentinel ( https:// ) wrote articles on her visit, and the visit was further publicized through the NASA Space Biology Facebook ( https:// ).

Later, McCaffrey Middle School Students presented their work on #PlantHabitat01 to the Galt School Board on (See post at: https:// ).

Ms. Lucchesi, her students, and Dr. Lewis visited NASA AMES Research Center, May 28, 2018. The students presented their research to NASA scientists, engineers, and managers. The students also handled small satellites, checked out our bone and signaling lab, learned about Astrobiology and Air Revitalization on ISS. (See post at: https:// ).

Ms. Lucchesi and one of her students traveled to Washington State University, Pullman to visit Dr. Lewis’ and Davin’s laboratory. They had hands-on training from the lab on what is being done with the Arabidopsis plants. (See post at: https:// )

The McCaffrey Middle School students were featured on a Good Day Sacramento segment between 8-10 AM, Thursday, October 25, 2018! (See Part 1: https:// and Part 2: https:// ), with the Lewis lab personnel included from Washington State University (WSU) (via teleconference).

3). On November 7, 2018 and January 3, 2019, the Pullman Droids, a Lego Robotics Team, visited Drs. Lewis and Davin to learn of their work on ISS.

4). Dr. Lewis gave seminars at Tarim University, Alar, Xinjian, China, June 15, 2018, and Southwest University for Nationalities, Chengdu, Sichuan, China, June 24, 2018, entitled “Approach to Lignification on Earth and on the International Space Station.”

5). The New Mexico Space Grant Consortium helped to fund a year-long collaboration between Dr. Hanson and the Explora science museum in Albuquerque. They developed and piloted a monthly course called "Extraterrestrial Botany" for around twenty students across the city who were in 5th through 9th grade. Ten sessions were developed and taught by a team of 2 post docs (1 female), two technicians (both female, 1 Hispanic), a female graduate student, and three undergraduates (2 female, 1 Hispanic), plus Dr. Hanson and two staff members at Explora. The project involved learning about challenges of space exploration, relevance for life on Earth, and the student assisted with data collection and analysis from the APH-01. Feedback from families and Explora was extremely positive and they would like to do more work with us.

6). Miss Bianca Serda, a Hispanic student with Dr. Hanson, gave an oral and 2 poster presentations. She is also the recipient of a NASA Space Life Science Training Program (SLSTP) fellowship at Ames Research Center (from June 10, 2019 to August 16, 2019).

• Serda B, Turpin M, Hudson P, Hanson D. “Plants in Space: Interactions between Morphology, Lignification, and Carbon Isotopic Composition.” Presented at the MARC Symposium, Albuquerque, NM, August 2018.

• Serda B, Turpin M, Hudson P, Hanson D. “Plants in Space: Interactions between Morphology, Lignification, and Carbon Isotopic Composition.” New Mexico Academy of Science Research Symposium at Albuquerque, NM, October 2018.

• Serda B, Turpin M, Hudson P, Hanson D. “Plants in Space: Interactions between Morphology, Lignification, and Carbon Isotopic Composition.” SACNAS Conference at San Antonio, TX, October 2018.

7). Fans of science in space now can experience fast-moving footage in even higher definition as NASA and ESA (European Space Agency) deliver the first 8K ultra high definition (UHD) video of astronauts living, working and conducting research from the International Space Station. https:// . One of the selected studies was our experiment carried out with the Advance Plant Habitat (APH) on ISS.

8) Our experiment has been showcased many many on the Space Biology Facebook page at: https://

9). Dr. Lewis and Dr. Davin were judges for the High School Student Poster competition at the annual ASGSR (American Society for

Gravitational & Space Research) meeting in Bethesda, MD, October 31 – November 3 2018.

**Task Progress:**

This reporting period focused on:
• Carrying out the second Experiment Verification Test (EVT). This was in order to further test conditions that will be employed on International Space Station (ISS).

• Updating the Experiment Requirements Document (ERD) for consideration and approval by the SLPS Program Executive for Space Biology at NASA Headquarters.

• Carrying out two 6-week Arabidopsis grow-outs on ISS.

1. Experiment Verification Test (EVT), Run 2

The second EVT was carried out in the Advanced Plant Habitat (APH) Ground Unit (S/N001) from 1/31/2018 to 3/14/2018.

