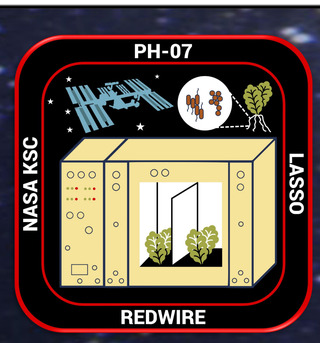
Preflight Definition and Verification Testing for the Plant Habitat-07 Experiment to Study Substrate Moisture Impacts on Lettuce Plant and Microbiome Development.

Gioia Massa[1], LaShelle Spencer[2], Blake Costine[2], Oscar Monje[2], Ani Dixit[2], Aaron Curry[2], Jacob Torres[3], Chad Vanden Bosch[4], Dave Reed[4], Thomas Tyson[4], Clayton Grosse[4], Ashleigh Ruggles[4], Denise Freeland[1], Nicole Dufour[1]

1. NASA, Kennedy Space Center, 2. LASSO II, Kennedy Space Center, 3. University of New Mexico, 4. Redwire Space Technologies, Inc.



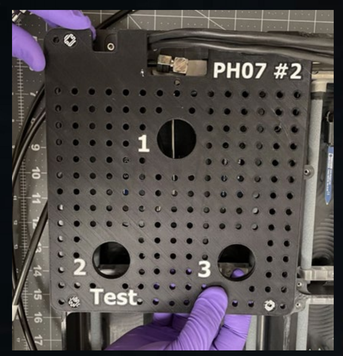
Introduction

Delivering adequate water and oxygen to root zones of crops growing in microgravity is challenging due to the complex behavior of fluids and gasses during spaceflight. Chronic excess (flood) or insufficient (drought) water levels, or intermittent watering and wilting of plants, leads to alterations in plant growth and impacts on the nutritional and microbial composition of those plants. PH-07 will apply controlled water stress to assess and quantify changes in plant growth and the microbiome of a well-tested food crop, 'Outredgeous' red romaine lettuce, grown in NASA's Advanced Plant Habitat (APH) on the ISS.

Preflight Definition Test

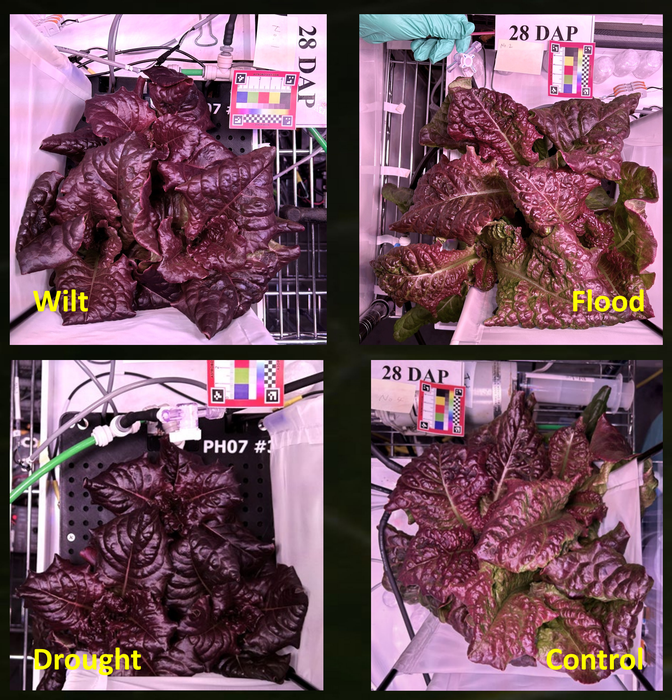
Ground testing was performed in controlled environment chambers at Kennedy Space Center.

Plant positions were defined to fall between porous tubes and sensors to allow for root extraction post-harvest. Root recovery is critical for understanding the microbiome in the root zone. Analog science carrier quadrants were constructed, and these were operated with standpipe irrigation control.



Analog quadrants were run in chambers set to mimic desired APH settings. Germination domes were tested, but a simpler solution of increasing RH and decreasing light during germination was determined for APH operations.

Plants were grown for 28 days and after several rounds of testing, moisture conditions were determined that would impose desired growth differences.



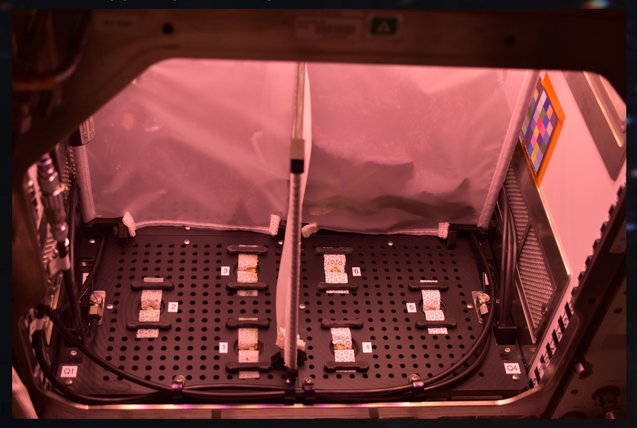
Clockwise from top left: Wilt treatment, Flood treatment, Control, and Constant Drought treatment. Note that both wilt and constant drought treatments show increased red coloration, an indication of enhanced anthocyanin.

Experiment Unique Equipment

Requirements drove development of unique equipment.

