



A Researcher's Guide to:

INTERNATIONAL SPACE STATION

# Plant Science



This International Space Station (ISS) Researcher's Guide is published by the NASA ISS Program Science Office.

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*Cover and back cover:*

- a. *View of Astronaut Peggy Whitson, Expedition 5 NASA International Space Station science officer, looking at the Advanced Astroculture Soybean plant growth experiment as part of Expediting the Process of Experiments to the Space Station Rack 4 located in the U.S. Laboratory/Destiny.*
- b. *Back Cover: Both images are Arabidopsis thaliana (Brassica family) plants grown under controlled conditions in a plant cultivation module in the BioServe Laboratories. Image courtesy of BioServe Laboratories.*

# The Lab is Open

Soaring 250 miles above Earth, the ISS is a modern wonder of the world, combining the efforts of 15 countries and thousands of scientists, engineers and technicians. The ISS is a magnificent platform for all kinds of research to improve life on Earth, enable future space exploration and understand the universe. This researcher's guide is intended to help potential researchers plan experiments that would be exposed to the space environment, while externally attached to or deployed from the ISS. It covers all the pertinent aspects of the space environment, how to best translate ground research to flight results and lessons learned from previous experiments. It also details what power and data are available on the ISS in various external locations.



*Cosmonaut Gennady Padalka harvests radishes from the Lada Plant Chamber (June 22, 2009).*





# Unique Features of the ISS Research Environment

- 1. Microgravity**, or weightlessness, alters many observable phenomena within the physical and life sciences. Systems and processes affected by microgravity include surface wetting and interfacial tension, multiphase flow and heat transfer, multiphase system dynamics, solidification, and fire phenomena and combustion. Microgravity induces a vast array of changes in organisms ranging from bacteria to humans, including global alterations in gene expression and 3-D aggregation of cells into tissue-like architecture.
- 2. Extreme conditions** in the ISS space environment include exposure to extreme heat and cold cycling, ultra-vacuum, atomic oxygen, and high energy radiation. Testing and qualification of materials exposed to these extreme conditions have provided data to enable the manufacturing of long-life reliable components used on Earth as well as in the world's most sophisticated satellite and spacecraft components.
- 3. Low Earth orbit** at 51 degrees inclination and at a 90-minute orbit affords ISS a unique vantage point with an altitude of approximately 240 miles (400 kilometers) and an orbital path over 90 percent of the Earth's population. This can provide improved spatial resolution and variable lighting conditions compared to the sun-synchronous orbits of typical Earth remote-sensing satellites.

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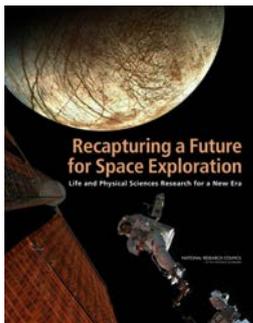
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# Why Use the ISS as \_\_\_\_\_ a Laboratory?

The progress in plant space biology over the past quarter century has greatly increased our understanding of how plants: respond to gravity; informed the design of advanced plant growth facilities; achieved the completed life cycle; and demonstrated that physiological processes necessary for biological life support are sustainable. In the process, the horticulture of plants in the unique environment of microgravity was being developed, and plant/microbe interactions were explored. The advances made during the decades of spaceflight experimentation have also identified critical gaps in our understanding of the role of gravity and the spaceflight environment on plant biology at the cellular, tissue, whole plant, and community levels.

In this context, the International Space Station is a unique platform where reduced gravity can be used to probe and dissect biological mechanisms in plants for understanding how terrestrial biology responds to gravity. This knowledge is important for supporting safe and long-term human habitation in space using bioregenerative life support, utilizing plants and microbial communities, and for reducing exploration risks to crews by designing countermeasures to problems associated with living in space. In addition, by using the facilities with centrifuges, scientists can investigate how plants respond to the reduced gravity environments on the Moon and Mars.

The National Research Council's 2011 Decadal Survey Report, "Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New



Era," (<http://www.nap.edu/catalog/13048.html>) recognized the significance of understanding plant responses in microgravity for both space exploration and terrestrial applications. The report had a number of recommendations for plant research including the following:

1. The establishment of a robust spaceflight program of research analyzing plant and microbial growth and physiological responses to multiple stimuli encountered in the spaceflight environments.
2. The establishment of a research program aimed at demonstrating the roles of microbial-plant systems in long-term life support systems.

A number of knowledge gaps were identified that are well-suited to the current capabilities of spaceflight hardware and analytical capabilities of the ISS. These gaps clearly point to the necessity of maximizing the science return from each spaceflight opportunity and the need for both basic research in transcriptional profiling, proteomic and metabolomic analysis in model systems. These as well as life cycle studies are necessary for developing horticultural techniques and validating environmental conditions to establish the feasibility of incorporating plants in bioregenerative life support systems. A partial listing of these research questions is included in Table 1.

Topic Area	Potential Research Questions Suitable for ISS Investigations
Gravity Sensing	<ul style="list-style-type: none"> <li>• What are the primary gravity receptors in leaf stem and root tissue?</li> <li>• What are intermediate signals at transcriptional, biomolecular and physiological levels?</li> <li>• What interactions with thigmotropic, phototropic and hydrotropic stimuli occur, and how are they differentiated?</li> </ul>
Plant Physiology	<ul style="list-style-type: none"> <li>• How do light and gravity responses interact?</li> <li>• Does the spaceflight environment induce stresses (<math>\mu</math>-gravity, elevated CO<sub>2</sub>, diffusion limited chemical exchange, root zone hypoxia, etc.), and what are primary signals and hormonal changes affecting development?</li> <li>• What are the effects of spaceflight environments on primary physiology: photosynthesis, respiration, transpiration, nutrition, and secondary metabolism?</li> <li>• How do the interactions of multiple environmental stimuli affect productivity and bioavailability of bioactive products?</li> <li>• How does the gravity effect on structural carbohydrates manifest itself over multiple generations?</li> <li>• Does microgravity affect genetic stability?</li> </ul>
Plant/Microbe Interactions	<ul style="list-style-type: none"> <li>• What aspects of the spaceflight environment regulate resistant and/or susceptibility to plant pathogen infection?</li> <li>• Is the gravity effect on virulence universal or species/strain specific?</li> <li>• How does the spaceflight environment affect the development of beneficial plant/microbe associations?</li> </ul>
Life Support Systems	<ul style="list-style-type: none"> <li>• How can horticultural approaches to sustained production of edible crops be implemented?</li> <li>• What are the effects of environmental stresses (lighting, CO<sub>2</sub>, root zone moisture, O<sub>2</sub>, trace gases) on productivity?</li> <li>• Are models of crop productivity developed in terrestrial conditions valid in spaceflight environments?</li> <li>• How do plants and microbes interact with physical and chemical life support systems?</li> </ul>

Table 1. Partial listing of research questions.

# Results from Past \_\_\_\_\_ Microgravity Research

## Microgravity as a Research Tool

The effects of gravity on the growth and development of plants have been the subject of scientific investigation for more than a century with the early work of Charles Darwin demonstrating the gravitropic response of roots (Darwin, 1881). The effect of spaceflight environment on plants has been studied since the early BioSatellite experiments in the 1960s (e.g., Johnson and Tibbitts, 1968). Subsequent investigations of plant responses in the spaceflight environment have allowed our understanding of the mechanisms of plant responses to gravity to be unraveled (Sachs, 1991; Bancaflor and Masson, 2003; Swarup and Bennet, 2009; Paul et al., 2013).

The microgravity environment has also been instrumental in understanding how the unique aspects of the spaceflight (microgravity, lack of convective currents) will affect biological life support systems for long-duration space missions (Ferland et al., 2002; Wolverton S.C., J.Z. Kiss. 2009. An update on plant space biology. *Gravitational and Space Biology* 22: 13-20. Monje et al., 2003; Wheeler, 2010).

