*Plant Cell Physiol.* 42(6): 657–664 (2001)


# Growth in Microgravity Increases Susceptibility of Soybean to a Fungal Pathogen

**Marietta Ryba-White [1], Olena Nedukha [2], Emmanuel Hilaire [3], James A. Guikema [4], Elizabeth Kordyum [2] and Jan E. Leach [1, 5]**

[1] *Plant Pathology Department, Kansas State University, Manhattan, KS 66506-5502, U.S.A.*
[2] *Institute of Botany, National Academy Sciences of Ukraine, 25004 Kiev, Ukraine*
[3] *National Jewish Medical and Research Center, Office of Intellectual Property and Technology Commercialization, Suite K734, 1400 Jackson Street, Denver, CO 80206, U.S.A.*
[4] *Division of Biology, Ackert Hall, Kansas State University, Manhattan, KS 66506-4901, U.S.A.*

**The influence of microgravity on the susceptibility of soybean roots to *Phytophthora sojae* was studied during the Space Shuttle Mission STS-87. Seedlings of soybean cultivar Williams 82 grown in spaceflight or at unit gravity were untreated or inoculated with the soybean root rot pathogen *P. sojae*. At 3, 6 and 7 d after launch while still in microgravity, seedlings were photographed and then fixed for subsequent microscopic analysis. Post-landing analysis of the seedlings revealed that at harvest day 7 the length of untreated roots did not differ between flight and ground samples. However, the flight-grown roots infected with *P. sojae* showed more disease symptoms (percentage of brown and macerated areas) and the root tissues were more extensively colonized relative to the ground controls exposed to the fungus. Ethylene levels were higher in spaceflight when compared to ground samples. These data suggest that soybean seedlings grown in microgravity are more susceptible to colonization by a fungal pathogen relative to ground controls.**

**Key words**: Ethylene — Microgravity — *Phytophthora sojae* — *Williams 82*.

Abbreviations: BRIC, Biological Research in Canister; CELSS, Controlled Ecological Life Support Systems; CUE, Collaborative Ukrainian Experiment; KFT, Kennedy Fixation Tube; KSC, Kennedy Space Center; OES, Orbiter Environmental Simulator; *SOYPAT*, soybean pathology; TEM, transmission electron microscopy.

## Introduction

The production of healthy, edible plants is a major component of the Controlled Ecological Life Support Systems (CELSS) which will house humans in the space environment. The need to regenerate food and water, produce oxygen and remove carbon dioxide from the air are crucial components of the CELSS due to the prohibitive costs of regular transport of these essential products from Earth (Nelson 1987). Because the spaceflight environment is not sterile, the potential for contamination of agricultural systems by microbial plant pathogens is a real danger. Microbes that can colonize plants frequently have been isolated from spaceflight hardware (Taylor et al. 1973, Taylor 1974, Brockett et al. 1978, Nelson 1987) and evidence of microbial contaminants colonizing and damaging plants has been documented in spaceflight experiments. For example, a recent report of contamination and damage of experimental wheat plants clearly demonstrated that plants grown in spaceflight are at risk of microbial invasion, even by an organism that normally does not cause disease on wheat (Bishop et al. 1997).

The implications of these observations for crop production in space are serious. The increased accessibility of plants to microorganisms may mean that the plants are not only more susceptible to recognized pathogens, but they also may be susceptible to pathogenic colonization by opportunistic microbes, i.e. organisms that are not normally pathogens to the plant that are present aboard the space shuttle or the international space station. Without information on why the plants are more susceptible, informed design of adequate growth facilities, establishment of optimal growth parameters and selection of suitable plant cultivars for sustainable crop production in space is impossible.

To date, no flight studies specifically designed to address susceptibility of plants to pathogens have been successfully completed. Thus, we developed an experimental system using *Phytophthora sojae*, the root rot pathogen, and its host soybean to systematically and quantitatively address whether or not soybean plants are more susceptible to disease when grown in spaceflight, and to begin to address how conditions of spaceflight might affect plant susceptibility to disease. The soybean pathology (*SOYPAT*) experiment was part of the Collaborative Ukrainian Experiment (CUE) that flew aboard Space Shuttle Columbia flight STS-87 in 1997. The soybean (host) and *P. sojae* (pathogen) disease interaction was selected for this study because of the amenability of the interaction to spaceflight conditions and the extensive documentation of the genetic and molecular mechanisms of the interaction (Keen and Yoshikawa 1983, Schmitthenner 1985, Schmitthenner 1989, Schmitthenner

[5] Corresponding author: E-mail, jeleach@ksu.edu; Fax, +1-785-532-5692.