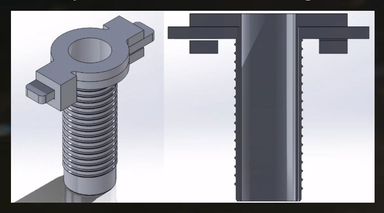
Dividers

- Treatments must be separated to study different microbiomes
- Shoot divider developed to allow air flow but keep plants contained to their quadrant and prevent leaf touching
- Divider installed on day 14 when exponential growth begins
- Divider assembled in 3 parts with partitions that can be removed individually during harvest
- Divider support posts snap into holes in APH science carrier lid



Root Extractor

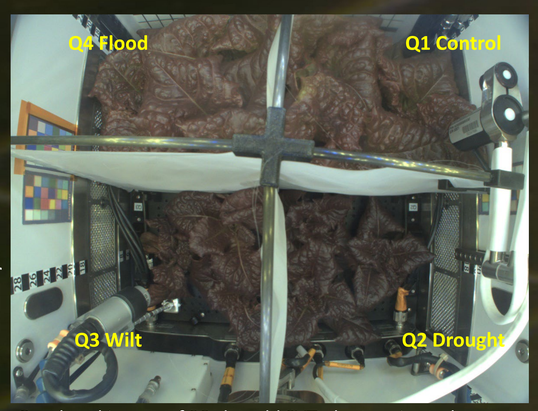
- Roots and substrate must be extracted for post-flight analysis
- Science carrier lid is sealed on Earth and cannot be opened in flight, preventing easy access to the interior
- Developed and iterated methods of extracting cores containing roots and substrates
- Must maintain containment of samples & remaining substrate
- Coring system screws through threaded holes in lid
- Outer cylinder remains in place, while inner core is removed
- Core sample is extracted into bag and frozen



Top: 3D printed iteration Right: Flight extractor
Bottom: Extraction during EVT

Science Verification Testing

Science Verification Testing (SVT) was conducted in the APH ground unit 1/30/24-2/27/24. This test helped refine success criteria and better understand moisture control in the APH hardware.



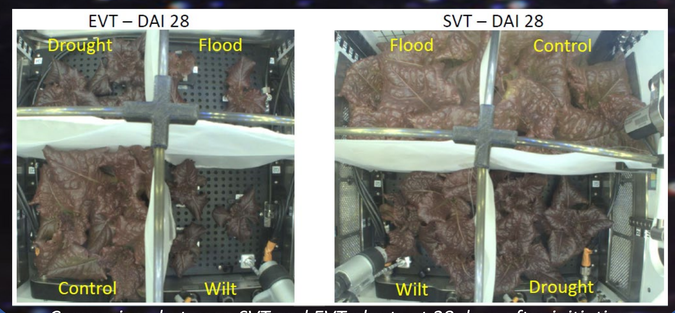
Overhead image of 28-day old SVT plants prior to harvest.

Experiment Verification Testing (EVT)

Flooded plants grew more than expected in SVT and wilt plants grew less than expected based on definition testing. Moisture control settings were slightly modified to better manage these conditions to desired levels during EVT.

EVT Summary

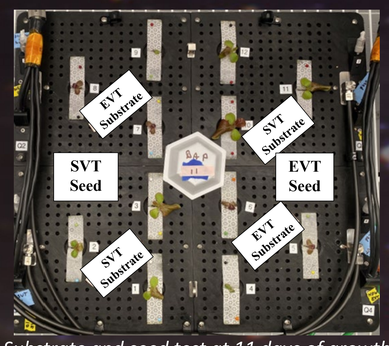
- New substrate was obtained between SVT and EVT
- EVT ran from 3/20/24-4/23/24
- Test extended to 35 days to better understand abnormalities in plant growth
- Plants showed unexpected stress beginning at 9 days
- Stress level varied by treatment, but was in all quadrants



Comparison between SVT and EVT plants at 28 days after initiation

Porous Ceramic Substrate Challenges

- Post-test analysis helped narrow in on porous ceramic substrate (Turface Proleague Elite®) as probable cause
- A short post-EVT growth test indicated stress with stunting and anthocyanin production
- Chemical analysis indicated high pH, EC, Ca, S, and SO_4
- Additional SVT substrate was located and validated for flight



Substrate and seed test at 11 days of growth

	EVT: Unsifted	EVT: Sifted	EVT: Washed	EVT: + Fertilizer	SVT: Washed	SVT: + Fertilizer	Fertilizer Only
Sulfate (ppm)	1212.8	1436.5	1032.7	1151.3	139.2	207.1	43.4
Calcium (ppm)	594.2	674.6	526.4	627.9	12.9	61.6	1
Sulfur (ppm)	507.06	586.84	459.29	34.23	55.24	85.07	18.1
EC (µS/cm)	2270	2460	2010	2970	379	1788	1328
pH	8.37	8.59	7.88	7.35	6.54	5.93	4.66

Chemical analysis of different substrate and fertilizer components of PH-07

Next Steps

- Three replicate 28-day growth tests on ISS
- Ground controls offset by 1 week delay
- Sample return (CRS-32) for postflight analysis
  - Plant chemistry
  - Food safety (cultural microbiology)
  - Microbiome analysis
  - Proteomics (Collaboration with Australian Centre of Plants for Space (P4S))
- Image analysis (Collaboration with P4S)
- Recommendations for optimal failure modes for future space crop production water/nutrient delivery systems

Acknowledgements

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