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INVITED REVIEW ARTICLE

Seeds in space

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Abstract

Since experimentation with plants began in space, a wide range of information has been gained regarding how this unique environment affects the biology of seeds. Seed biology experiments in this milieu have addressed aspects of seed storage, seed germination and metabolism, seedling orientation and seed production by flowering plants. Construction of hardware that provides a suitable growth environment in microgravity has been especially challenging because of the consequences posed by microgravity for fluid and gas distribution around the plant. Fluid shifting causes seed hydration kinetics to occur at a faster rate in microgravity than in 1 *g*; however, it also induces hypoxic metabolism during the seed germination process. In the absence of a detectable gravitational force, seedling roots grow according to their embryonic orientation and then initiate random walk movements. Light and oxygen gradients are the primary stimuli that orient root growth in this environment. For seed development to occur in spaceflight, well-ventilated growth chambers are necessary to support the carbohydrate supply needs of the developing embryos, and to provide the necessary humidity gradient for anthers to successfully dehisce and release pollen. The dry weight of seeds formed in space is lower than that in ground controls, and seed storage reserves are altered. Seed storage phenomena in spaceflight depend on whether or not oxygen and moisture are present – if not, viability exceeds that of seeds stored under comparable conditions on the ground. Because of the key role to be played by seeds in future advanced life support scenarios in space, more research is needed on the implications of this unique environment for seed biology.

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Introduction: Why study seeds in space?

Viewed as the final frontier for mankind, space combines the elements of adventure, discovery and manifest destiny with a homesteading challenge. Seeds, the quintessential symbol of our self-reliance and the legacy of our agricultural heritage, have played an important symbolic role during man's first tentative voyages away from the Earth. Thirty-four cities across the United States are home to 'Moon trees': sycamores, pines, firs or redwoods grown from the 500 seeds that were orbited around the Moon in the personal belongings of Stuart Roosa in 1971 (Fig. 1). Thousands of young people have felt their own lives transformed when they were asked to grow tomato plants from seeds that had been in space for 6 years.[1] Another element of this metaphysical link between humans and seeds in the new frontier of space lies in the psychological comfort of watching a seed germinate and grow, even in the remote confines of a spacecraft far from other signs of life. This review will focus on the basic biology of seeds in the environment of space. As is the case on

[1]SEEDS (Space Exposed Experiment Developed for Students) was a cooperative project between NASA and the George W. Park Seed Company. Approximately 132,000 SEEDS kits containing Rutger's tomato seeds that had flown in space for nearly 6 years on the LDEF (Long Duration Exposure Facility), as well as similar seeds that had been stored in a climate-controlled warehouse for the same time period, were sent to schools in every state and 30 foreign countries. Student researchers compared germination and growth characteristics of the space-exposed and Earth-based seeds and returned data to NASA for analysis. The students reported very little difference between the two seed groups (Grigsby and Ehrlich, 1991).

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Earth, the impetus behind this basic research has its origins in meeting the physical as well as the intellectual needs of humans. Just as plants are the major component of our agroecosystem here on Earth, they are envisioned to carry out a similar role during long-duration space exploration and/or colonization, as the cornerstone of a bioregenerative life support system (BLSS). While providing a food source, plants would also regenerate air and water in these closed, energy-conserving settings (NRC, 1997) (Fig. 2). Using plants especially developed to maximize harvest index, and to minimize production time and energy input, it is estimated that a growing area of 20–50 m^2 would be able to provide the nutritional needs of one person on a continuing basis (NRC, 1997). At the same time, this growing area would provide two times the CO_2 scrubbing and oxygen replenishment needs of the person, and more than four times the water recycling requirements (purification through plant transpiration and subsequent condensation). The Bio-plex test bed at Johnson Space Center is being developed to integrate these highly efficient plant modules with the other biota of the BLSS: the microbial bioreactors (used for resource recovery) and crew members (Henninger, 2001). Long-duration tests with the Bio-plex will provide data to build modelling and computer control capabilities. The BLSS concept and basic research leading into it have already modified our views on the limits of yield in conventional crop plants such as wheat (Table 1).

Figure 1. Moon tree (a sycamore) at Goddard Space Flight Center in Greenbelt, MD, grown from a seed carried around the moon in 1971 by the Apollo 14 Command Module. The seed was one of many taken aboard Apollo to study the effects of prolonged weightlessness on seed germination and seedling growth. Tests conducted by the Forest Service determined that nearly all of the seeds germinated successfully, and after over 30 years there is no discernible difference between trees from these seeds and those from their Earth-bound counterparts. Image courtesy of Dave Williams and Jay Friedlander, National Space Science Data Center, NASA.

For the BLSS concept to work, the basic physiological and developmental processes of all organisms concerned must be confirmed in the spaceflight environment. In part because of their ease of storage and small space requirements, seeds and seedlings have been the objects of many spaceflight experiments dealing with aspects of germination and seedling tropisms. As hardware for maintaining plant growth in the spaceflight environment became available and its functionality improved, investigators were able to study aspects of seed development during spaceflight. Finally, a small collection of

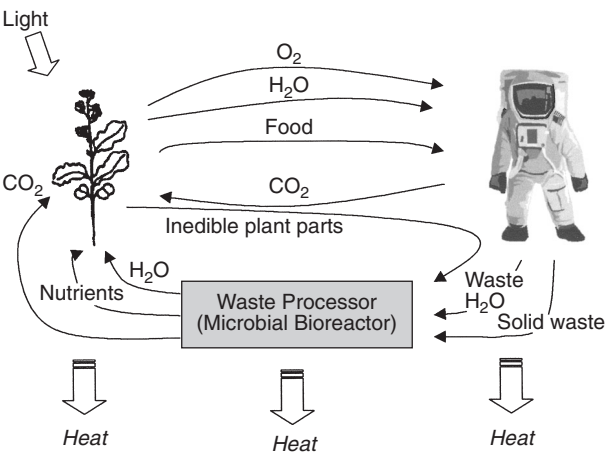

Figure 2. Principal relationships in a bioregenerative life support system (BLSS) that would be used to support human habitation of remote outposts in space. Plants play an integral role by regenerating the atmosphere and recycling water while providing food for the crew. Modified from NRC (1997).

