



Plant Science in Reduced Gravity: Lessons Learned

Gary W. Stutte, Ph.D.¹ Oscar Monje, Ph.D.¹ and Raymond M. Wheeler Ph.D.²

¹ ESC, Team QNA, MC ESC-24; ²NASA Surface Systems Office, MC NE-S.

Kennedy Space Center, FL 32899

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Abstract

- The effect of gravity on the growth and development of plants has been the subject of scientific investigation for over a century. The results obtained in space to test specific hypotheses on gravitropism, gene expression, seed formation, or growth rate are affected by both the primary effect of the microgravity and secondary effects of the spaceflight environment.
- The secondary effects of the spaceflight environment include physical effects arising from physical changes, such as the absence of buoyancy driven convective mixing, altered behavior of liquids and gases, and the environmental conditions in the spacecraft atmosphere. Thus, the design of biological experiments (e.g. cells, plants, animals, etc.) conducted in microgravity must account for changes in the physical forces, as well as the environmental conditions, imposed by the specific spaceflight vehicle and experimental hardware.
- In addition, researchers must become familiar with other aspects of spaceflight experiments: payload integration with hardware developers, safety documentation and crew procedures, and the logistics of conducting flight and ground controls. This report reviews the physical and environmental factors that directly and indirectly affect the results of plant science experiments in microgravity and is intended to serve as a guide in the design and implementation plant experiments in space.

Decadal Survey

- Understanding how terrestrial biology responds to micro/partial gravity will reduce exploration risks to crews by designing countermeasures to problems.
- ISS is a platform where reduced gravity can be used to probe and dissect biological mechanisms.
- Can life survive/thrive outside Earth? What limits life in the universe?

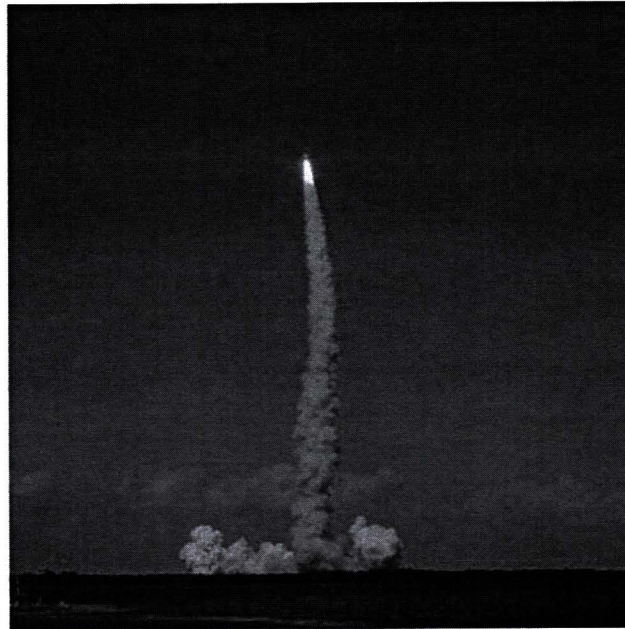
Decadal Survey

- Insights into plant and microbe responses to reduced gravity enhances our knowledge about us due to structural and functional similarities among Earth life forms.
- This knowledge is important for supporting safe and longterm human habitation in space using bioregenerative life support.
 - **Sensory mechanisms:** Gravity sensing and response to mechanisms in plants, Gravity and mechanical sensing in microbes, Radiation effects on plants/microbes
 - Plant/microbial growth under altered atmospheric pressures
 - **Spaceflight syndromes:** Response to integrated spaceflight environment, microbial ecosystems and environments, changes in virulence of pathogens.
 - Plant – Microbe Interaction, Role in Life support systems