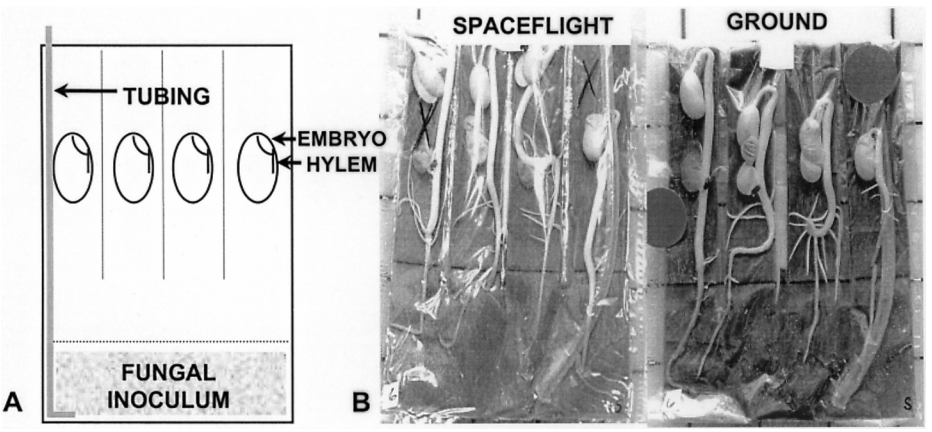


**Fig. 1** Soybean seedlings in modified growth pouches. (A) Schematic drawing of modified germination pouch design and seed orientation. (B) Seedlings from day 7 harvest. In both spaceflight and ground-growth locations, seedling roots inoculated with *P. sojae* are brown in color and the tissues are macerated. Uninoculated roots were not discolored or macerated (not shown).

et al. 1994, Enkerli et al. 1997, Graham and Graham 1999). In addition, soybean seedlings were used to determine the effects of growth in microgravity on soybean physiology (Brown and Huber 1987, Brown and Huber 1988, Brown and Piastuch 1994, Brown et al. 1995, Hilaire et al. 1996, Kuznetsov et al. 1997). Using this experimental system, we demonstrate that when grown in conditions of spaceflight, soybeans are more susceptible to colonization by *P. sojae*.

## Materials and Methods

*Pouch design*

Plastic growth pouches ('cyg' pouch; Mega International, Minneapolis, MN, U.S.A.) were adapted to ensure proper directional growth such that seedling roots would become infected during spaceflight. The paper lining was trimmed and the plastic heat-fused to create troughs (2×8 cm with 2 mm between each trough) to guide root growth toward fungal inoculum after germination. One end trough per pouch contained a piece of tygon tubing that was fitted with a blunt-end needle to allow introduction of the fungal inoculum to the bottom of the pouch (Fig. 1A).

*Seed preparation and sowing*

Prior to sowing, seeds were surface-sterilized by submersion in 70% ethanol for 1 min, soaked for 2 min in 15% sodium hypochlorite, rinsed twice in sterile distilled water and air-dried in a laminar flow hood (24°C). Seeds were positioned at 4 cm from the top of the germination paper and were fixed into position with Guar gum (Sigma #G-4129) with the embryos all facing the same side of the pouch (Fig. 1A). The pouches were prepared 1 week ahead of the scheduled flight date and stored at 4°C.

*Fungal inoculum*

*Phytophthora sojae* strain B8R25–81–12 (a gift from A. Schmitthenner, Ohio State University) was cultured on Lima Bean agar (Difco #0117–17–1) for 7 d. This strain is virulent (causes disease) on soybean cv. *Williams 82*. Six 1-$cm^2$ blocks (consisting of agar, fungal hyphae and spores) were cut from the leading edge of the fungal mat on one agar plate and were macerated in 5.4 ml sterile $H_2O$. The fungal macerate was mixed with 24 ml of a 0.5% lima bean agar slurry. At 24 h prior to launch, sterile $d_2H_2O$ (3 ml) was introduced through the tygon tubing into each pouch to wet the germination paper. This was immediately followed by introduction of the fungal slurry (5 ml). The slurry was spread evenly across the pouch bottom. Mock-inoculum was prepared as above, but without the fungal-infested agar blocks. The pouch opening was folded and taped shut.

*Experimental design and ground control*

Soybean seeds (cv. *Williams 82*, provided by A. Schmitthenner) were sown in the modified growth pouches. At 24 h before launch, the six prepared pouches (three uninoculated and three inoculated) were placed in each of four Biological Research in Canisters (BRICs; Fig. 2C). The BRICs were placed in the mid-deck locker of U.S. Space Shuttle Columbia (mission STS-87). One pouch per treatment per replication was harvested at 3, 6 and 7 d after launch and each pouch was photographed prior to removal of plant material. The complete experiment was duplicated at 1×*g* in the Orbiter Environmental Simulator (OES) at the Kennedy Space Center (KSC). The ground control was initiated 24 h post-launch to enable reproduction of spacecraft environmental conditions (cabin temperature and $CO_2$). An electronic temperature gauge was placed inside one of the spaceflight BRICs 24 h prior to launch to adjust for daily temperature changes (30 min increments) during the experiment. No significant deviations were observed between ground and spaceflight temperature conditions.

*Gas sampling*

A gas-tight syringe was used to remove 20 cc of gas from sampling ports on two of the four BRICs to monitor $CO_2$ and ethylene output from the seedlings. Samples were taken immediately after placing the pouches into the BRICs prior to launch, and prior to each harvest (flight days 3, 6 and 7). Samples from the flight and ground experiments were stored in the syringes until the space shuttle returned to earth, and were subsequently analyzed for $CO_2$ and ethylene by gas chromatography (performed by Barbara Peterson, KSC chemistry laboratory) as described in Hilaire et al. (1996).