Science Carrier Configuration and Plant Harvest:

• Each quadrant of the Science Carrier was filled with media consisting of approximately 1.6-1.7 L of washed and autoclaved 1-2 mm arcillite which contains 7.5 g/L of Type 180, 18-6-8 nutricote slow release fertilizer. Five to seven seeds each (in individual groupings) of A. thaliana WT, and transgenic adt5, adt4/5, adt3/4/5/6, WT/CCM, and adt3/4/5/6/CCM lines were adhered (using guar gum) to the gauze material in each of the 48 locations in the Science Carrier. The location of each line in each quadrant was randomly determined using a stratified randomized design.

• The Science Carrier was next installed into the APH Ground Unit (S/N001) and water was supplied to each of the four quadrants on January 31, 2018. Pictures were taken both from the side and the top of the APH and thereafter each day during the growth period.

• Fourteen days later (i.e., February 14, 2018), seedlings were thinned out so that only one remained in each spot). Prior to thinning, Pulse-Amplitude Modulated (PAM) measurements were carried on all seedlings using a FluorPen.

• After four-weeks growth (February 28, 2018), PAM measurements were carried on plants located in the front two rows of Quadrants 1 and 4 with the FluorPen and half of the plants in the Science Carrier (24) were harvested.

• The remaining plants were harvested on March 14, 2018.

• As before, for both harvests, plants were collected from the left to right and from the front to the back of the Science Carrier, separated into stem and rosette leaf samples, transferred into labelled foil packets, weighed, and then frozen at –150°C using a conditioned cold block in a mini cold bag. Following harvest of the last plant, specimens were transferred into a –80°C freezer where they were kept frozen until shipped to Washington State University (WSU) on dry ice, where they were again stored at –80°C.

Lignin Analyses:

• In order to determine if the Arabidopsis plants grown in the APH for the second EVT had attained the same level of maturity and lignification as those previously grown in the greenhouse at WSU, lignin compositions were estimated in a subset of plants. Thus, stems from WT, and mutants, adt5, adt4/5, and adt3/4/5/6, were freeze-dried, ground to a powder (in a mortar by means of a pestle), with cell wall residues next obtained as routinely performed in our laboratory. The cell wall residues of these selected plants were next subjected to thioacidolysis to estimate lignin amounts. Lignin levels were within the ranged observed in plants grown in the WSU greenhouse.

2. Experiment Requirements Document (ERD)

In collaboration with the Payload Development Team and upon completion of two Experiment Verification Tests (EVTs), the Experiment Requirements Document (ERD) was updated. On April 26, 2018, the ERD was presented to the SLPS (Space Life & Physical Sciences) Program Executive for Space Biology at NASA Headquarters who gave his approval to proceed with the flight experiment.

3. Flight Preparation

Two Science Carriers (SN003 and SN004) were prepared (April 26, 2018) by Kennedy Space Center (KSC) personnel as described above for EVT Run 2 and seeded on May 9 and 10, 2018. Again, the location of each line in each quadrant was randomly determined using a stratified randomized design for both Science Carriers.

These were then packed and shipped custom critical to Wallops, VA on May 11, 2018 to be loaded on resupply spacecraft Cygnus.Launch of Orbital ATK Antares rocket for OA-9 mission was on May 21, 2018 at 4:39 a.m. EDT. Cygnus connected with ISS on May 24, 2018 at 1:13 a.m. EDT. For the corresponding ground controls, two other Science Carriers (SN005 and SN006) were prepared in a similar way a week later.

4. First Grow-out on ISS

This was initiated by astronaut Ricky Arnold on June 8, 2018 (Day 0). While many seeds germinated, some sites showed no apparent germination. On June 22, 2018 (Day 14) FluorPen photosynthesis measurements were carried out, followed by thinning of the Arabidopsis seedlings to one per spot. Following thinning, several plants unexpectedly died. Overall, ISS APH Arabidopsis plants grew slightly slower than the ground control plants.

The consequence of spaceflight environment effects (reduced plant number, and somewhat smaller size, resulted in only one harvest at 45 days being possible, i.e., in order to ensure sufficient plant material for our multi-omics analysis. FluorPen photosynthesis measurements were, however, carried out after 31 days (4 weeks and 3 days) of growth (July 9, 2018). Plants were harvested on ISS on July 23, 2018, and immediately frozen and stored in the GLACIER freezer at –160 °C (as already done for the thinnings). A provisional explanation to the spaceflight environment/microgravity effect observed for the first grow-out was that the water introduced into the Science Carrier did not efficiently reach all 48 sites equally. This could have been caused by small bubbles in the water lines. Alternatively, it may have been partly due to intermittent partial lighting failures in the ISS APH.