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Table 1. High yields of wheat crops in controlled environments as would be used in a bioregenerative life support system (BLSS) (adapted from Salisbury and Bugbee, 1988). The higher yield was achieved primarily by using high planting densities along with full sunlight equivalent, optimal temperature and a cultivar selected for controlled environment hydroponic culture

	Time to harvest (d)	Edible dry biomass ($g\ m^{-2}$)	Harvest index (%)	Average growth rate ($g\ m^{-2}\ d^{-1}$)
High average field yield	120	500	45	4.2
World record yield	140	1450	45	10.4
Utah State University BLSS	79	4760	44	60.3

BLSS, bioregenerative life support system.

experiments has addressed questions of seed storage in the spaceflight environment. This review will begin with descriptions of the spaceflight environment and the platforms that have been used for experiments on seeds in space. Findings from experiments on seed germination during spaceflight will then be reviewed, followed by a discussion of seed development during spaceflight. Seed storage experiments will then be discussed, and the review will conclude by considering the unique physical challenges of the spaceflight environment, and the consequences for future applications. Ancillary horticultural studies, such as requirements for germination in microgravity (Levine and Piastuch, 1999), use of light-emitting diodes (LEDs) as an energy source for plants (Bula *et al*., 1991), use of plant fibre as a formed germination matrix (Morrow *et al*., 2000), and water and nutrient delivery techniques for use in microgravity (Porterfield *et al*., 2000a) and fractional gravity settings (Johnson *et al*., 1995), will not be discussed.

Spaceflight

Orbital altitudes for human spaceflight vary between 120 and 360 miles (192–576 km) above the surface of the Earth. At this distance from the Earth, the gravitational field is still about 90% of its strength at the surface. However, spacecraft in orbit around the Earth are in a state of continuous free-fall. For this reason, the gravitational force experienced inside orbiting spacecraft is 10^{-4}–10^{-6} g, and is commonly called 'microgravity'. For the purposes of this review, microgravity will be considered the primary attribute of the spaceflight environment.

A whole range of experiment platforms have provided access to the spaceflight environment. Included in this are orbiting spacecraft such as the shuttle orbiter, the Mir space station, the planned research facilities on the International Space Station (ISS), unmanned satellites, sounding rockets and aircraft flying in parabolic arcs. Another source of microgravity within the Earth's gravitational field is drop towers, but these have not been used for research germane to seed biology because the low-gravity duration is only a few seconds (Krikorian and Levine, 1991). Environments providing fractional g-levels, as would be encountered on the Moon and Mars, have been recreated by centrifuges inside orbital spacecraft and have been used for experiments that investigate gravity perception thresholds in seedlings (Perbal and Drissecole, 1994).

The duration and quality of microgravity exposures varies greatly in these seed-biology experiments (Table 2). The longest seed-biology experiments in microgravity were surely the seed storage tests on the Long Duration Exposure Facility (LDEF), which lasted 69 months (Kahn and Stoffella, 1996). Long-duration plant reproduction experiments were conducted on the Mir station, where microgravity periods were often disturbed due to vibration and docking activities. On the shuttle orbiter, which has been the site of many seed germination experiments, g-forces range between 10^{-3} and 10^{-5} g. Very short-duration experiments examining the mechanisms of gravity sensing and microgravity effects on the physical environment around seeds and seedling roots have been performed on sounding rockets (2–6 minutes) and during parabolic flights of aircraft (20 seconds).

Other physical aspects of the spaceflight environment may include radiation and/or vibration and transient exposure to high-g (Krikorian and Levine, 1991). The latter occur during launch and

Table 2. Types of seed-biology experiments conducted in space to date, platforms supporting the experiment, and the duration of the microgravity (10^{-4}–10^{-6} g) exposure

Experiment type	Platform	Duration of microgravity
Seed storage	Long Duration Exposure Facility	6 years
Seed-to-seed	Mir space station	4.5 months
Seed development	Shuttle orbiter	6–15 days
Germination	Shuttle orbiter	2–8 days
Seed environment	KC-135 parabolic flight	20 seconds

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re-entry of the rockets or spacecraft. Although radiation exposure is minimized through shielding during manned spaceflight, it cannot be completely avoided. For a 6-d shuttle experiment studying reproductive development in *Arabidopsis* that will be discussed later, the radiation experienced by the plants was in the range of 35–39 mrad (Kuang *et al*., 1995). In terrestrial seed development and germination studies, this is substantially below the dose considered important (Daly and Thompson, 1975). In other experiments, high radiation is a desired attribute of the spaceflight environment and minimal or variable shielding is employed, as was the case in the seed storage experiment on the LDEF, during which radiation averaged 100 mrad d^{-1}.

Germination and seedling establishment

The microgravity environment has been an excellent tool for examination of the role of gravity in plant growth and development. A favourite experimental system for these studies has been the germinating seed. Dark-grown seedlings coupled with the availability of on-board centrifuges have been used to elucidate elements of gravitropism and have helped to distinguish the processes of graviperception and graviresponse. Investigators have found the microgravity environment to be a good setting for studying other tropisms, such as phototropism and oxytropism, because of the absence of the confounding growth response relative to the gravity vector. Ultrastructural and biochemical studies of germinating seeds have led to a large body of information regarding spaceflight effects on reserve mobilization in seeds during germination. Because the latter phenomenon is so closely tied to environmental attributes, the physical surroundings of germinating seeds will be discussed first.

Hardware for seedling studies, and microgravity effects on the seedling environment

Seed germination studies have been conducted in a variety of small, closed or passively ventilated chambers, which typically provide a dark environment for the germination event. Seeds may be launched with moisture already supplied, or germination may be initiated during spaceflight by injection of water. In the latter case, some early experiments were no doubt compromised by the use of galley water, which contained the antimicrobials iodine (US platforms; Janik *et al*., 1988) or silver (Soviet platforms; Nechitailo and Mashinsky, 1993).

Seedlings have also been grown in lighted, ventilated chambers as part of the growth sequence to produce older plants. The root zone of one such chamber, the Svet greenhouse used on Mir (Bingham *et al*., 1996), is shown diagrammatically in Fig. 3, to illustrate one of the important differences of the physical environment for seedlings in microgravity. Dark shading indicates the greatest amount of water in the substrate, and indicates the fluid shift that occurs in the root zone in the microgravity environment. As a result of this difference in fluid distribution, the hydration kinetics for media and the seeds planted in them change in microgravity. Seed germination is typically advanced as a result (Brown *et al*., 1992).