The past quarter century has provided remarkable progress in our understanding of how to grow plants in the unique environment of space and progressed from simple, short-duration experiments with limited environmental control to complex, long-duration experiments with sophisticated environmental control and monitoring. The complex effects of the spacecraft environment on nutrient and water uptake, temperature control and diffusion gradients have been identified; the effects of gravity on signal perception and transcription are being unraveled, and technology to support long-duration, multiple-generation plant growth is being developed.

This report provides a general overview of the results from these spaceflight studies and identifies the unique aspects of the spaceflight environment that lend themselves to scientific investigation and understanding of space plant biology.

## Results from Microgravity

Studies from satellites in the 1960s showed that photosynthesis proceeded as expected in space (Ward et al., 1970) and that plants (peppers) developed leaf epinasty in a free-fall environment (Johnson and Tibbitts, 1968). The photosynthetic observations were later confirmed in a more thorough study as part of the first plant experiment flown on the ISS (Monje et al., 2005, Stutte et al., 2005). The observation of leaf epinasty in space confirmed the long-observed linkage between gravity and ethylene mediated responses observed on horizontal clinostats and when plants were gravitationally disoriented (Denny, 1936; Leather et al., 1972). Further analysis of the epinasty data showed that the extent of leaf curling for peppers in space and on horizontal clinostats was different, yet confirmed its occurrence under both conditions (Brown et al., 1974).

A long-standing challenge for space biological research was to determine whether plants could successfully complete a life cycle in space—a so-called “seed-to-seed” experiment. This was first demonstrated with *Arabidopsis* by Merkys et al. (1984) on the Russian Salyut-7 and then later by Link et al. (2003) as well as with other species including *Brassica rapa* (Musgrave et al., 2000) and wheat (Sytchev et al., 2001), which were both carried out on the Russian Mir Space Station. Subsequent studies on the International Space Station using the “Lada” plant chamber demonstrated successive generations of pea (*Pisum sativum*) plants (Sytchev et al., 2007).

Other researchers used the free-fall environment of spaceflight to finally test Charles Darwin’s hypothesis that circumnutation of growing plant stems is an endogenous rhythm rather than just gravitational overshoot of the plumb-line following by continuous self-correction (Darwin, 1881). Brown et al. (1990) reported that sunflower hypocotyls showed circumnutation in weightlessness but at a different frequency than in 1-g. Thus, a spaceflight experiment was needed to answer this more than 100-year-old question. (Johnson A., Solheim B.G.B. Iverson, T.-H. (2009) Gravity amplifies and microgravity decreases circumnutations in *Arabidopsis thaliana* stems: results from a space experiment. *New Phytologist* 182, 621-629. Others have taken advantage of the free-fall environment in low-Earth orbit to carefully study plant phototropic responses (Heathcoat et al., 1995; Kern and Sack, 1999; Millar et al., 2010) and other fundamental influences on plant growth and development such as magnetic fields (Hasenstein et al., 2005) or water-potential gradients on root growth (Takahashi et al., 2003).

In addition, by preserving plant tissue retrieved from spaceflight, space biologists have been able to detect differential expression of genes between the  $\mu$ -g and 1-g environments and draw inferences on the types of enzymes and metabolic responses that might occur in plants while growing in space (Paul et al., 2001). Spaceflight testing has also allowed assessment of amyloplast (statolith) positions in gravity-sensing organs and tissues in microgravity environments (Kern et al., 2001).

## Opportunities on ISS for Plant Research



*Figure 1. Wheat cv. USU Apogee grown in Biomass Production System during Photosynthesis Experiment and System Testing and Operation experiment during International Space Station Increment IV.*

Research on the ISS offers the opportunity to study how gravity and other factors influence the physical and biological processes in plant biology. It also provides the opportunity to exploit these findings to advance the understanding of basic phenomena and to promote the commercialization of these results. The designation of the U.S. portion of the ISS as a National Laboratory (NL) by the U.S. congress in 2005 opened the door to use this unique research environment by government agencies, academic institutions, private foundations, and commercial interests.



The ISS NL provides a variety of purpose-built, state-of-the-art equipment to enable research in the life sciences. These include access to multipurpose facilities with supporting hardware fitted with standard interfaces for power, data, cooling, water, and other critical resources to maintain and monitor an experiment. Cold stowage facilities (down to  $-80^{\circ}\text{C}$ ) exist to maintain samples collected on ISS NL and a work environment suited to contain samples and conduct research with liquids and/or hazardous materials. There is specialized hardware for conducting plant research aboard the ISS NL that has been developed by NASA, international partners and commercial payload providers.

# Lessons Learned

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## Physical Phenomena and Primary Effects of Microgravity

Our understanding of how the spaceflight environment affects growth, development and physiology of plants can be traced to the earliest days of space exploration. On Biosatellite II (launched 7 September 1967), wheat (*Triticum sativum* L.) seedlings were examined for orientation, morphogenesis and biochemical changes (Gray and Edwards, 1968), and leaf orientation of young pepper (*Capsium annum*) leaves was monitored (Johnson and Tibbitts, 1968). The research developed since that time revealed that plants can perceive microgravity as a stress either directly via effects on biological process (e.g., movement of gravity sensing statoliths) or indirectly because of secondary effects of the spaceflight environment. For example, there is an absence of buoyancy-driven convective currents in microgravity, resulting in thicker boundary layers on plant surfaces. This increase in boundary layer thicknesses affects rates of gas exchange, rate of evaporative cooling, and heat transfer through aerial organs of plants.

The lack of gravity and lack of convective mixing also has implications for the movement of water, oxygen and solutes through the root zone. This occurs directly because of the movement of water and solutes in root zones by diffusion (Porterfield, 2002), and indirectly by reducing the rate of evapotranspiration from the leaves (Monje et al., 2003). As a consequence, most plants returned from microgravity experience some degree of hypoxia stress (Stout et al., 2001). In addition, the reduction in evapotranspiration, combined with diffusion limited movement of solutes, can affect nutrient uptake by the roots (Wolff et al., 2013).

The series of experiments by Musgrave and co-workers from 1997 to 2002 illustrates how spaceflight improved our understanding of the impact of secondary effects of microgravity and physiological responses measured in space. Prior to her work, the only positive result in completing plant reproduction in a spacecraft was achieved on Salyut-7 in a miniature plant growth chamber called Phytton (Merkys et al., 1984).

In 1993, a series of experiments Chromosome and plant cell division in space (CHROMEX) studied the apparent sensitivity of reproductive events to the spaceflight environment 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, CO<sub>2</sub> 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 cabin air that had been filtered to remove volatile organic compounds (VOCs). Under these conditions, the spaceflight plants finally exhibited reproductive development comparable to the ground controls with immature seeds similar to those from the ground control plants (Musgrave et al., 1997).

This example showed that many physical changes will occur in the plant's environment because of microgravity. Because microgravity changes the behavior of fluids and gases, protocols established for successfully growing plants on the ground do not necessarily provide the same physical environment for plants growing in-orbit. The growth conditions provided in CHROMEX-03 allowed ground control plants to complete their early reproductive development, but the same protocol resulted in aborted development at an early stage in spaceflight. Since subsequent modifications of the gaseous environment in CHROMEX-04 (CO<sub>2</sub> enrichment) and CHROMEX-05 (gas flow-through) allowed reproduction to proceed normally during spaceflight, it was concluded that the gaseous environment around the spaceflight plants in CHROMEX-03 was inadequate to support continued reproductive development (Musgrave et al., 2000).

## ISS Atmospheric Conditions

The atmospheric composition of ISS can differ from terrestrial environments (e.g., drier [30 to 50 percent relative humidity (RH)], CO<sub>2</sub> enriched [3,000 to 7,000 ppm], warm [ $>23^{\circ}\text{C}$ ]), and contains many VOCs. Evaluation of both in-flight and post-flight cabin air quality samples from the ISS demonstrates that even though onboard air revitalization and control systems can maintain acceptable cabin air quality, significant spatial and temporal effects still occur (Perry and Peterson, 2003). Such effects are directly influenced by ISS's configuration and operational activities, the functional status of trace contaminant control equipment and equipment failures. Chief contributors to the total trace contaminant load include methane, alcohols and organosilicones. Other minor contributors include ketones, halocarbons, hydrogen, and carbon monoxide.