*Tissue sampling*

One pouch per treatment was removed from each of the four BRICs on days 3, 6 and 7 after launch. After being photographed, two whole seedlings from each treatment were removed from each pouch

and placed into a KSC fixation apparatus [Kennedy Fixation Tube (KFT); Fig. 2A, B]. Fixation was initiated by flooding the chamber with 15 ml of fixative solution (2% glutaraldehyde/2% paraformaldehyde in PIPES buffer pH 7.4). The seedlings were bathed in the fixative inside the KFTs until landing. The two remaining seedlings were placed in a gaseous $N_2$ freezer, but these samples were lost to the experiment due to a hardware malfunction.

*Microscopy*

Six hours after space shuttle landing, seedlings were removed from the fixation apparati and placed in fresh fixative. The tissues were washed in 50 mM PIPES pH 7.4 for 15 min, stained in 0.05% phloxine B dye dissolved in the same buffer for 10 min at 24°C, and finally rinsed in the PIPES buffer. The seedling lengths were measured and roots photographed. Each seedling from the day 6 harvest was divided into various root zones, including: (1) root apex with meristem, (2) zones of elongation and differentiation, (3) root hair zone, (4) area between first and second lateral roots, (5) hypocotyl hook and (6) cotyledon. Sections were examined under a dissecting microscope for tissue integrity and evidence of fungal invasion prior to embedding. Using 2.5 µm tissue sections and a light microscope, the percentage of soybean cells with associated fungal structures (number of soybean cells with fungal structures associated per total number of cells) was determined. This included the presence of these structures within and between the layers of cells. An average of 100 cells per root zone was counted.

Portions of divided tissues were processed for embedding in epoxy resin (Epon) for ultrastructural analysis of tissue. In this case, the tissues were post-fixed in 2% $OsO_4$ in PIPES buffer pH 7.4 at 4°C for 12–14 h and then washed with water (4°C) for 15 min. Fixed tissues were dehydrated in a graded ethanol series (30% for 15 min; 50% for 15 min; 70% for 15 min; 80% for 15 min; 90% for 15 min; 100% for 30 min; 100% for 30 min) followed by two changes of 100% acetone (1 h each). The tissues were infiltrated with mixtures of acetone and epoxy resins (Epon 812, 3 ml; Araldit M, 2 ml; Epon DDSA, 5 ml; Epon DMP, 10 drops) in a graded series [ratio acetone : resin (v/v) = 3 : 1, 1 : 1 and 1 : 3 for 24 h], then infiltrated with fresh epoxy resin for 24 h and polymerized for 24 h at 35°C followed by 24 h at 60°C.

Tissue blocks were sectioned [2.5 µm for light microscopy and 95 nm for transmission electron microscopy (TEM)] with a Reichert Ultra Microtome. The sections for light microscopy were stained for 1–2 min with 1.0% toluidine blue in 1.0% sodium borate, and rinsed before viewing with an Olympus BX40. For TEM, sections were stained with 2% uranyl acetate in 25% ethyl alcohol followed by Reynold's lead citrate, and analyzed with a Philips CM-100 transmission electron microscope.

To count fungal oospores, tissues were dehydrated in an ethanol series (50%, 70%, 85%, 95% and 3× 100%) for 30 min each. They were then infiltrated in a graded series [ratio ethanol : xylene (v/v) = 3 : 1, 1 : 1 and 1 : 3 for 30 min] followed by 100% xylene for 30 min, fresh 100% xylene for 60 min, a 1 : 1 ratio of xylene and paraffin overnight at 37°C and then in pure paraffin at 60°C (3×30 min). The tissues were placed into molds and left at room temperature to harden. Ten micron sections were made using a microtome (Spencer Lens Co. #820), and stained with aniline blue (Eschrich and Currier 1964) prior to viewing under a blue light epifluorescence microscope (Zeiss standard microscope with IV FL condensor).

*Assessment of disease symptoms and fungal invasion*

Photographs from each harvest were used to determine the average seedling length, number of lateral roots per pouch for the four replications and the visual symptoms (percentage of brown and macerated tissue). The occurrence of disease symptoms was expressed as the percentage of root showing brown and macerated tissues. Comparisons of the extent of fungal colonization of the tissue were obtained using light microscopy to assess the amount of fungal structures (i.e. mycelium and/or reproductive structures) in or near cells of spaceflight vs. ground treatments. Oospores were counted within a 2 $mm^2$ area inside each root zone in three replications of spaceflight-grown tissue and four of the ground-grown tissue. Ultrastructural studies using TEM were performed to assess integrity of host and pathogen cellular structures, relative to ground controls.