A related consequence of microgravity is the propensity for hypoxia to develop in the root zone. This may be seen as a combination of enhanced water content in the matrix surrounding a seed (thereby impeding access to oxygen) and the uniform coating

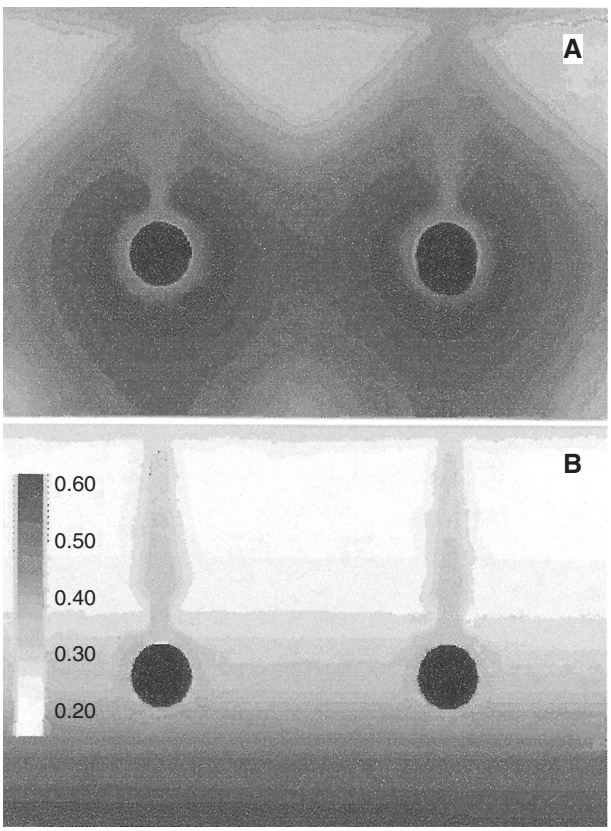

Figure 3. Hydration patterns for a particulate substrate in the root module of the Svet greenhouse in microgravity or 1 *g*. Water is supplied through two porous tubes, shown in cross-section in the diagram. In 1 *g*, maximum moisture occurs at the bottom of the root module (B), while in microgravity, high moisture levels occur closer to the surface and surround the input tubes (A). This difference in water distribution has led to observations of accelerated seed germination kinetics during spaceflight, due to enhanced hydration. Figure courtesy of Scott Jones and Gail Bingham, Utah State University.

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of matrix pores by water films (thereby restricting movement of gases throughout the matrix) (Fig. 4). Although there have been numerous reports of root-zone hypoxia in space-grown plants, based on ultrastructural (Krikorian and O'Connor, 1984; Slocum *et al*., 1984; Porterfield *et al*., 1997; Stout *et al*., 2001) and enzyme studies (Porterfield *et al*., 1997; Stout *et al*., 2001), this phenomenon was recently confirmed by Porterfield *et al*. (2000b) using direct measurement of oxygen bioavailability with microelectrodes during parabolic flight.

Seedlings developing in moist atmospheres, and the aerial portions of seedlings rooted in a matrix, also exhibit gas exchange limitations in microgravity. Buoyancy-driven convective air movement is lacking in microgravity, leading to the formation of stagnant air layers around seedlings growing in closed containers (Musgrave *et al*., 1998). This may result in the localized build-up of seedling-produced contaminants, such as ethylene, or changes in metabolic gas levels.

Similar to the situation with water supply additives in spacecraft that adversely affected seedling growth, the spacecraft cabin atmosphere may have unique attributes that influence seedling development (Krikorian and Levine, 1991). Many seedling studies have been undertaken on the US shuttle, where the operating atmosphere is typically 21% O_2, 1000 p.p.m. CO_2, with trace amounts of ethylene. The Mir station ran at 21% O_2, 6000–10,000 p.p.m. CO_2 and 0.8–1.3 p.p.m. ethylene, and many observations of plants from Mir were consistent with exposure to excessive amounts of ethylene (Halstead and Dutcher, 1987). When the orbiter would dock with Mir during the Shuttle–Mir era, investigators began seeing the ethylene 'triple response' by seedlings in their shuttle-based experiments as well, presumably because of the atmosphere exchange between the two spacecraft (Kiss *et al*., 2000).

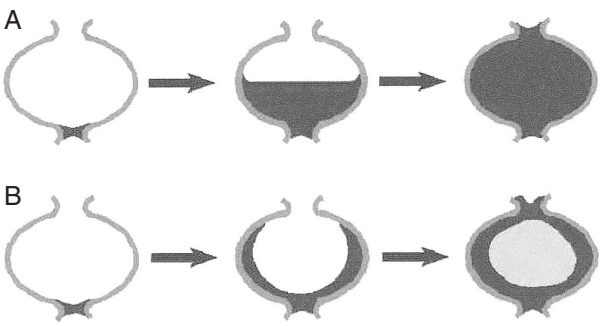

Figure 4. As the moisture content of a substrate increases, gas-filled pores are isolated from one another in microgravity due to a uniform water-film coating on the inner surface of the pore. This results in root-zone hypoxia at a lower water content of the substrate in microgravity (B) than in 1 *g* (A). Figure courtesy of Scott Jones and Gail Bingham, Utah State University.

Cellular organization and reserve mobilization

A number of gravity-dependent phenomena occur at the scale of the single cell (Todd, 1989), and these have consequences for the seed germination sequence during spaceflight. Shortly after imbibition, changes are already manifest at the ultrastructural level in seeds growing under spaceflight conditions. Moore (1990) found that collumella cells of *Brassica perviridis* seedlings grown in microgravity had more lipid bodies, less starch and fewer dictyosomes than in 1 *g* (Table 3). This suggests a general reduction of storage reserve mobilization in the *Brassica* cells in microgravity.

Modification in mitochondrial shape and size, similar to changes caused by hypoxia, have been noted in spaceflight seedling material (Slocum *et al*., 1984; Moore, 1990), along with a general decrease in amyloplast starch reserves (Volkmann *et al*., 1986; Moore, 1990). Brown *et al*. (1996) reported lower starch concentrations, lower activity of the starch synthetic enzyme ADP glucose pyrophosphorylase and higher activity of the starch-degrading enzyme, starch phosphorylase, relative to the ground controls

Table 3. Volume of columella cell constituents from *Brassica perviridis* seedlings when grown in 1 *g*, microgravity, or in two devices, the clinostat and the fluid-filled slow-turning lateral vessel (STLV), used to mimic microgravity conditions (adapted from Moore, 1990)

	1 *g*	Microgravity	Clinostat	STLV
Percent of volume occupied by				
Nucleus	9	8.0	8.2	7.5
Vacuole	34	31	35	34
Hyaloplasm	41	48	41	46
Mitochondria	7.0	4.4*	6.6	4.4*
Plastids	6.3	3.2*	6.7	3.2*
Dictyosomes	2.6	0.5*	2.3	0.8*
Lipid bodies	0.4	4.5*	0.5	6.1*
Relative volume of starch/amyloplast	57	35*	52	39*

* Significantly different from values at 1 *g*.

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in dark-grown soybean seedling cotyledons. Kuznetsov *et al.* (1997), using magnetograviphoretic analysis, showed that starch grains from space-flown soybean tissue were 20–50% smaller than the ground controls, and also showed a difference in magnetic susceptibility, indicating potential alterations in chemical composition. Similarly, Cook *et al.* (1998) found that space-grown potato tubers have more and smaller starch grains.