*Figure 2. Shannon Lucid inspects wheat grown in Svet plant growth chamber on Mir.*

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. Super dwarf was grown for an entire life cycle, resulting in approximately 300 sterile heads because of 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 also affected the growth and morphology of *Arabidopsis* seedlings 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, suggesting that the shuttle air was contaminated during docking with Mir. Kiss et al. (1999), and it was observed that two striking features of *Arabidopsis* seedlings developed in spaceflight: anomalous hypocotyl hook structure (Fig. 2) and a higher density of root hairs. Experiments subsequently confirmed that these responses were similar to those induced by ethylene, or ethylene analogs and suggested that ethylene-like compounds were present in the spacecraft cabin.

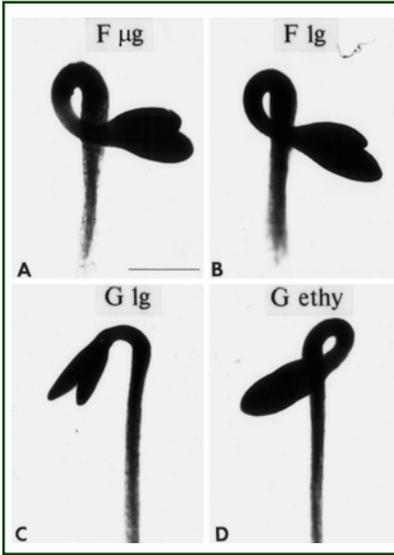


Figure 3. Light micrographs of hypocotyls of *Arabidopsis* seedlings from the F  $\mu$ g (A), the ground controls (B = F 1 g; C = G 1 g), and the ethylene control (D = G ethy). Note the anomalous hook that developed in hypocotyls of the light seedlings (both F  $\mu$ g and F 1g) and in the ethylene ground control. A typical hypocotyl hook, characteristic of dark-grown seedlings, developed in G 1-g seedlings. Bar = 500  $\mu$ m (Kiss et al. 1999).

Currently, the ISS is required to maintain ethylene below 0.05 ppm. However, ethylene is seldom measured and requires specialized 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. During rapid growth, the ethylene production rates from lettuce and wheat plants range between 1.6 -2.5 nmol m<sup>-2</sup> d<sup>-1</sup> but could be as high as 93 nmol m<sup>-2</sup> d<sup>-1</sup> for tomatoes during fruit ripening (Wheeler et al., 2004). Plant growth chambers like the Svet on Mir, Lada on ISS, and Veggie that circulate cabin air for cooling, providing CO<sub>2</sub> and humidity control remain exposed to ethylene from the spacecraft. Apparently, the current ethylene control on the ISS is sufficient to allow seed formation as seed-to-seed experiments have been recently accomplished using the Lada open-chamber system on ISS (Sychev et al., 2007).

## Gravity

Orbiting spacecraft are ideal platforms for studying direct effects of weightlessness (microgravity) on living organisms. In reality, spacecraft such as the ISS are still under about 90 percent of Earth's gravitational pull, but their continuous free-fall provides a unique environment for scientific research. Ideally, it would be best to have centrifugation capabilities along with the spaceflight microgravity treatments to provide side-by-side 1-g or fractional g comparisons, and this approach has been used in the past (e.g., Brown et al., 1990) and exists with research hardware such as the European Modular Cultivation System (EMCS). Reduced or fractual



Figure 4. Water droplet on pea leaf grown in Lada chamber on ISS. The lack of buoyancy-driven convection in microgravity alters the behavior of fluids and gases at the leaf interface.

gravity can be used to study plants in the gravity levels found on the moon and Mars (Kiss J.Z., P. Kumar, R.N. Bowman, M.K. Steele, M.T. Eodice, M.J. Correll, R.E. Edelman. 2007. Biocompatibility studies in preparation for a spaceflight experiment on plant tropisms (TROPI). *Advances in Space Research* 39: 1154-1160). However, the results obtained in space to test specific hypotheses on gravitropism, gene expression, seed formation, growth rate, etc., can be compromised by secondary effects caused by changes in the physical environment compared to 1 g. As noted earlier, 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 in microgravity are responsible for phase separation and for dominance of capillary forces (Porterfield, 2002).

Thus, the 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) potential effects of spacecraft atmosphere, and 3) hardware-specific limitations (ventilation, light level, CO<sub>2</sub> supply, humidity and temperature control, and ethylene removal; Monje et al., 2003; Wolff et al., 2013).

## Lack of Convection

In the presence of gravity, buoyancy-driven thermal convection induces the movement of fluids surrounding plant organs and tissues (e.g., hot air rises and cold air sinks). This movement enhances mass and heat transport between the tissues and the surrounding bulk-air (Porterfield, 2002). In space, plants without adequate ventilation will become surrounded by stagnant air layers that may translate into significant effects on plant metabolism. At 1 g, the boundary layers are thin enough so that metabolic processes such as respiration and transpiration are rarely diffusion-

limited (Monje et al, 2003). In microgravity, the thickness of these boundary layers increase and heat-and-mass transfer is sustained only by molecular diffusion when there is no forced air convection.

The supply and removal of metabolic gases ( $O_2$ ,  $CO_2$ ,  $H_2O$ , and ethylene) to and from plant organs (seeds, flowers, shoot tips, leaves and roots) also becomes diffusion limited, which can lead to plant stress and reduced growth because most biological processes (e.g. photosynthesis and respiration) quickly exceed the supply rates that can be achieved by diffusion alone (Porterfield 2002; Monje et al., 2003). These secondary effects of microgravity operate by limiting heat and mass transport across boundary layers. They affect shoot and root organs. However, roots are affected by an additional factor, microgravity-induced moisture redistribution

within the root media, which further reduces the availability of oxygen to roots (Jones and Or, 1998; Liao et al, 2004). In the absence of convective mixing, the secondary effects associated with increased resistance across the boundary layer for gas exchange, reduced capacity of evaporative heating, and accumulation of volatiles in localized areas require that careful attention be paid to the design of ground control experiments and appropriate selection of flight hardware.

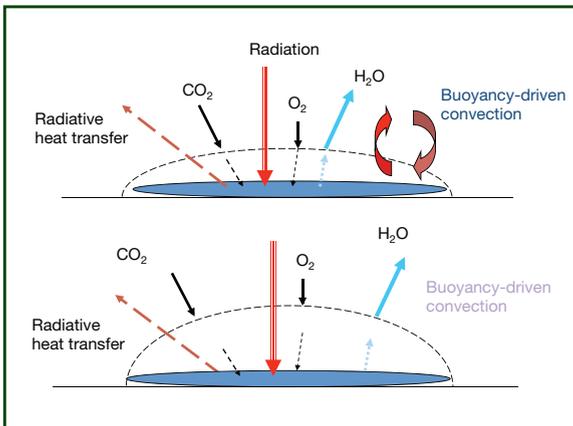


Figure 5. On Earth, buoyancy-driven convective mixing results in the boundary layers around plant organs being smaller than in microgravity. Under 1-g conditions, the boundary layers are usually small enough that gas exchange is not diffusion limited. In contrast, these processes are often diffusion limited in  $\mu$ -g.