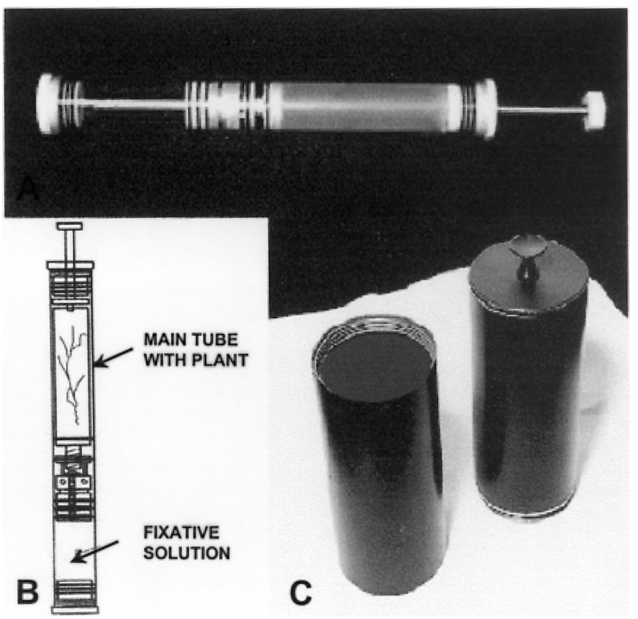


**Fig. 2** Hardware used for the *SOYPAT* experiment. (A) The Kennedy Fixation Tubes (KFT) were designed to carry and release fixation fluid used to preserve harvested tissue in spaceflight and on the ground. (B) The KFT was comprised of two main parts: the fixative chamber where fixative was loaded preflight and the main tube where harvested plants were placed for fixation. To fix a plant sample, the sample was placed into the plant tube. A plunger was used to push the expansion plug into the fixative, which forced the fixative through an opening at the bottom of the plant tube. (C) The BRIC housed the *SOYPAT* experiment. Each BRIC was 8.2 cm (diameter) by 31.2 cm (height), made of anodized-aluminum with a screw-top lid containing four light-tight, passive pressure relief vents (1.5 mm in diameter) used as gas sampling ports. Eight containers were used altogether (four each for spaceflight and ground) and each contained nine pouches.

## Results and Discussion

*Disease development in spaceflight*

To evaluate susceptibility of soybean to *P. sojae* in both environments (spaceflight and ground), we measured (a) root length, (b) number of lateral roots and (c) disease symptom expression. *Phytophthora* root rot is a vascular disease of soybean. Symptoms include browning and maceration of infected tissue, reduction in root length and number of lateral roots. Spaceflight-grown soybean roots inoculated with *P. sojae* exhibited more symptoms than did the ground control. Uninoculated controls at both locations displayed no symptoms char-

**Table 1** Percent of soybean cells with associated structures of the pathogen *P. sojae* at 6 d after launch

| Root tissue | Soybean cells with associated fungal structures (%) [a] | |
|---|---|---|
| | Spaceflight | Ground |
| Apex | | |
| Collumellar | 34 | 0 |
| Meristem | 33 | 0 |
| Elongation zone | | |
| Epidermis | 21 | 0 |
| Exodermis | 31 | 0 |
| Cortex | 22 | 0 |
| Root hair zone | | |
| Epidermis | 4 | 4 |
| Exodermis | 0 [b] | 0[b] |
| Cortex | 100 | 13 |
| Phloem | 100 | 9 |
| Xylem | 50 | 5 |

[a] Numbers represent percent of cells where fungal fragments were found either inside the cell or in the intercellular spaces adjacent to cells of infected soybean tissue. Roots were divided into four zones and 50–100 cells were examined inside each zone. The lateral root zone did not have significant infection across replications and data are not presented.

[b] Cells of the exodermis were damaged, but no fungus was detected.

acteristic of *Phytophthora* root rot and no symptoms were found in the day 3 uninoculated or inoculated tissue. However, disease symptoms were observed by the 6 and 7 day harvests. Seventy-one percent of the inoculated spaceflight-grown roots on soybean seedlings exhibited symptoms as compared with 56% of ground-grown seedlings.

The formation of oospores, which are the fungal sexual reproductive spores, as well as the overwintering structure of *P. sojae*, was assessed in the roots from the day 7 harvest. Oospores were found along the epidermis as well as in the cortex of the root. Most oospores were located in the elongation and root hair zones (Fig. 4c). More oospores were observed in spaceflight-grown seedlings (7±4 per 2 $mm^2$) than in the ground controls (3±1 per 2 $mm^2$).

The extent of ramification of fungal hyphae and formation of fungal structures (haustoria, oospores) in soybean tissues were quantified by determining the percentage of soybean cells with associated fungal fragments using light microscopy (Table 1). Fungal colonization of roots was evaluated using the day 6 harvested roots because this tissue was less macerated than the day 7 harvest and therefore, easier to section. Because some tissues were lost during sampling, only two to three of the four replications were evaluated.

In contrast to colonization in unit gravity, which occurred predominantly in the root hair zone, infection of the spaceflight-grown soybean seedlings was observed in most root zones, including the apex, meristem, zone of elongation and root hair zone. No colonization of the lateral root zone was observed in both gravity treatments. More haustoria and intercellular hyphae were observed in spaceflight- than ground-grown tissues (Table 1).

Based on examination by electron microscopy, no detectable difference in the appearance of the fungal structures in tissues grown in spaceflight compared to unit gravity was observed (Fig. 3A, B). Intercellular hyphae and fungal haustoria appeared identical from spaceflight- and ground-grown tissues. The formation of haustoria in the susceptible interaction is evidence of establishment of fungal parasitism.