Prior to the availability of orbital platforms, classical gravitational biology often employed clinostats to mimic effects that might occur in microgravity. With a rotation rate that prevents the proper function of the plant's gravisensing machinery, clinostats operate on a principle of gravity confusion (Sievers and Hejnowicz, 1992). For attributes such as the physical distribution of organelles within the cytoplasm, the clinostat effectively mimicked microgravity (Moore, 1990). However, changes in reserve mobilization caused by microgravity were not reproduced. Interestingly, a type of bioreactor used for growing three-dimensional tissues, the slow-turning lateral vessel (STLV), was able to reproduce both suites of changes (Table 3). It may be that the fluid-filled vessel, in which oxygen is supplied to tissues by diffusion, recreated the effects of stagnant air layers that would surround the seedling in microgravity. Walther *et al.* (1996), working with yeast, showed that the induction of alcoholic fermentation, indicative of stagnant zones around cells, was much more prevalent in their space-flown, aerated, stirred bioreactor than in their stationary bioreactor maintained at 1 ***g***.

Use of germinating seeds to study tropisms in microgravity

Seedlings growing in microgravity demonstrate directional growth that is predetermined by the orientation of the axis as it resides within the seed coat. This automorphism (Sievers and Hejnowicz, 1992) is characterized by the angle between the primary organs and the embryo axis, as shown by the primary roots of cress (Volkmann *et al.*, 1986) germinated in the Spacelab D-1 mission. Antonsen and Johnsson (1998) and Johnsson *et al.* (1996) have shown that there is a random walk component to root growth in microgravity that is more or less prominent during different stages of root growth.

The microgravity environment is very useful as a means of understanding the relationship between gravitational stimulus and response in seedlings (Kiss, 2000). Using time-lapse photography, Perbal and Drissecole (1994) followed the gravitropic curvature of seedlings of lentil (*Lens culinaris*) grown in microgravity and stimulated on the 1-***g*** centrifuge for 5–60 minutes. In space the initial rate of curvature, as well as the amplitude of curvature, varied as a function of the quantity of stimulation (***g*** min). Based on extrapolation from the data generated in these experiments, the time necessary for the ***g*** stimulus to be perceived by the root (presentation time) was calculated to be 27 s. Following the removal of a ***g*** stimulus, autotropism or 'autotropic straightening' of the root occurs (Stankovic *et al.*, 1998), as the root straightens to grow in the unstimulated direction.

Another elegant use of the microgravity environment has been to test the perception thresholds of various gravitropism mutants. Seedling studies with starchless mutants of *Arabidopsis* investigated the starch–statolith hypothesis of gravity-sensing (Kiss *et al.*, 1997, 2000). Recently, Vitha *et al.* (2000) have used starchless mutants of *Arabidopsis* to probe the relative strength of gravitropic and phototropic responses of seedling roots.

In the absence of gravity, the oxytropic behaviour of *Arabidopsis* roots was clearly observed during a spaceflight experiment on STS-68 (Shuttle Transport System) when roots grew out of a hypoxic agar medium. Working subsequently with an agravitropic pea mutant, Porterfield and Musgrave (1998) showed that seedling root growth will orient to zones of higher oxygenation all along the gradient between 0 and 21%, even when no metabolic response to hypoxia is elicited. Takahashi *et al.* (1999) demonstrated positive hydrotropism in the lateral roots of cucumber seedlings growing in microgravity. In a 1-***g*** setting this growth response toward moister regions of the substrate is masked by gravity, which orients the growth of lateral roots perpendicular to the primary root.

Seed development

Because of general problems of growing plants in microgravity, researchers had been unable to confirm or disprove any specific role for gravity in plant reproduction. The extended periods of plant growth necessary for reproduction to occur were not possible, and plants frequently died in the transition from the vegetative to the reproductive stage (Halstead and Dutcher, 1984, 1987; Nechitailo and Mashinsky, 1993). One member of the family *Brassicaceae*, *Arabidopsis thaliana*, has been the most extensively studied species in this research area (Table 4) because its compact size, low light requirement and short life cycle make it amenable to experimentation in the small growth chambers that have been developed for use on orbital platforms (Krikorian and Levine, 1991). However, as will be described below, more detailed information on seed development in space has been generated by using the larger plants, *Brassica* sp. and wheat.

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Several attempts to grow plants through a complete life cycle in space were unsuccessful because of delayed development (Kordyum *et al*., 1983; Mashinsky *et al*., 1994). Partial or total sterility of the reproductive material that eventually did develop has been observed in *Arabidopsis* (Kordyum *et al*., 1983; Merkys and Laurinavicius, 1983). Even when *Arabidopsis* plants were pre-grown to the flowering stage on Earth and allowed to form seeds on orbit, only 55% of seeds were fertile (27% aborted and 18% had non-viable embryos) (Parfenov and Abramova, 1981).

The first successful complete plant life cycle in microgravity occurred in 1983 with *Arabidopsis thaliana* on board Salyut 7 in a miniature plant growth chamber called Phyton 3. The plants had grown from seed planted on orbit, and, although development was delayed, they flowered and produced new seed. However, reports on the returned material described a large proportion of empty seed, and a high number of embryonic lethals in the seeds that were produced in space (Merkys and Laurinavicius, 1983) relative to the ground controls.

Recently, an experiment in the Svet greenhouse on the Mir station showed promise that a full life cycle may have been completed in wheat as well (Salisbury *et al*., 1995); however, when the plants were returned to Earth, all of the heads were found to be devoid of seeds (Strickland *et al*., 1997). Subsequent ground-based experiments suggested that the reproductive function of the wheat variety used for the experiment, 'Super Dwarf', was impaired by the high levels of ethylene that prevailed on the Mir station. When the experiment was repeated using a dwarf wheat cultivar that is less sensitive to ethylene, 'Apogee', viable pollen was produced and seeds were formed (Levinskikh *et al*., 2000).

These previous studies on plant reproduction in space have been summarized in Table 4 to highlight differences in plant material and ventilation capability of the plant growth hardware. Nine different types of flight hardware have been used to grow plants in space for studies on plant reproduction. Additional details on the hardware that has been used for experiments on plant reproduction may be found in a recent review by Porterfield *et al*. (2002). Of the 13 experiments described in Table 4, the six in which set seed occurred all had some type of ventilation with outside cabin air. As detailed below, in a series of experiments in the Plant Growth Unit (PGU), we have determined that the composition of the gaseous environment is very important to the success of reproductive development in spaceflight.