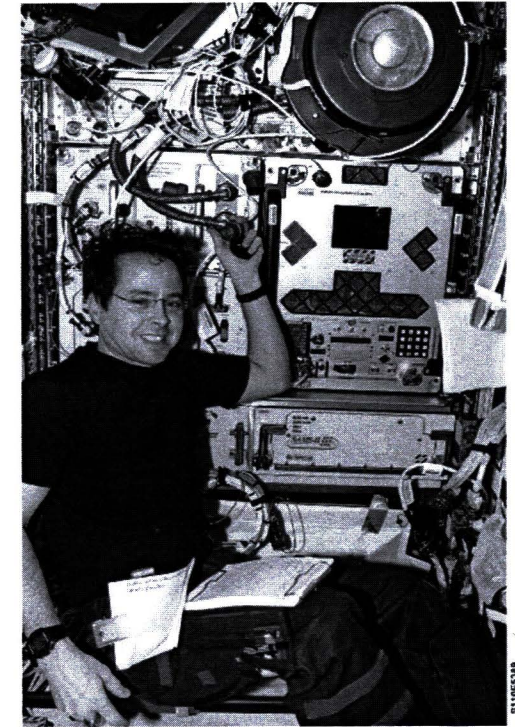
Flight Experiments



Ground Experiment
KSC



Launch Payload
KSC



Flight Experiment
ISS

Plant Growth in Space

- The past quarter century has provided remarkable progress in our understanding of how to grow plants in the unique environment of space, and have progressed from simple, short duration experiments with limited environmental control to complex, long-duration experiments with sophisticated environmental control and monitoring capabilities.
- The understanding of the complex effects the lack of convective mixing on nutrient and water uptake, temperature control, and diffusion gradients is being unraveled, the subtle effects microgravity on localized micro-climates in the atmosphere have been discovered, and the technology to support long-duration, multiple generation studies has been developed.
- The result of these incremental advances has been the inability of scientist to achieve seed germination and growth to the successful completion of seed to seed life cycle experiments for multiple species.

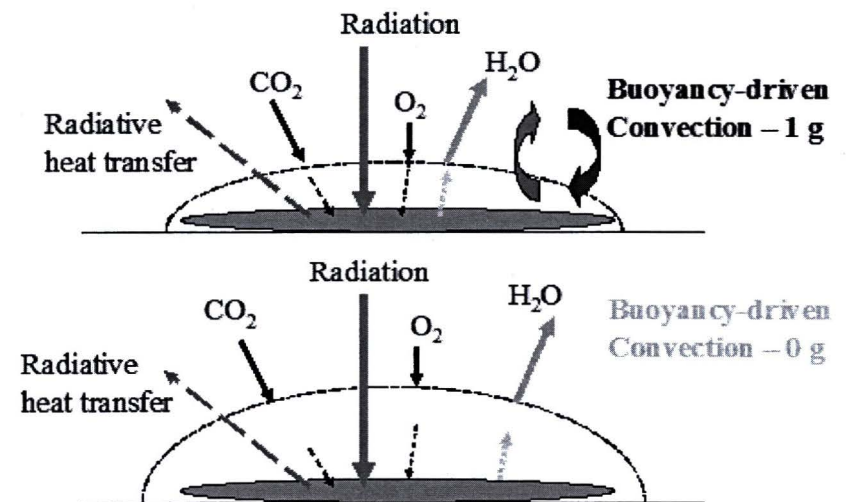
Example: Seed-to-Seed Expts

- Mary Musgrave from 1997-2002 illustrates how spaceflight experiments testing the space biology of reproduction have improved our understanding of the impact that secondary effects of microgravity may have on physiological responses measured in space.
- In 1993 a series of experiments (CHROMEX) to investigate the apparent sensitivity of reproductive events to the spaceflight environment were initiated using the Plant Growth Unit (PGU), a mid-deck locker payload on the Space Shuttle.
- *Arabidopsis thaliana* (L.) Heynh was selected for use in these experiments because of its compact size, low light requirement, and short life cycle. Early events in reproductive development were studied: gametophyte development, pollination, fertilization, and early embryogenesis during three flight experiments: CHROMEX-03 on STS-54 (6 d), CHROMEX-04 on STS-51 (10 d), and CHROMEX-05 on STS-68 (11 d).
- In CHROMEX-03, plants were grown in closed plant growth chambers (PGCs), and male and female gametophyte development aborted at an early stage in the flight material. In CHROMEX-04, CO₂ enrichment was provided to the closed PGCs and reproductive development proceeded normally until the pollination stage, when there was an obstacle to pollen transfer in the spaceflight material. In CHROMEX-05, an air-exchange system was used to ventilate the PGCs with VOC-free cabin air. Under these conditions, the spaceflight plants finally exhibited reproductive development comparable to the ground controls, and immature seeds that were similar to seeds from the ground control plants were produced.