This first set of samples was brought back to Earth (Splashdown in the Pacific Ocean, 01-13-2019), with transport and delivery to IBC/WSU on 01-15-2019). Our multi-omics analyses are now beginning. The ground control at KSC was initiated a week later (June 15, 2018; Day 0), with FluorPen photosynthesis measurements/thinning on June 29, 2018 (Day 14), FluorPen photosynthesis measurements on July 13, 2018 (Day 28) and final harvest on July 27, 2018 (Day 42). Plants were transported/delivered to IBC/WSU on 01-25-2019.

5. Second Grow-out on ISS

The second grow-out was initiated by astronaut Serena Auñón-Chancellor on September 18, 2018 (Day 0). The watering step was modified, with much improved germination occurring at 47 of 48 sites by 14 days.

Following FluorPen photosynthesis measurements, the subsequent thinning operation on ISS (October 2, 2018) apparently adversely affected growth/development of some Arabidopsis plants with several dying. This did not occur with KSC APH ground controls. FluorPen measurements were next carried out on October 19, 2018 (Day 31), with slightly slower growth again being observed in the ISS APH facility. To mitigate this, only a harvest at ~6 weeks (November 2, 2018; Day 45) was carried out. Thinnings and 6 week old harvested plants were immediately frozen and stored in the GLACIER freezer at –160 °C. These will provisionally be returned in late spring 2019. The ground control was initiated at KSC a week later (September 25, 2018; Day 0), with FluorPen photosynthesis measurements/thinning occurring on October 9, 2018 (Day 14), FluorPen photosynthesis measurements on October 26, 2018 (Day 31), and final harvest was on November 9, 2018 (Day 45).