Table 4. Experiments on plant reproduction during spaceflight have utilized a variety of plant materials and growth chambers.[a] The results of these experiments have been reviewed elsewhere (Halstead and Dutcher, 1984, 1987; Nechitailo and Mashinsky, 1993; Musgrave *et al*., 1997)

Starting material	Chamber	Ventilation	Flowers	Seeds	Reference
Plants or seedlings					
Dicotyledons					
Arabidopsis	(Kosmos 1129)[b]	Open	+	+	Parfenov and Abramova (1981)
	PGU[c] (STS-54)	Closed	+	–	Kuang *et al*. (1995)
	PGU (STS-51)	Closed + CO_2	+	–	Kuang *et al*. (1996a)
	PGU (STS-68)	Active	+	+	Kuang *et al*. (1996b)
Brassica	PGF[c] (STS-87)	Active	+	+	Kuang *et al*. (2000a)
Monocotyledons					
Epidendrum	Malachite (Salyut 6)	Open	–[d]	–	Nechitailo and Mashinsky (1993)
Seeds					
Dicotyledons					
Arabidopsis	Svetoblok (Salyut 6)	Closed	+	–	Kordyum *et al*. (1983)
	Phyton (Salyut 7)	Passive	+	+	Merkys and Laurinavicius (1983)
Pisum	Oasis (Salyut 6)	Open	–	–	Nechitailo and Mashinsky (1993)
Brassica	Svet (Mir)	Open w/fan	+	+	Kuang *et al*. (2000b)
Monocotyledons					
Triticum	Svetoblok M (Mir)	Passive	+	–	Mashinsky *et al*. (1994)
	Svet (Mir)	Open w/fan	+	–	Strickland *et al*. (1997)

[a] Sporadic flower formation also occurred in the PGU (equipped with an air-exchange system) on STS-29 during an experiment designed to study spaceflight effects on chromosomes using aseptically cultivated plantlets of *Haplopappus gracilis* (Nutt.) (Levine *et al*., 1990).

[b] Plexiglas beaker containing moist soil.

[c] PGU, Plant Growth Unit; PGF, Plant Growth Facility.

[d] Plants were taken to the Salyut station in blossom but no additional flowers were formed on orbit.

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Embryo development in space: role of the gaseous environment

Prior to experiments with embryo development during seed formation in space, Krikorian sought to explore effects of *g*-unloading in somatic embryos raised in tissue culture by using karyological indicators. Physical challenges to embryoids that initiate from cellular embryo initials may be analogous to those encountered during *in situ* embryo development. Krikorian showed that, in the case of carrot and day lily, the younger the embryoids are in the developmental progression, the more sensitive they are to the spaceflight environment (reviewed in Krikorian, 1998). Physical attributes of the spaceflight environment, such as lack of convection, lack of buoyancy and the concomitant role of surface tension, combine to effect 'space stress' for these small units that is expressed at the level of the nucleus (Krikorian, 1996).

When we began to study spaceflight effects on reproductive development in *Arabidopsis*, we chose to work with pre-grown plants because of the large body of literature that indicated that vegetative development was often retarded during growth in microgravity. *Arabidopsis* takes about 45 d to complete a full life cycle. For experiments in the PGU, plants were pre-grown on an agar-solidified nutrient medium that had been developed for use on Biosatellite (Brown *et al*., 1976) and were 13 d old at the time of launch. During the subsequent time on orbit, flower buds appeared and early events in reproductive development occurred. In this system, even during short-duration shuttle flights, it is possible to study spaceflight effects on the complex processes of mitosis, meiosis, cell division, tip growth of cells, tissue differentiation and organ formation.

In our three experiments with the PGU, plants developed at the same rate in spaceflight and ground control, and initiated the same number of flowers. Approximately 500 flowers were available from each experiment (Musgrave *et al*., 1997). The differences in success of subsequent reproductive development in microgravity were related to variations in the gas phase of the plant growth hardware, as described below and summarized in Table 5.

In the experiment Chromex-03 on STS-54, growth chambers were closed, and reproductive development aborted at an early stage in both the male and female tissues of the spaceflight material. Pistils were collapsed and the ovules were empty. No viable pollen was observed using a functional test (Heslop-Harrison *et al*., 1984), and young microspores were deformed and empty (Kuang *et al*., 1995). Analysis of foliage indicated that the spaceflight material had significantly lower carbohydrate levels than the ground controls (Musgrave *et al*., 1998). Flowering and seed production are known to be hindered by insufficient carbon reserves in the plant. Thus, we hypothesized that the failure in reproductive development in this case was attributable to some limitation on the whole-plant physiological performance rather than on the reproductive apparatus *per se*.

Supplementation of the gas phase of the closed plant growth chambers with high carbon dioxide (8000 p.p.m.) in Chromex-04 (STS-51) overcame this early abortion, and material developed normally up through the stage of mature pollen and embryo sacs. Pollen was viable and well-formed, although a filmy material inside the tapetum appeared to restrict the release of pollen from the anthers (Kuang *et al*., 1996a). Fertilization did not occur in these flowers and scanning electron microscope (EM) analysis of stigmatic surfaces post-flight revealed that no pollen had been transferred from the anthers to the stigmatic papillae.

In Chromex-05 on STS-68, an air-exchange system provided a flow of filtered cabin air through the plant growth chambers, and development proceeded normally in orbit through the stage of immature seeds. These seeds were comparable to those

Table 5. Summary of experiments on early reproductive development in *Arabidopsis thaliana* (Chromex-03, -04, and -05) on STS-54, STS-51 and STS-68

Experiment	Duration	Chamber configuration	Early reproduction	Pollination/seeds
Chromex-03	6 days	Sealed chambers	Pollen and embryo sac aborted	Pollen non-viable[a]
Chromex-04	10 days	Sealed chambers + CO_2	Androecium and gynoecium normal	No pollen transfer[b]
Chromex-05	11 days	Continuous air flow	Androecium and gynoecium normal	Normal[c]

[a] As determined post-flight by fluorescein diacetate staining. Refer to Kuang *et al*. (1995) for complete details on reproductive development in these plants.
[b] As determined post-flight by scanning and transmission electron microscopy. Refer to Kuang *et al*. (1996a) for complete details on reproductive development in these plants.
[c] Refer to Kuang *et al*. (1996b) and Musgrave *et al*. (1997) for details.

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produced in the ground control material in their morphology and size (Kuang *et al*., 1996b). The most mature seeds from these plants contained completely developed embryos with seed coats. Tissue development included radicle, hypocotyl, meristematic apical tissue and differentiated cotyledons (Fig. 5). Protoderm, procambium and primary ground tissue had differentiated in these embryos. Starch and protein reserves were deposited in the embryos during tissue differentiation in a manner quantitatively and qualitatively similar to that in the ground control, and the seed coat was developing properly (Kuang *et al*., 1996b). This was the first instance in which seed development in microgravity (at least for a period of development up to 10 d after pollination) was comparable to that in ground controls.