## Gas Exchange

A number of experiments have investigated the effects of microgravity on gas exchange in plants (Ward et al., 1970; Tripathy et al., 1996; Stutte et al., 2005). The early spaceflight experiments were conducted in poorly ventilated growth chambers, and this resulted in net decreases in growth and apparent photosynthetic

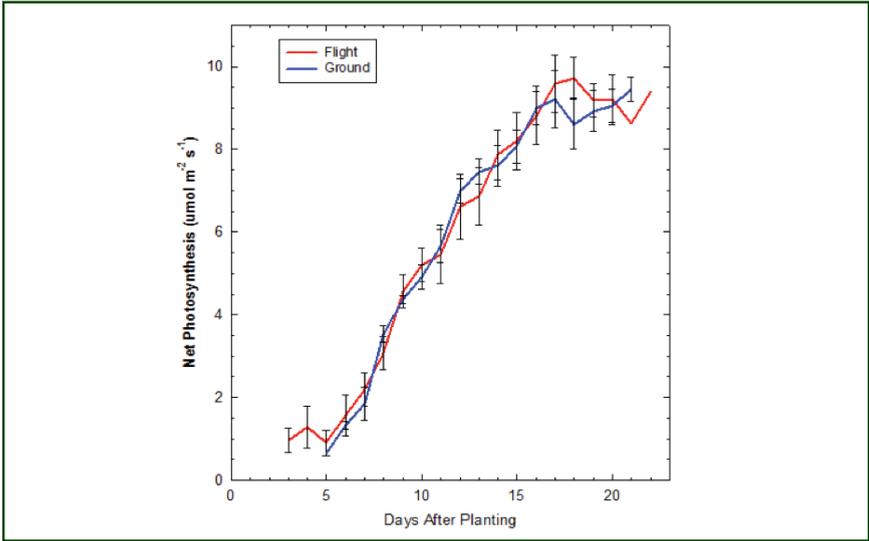


Figure 6. Net photosynthesis of wheat cv. USU Apogee was maintained at same rates in microgravity as 1 g when environmental conditions were maintained to minimize boundary layer effects (Monje et al. 2005).

rates. A series of parabolic experiments by Kitaya et al. (2003) suggested this could be mitigated by increasing air velocity across the leaf, thus reducing size of the boundary layer. In similar parabolic flights, the photosynthetic rates of barley leaves decreased by 13 to 20 percent when exposed to low air velocity ( $<0.2 \text{ m s}^{-1}$ ) and microgravity (Kitaya et al., 2004).

During the International Shuttle-Mir Greenhouse Project, CO<sub>2</sub> and water vapor fluxes of wheat cv. Superdwarf were measured with the Gas Exchange Measurement System in Svet and together with soil moisture data; this provided simultaneous measurements of photosynthesis, transpiration and water balance in space (Monje et al., 2000; Ivanova, 2002). However, those measurements were made without environmental control as the Svet chamber used CO<sub>2</sub> supplied from cabin air, which fluctuated between 3,000 and 9,000 ppm.

The gas exchange measurements during the Photosynthesis Experiment and System Testing and Operation (PESTO) plant experiment were made under tightly controlled light, air temperature, CO<sub>2</sub> concentration, root zone moisture, and

humidity. The Biomass Production System (BPS) hardware used for the PESTO experiment was able to effectively control air velocity across the leaf, and there were no differences in stomatal resistance, gas exchange rates or evapotranspiration between flight and ground controls (Stutte et al., 2006). This suggests that gas exchange is not affected directly by microgravity, but indirectly by the lack of buoyancy-driven convective currents that limit diffusion of gases to the leaf (Porterfield, 2002; Monje et al., 2005). As such, it is important to ensure that a sufficient rate of air mixing is occurring in the plant chamber to prevent confounding of results by the secondary effects of the spaceflight environment.

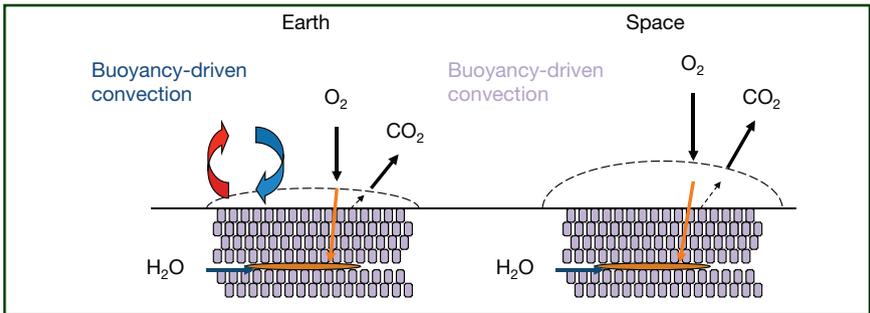


Figure 7. The absence of buoyancy-driven convection in microgravity results in a barrier at the soil/atmosphere interface that limits the diffusion of O<sub>2</sub> into the root zone.

## Root Zone Aeration

Oxygen can fall to low concentrations within plant tissues either because of environmental factors that decrease the external oxygen concentration or because the movement of oxygen through the plant tissues cannot keep pace with the rate of consumption. The lack of buoyancy-driven convection in microgravity may inhibit oxygen transport to roots by increasing the boundary layers surrounding the root zone thereby making oxygen supply diffusion limited (Porterfield, 2002; Liao et al., 2004; Monje et al., 2004). Hypoxia can develop when root respiration exceeds the rate of diffusion-limited oxygen supply (Porterfield, 2002). Root zone hypoxia during spaceflight can cause changes in mitochondrial ultrastructure, decreases in tissue starch reserves (Moore, 1990; Kordyum, 1994), and increased root alcohol dehydrogenase activity (Porterfield et al., 1997; Stout et al., 2001). Although transcript levels of genes involved in glycolysis and fermentation pathways, ethylene synthesis and perception, calcium signaling, nitrogen utilization, and alkaloid



synthesis of *Arabidopsis thaliana* are significantly altered in response to oxygen limitation at 1 g (Liu et al., 2005); roots exposed to microgravity did not exhibit differential expression of genes associated with hypoxia (Paul et al., 2005).

### Root Zone Fluid Dynamics

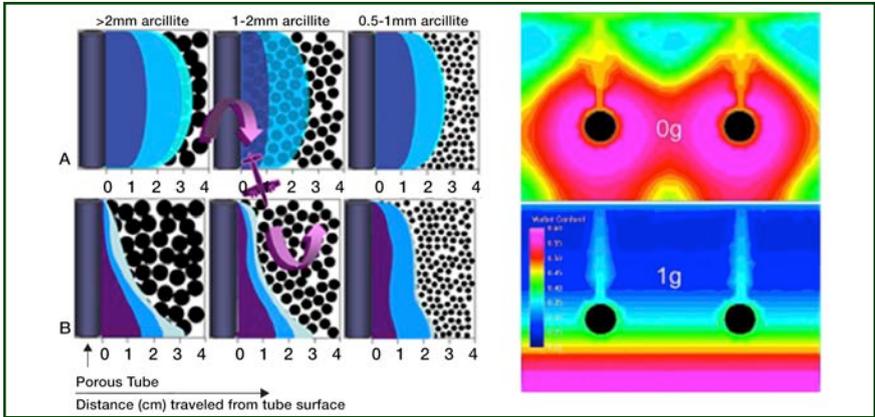


Figure 8. Moisture distribution in media is different in microgravity that at 1 g. The picture on the left shows the effect of particle size on moisture distribution between the 0 and 1.8 g during a parabolic flight. The image on the right shows the distribution of water around a porous tube under 0-g (upper) and 1-g (lower) conditions.

Plants grown in chambers aboard spacecraft have been supported in various rooting media employing both passive (agar, phenolic foam) and active (zeolites, clays, and porous tubes) means for delivering water and nutrients (Morrow et al., 1994; Dreschel et al., 1994; Steinberg and Henninger, 1997; Hoehn et al, 2000; Jones and Or, 1998). Agar and foams have been used during short-duration flights (seven days) or when very small seedlings are used. In longer-duration experiments (7 to 60 days), plants become large and the use of root modules filled with porous substrates and active moisture control is required. The porous media provides a network for root support, facilitates liquid and nutrient supply and provides capacitance. Root and microbial respiration consume oxygen and generate carbon dioxide, creating gradients between the chamber bulk air and the air within the porous media. The challenge in systems utilizing porous media is to provide sufficient water to the plants without filling the air spaces, which prevents proper aeration. Higher water content leads to reduced gas exchange because diffusion of gases through water is four orders



of magnitude slower than diffusion through the air. The gas diffusion process near roots is dependent on air-filled pores and is often described in terms of a gas diffusion coefficient. Measurement and modeling of porous media physical characteristics are needed to design and model improved plant rooting environments for space (Jones and Or, 1998; Scovazzo et al., 2001, Jones et al., 2003).