| | |
|---|---|
| **Bibliography Type:** | Description: (Last Updated: 04/30/2024) |
| **Abstracts for Journals and Proceedings** | Lewis NG, Costa, MA, Moinuddin SG, Davin LB, Richards J, Monje O, Levine H, Onate B, Dufour N, Carver JA, Hanson DT, Turpin M, Lipton MS, Hixson KK, Starkenburg SR, Sayre RT. "Arabidopsis GRO (Gravitational Response Omics): Plant Habitat (PH-01)." Presented at the 34th Annual Meeting American Society for Gravitational and Space Research, Bethesda, MD, October 31-November 3, 2018.<br>34th Annual Meeting American Society for Gravitational and Space Research, Bethesda, MD, October 31-November 3, 2018. , Nov-2018 |
| **Abstracts for Journals and Proceedings** | Turpin M, Hanson D, Lewis NG, Davin LB. "Gravitational effects on the growth of modified Arabidopsis thaliana in the ISS Advanced Plant Habitat." Presented at the 34th Annual Meeting American Society for Gravitational and Space Research, Bethesda, MD, October 31-November 3, 2018.<br>34th Annual Meeting American Society for Gravitational and Space Research, Bethesda, MD, October 31-November 3, 2018. , Nov-2018 |
| **Significant Media Coverage** | Rickey T. "Article on PI's research: 'A matter of gravity: Understanding how plants grow in space.' " Molecular Bond, EMSL (Environmental Molecular Sciences Laboratory) Newsletter, June 29, 2018. See article at https://mailchi.mp/pnnl/emsls-molecular-bond-june-1?e=778eef759d , Jun-2018 |
| **Significant Media Coverage** | Watson KE. "Astronaut Williams described working with PI's plants on ISS: 'McCaffrey students visited by space explorer: Astronaut Sunita Williams shares her experiences.' " The Galt Herald, April 18 2018. See article at http://www.galtheraldonline.com/news/mccaffrey-students-visited-by-space-explorer/article_144aa81a-4334-11e8-842f-f7106ee5d2d7.html , Apr-2018 |
| **Significant Media Coverage** | Bays J. "Astronaut Williams described working with PI's plants on ISS: 'NASA astronaut Sunita Williams nudges Galt students to reach for the stars.' " Lodi News-Sentinel, April 14 2018. See article at https://www.lodinews.com/news/article_982e5434-3faa-11e8-b4e1-db589601c0df.html?mode=story , Apr-2018 |
| **Significant Media Coverage** | Radio Sputnik. "Growing Plants in Antarctica 'Open Way' for Distant Space Missions - Analyst." Antarctica has yielded its first vegetable crops, grown without soil or daylight. Scientists grew the greens at the Neumayer-Station III inside a high-tech greenhouse. The project aims to help cultivate fresh food on other planets. Sputnik discussed the significance of the scientists' success with Norman G. Lewis, regents' professor at the Institute of Biological Chemistry, Washington State University." Radio, April 23, 2018. Listen interview at https://sputniknews.com/analysis/201804121063464838-iraq-antarctica-iraq-plants-growth/ , Apr-2018 |
| **Significant Media Coverage** | Ferguson W. "Article on PI's research: 'WSU research heads to International Space Station.' " WSU Insider. May 16, 2018. See article at https://news.wsu.edu/2018/05/16/wsu-research-heads-international-space-station/ , May-2018 |
| **Significant Media Coverage** | Jackson S. "Article on PI's research: 'WSU research off to space.' " The Spokesman Review, May 18, 2018. See article at http://www.spokesman.com/stories/2018/may/18/wsu-research-off-to-space/ /, May-2018 |
| **Significant Media Coverage** | Cary A. "Article on PI's research and sharing to Pacific Northwest National Laboratory researchers: 'Plants from outer space are coming to Richland.' " The Tri-City Herald, May 18 2018. See article at https://www.tri-cityherald.com/news/local/pacific-northwest-national-lab/article211396464.html , May-2018 |
| **Significant Media Coverage** | Boyle A. "Article on PI's research: 'Small seeds on Cygnus cargo ship could lead to giant leap for farming in space.' " GeekWire, May 19, 2018. See article at https://www.geekwire.com/2018/small-seeds-cygnus-cargo-ship-lead-giant-leap-growing-plants-space/ , May-2018 |
| **Significant Media Coverage** | Doughton S. "Article on PI's research aboard ISS: 'Look up to catch stellar views of the International Space Station this week.' " The Seattle Times, May 23, 2018. See article at https://www.seattletimes.com/seattle-news/science/look-up-to-catch-stellar-views-of-the-international-space-station-this-week/ , May-2018 |
| **Significant Media Coverage** | Snyder A. "Article on PI's research: 'These mutant seeds may help us garden in space – and on Earth.' " AXIOS, May 20, 2018. See article at https://www.axios.com/mutant-seeds-nasa-mission-growth-molecule-ccb7c095-f38d-469c-ac33-2598f4fcce11.html , May-2018 |
| **Significant Media Coverage** | Whisenant H. "Interview with PI Lewis: 'Plants in space: Not your average garden variety cosmos.' " Northwest Public Broadcasting (PBS). June 8, 2018. Listen to interview at https://tinyurl.com/y8dlqsd6 , Jun-2018 |
| **Significant Media Coverage** | Feustel AJ (Drew). "Twitter cast: 'Happy Friday everybody! @astro_ricky and I wanted to show you some of the plants we've been growing on @Space_Station.' about PI's research." Twitter. June 22, 2018. Listen at https://twitter.com/Astro_Feustel/status/1010236767251894272 , Jun-2018 |
| **Significant Media Coverage** | Feustel AJ (Drew). "Video on Twitter: 'It's been a while since we've checked in on @astro_ricky and the plants he's growing here on @Space_Station. Anyone else growing the same plants on Earth right now?' about PI's plants growing on ISS." Twitter. July 9, 2018. Listen at https://twitter.com/Astro_Feustel/status/1016430287083974657 , Jul-2018 |
| **Significant Media Coverage** | Feustel AJ (Drew). "VIDEO from space, harvest Day! Farmer's almanac update on International Space Station. The plants continue to grow under the watchful eye of #AstroFarmer @astro_ricky! Twitter video of PI's plants on ISS." Twitter. July 23, 2018. Listen at https://twitter.com/hashtag/AstroFarmer?src=hash , Jul-2018 |
| **Significant Media Coverage** | WSU Insider. "PI's plant experiments first 8K ultra-high-definition video: 'Experience space station research in high res video.' " WSU Insider, November 9, 2018. See article at https://news.wsu.edu/2018/11/09/experience-high-res-science-first-8k-nasa-space-station-footage/ , Nov-2018 |