This opportunity to perform a series of spaceflight experiments and change variables incrementally, as one would in a laboratory experiment, has provided unique insight into the importance of metabolic components of the gas phase for normal reproductive development. In our first experiment with closed plant growth chambers in the PGU, the abortion of reproductive development appeared to be similar to that in a previous experiment using Svetoblok (Kordyum *et al*., 1983). However, when closed chambers were supplemented with high carbon dioxide (Chromex-04), pollen and embryo sacs were indistinguishable from the ground control. The obstacle to seed formation in the Chromex-04 experiment was a lack of pollen release from the anthers. The results have been especially intriguing because they imply the existence of stagnant air zones

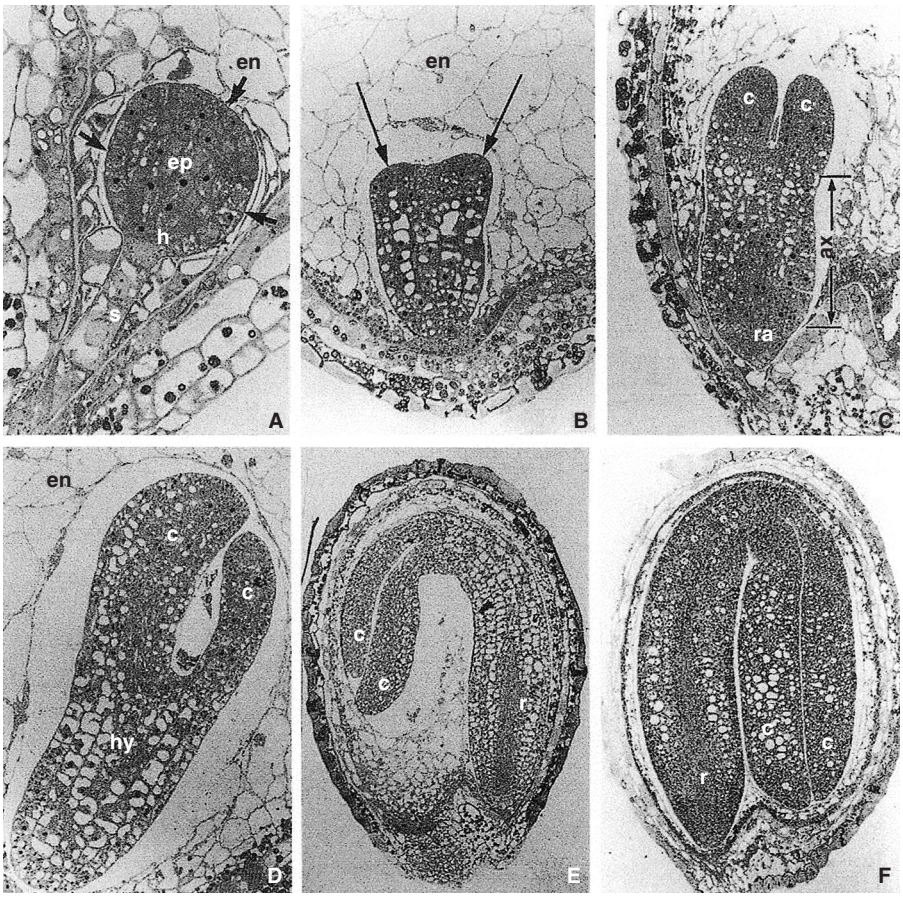

Figure 5. Representative embryos at different developmental stages in *Arabidopsis* plants grown under spaceflight conditions on STS-68. (A) Late globular or transition stage. en, endosperm; ep, embryo proper; s, suspensor; h, hypophysis cell; arrowheads, protoderm. (B) Cotyledon initiation stage. arrows, cotyledon primordia; en, endosperm. (C) An early linear cotyledon stage. ax, hypocotyl axis; c, developing cotyledon; ra, radicle apical meristem. (D) Late linear cotyledon stage. c, cotyledon; en, endosperm; hy, hypocotyl. (E) Curled cotyledon stage; c, cotyledon; r, radicle. (F) Mature embryo. c, cotyledon; r, radicle. Figure reproduced with permission from Kuang *et al*. (1996b), © *Annals of Botany*.

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in microgravity that lead to a shortage of carbon dioxide and excess water vapour around the plant. Deficiency in CO_2 resupply would limit photosynthesis and subsequently result in the reduction in carbon reserves that was observed in the spaceflight material in Chromex-03 (Musgrave *et al*., 1998). Flow-through of cabin air was necessary to permit the full reproductive sequence to occur in orbit (Kuang *et al*., 1996b), despite the fact that both hardware configurations used previously (closed chambers) had been sufficient to support reproductive development at 1 ***g***.

Storage reserve deposition in microgravity

This series of experiments provided the first evidence that spaceflight changes the environment of plants in ways that are detrimental to normal reproductive development. The lack of convective air movement in microgravity makes plant growth in closed chambers problematic because metabolic gases (in this case, CO_2) cannot be resupplied. Using this finding regarding the importance of gas resupply for successful plant development in microgravity, we successfully grew a closely related plant, *Brassica rapa*, through its full life cycle, using a well-ventilated chamber on the Mir space station (Musgrave *et al*., 2000). Interestingly, although plant development, flowering, pollination and early seed development occurred normally, significant changes occurred during late stages of seed development in microgravity.

The first external manifestation of developmental differences during seed maturation occurred about 20 d after pollination as siliques were beginning to ripen. *Brassica* silique ripening on Mir consistently occurred from the tip of the silique to the point of attachment on the plant (Fig. 6A), compared to the uniform ripening pattern seen in 1 ***g*** (Fig. 6B). This suggests that a metabolic gas gradient develops inside the silique in microgravity due to the absence of buoyancy-driven convection (Kuang *et al*., 2000b).

Seeds of *Brassica rapa* produced on the Mir space station were smaller and weighed less than ground control seeds (Table 6). Although cell size in the cotyledons was not significantly lower in the spaceflight material than in the ground control, the cotyledons contained substantially fewer cells. Storage reserves were significantly altered as well, with smaller protein bodies and a retention of starch at maturity in the spaceflight cotyledons. The spaceflight-induced differences in storage reserves were already manifest in the cotyledon cells of developing *Brassica* seeds at 16 d post-pollination, as was found in our experiment on STS-87. At this stage, starch grains were still evident in the ground control material, but many more were present in the spaceflight cotyledon cells. Protein bodies were already well developed in the ground control material, while lipid bodies and starch were the major storage reserves in the spaceflight tissue (Fig. 7).