In space, water flow and distribution in porous media are affected by changes in buoyancy, dominance of capillary forces and particle rearrangement, which can be affected by vibration during launch (Ivanova and Dandolov, 1992; Podolsky and Mashinsky, 1994; Heinse et al., 2007). Figure 8 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-centimeter-deep root modules of the Svet plant chamber (Jones and Or, 1999). When water was added to substrates using porous tubes, the shape of the wetting fronts was 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 in turn may contribute to poor aeration.

The moisture distribution of the Svet root module, obtained from data collected by an array of moisture sensors (right), clearly shows that water collects at the bottom of the root module in 1 g. 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. This example illustrates how a difference in gravitational

force can result in significant offsets in control parameters developed on Earth because of these shifts in water distribution (Jones et al., 2003). These effects can be partially offset through appropriate selection of rooting media and soil moisture potential. For example, in the PESTO experiment, these effects were mitigated by using 1 to 2 mm arcillite media and 3-centimeter-deep root modules (Monje et al., 2005; Stutte et al., 2005).

## Temperature Control

Heat and mass transfer between plants and the ambient air surrounding them occurs through boundary layers. Plant organs (leaves, growth and reproductive

structures) are heated by incident radiation from the lighting system. Heat is then dissipated by heat transfer across boundary layers surrounding the plants and by evaporative cooling from transpiration.

In microgravity, without any forced air circulation, heat and mass transfer are sustained only by diffusion as buoyancy-driven convection ceases.

Parabolic flights have been used to determine the effect of gravity on the surface temperature of leaves (Kitaya et al., 2003). Researchers observed that the leaf temperature of sweet potatoes and barley increased rapidly by 1.9°C and 1.3 C, respectively, during the 20 s of microgravity in each parabola. They observed that leaf boundary layer conductance to sensible heat exchange decreased by 5 percent when gravity decreased from 1.0 to 0.01 g at an air velocity of 0.2 m s<sup>-1</sup>. In contrast, leaf temperatures decreased by 0.5°C during the 2 g portions of the parabolas because increasing gravity levels reduce the thickness of the boundary layers and enhance buoyancy-driven convection. They concluded that reduced convection resulted in less leaf-to-air heat exchange at lower gravity levels because of reduced evaporative cooling. The leaf temperatures increased by smaller amounts when the air velocity over the leaves was raised from 0.2 m s<sup>-1</sup> to 1.0 m s<sup>-1</sup> (Kitaya et al., 2001).

## Lighting

Various types of electric-lighting approaches have been used to grow plants in space. Many plant chambers have used small fluorescent lamps, which provide a broad spectrum for photosynthesis and photomorphogenesis and have a vast literature on their use in controlled environments (Withrow and Withrow, 1947; Sager et al., 1982). Examples of plant chambers that used fluorescent lighting include: NASA PGU; NASA's Plant Growth Facility (PGF); the Russian Svet chamber used on Mir; the Russian Lada chamber on the ISS; NASA's (Orbitec) Biomass Production System (BPS); and the BioServe commercial Plant Growth Bioprocessing Apparatus. Another NASA-funded commercial group, the Wisconsin Center for Automation and Space Robotics (WCSAR), proposed the concept of using light-emitting diodes (LEDs) to provide lighting in space (Bula et al., 1991; Barta et al., 1992). LEDs are solid state devices that do not radiate much heat (i.e., long wave), are easily dimmable and have a long operating life; hence, LEDs had a lot of appeal for space applications. Moreover, they do not contain mercury vapor found in fluorescent lamps, which can be a safety concern in space. The WCSAR group and partner company Quantum Devices, Inc. patented the concept for using LEDs to grow plants (Ignatius et al., 1991). WCSAR subsequently used LEDs in their Astroculture plant chambers in the



*Figure 9. The Advanced Astroculture designed and built by the Wisconsin Center for Space Automation and Robotics was one of the first plant-growth facilities to use Light Emitting Diodes on the International Space Station.*

1990s and early 2000s (Morrow et al., 1995), and other groups such as the European Space Agency (for the EMCS chamber) and NASA (for the ABRs) chamber have also used LEDs.

A challenge with LEDs is that the spectral output is very narrow, e.g., approximately 25 nm half band width, and thus providing adequate spectral combinations became the focus of research.

Subsequent testing, much of which was focused on space applications, demonstrated that both red and blue lights were essential for normal growth and development for many species (Hoenecke et al.,

1992; Kim et al., 2007). Blue becomes especially important in the  $\mu$ -gravity of space to orient plants through phototropic responses. Since their early use for growing plants in the 1990s, LED electrical efficiencies have improved by nearly an order of magnitude, especially blue LEDs (Bourget, 2008) and thus have even greater appeal for space applications.

Regardless of the source, the requirement for light to sustain plant growth makes plant chambers and plant testing in space more challenging for thermal management. Even though some of the light is fixed by the plants into biochemical energy through photosynthesis, from a thermal management perspective, nearly all of the power required to generate the lighting in a plant chamber ultimately becomes heat. Hence, plant chambers and experiments for space should have adequate air mixing and sufficient cooling capacity to maintain temperature control. This often translates into higher electric power requirement for conducting plant research in space when it requires light. (Note: dark experiments, such as germination studies, would not require such high power budgets.)

The majority of plant experiments to date have been conducted at fairly low to moderate light levels ( $75\text{-}300 \mu\text{mol m}^{-2} \text{s}^{-1}$  of photosynthetically active radiation) because of constrained power allotments to spaceflight plant growth chambers. The

results from the PESTO flight experiment indicate that there is no difference in the rates of photosynthetic carbon uptake, water loss via transpiration growth between flight and ground plants at moderate light levels ( $\sim 300 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and saturating  $\text{CO}_2$  concentration (Stutte et al., 2005). These findings suggest that plants stand water purification, and food production rates will not change in space because the underlying biological processes operate at the same rates as in 1 g at those light levels (Monje et al., 2005). However, this does not mean that direct and indirect effects of microgravity cannot affect plant growth at higher, more demanding light levels (e.g., 600 to 900  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ).

In previous studies, a significant (approximately 17 percent) reduction in whole-chain electron transport was observed in chloroplast extracts from plants raised in microgravity at high light levels (Tripathy et al., 1996). These higher light levels translate into faster plant growth rates, which result in proportionally higher rates of water demand and greater rates of respiratory  $\text{O}_2$  consumption by both shoots and roots. In turn, these higher rates impose greater loads (e.g., greater water supplied to the root zone, greater dehumidification capacity, and increased oxygen consumed in respiration) that could cause plant stress if the flight hardware cannot control or sustain mass exchange rates. The Plant Habitat (PH), currently under development, is designed to accommodate plant experiments at higher light intensities. Its development has demanded improved technologies to provide higher capacity water supply and condensate recovery, heat rejection, and  $\text{CO}_2$  control because the higher light and accelerated plant growth impose larger demands on chamber subsystems.

## Approaches to Mitigate Effects of Microgravity Environment

### Biological Approaches

In order to mitigate the secondary effects of the spaceflight environment, a number of biological approaches can be taken. These include the selection of plant material that is more resistant to the stress. During the International Shuttle-Mir Greenhouse Project, Salisbury (1997) grew wheat cv. Superdwarf in the Svet chamber for an entire life cycle, but only sterile wheat heads were obtained because of exposure to the 0.4 ppm ethylene present in the Mir atmosphere (Campbell et al., 2001). Musgrave et al. (2000) conducted the Greenhouse 3 seed-to-seed experiments with Brassica in 1997 in spite of exposure to 1.1 ppm ethylene. Ethylene exposure experiments with wheat cv. Apogee during the ground testing found that Apogee was more resistant to ethylene than Superdwarf. When Apogee was substituted for Superdwarf in the Greenhouse 4 experiment, seed-to-seed experiments with wheat were possible on Mir even though only few seeds per head

were produced at 1 ppm ethylene (Sytchev et al., 2001).