Spaceflight Environment

Spacecraft are ideal platforms for studying direct effects of the lack of gravity on metabolism. However, the results obtained in space to test specific hypotheses on gravitropism, gene expression, seed formation, or growth rate may be compromised by secondary effects caused by changes in the physical environment compared to 1 g. The absence of gravity induces a number of physical effects that alter the microenvironment surrounding plants and their organs. These effects include increased boundary layers surrounding plant organs and the absence of convective mixing of atmospheric gases. In addition, altered behavior of liquids and gases is responsible for phase separation and for dominance of capillary forces in the absence of gravitational forces.

For example, in the absence of forced convection the lack of buoyancy-driven convection during spaceflight leads to thicker boundary layers in the direct contact with a plant, so the exchange of gases with the atmosphere and of water and solutes within the root zone is limited by diffusion processes and thus much slower than in a normal gravity environment (Porterfield, 2002).



Experiment Design

Design of biological experiments (e.g. cells, plants, animals, etc) conducted in microgravity must account for:

- 1) changes in gravity-dependent fluid and gas behavior,
- 2) the bulk atmospheric composition of the spacecraft (i.e. 6000 ppm CO₂, 30-50% RH, ethylene < 50 ppb, and other trace contaminants on ISS), and
- 3) hardware-specific limitations (ventilation, light level, CO₂ supply, humidity and temperature control, and ethylene removal) imposed by implementing the experiment within a given experimental platform.

Nevertheless, once the plant hardware used to grow the organisms is able to mitigate the effects of secondary effects of microgravity using adequate illumination, ventilation, VOC control, temperature and humidity control, and active root zone moisture control then plants grow and are able to complete their life cycles (Monje et al., 2003; Wolff et al., 2012).

Root Zone – Moisture Control

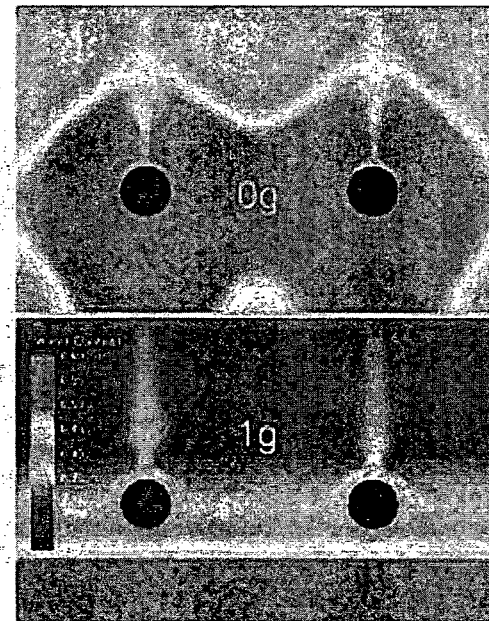
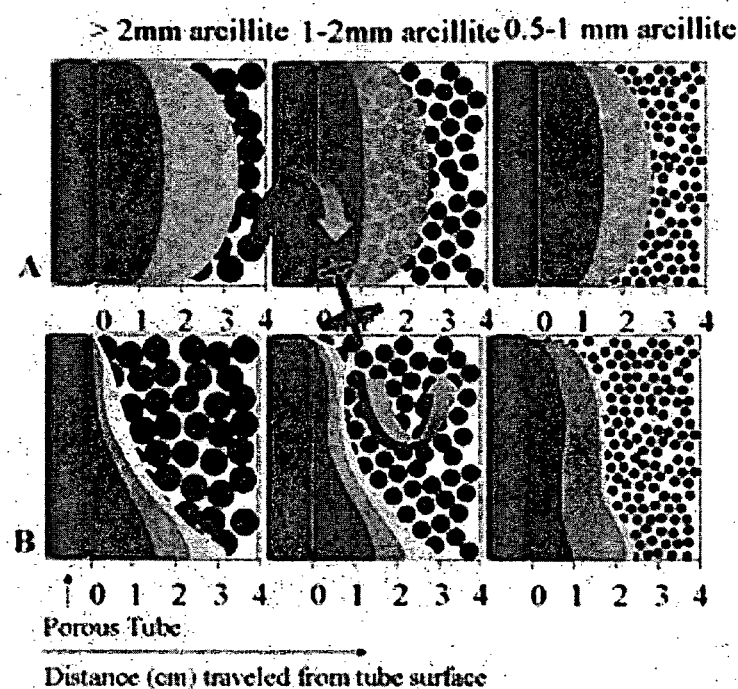
- In space, water flow and distribution in porous media is affected by changes in buoyancy, dominance of capillary forces, particle rearrangement, and vehicle vibration during launch (Ivanova and Dandolov, 1992; Podolsky and Mashinsky, 1994; Heinse et al., 2007). The figure below shows results from water addition experiments conducted during parabolic flights in preparation for the PESTO experiment (left), and a simulation of how gravity affects root zone water distribution (right) in the 15 cm deep root modules of the Svet plant chamber (Jones and Or, 1999). When water is added to substrates using porous tubes, the shape of the wetting fronts is gravity dependent. In gravity, water drains along the gravity vector leaving the rooting media moist, but well-aerated. Substrates with smaller particle sizes only partially overcome the pull of gravity because of capillary forces. In space, water distribution within rooting media is more homogenous because capillary forces dominate, which may contribute to poor aeration.
- The moisture distribution of the Svet root module, obtained from data collected by an array of moisture sensors, clearly shows that water collects at the bottom of the root module in 1g. In contrast, the same amount of water is more evenly distributed throughout the root module in microgravity. This behavior can lead to hypoxia during spaceflight if root zone moisture is controlled using setpoints derived during ground-based experiments.