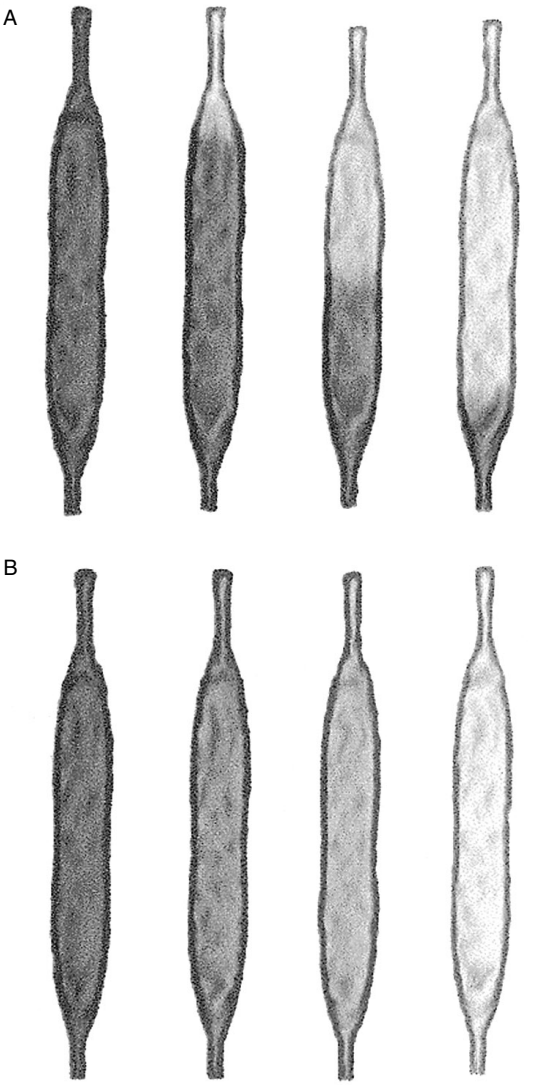

Figure 6. *Brassica* silique ripening occurred basipetally in microgravity (A) during the Greenhouse 3 experiment on Mir, compared with the usual overall ripening pattern seen in the 1-***g*** ground control (B). Figure reproduced with permission from Kuang *et al*. (2000b), © *Annals of Botany*.

To summarize, the results of these two spaceflight experiments, studying seed development in *Brassica*, indicate that development in microgravity causes major shifts in the storage reserve composition. The normal developmental process by which the starch that is stored initially in young seeds is converted into the lipid and protein storage reserves is delayed or disrupted in microgravity. This finding has significance for: (1) the vigour of plants arising from seeds produced in microgravity; (2) the nutritional quality of the seeds produced in microgravity; and

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Table 6. The comparison between characteristics of the mature *Brassica rapa* seeds produced in space on Mir and the corresponding ground control seeds (modified from Kuang *et al*., 2000b)

	Seed source	
Characteristics	Spaceflight	Ground control
Seed weight (mg)		
Expt. 1	1.1*	1.6
Expt. 2	1.3*	1.7
Cell number/cotyledon × 10^2	10.3*	60.0
Cell size (μm^2)	303	435
Protein bodies		
Number per cell	3.4	2.8
Size (μm^2)	23.9*	42.4
Size ratio (pb area/cell* size)	26.1	33.2
Starch grains per cell	2.7	0

* Significantly different from ground control, $P < 0.05$

hence (3) the feasibility of using plants in a microgravity setting to provide biological life support functions for humans on long-duration missions. A further point of basic significance of the finding is a demonstration of the role of gravity in shaping the physical environment that defines the biological process of seed ripening.

Consequences of development in microgravity for seed performance

In the Greenhouse 3 experiment on the Mir station, seeds produced in space were replanted alongside seeds brought from Earth, allowing comparison of first- and second-generation plant growth. Plants were significantly smaller when grown from seeds produced in spaceflight. The post-flight ground control confirmed that this was a consequence of seed production in microgravity, rather than a result of hardware or Mir atmosphere characteristics (Fig. 8) (Musgrave *et al*., 2000).

A similar test of seed performance was accomplished using the immature seeds produced on STS-87 and an embryo rescue procedure described by Kuang *et al*. (2000a). Siliques produced during spaceflight were removed from the plants post-flight and cultured for 1 week, to allow the embryos to reach the stage at which they could subsequently

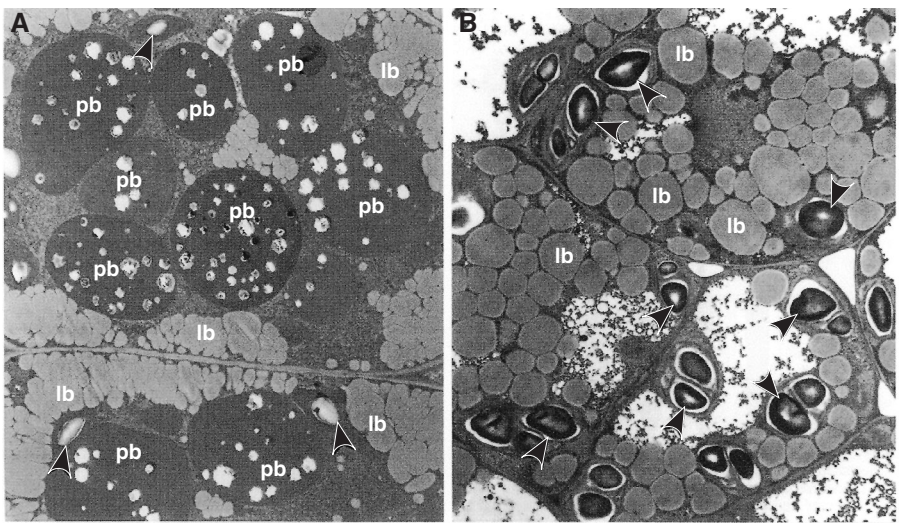

Figure 7. Electron micrographs showing the ultrastructure of cotyledon cells of immature *Brassica rapa* seeds 15 d after pollination, from the STS-87 shuttle experiment. Note that although the ground control (A) and spaceflight seeds (B) are the same age, the internal structure of the cells is different. In the ground control seeds (A), large protein bodies (pb) have formed, and lipid bodies (lb) and starch grains (arrowheads) are present. In the space-produced seeds (B), no protein bodies were observed, but lipid bodies (lb) and numerous large starch grains (arrowheads) are major storage reserves. Figure reproduced from Kuang *et al*. (2000b), © *Annals of Botany*.

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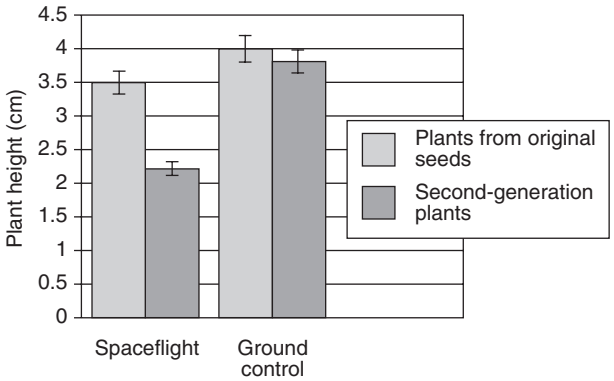

Figure 8. Effect of seed production in space on plant height in the subsequent generation. *Brassica rapa* seeds were taken to the Mir space station and sown. Plants were pollinated and seeds were collected and replanted alongside Earth-produced seeds while in orbit. Second-generation plants were significantly smaller than those grown from seeds brought from Earth. Second-generation plants grown from seeds produced during the high-fidelity ground control were the same size as plants grown from the starting seeds (right-hand bars). Modified from Musgrave *et al*. (2000).

develop into plants, without going through the usual dry-down stage that accompanies seed maturation. The E_1 plants obtained from spaceflight embryos (15 d after pollination) using this technique showed a marked difference in the rate of development, lagging behind the ground controls by 12 d.