*Effects of spaceflight on plant growth and development*

In both the flight experiment and ground controls, we obtained 100% seed germination and less than 2% of the uninoculated seedlings exhibited microbial contamination. Photographs taken at all three harvest dates were used to monitor growth during the *SOYPAT* experiment as well as to determine the effects of growth in ground and spaceflight conditions on disease development (Fig. 1B).

Upon measurement of the day 7 soybean roots, differences between locations in tissue growth and development were observed (Table 2). Although no significant differences in root lengths were seen in both the uninoculated and inoculated tissues between gravity treatments, a significantly higher number of lateral roots were present in the ground-grown uninoculated soybean roots than in the spaceflight-grown tissues. Inoculated tissues demonstrated no difference in lateral root numbers between gravity treatments.

**Table 2** Effects of spaceflight and inoculation with *P. sojae* on soybean root length and the number of lateral roots

| | Treatment and environment [a] | | | |
|---|---|---|---|---|
| | Uninoculated | | *Phytophthora sojae* inoculated | |
| | Spaceflight | Ground | Spaceflight | Ground |
| Root length (cm) | 9.3±1.4 (a) | 9.8±0.8 (a) | 8.3±0.8 (b) | 5.7±0.9 (b) |
| Number of lateral roots | 8±2 (a) | 17±1 (b) | 3±2 (c) | 1±0 (c) |

[a] LS means was conducted on data by rows using four replications with 3–4 plants per replication. Letters in parentheses indicate differences observed at $p = 0.05$.

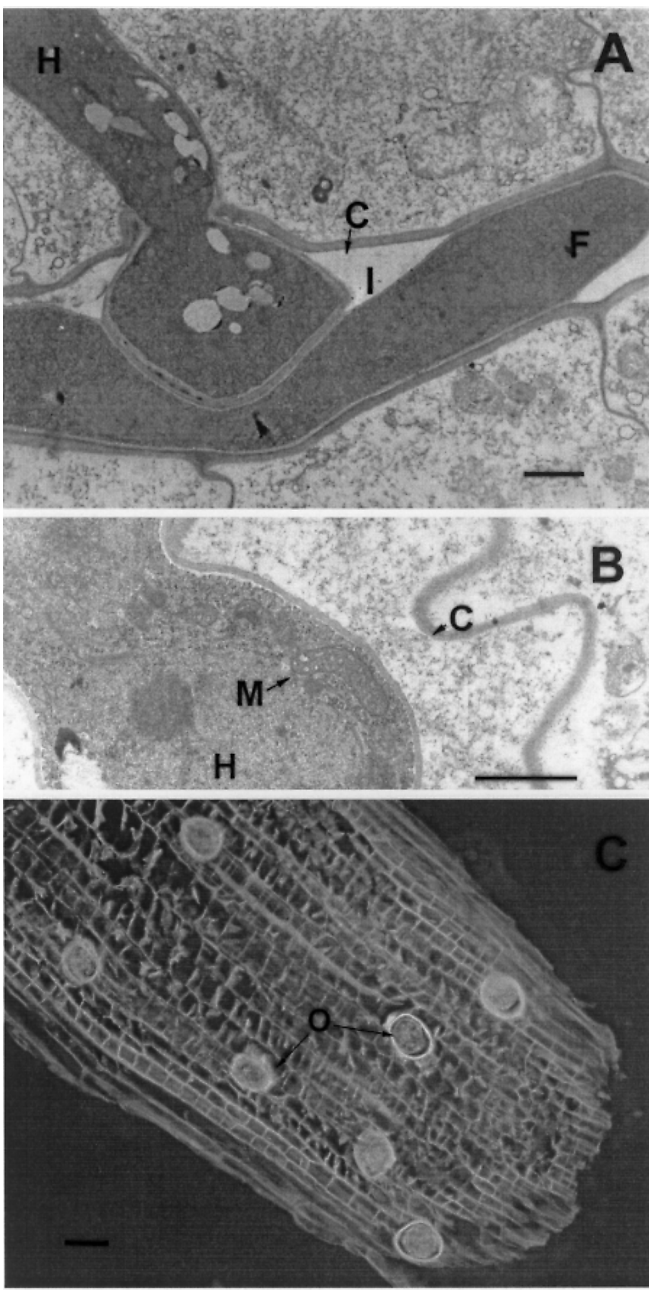


**Fig. 3** Micrographs of fungal structures located inside infected soybean root tissue during spaceflight. Sections (2.5 μm) of epon-embedded soybean (A and B) were stained with 2% uranyl acetate in 25% ethyl alcohol followed by Reynold's lead citrate, and analyzed with a Philips CM-100 transmission electron microscope. Bar = 2 μm. (C) Sections (10 μm) embedded in paraffin were stained with aniline blue and viewed under a blue light epifluorescence microscope (Zeiss standard microscope with IV FL condensor). Bar = 20 μm. C, Plant cell wall; F, fungal hypha; H, haustorium; I, intercellular space; M, mitochondria; O, oospores.