Alternatively, the inclusion of lines of plant material with a range of responses to stress can be used. This is a particularly powerful approach when defined genetic material is available, allowing for the use of biomolecular tools to differentiate between direct and indirect effects of microgravity (Muday et al., 2008). Kiss and co-workers (1999, 2000, 2012) performed spaceflight experiments (Biorack, TROPI-1; TROPI-2) to address issues associated with starch-stanolith theory of gravity perception and to study phototropism in microgravity. This series of experiments allowed the genetic and gravitropic responses to be decoupled and indicated that the responses in microgravity are consistent with the statolith model of gravity perception. The Kiss group also was able to use microgravity to identify a novel red-light-based phototropism in plants (Miller et al 2010; Kiss J.Z., P. Kumar, R.N. Bowman, M.K. Steele, M.T. Eodice, M.J. Correll, R.E. Edelmann. 2007. Biocompatibility studies in preparation for a spaceflight experiment on plant tropisms (TROPI). *Advances in Space Research* 39: 1154-1160). Similarly, Hoson et al. (2009) used tubulin mutants of *Arabidopsis* to differentiate the effects of microgravity from developmental signals for cell wall properties of microgravity grown tissues.

## Engineering Approaches

The designs of the latest generation of plant growth chambers (e.g., EMCS, ABRS and PH) under development have incorporated sophisticated engineering controls for CO<sub>2</sub> concentration with active addition and/or removal of CO<sub>2</sub> from the plant growth chamber, maintenance of relative humidity through the use of porous tube or plate technology, and removal of VOCs such as ethylene, with selective absorbents or photo-catalytic scrubbers. Air flow through the chambers is maintained to minimize the effects of microgravity on boundary layer formation, without inducing thigmomorphogenic effects on the plants, and automatic water/nutrient delivery systems to mitigate microgravity effects on water and oxygen movement throughout the media. The ability to control these parameters in situ provides the opportunity to fully investigate the role of the spaceflight environment, and coupled with biological control measures, to differentiate the unique effects of microgravity from the secondary environmental effects of the spaceflight environment.

# Research Facilities on ISS and How to Choose Them

## Plant Growth Facilities

### The Advanced Biological Research System (ABRS)

The ABRS is a single, mid-deck, locker-sized plant growth unit that is split into two growing compartments, each 260 cm<sup>2</sup> in growing area (Levine et al., 2009). Each of the growing compartments is independently controlled, with an LED lighting system capable of providing up to 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR and has temperature, RH and CO<sub>2</sub> control for independent control of the internal atmosphere. ABRS allows for imaging the experiment from three cameras installed in the chamber, and one of the compartments also has the capability to image green-fluorescent, protein-modified plants or other organisms.

### Biological Research in Canisters (BRIC)

BRIC canisters provide a carrier to house Petri plates of various sizes to accommodate study of tissue or cell cultures, microbes or other organisms that could be contained in a dark environment. A series of BRIC hardware exists to accommodate various experiment requirements. These include: the BRIC-60, which has two compartments that each have the capacity for 12 60-mm Petri dishes; BRIC-100, which is a single anodized-aluminum cylinder that can accommodate up to nine 100 mm Petri plants; and the BRIC-100 (VC), which has additional structural support for vacuum containment of specimens.

### BRIC Petri Dish Fixation Unit (BRIC/PDFU)

A more sophisticated version of using Petri dish type studies can be carried out using the BRIC/PDFU hardware, which can also accommodate in situ fixation of the tissue with chemical fixatives and provide unilateral stimuli of red LED light during growth (Kern et al., 1999). The PDFU is a specialized holder for a 60-mm Petri dish and reservoir for the containment and delivery of fixative. Each BRIC canister can contain up to six individual PDFUs.

### Commercial Generic Bioprocessing Apparatus (CGBA)

The Commercial Generic Bioprocessing Apparatus (CGBA) provides programmable, accurate temperature control for applications ranging from cold stowage to customizable incubation. The CGBA is used for experiments on cells, microbes, and plants. The CGBA provides temperature control for a variety of applications



ranging from cold stowage to customizable incubation. The CGBA can be used in a variety of biological studies, such as protein crystal growth, small insect habitat, plant development, antibiotic-producing bacteria, and cell culture studies.

### European Modular Cultivation System (EMCS)

The EMCS provides temperature and atmosphere control, water supply, illumination, imaging, and variable g-levels on two independent centrifuge rotors. This is the only plant growth facility that has variable g control capability in-orbit. Experiment Containers (EC) hold experiment unique equipment (EUE) to support particular experiments; there are four ECs per centrifuge rotor. Ames Research Center has developed EUE for the EC's, which consist of five seed cassettes with cover heaters for imaging clarity, LED lighting, hydration bellows, hydration pumps, control circuit boards, and an air circulation fan. The EMCS computer commands the heaters, LEDs, and hydration system in the EC/EUEs during the experiments. (Kiss J.Z., P. Kumar, R.N. Bowman, M.K. Steele, M.T. Eodice, M.J. Correll, R.E. Edelman. 2007. Biocompatibility studies in preparation for a spaceflight experiment on TROPI. *Advances in Space Research* 39: 1154-1160). The seed cassettes can be removed by the crew for sample processing, e.g., freezing (Brinkman, 2005).

### Lada

The Lada plant chamber is housed in the Russian module of the ISS and owned and operated by the Russian Institute for Biomedical Problems. The Lada chamber provides about 0.034 m<sup>2</sup> of growing area with fluorescent lighting and a sub-irrigated root module. The Lada chamber is open to the cabin atmosphere and uses cabin air for CO<sub>2</sub> supplement and thermal and humidity control (Sytchev et al., 2007).

### Plant Experiment Unit / Cell Biology Experiment Facility (PEU/CBEF)

The PEU Series is equipment dedicated to life-science experiments on ISS. Of the four types of PEU developed on KIBO, the PEU installed in the CBEF provides general environment controls such as temperature, humidity and CO<sub>2</sub> concentration. The PEU, connected to the external PC, performs various functions required in life science experiments including: culture-medium exchange, water delivery, air circulation, data acquisition (temperature, humidity), video capture, and sample fixation.

## Plant Habitat (in development)

The PH provides greater area (0.17 vs. 0.026 m<sup>2</sup>), height (48 vs. 13 to 20 cm) and higher light levels (600-900  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) than previous flight chambers, which enables the study of a wider range of plant species than before. The PH is being designed to temperature (approximately 18 to 30°C), humidity (50 to 90 percent RH) and CO<sub>2</sub> (400 to 5,000 ppm) control as well as providing scrubbers to remove ethylene. Standard instrumentation will monitor and record the environment within the growth chamber, e.g., temperature, humidity, CO<sub>2</sub>, and O<sub>2</sub> concentrations as well as record root zone moisture content, temperature, and O<sub>2</sub> concentration. The larger PH can still accommodate growth of model plant species (e.g., Arabidopsis) and would provide nondestructive measurements of growth (i.e., photosynthetic rates).

## Vegetable Production System (Veggie)

The Veggie plant growth chamber is scheduled for its initial spaceflight test in the fall of 2013. The Veggie is a simple, expandable plant growth unit with a LED (red, green, blue) lighting system that can provide up to 350  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of PAR (Stutte et al., 2011). Air temperature, humidity, and CO<sub>2</sub> control are achieved by drawing cabin air through the system. The total plant growing area is 0.13 m<sup>2</sup>, and water is provided passively to packets of rooting media using capillary connections to a reservoir.

## Support Facilities

The International Space Station has a variety of multidisciplinary laboratory facilities and equipment available to support the National Laboratory operations. These capabilities have been built by NASA and its International Partners and can be made available on a time-shared basis to other U.S. government agencies and private entities to pursue their own mission-driven research and applications on the ISS.

In addition to the NASA-supported hardware to support plant experiments (ABRS, BRIC, Lada), the EMCS and Commercial Generic Bioprocessing Apparatus are available to support plant growth experiments. Temperature-controlled stowage of samples is available in Minus Eighty-Degree Laboratory Freezer for the ISS and General Laboratory Active Cryogenic ISS Experiment Refrigerant facilities; fixation of tissues for microscopic and/or molecular analysis can be achieved with Biotube



and Kennedy Space Center Fixation Tubes. There is a whole suite of facilities and instrumentation currently available to support plant experiments on the ISS including the Microgravity Science Glovebox for handling hazardous materials and Light Microscope Module for analysis of samples in space. In addition to NASA provided hardware, facilities from experiment implementation partners are also available such as the Nano-racks Microscopes and Nano-racks Plate Reader.