Atmospheric Composition of ISS

- The atmospheric composition of ISS is vastly different than that of terrestrial environments (e.g. drier (30-50% RH), CO₂ enriched (6000 ppm), warm (23 °C), and contains many VOCs (ethylene (<50 ppb), ethanol (5 ppm), acetone (<1 ppm)). Evaluation of both in-flight and post-flight cabin air quality samples from the *ISS* demonstrates that even though the on-board contamination control systems and passive controls implemented to minimize contamination sources are maintaining acceptable cabin air quality, significant spatial and temporal effects do occur (Perry and Peterson, 2003). Such effects are directly influenced by the station's configuration, the operation of on-board contamination control equipment, and equipment failures. Chief contributors to the total trace contaminant load include methane, alcohols, and organosilicones. Minor contributors include ketones, halocarbons, hydrogen, and carbon monoxide.
- The atmospheric composition may have direct effects on plant growth when the plant experiments are exposed to cabin air. During the International Shuttle-Mir Greenhouse Project wheat cv. Superdwarf was grown for an entire life cycle, resulting in ~300 sterile heads due to high ethylene (0.4 ppm during anthesis) (Campbell et al., 2001). This was the first case identifying ethylene, a plant hormone, as a deleterious contaminant found on spacecraft.
- Ethylene was also shown to have affected the growth and morphology of *Arabidopsis* seedlings from a gravitropic study conducted in Biorack during STS-84 (Guisinger and Kiss, 1999), the sixth Shuttle/Mir docking mission. Air samples of Shuttle air taken during the mission revealed the presence of up to 1.6 ppm ethylene suggested that the Shuttle air was contaminated during docking with Mir.
- Currently, the ISS is required to maintain ethylene below 0.5 ppm. However, ethylene is seldom measured and requires complex analytical instrumentation to detect. Thus, most plant chambers utilize engineering controls (e.g. potassium permanganate sorbents like Purafil© or photocatalytic oxidation) for removing ethylene produced by the plants growing within.

Moisture Redistribution

This example illustrates how a difference in gravitational force can result in significant offsets in control parameters developed on earth for optimum plant growth, due to these shifts in hydrostatic water distribution (Jones et al., 2003). In PESTO, these effects were mitigated by using 1-2 mm arcillite and 3 cm deep root modules.



Conclusions

- ISS is a unique platform for studying plant responses to reduced gravity – important for Life Support
- Experiment outcomes influenced by:
 - Response to lack of gravity.
 - Effects from spaceflight environment
- Must control: ventilation, light level, CO₂ supply, humidity and temperature control, and ethylene.
- Must address: payload integration with hardware developers, safety documentation and crew procedures, and logistics of conducting flight and ground controls.