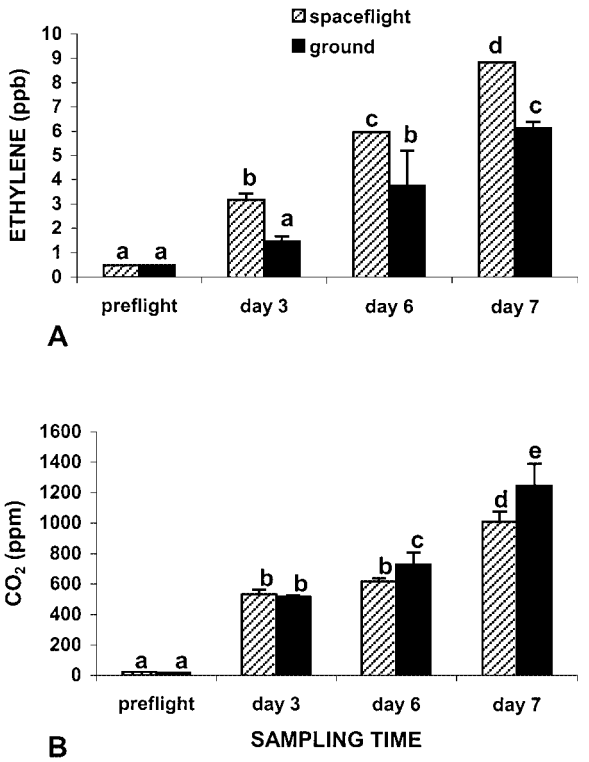


**Fig. 4** Gas sampling. Ethylene (A) and $CO_2$ (B) levels from each BRIC at preflight and at each harvest. Mean spaceflight ethylene levels were higher than ground controls by day 3. These levels continued to increase throughout the experiment to day 7 in both locations, but were always higher in spaceflight. Mean $CO_2$ levels increased in both locations by day 3 at the same level. By day 6, $CO_2$ levels in the ground BRICs had risen to a significantly higher level than those in spaceflight and remained higher at day 7. (Error bars represent the SE; different letters indicate significant differences at $p = 0.05$).

Gas samples were collected from two of the four BRICs at preflight and immediately preceding subsequent harvest times. Gases were kept in gas-tight syringes until post-landing analysis on Earth. Spaceflight ethylene levels were higher than ground controls by day 3. These levels continued to increase throughout the experiment to day 7 in both gravity treatments, but were always higher in spaceflight (Fig. 4A). In comparison, $CO_2$ levels increased in both unit gravity and microgravity by day 3 to the same level. By day 6, $CO_2$ levels in the ground BRICs had risen to a significantly higher level than those in spaceflight (Fig. 4B).

The *SOYPAT* experiment demonstrated that soybean plants grown in spaceflight are more susceptible to invasion by a fungal pathogen than plants grown on the ground. Roots inoculated with *P. sojae* exhibited more disease symptoms (brown discoloration and tissue maceration) when grown in spaceflight than at 1×*g*. Colonization of roots by *P. sojae* was observed to be more extensive in spaceflight-grown tissues than in ground-grown tissues because more fungal structures

were found associated with plant cells (Table 1) and the area of the root colonized by the fungus was greater in spaceflight samples. For example, more fungal structures were observed in the stele (vascular tissue) of spaceflight-grown roots, indicating the fungus had penetrated deeper into the tissues than in the ground-grown tissues.

The findings from this experiment raise the important question of 'Why are the spaceflight-grown soybean roots more susceptible to pathogenic colonization?'. There are several possibilities for these results. Alterations have been observed in plant cell wall structure, including reduced cellulose and lignin content when grown in microgravity (Cowles et al. 1989, Nedukha 1996). Thinner walls may be less able to withstand pathogen attack. Starch content in space grown plants is reduced relative to ground controls (for a review see Brown et al. 1995), indicating there are more soluble sugars in space tissue. *Phytophthora sojae* penetrates directly through the epidermal cell layers of the root tissue and then colonizes parenchyma cells, thus structural changes in the cell wall and sugar content of these tissues in microgravity may allow for more rapid and extensive parasitism by this pathogen. Another possibility is that spaceflight may affect growth of the fungal pathogen. Although our experimental design did not allow calculation of the rate of growth of the fungus, we observed no detectable morphological differences in the fungal strain between spaceflight or unit gravity conditions, based on analysis by TEM (Fig. 3).

Elevated levels of both ethylene and $CO_2$ have been shown to influence plant growth (Krenzer and Moss 1975, Sionit et al. 1980, Mortensen 1987, Levine et al. 1990, Merkys 1990, Gallegos et al. 1995, Hilaire et al. 1996) and elevated levels of ethylene have been demonstrated to enhance disease development (Abeles et al. 1992). Hilaire et al. (1996) found that root lengths of soybean seedlings grown inside the BRIC hardware on a clinostat increased 2-fold over those in stationary BRICs and that this increase was correlated with doubled ethylene levels inside the BRIC. Increases in $CO_2$ concentrations up to 1,200 ppm (0.12%) in air have been reported to result in enhanced growth of plants on earth (Krenzer and Moss 1975, Sionit et al. 1980, Mortensen 1987, Merkys 1990). Closed systems (glasshouses or growth chambers) can easily exceed these concentrations. Reuveni and Bugbee (1997) looked at the effects of six $CO_2$ concentrations on plant growth in two cultivars of wheat. They found that levels up to 1,000 ppm increased seed yield, vegetative biomass and number of seeds per head but concentrations from 1,000 to 10,000 ppm had a decreasing effect on these characteristics (that is, with increasing concentrations of $CO_2$, the measured traits did not differ from those observed at ambient $CO_2$ concentrations).