The most up-to-date descriptions of these facilities and their capabilities can be found at a number of different websites including the NASA ISS facilities website at [http://www.nasa.gov/mission\\_pages/station/research/facilities\\_category.html](http://www.nasa.gov/mission_pages/station/research/facilities_category.html), and NASA's Fundamental Space Biology Science Plan at [http://www.nasa.gov/pdf/541222main\\_10-05-17%20FSB%20Sci%20Plan-Signed\\_508.pdf](http://www.nasa.gov/pdf/541222main_10-05-17%20FSB%20Sci%20Plan-Signed_508.pdf).

## Plant Science Hardware and Facilities on ISS

	ABRS	BRIC	BRIC / PDFU	CGBA	EMCS	Lada	PEU/CBEF	PH	Veggie
	Advanced Biological Research System	Biological Research in Canisters	Biological Research in Canisters/ Petri Dish Fixation Unit	Commercial Generic Bioprocessing Apparatus	European Modular Cultivation System	Greenhouse chamber	Plant Experiment Unit / Cell Biology Experiment Facility	Plant Habitat	Vegetable Production System
Doc. link	ABRS	BRIC	BRIC/PDFU	CGBA	EMCS	N/A	BEU / PEU	N/A	Veggie****
Contact	Jose Camacho; Bryan Onate	Howard Smith	Howard Smith	Stefanie Countryman	Ulrich M. Kuebler	Gail Bingham; Vladimir N. Sychev	Sachiko Yano	Bryan Onate	Thomas Crabb; Bryan Onate
Area (cm2)	2 x 260	BRIC-100 9 x 80; BRIC-60 12 x 30	6 x 30	750	8 EC*** X 96	340	273	1700	1300
Light Intensity (umol/m2s)	400	N	N	250	75 W/ m2s	250	110 - 190	1000	300
Lighting	LED	N	N	Fluorescent	LED	Fluorescent	LED	LED	LED
Temperature Control (°C)	Y	N	N	Y	Y	Y	Y	Y	N
CO <sub>2</sub> Control (%)	Y	N	N	Y	Y	Y	N	Y	N
Humidity Control (%)	Y	N	N	Y	Y	Y	Y	Y	N
Centrifuge	N	N	N	N	Y (0-2g)	N	Y	N	N
Power to EUE	Y	N	N	N	Y	Y	Y	Y	N
Data	Y	N*	N*	Y	Y	Y	Y	Y	N
Video	Y	N	N	Y	Y	Y	Y	Y	N
Lighting	Y	N	N	Y	Y	Y	Y	Y	Y

Table 2. \* HOBO data logger access data postflight.

\*\* BRIC-60, BRIC-100VC, BRIC-Opti use 60 mm and 100 mm petri dishes; Several BRICs can be flown per mission.

\*\*\* EC - Experiment Container.

\*\*\*\* Still in process of flight qualification. Currently scheduled for a test on ISS for SpaceX 3.

# Developing and Flying Research to ISS

## Process for Payload Development and Implementation

The recommendation of the NRC Decadal Survey (2011) was that all future spaceflight research be hypothesis driven. It is thus essential that principal investigators (PIs) understand the constraints of spaceflight and incorporate steps to mitigate the confounding effects identified in the previous sections. The extent and impact of these effects will vary according to the specific experiment and the operational characteristics of the flight hardware selected to support the payload.

Once an experiment is selected for development as a spaceflight payload, the PI will be working with a payload integrator or hardware developer to identify the specific laboratory requirements necessary to support the experiment and develop any experiment unique equipment necessary to support the payload. It is highly recommended that the PI conduct experiments in the actual flight hardware under configuration control conditions similar to those anticipated in flight prior to launch. This allows any issues unique to the flight hardware and mission-specific constraints to be identified, and mitigated, prior to launch.

The PI is also advised to seek the advice of researchers who have conducted spaceflight experiments for tips on how to overcome hardware and mission-specific issues and tricks on how to implement solutions during flight. Any issues that are identified during preflight testing that may limit success should be mitigated and the solution tested in an appropriate environment such as parabolic flights, closed systems or reduced pressure facilities prior to flight. It is also advisable, where appropriate, to identify possible failure modes and develop responses to those events prior to launch. Once these constraints have been identified and mitigated, then the experiment configuration for flight should be fixed.

When necessary, the PI will work with the payload integrator to develop crew procedures, establish timelines for those operations and provide them to the Astronaut Office for validation and crew training purposes. It is highly recommended that the PI initiate these discussions early in the experiment development process to determine what are the timelines for meeting particular milestones. The payload integrator and/or hardware developer will also work with the PI to determine stowage requirements, number of spare parts, develop experiment unique equipment, and identify special hazards or constraints that will affect the success of the experiment. The PI will also need to make a decision of

whether to conduct a synchronous or asynchronous ground control, and determine where these experiments will be performed.

If using live specimens, the PI should anticipate various launch scrub scenarios and develop contingency plans to reduce the effects of launch delays on science objectives. The PI will also need to determine where post-landing operations will occur, and it is also advisable to determine what must be done at landing immediately upon receipt of samples and which operations can be deferred and conducted in the PI's home laboratory.

## Environmental Conditions on ISS

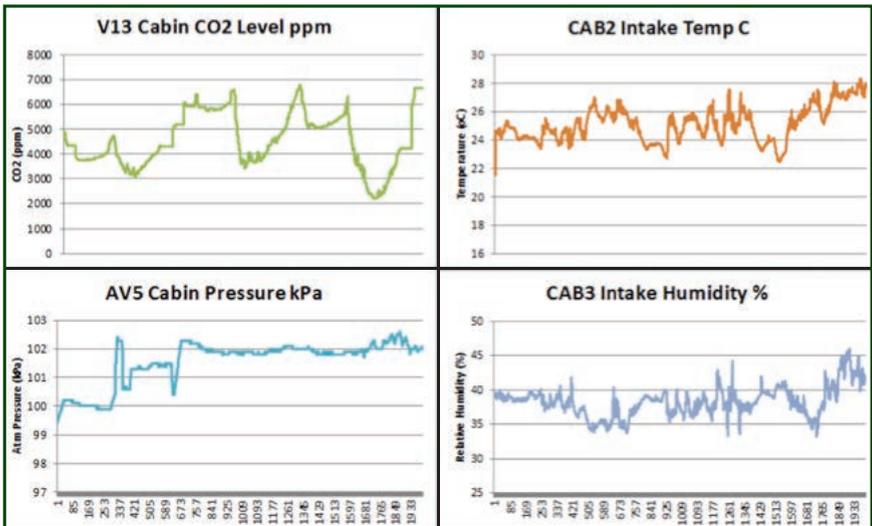
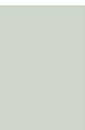


Figure 10. ISS ambient CO<sub>2</sub>, pressure, temperature and humidity levels recorded by the Biomass Production System flight hardware on flight days 67, 68 and 69 (13-15 June, 2002) of the Photosynthesis Experiment and System Testing and Operation experiment during ISS Increment IV.

The ambient environment of the ISS can fluctuate significantly, depending upon operation, equipment anomalies and crew activities. Typical ISS control parameters are temperatures between 22 and 28°C, relative humidity at 30 to 45



percent and CO<sub>2</sub> between 3,000 and 7,000 ppm. The absence of buoyancy-driven convection has the potential for significant spatial variation in the spacecraft to occur. It is desirable to obtain sensor data on ambient conditions as near to the experimental payload as possible to increase the fidelity of the ground controls. Figure 9 illustrates typical variation in ISS CO<sub>2</sub> concentration, cabin pressure, air temperature and relative humidity recorded during the PESTO experiment during ISS Increment IV.