The quantity of seed storage substances accumulated during late development determines the vigour of the subsequent generation. The consequence of diminished storage reserve deposition in cotyledon cells in microgravity is the significant reduction in seed weight and subsequent seedling vigour observed in the *Brassica* seeds produced on Mir (Fig. 8; Table 6). A similar reduction in seed size and subsequent seedling vigour has been reported for wheat seeds produced on the Mir station (Levinskikh *et al*., 2000), suggesting that this phenomenon is not restricted to *Brassica*, and may be a general consequence of seed development in microgravity.

Nutritional quality of seeds produced in microgravity and the implications for BLSS

In the case of *Brassica*, a seed normally rich in oil and protein, the retention of starch as a major storage reserve at maturity has significant consequences for the nutritional quality of seeds that would be produced in microgravity. Biochemical analysis of dry seeds from commercial *Brassica* indicates that about 40% of the dry weight is protein (Robbelen and Thies, 1980). We found that the total size of protein bodies represented about 26% of the cell in space-produced seeds, compared with 33% in the ground-produced seeds (Table 6). *Brassica* has been proposed as a candidate crop for a biological life support system because of its value as an edible oil and protein source (Frick *et al*., 1994). If microgravity causes such substantial changes in the composition of seed storage reserves in other species, as we have found with *Brassica*, proposed nutritional roles of individual species will have to be reconsidered. Furthermore, the reduction in seed size and the vigour of subsequent generations of plants caused by microgravity threatens the application of plants in biological life support scenarios that might be proposed for use in transit vehicles.

Seed storage

A number of seed biology experiments were flown aboard the Long Duration Exposure Facility (LDEF) and were designed to investigate seed storage phenomena in spaceflight. LDEF was a cylindrical frame used for the attachment of various experiment payloads, launched from the Challenger orbiter on 6 April 1984. During orbit the seeds experienced a g force less than 1 millionth of that at Earth's surface, and temperatures inside the canisters ranged from –23°C to 35°C. Radiation levels at the orbital altitude of the LDEF were approximately 100 mrad d^{-1} and, over the course of the experiment (nearly 6 years), the measured radiation dose was 2.66–6.48 Gy (Frank *et al*., 1993). Total mean dosages for flight and ground control thermoluminescent dosimetry wafers were 210 and 0.9 rad, respectively (Schuerger *et al*., 1991).

Seed performance following storage on LDEF was compared with the performance of seeds in controlled storage on the ground. Two million seeds (representing 106 species) were sent on LDEF by Park Seed in either sealed or vented canisters. There was a better survival rate of seeds in the sealed canister in space than in the storage facility at Park Seed, and at least some seeds in each of the vented canisters survived the exposure to vacuum for almost 6 years. The observed mutations were very low (Alston, 1991). In contrast, Mei *et al*. (1994) reported a range of somatic mutations in plants developed from 500 *Zea mays* grains flown on LDEF, including white-yellow stripes on leaves, dwarfing and changes in leaf sheath or seedling colour. The incidence of the white-yellow mutation in the spaceflight grains could be matched by exposure to 635 cGy of gamma-rays on the ground.

Hammond *et al*. (1996) was one of the university groups to conduct an analysis of the LDEF tomato seeds that had been broadly distributed to schoolchildren. Twelve and a half million Rutger's tomato seeds, provided by the Park Seed Company, were sealed in five aluminium canisters at 0.1013 MPa and 15% relative humidity. The space-exposed

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seeds germinated sooner than the Earth-based seeds and also grew at a faster rate. EM analysis of the seeds showed the tissues to be more porous than those of the Earth-based control. Kahn and Stoffella (1996) also found no evidence of adverse germination, emergence and fruit yield due to space exposure of the tomato seeds. Rice grains (Bayonove *et al*., 1994), used in an electron spin resonance (ESR) study of free-radical formation, and *Arabidopsis* seeds, included as radiobiological indicators (Zimmermann *et al*., 1994), were also flown on LDEF. The free radical concentration found in rice grains post-flight depended on the irradiation doses and whether or not they were delivered in the presence of oxygen (Bayonove *et al*., 1994). Similarly, the loss of viability in *Arabidopsis* seeds caused by cosmic ionizing radiation exposure varied according to the position of the samples on the LDEF (and hence absorbed radiation dose) (Zimmermann *et al*., 1994). Schuerger *et al*. (1991) characterized the microorganisms borne on seeds of seven different crop species that survived the long periods of spaceflight exposure on LDEF, and found bacterial diversity and relative abundance to be similar for the LDEF and ground control samples.

Seeds in the unique spaceflight environment

From its inception, gravitational biology research has had both a mission-driven component and an element of basic scientific enquiry. In no case has this dual approach been more apparent than in the subject area of seed biology. Because of the plans to use plants as an integral component of life support systems for human outposts in space, seed biology becomes a topic of critical importance to NASA.

At the same time, the microgravity environment presents a unique setting for seed biology research. Our understanding of the tropic behaviour of seedling roots and shoots has been greatly advanced by access to an environment that lacks one of the major stimuli eliciting a tropic response. Coupled with the ability to manipulate gravity doses experimentally, access to a laboratory in space has resulted in a strong literature regarding gravity perception and signal transduction.

Because the physical environment of plants is so dependent upon the behaviour of fluids and gases, microgravity presents a suite of horticultural challenges. Plant growth hardware design is evolving to provide plants growing in space with a satisfactory physical environment despite the unusual behaviour of fluids and gases. Microenvironments within plant structures, such as the air space around seeds developing inside a pod, also change in the spaceflight environment. These changes have adverse affects on seed quality and, thus, have important consequences for the role of plants in bioregenerative life support systems, as well as implications for dynamics within the microenvironment of the developing seed.

Just as *g*-threshold studies have been carried out with young seedlings to better understand signal perception and transduction, studies with fractional-*g* will help to elucidate the role of gravity in shaping the physical microenvironments that dictate both the metabolism of germinating seeds and the deposition of storage reserves in developing seeds. As facilities for this type of research become available, an approach of using plants as phytometers (Porterfield *et al*., 1997; Paul *et al*., 2001; Stout *et al*., 2001) coupled with small-scale environmental measurements (Porterfield *et al*., 2000b) will provide valuable information that will both advance our basic understanding of the role played by these microenvironments and contribute to defining the capability of plants to function in future bioregenerative life support systems.

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