Visser et al. (1997) measured the effect of $CO_2$ concentration in flood-stressed plants on root elongation, and did not see any effect on root elongation either at concentrations of up to 10% of ambient air or in the complete absence of $CO_2$. In the *SOYPAT* experiment, $CO_2$ levels reached 1,300 ppm and ethylene levels reached 6 ppb inside BRIC's on the ground compared to 1,000 ppm and 9 ppb, respectively, inside BRIC's during spaceflight. We did not observe significant differences in soybean root or seedling lengths between locations. It is likely, given the findings of Reuveni and Bugbee (1997), that the $CO_2$ concentrations, though lower in spaceflight than on the ground, were not sufficiently different to have an effect on plant growth.

Hilaire et al. (1996) saw differences in soybean growth between clinostat and stationary-grown plants beginning at 2 d after planting, and attributed these differences partially to increased ethylene levels, which were 25% higher in the clinostat environment at that time point than stationary grown plants. At the initiation of their experiment, the ethylene levels were 8 ppb and the $CO_2$ levels were 1,200 ppm. They maintained a temperature of 20°C throughout their experiment, whereas the average temperature of *SOYPAT* was 25°C. At the end of *SOYPAT*, we observed that ethylene levels were 25% higher in BRICs with spaceflight than ground-grown plants, but we did not observe significant differences in root or shoot length. Had the *SOYPAT* experiment been extended with the plants exposed to these elevated levels for a longer duration, we may have observed a difference in growth.

Microgravity could be responsible for the increased levels of ethylene observed in the spaceflight location. Stress ethylene is produced by injured plant cells and among other effects, enhanced disease development is a characteristic that plants demonstrate in response to stress ethylene (Abeles et al. 1992). We observed more haustoria and intercellular hyphae in spaceflight- than ground-grown tissues (Table 1). Therefore, we speculate that the increased ethylene contributed to the increased pathogen colonization seen in inoculated spaceflight plants.

Significantly fewer lateral roots were observed in uninoculated spaceflight-grown than ground-grown tissues in *SOYPAT* (Table 2). Visser et al. (1997) looked at the effects of stress ethylene produced by plants under flooding conditions. Even though ethylene levels were much lower (0.5 ppb) than those measured in the spaceflight treatment (9 ppb), primary lateral roots of *Rumex thyrsiflorus* and *Rumex palustris* exposed to 0.5–1.5 ppb of ethylene were shorter than those grown in non-stress ethylene-inducing conditions. The results from this report suggest that ethylene may have contributed to the reduced number of lateral roots that were observed on uninoculated soybean seedling roots in spaceflight in the *SOYPAT* experiment. It is interesting that no significant difference in root length, number of lateral roots or disease symptom expression was observed between the diseased plants in both locations; the only significant differences were observed between uninoculated plants at the two locations. It is possible that the high $CO_2$ and high ethylene levels moderated the root shortening effect of the fungus in spaceflight, so that the lengths were the same as in the inoculated ground control.

Effects of ethylene on the fungus are unknown. Along with plants, fungi also produce ethylene under conditions of stress (Ilag and Curtis 1968). However, increased ethylene present during fungal infection of plants is generally attributed to the plant (Abeles et al. 1992). We can only conclude that *P. sojae* was able to cause disease on the soybean host in the presence of ethylene levels that approached 9 ppb. Chalutz and DeVay (1969) observed that at ethylene levels of 9.2 ppm, *Ceratocystis fimbriata*, the causal agent of fungal black rot of potato, was not affected inside the host. This amount is 1,000-fold more than the ethylene content in the *SOYPAT* BRIC. Not only did *P. sojae* cause disease in both gravity treatments under increasingly elevated levels of $CO_2$ and ethylene, but the pathogen also spread further into the spaceflight tissues. This observation supports the suggestion that high ethylene levels may not negatively affect fungal growth but do have severe impact on plant performance.

There were several limitations to the *SOYPAT* experiment: although conditions between ground and spaceflight were kept as similar as possible, physical conditions that we did not measure between the two environments may have varied. One way to avoid this problem would be to run $1\times g$ treatments in a centrifuge on board the space station. Unfortunately, since the experiment could not be repeated over time, we could not confirm the generality of our conclusions.

The fact that plants are more susceptible to invasion by a pathogen in spaceflight conditions is intriguing and alarming. The *SOYPAT* experiment was a first step in addressing this important issue. If we are to sustain life on a space station or in spaceflight for long-term space exploration, healthy food production is essential. Relative to our understandings of plant growth, development and metabolism in unit gravity, our knowledge of these processes in microgravity is in its infancy. For example, it is not yet known whether higher plants, which have evolved in a unit gravity-imposed environment, can pass normally through all phases of development, tissue differentiation and reproduction through consecutive generations in microgravity (Krikorian and Levine 1991). But even more limited is our understanding of how plants interact with other organisms in microgravity, and as a result, we have no plans for how to control plant diseases in this exotic environment. Future research in microgravity is needed to evaluate the real consequences of these findings.