# Sponsorship Opportunities

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## What Should Principal Investigators Know About Conducting Research on ISS?

Supporting research in science and technology is an important part of NASA's overall mission. NASA solicits research through the release of NASA Research Announcements (NRA), which cover a wide range of scientific disciplines. All NRA solicitations are facilitated through the Web-based NASA Solicitation and Proposal Integrated Review and Evaluation System (NSPIRES) <http://nspires.nasaprs.com/external/>. Registering with NSPIRES allows investigators to stay informed of newly released NRAs and enables submission of proposals. NSPIRES supports the entire lifecycle of NASA research solicitations and awards, from the release of new research calls through the peer review and selection process.

In planning the scope of their proposal, investigators should be aware of available resources and the general direction guiding NASA research selection. NASA places high priority on recommendations from the 2011 National Research Council's NRC Decadal Survey, which placed emphasis on hypothesis-driven spaceflight research. In addition, principal investigators (PI) should be aware that spaceflight experiments may be limited by a combination of power, crew time, or volume constraints. Launch and/or landing scrubs are not uncommon, and alternative implementation scenarios should be considered in order to reduce the risk from these scrubs. Preliminary investigations using ground-based simulators may be necessary to optimize procedures before spaceflight. Also, many experiments require unique hardware to meet the needs of the spaceflight experiment. To understand previous spaceflight studies, prospective PIs should familiarize themselves with the NASA ISS Program Science Office database, which discusses research previously conducted on the ISS, including that of the International Partners. A detailed catalog of previous, current, and proposed experiments, facilities, and results, including investigator information, research summaries, operations, hardware information, and related publications is available at [www.nasa.gov/iss-science](http://www.nasa.gov/iss-science) through the NASA ISS Program Office. Additionally, details pertaining to research previously supported by the Space Life and Physical Sciences Research and Applications Division of NASA's Human Exploration and Operations Mission Directorate can be located in the Space Life & Physical Sciences Research and Applications Division Task Book in a searchable online database format at: <https://taskbook.nasaprs.com/Publication/welcome.cfm>.

## Process for Payload Development and Implementation

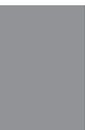
Following selection of an experiment for spaceflight, the PI will work with a payload integrator or hardware developer to define the most suitable hardware, and determine if hardware needs to be created or modified. The research team in combination with payload integrations will establish the specific laboratory requirements needed to support the experiment. Through these collaborative efforts, concerns such as crew procedures and crew training, the need for spare parts and/or contingencies involving hardware, and stowage requirements of the samples will be addressed and resolved. It is highly recommended that the PI perform a series of investigations using the identical hardware and under configuration and control conditions similar to those anticipated inflight prior to the launch. This will prevent unforeseen issues with the hardware and allow specific mission constraints to be defined, and mitigated, prior to the experiments implementation once aboard the ISS. It is also within this time frame that the science team needs to characterize the details involved with their synchronous ground controls. The PI's team should also have finalized all post-landing procedures, including sample preservation, storage, and transport, and data acquisition prior to the launch.

Another option to flying your experiment is through the Center for the Advancement of Science in Space (CASIS) (<http://www.iss-casis.org>). CASIS is a nonprofit organization tasked by U.S. Congress and NASA with promoting and enabling research on ISS. CASIS can be used for all stages of payload development and can match PIs with implementation partners (table below) who can provide heritage hardware or new flight packages:



<b>Company</b>	<b>Contact Information</b>
The Aerospace Corporation	<a href="http://www.aero.org">www.aero.org</a>
Astrium North America	<a href="http://www.astrium-na.com">www.astrium-na.com</a>
Astrotech Corporation	<a href="http://www.astrotechcorp.com">www.astrotechcorp.com</a>
Aurora Flight Sciences	<a href="http://www.aurora.aero">www.aurora.aero</a>
Bionetics Corporation	<a href="http://www.bionetics.com">www.bionetics.com</a>
Bioserve	<a href="http://www.colorado.edu/engineering/BioServe">www.colorado.edu/engineering/BioServe</a>
Boeing	<a href="http://www.boeing.com">www.boeing.com</a>
CSS-Dynamac	<a href="http://www.css-dynamac.com">www.css-dynamac.com</a>
Hamilton Sundstrand	<a href="http://www.hamiltonsundstrand.com">www.hamiltonsundstrand.com</a>
Jamss America	<a href="http://www.jamssamerica.com">www.jamssamerica.com</a>
Kentucky Space, LLC	<a href="http://www.kentuckyspace.com">www.kentuckyspace.com</a>
MDA	<a href="http://www.mdacorporation.com">www.mdacorporation.com</a>
MEI Technologies	<a href="http://www.meitechinc.com">www.meitechinc.com</a>
Nanoracks LLC	<a href="http://www.nanoracks.com">www.nanoracks.com</a>
Orbital Technologies Corporation	<a href="http://www.orbitec.com">www.orbitec.com</a>
Paragon TEC	<a href="http://www.paragontec.net">www.paragontec.net</a>
Qinetiq	<a href="http://www.qinetiq-na.com">www.qinetiq-na.com</a>
Space Systems Concepts, Inc.	<a href="http://www.space-concepts.com">www.space-concepts.com</a>
Space Systems Research Corporation	<a href="http://www.spacesystemsresearch.com">www.spacesystemsresearch.com</a>
Tec-Masters, Inc.	<a href="http://www.tecmasters.com">www.tecmasters.com</a>
Techshot	<a href="http://www.techshot.com">www.techshot.com</a>
Teledyne Brown Engineering, Inc.	<a href="http://www.tbe.com">www.tbe.com</a>
Thales Alenia Space	<a href="http://www.thalesgroup.com/space">www.thalesgroup.com/space</a>
UAB	<a href="http://www.uab.edu/cbse">www.uab.edu/cbse</a>
Wyle Integrated Science and Engineering	<a href="http://www.wyle.com">www.wyle.com</a>
Zin Technologies	<a href="http://www.zin-tech.com">www.zin-tech.com</a>

Table 3. Implementation Partners for Flight Experiments on the ISS.



## Funding Opportunities and Points of Contact

There are various avenues that can result in funding for research to be conducted on the ISS, and the source of funding often dictates the availability of launch opportunities. Generally, funding for research is awarded through NASA-sponsored research announcements (NRAs), ISS National Laboratory awards through other government agencies, private commercial enterprise, nonprofit organizations, and research awards sponsored by the ISS International Partners. It is not the responsibility of a researcher awarded an ISS flight experiment to fund costs associated with launch or the ISS laboratory facilities. Greater detail concerning current funding opportunities for ISS research can be found through the NASA ISS research website: [http://www.nasa.gov/mission\\_pages/station/research/ops/research\\_information.html](http://www.nasa.gov/mission_pages/station/research/ops/research_information.html).

The NASA Solicitation and Proposed Integrated Review and Evaluation System (NSPIRES) can be accessed via: <http://nspires.nasaprs.com/external/>.

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# Acronyms

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ABRS	Advanced Biological Research System
BPS	Biomass Production System
BRIC	Biological Research in a Canister
CASIS	Center for the Advancement of Science in Space
CBEF	Cell Biology Experiment Facility
CGBA	Commercial Generic Bioprocessing Apparatus
CHROMEX	Chromosome and Plant Cell Division in Space
EC	Experiment Container
EMCS	European Modular Cultivation System
EUE	Experiment Unique Equipment
ISS	International Space Station
LED	Light-emitting Diode
NL	National Lab
NSPIRES	NASA Solicitation and Proposal Integrated Review and Evaluation System
PDFU	Petri Dish Fixation Unit
PESTO	Photosynthesis Experiment and System Testing and Operation
PEU	Plant Experiment Unit
PGC	Plant Growth Chambers
PGF	Plant Growth Facility
PGU	Plant Growth Unit
PH	Plant Habitat
PI	Principal Investigator
RH	Relative Humidity
TROPI	Tropism in Plants
VOC	Volatile Organic Compounds
WCSAR	Wisconsin Center for Automation and Space Robotics

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