## Acknowledgements

We wish to thank the crew of the STS-87 mission (November 19 to December 5, 1997) and especially payload specialist Colonel Leonid Kadeniuk and Dr. Yaroslav Pustovyi for their help in conducting the flight and ground experiments, respectively. Our thanks are also extended to the mission support personnel at KSC and to Marla Pyle for technical help. We would also like to thank Dr. A. Schmitthenner for providing the soybean seeds and *P. sojae* cultures and Dr. A. Schuerger for helpful discussions and technical advice. This work was supported by NASA grants NAG10–0142, NAGW-2328, the Kansas NASA EPSCoR program and the Kansas State University Agricultural Experiment Station.

## References

Abeles, F.B., Morgan, P.W. and Saltveit, M.E., Jr. (1992) Stress ethylene. *In* Ethylene in Plant Biology, 2nd edition. Academic Press, San Diego, CA, U.S.A.

Bishop, D.L., Levine, H.G., Kropp, B.R. and Anderson, A.J. (1997) Seedborne fungal contamination: consequences in space-grown wheat. *Phytopathology* 87: 1125–1133.

Brockett, R.M., Ferguson, Z.J.K. and Henney, M.R. (1978) Prevalence of fungi during Skylab missions. *Appl. Environ. Microbiol.* 36: 243–246.

Brown, C.S., Hilaire, E., Guikema, J.A., Piastuch, W.C., Johnson, C.F., Stryjewski, E., Peterson, B.V. and Vordermark, D.S. (1995) Metabolism, ultrastructure and growth of soybean seedlings in microgravity: results from the BRIC-01 and BRIC-03 experiments. *ASGSB Bull.* 9: 93.

Brown, C.S. and Huber, S.C. (1987) Photosynthesis, reserve mobilization and enzymes of sucrose metabolism in soybean (*Glycine max*) cotyledons. *Physiol. Plant.* 70: 537–543.

Brown, C.S. and Huber, S.C. (1988) Reserve mobilization and starch formation in soybean (*Glycine max*) cotyledons in relation to seedling growth. *Physiol. Plant.* 72: 518–524.

Brown, C.S. and Piastuch, W.C. (1994) Starch metabolism in germinating soybean cotyledons is sensitive to clinorotation and centrifugation. *Plant Cell Env.* 17: 341–344.

Chalutz, E. and DeVay, J.E. (1969) Production of ethylene in vitro and in vivo by *Ceratocystis fimbriata* in relation to disease development. *Phytopathology* 59: 750–755.

Cowles, J.R., LeMay, R., Jahns, G., Scheld, W.H. and Peterson, C. (1989) Lignification in young plant seedlings grown on earth and aboard the space shuttle. *In* Plant Cell Wall Polymers: Biogenesis & Biodegradation. Edited by Lewis, N.G. and Paice, M.G. pp. 203–213. American Chemical Society, Washington, DC, U.S.A.

Enkerli, K., Hahn, M.G. and Mims, C.W. (1997) Ultrastructure of compatible and incompatible interactions of soybean roots infected with the plant pathogenic oomycete *Phytophthora sojae*. *Can. J. Bot.* 75: 1493–1508.

Eschrich, W. and Currier, H.B. (1964) Identification of callose by its diachrome and fluorochrome reactions. *Stain Tech.* 39: 303–307.

Gallegos, G.L., Hilaire, E., Peterson, B.V., Brown, C.S. and Guikema, J.A. (1995) Effects of microgravity and clinorotation on stress ethylene production in two starchless mutants of *Arabidopsis thaliana*. *J. Gravi. Physiol.* 2: 153–154.

Graham, T.L. and Graham, M.Y. (1999) Role of hypersensitive cell death in conditioning elicitation competency and defense potentiation. *Plant Physiol.* 105: 571–578.

Hilaire, E., Peterson, B., Guikema, J. and Brown, C. (1996) Clinorotation affects morphology and ethylene production in soybean seedlings. *Plant Cell Physiol.* 37: 929–934.

Ilag, L. and Curtis, R. (1968) Production of ethylene by fungi. *Science* 159: 1357–1358.

Keen, N.T. and Yoshikawa, M. (1983) Physiology of disease and nature of resistance to *Phytophthora*. *In Phytophthora*: Its Biology, Taxonomy, Ecology and Pathology. Edited by Erwin, D.C., Bartnicki-Garcia, S. and Tsao, P.H. pp. 279–287. APS Press, St. Paul, MN, U.S.A.

Krenzer, E.G., Jr. and Moss, D.N. (1975) Carbon dioxide enrichment effect upon yield and yield components in wheat. *Crop Sci.* 15: 71–74.

Krikorian, A.D. and Levine, H.G. (1991) Development and growth in space. *In* Plant Physiology: a Treatise, Vol. 10. Edited by Steward, F.C. and Bidwell, R.G. pp. 491–555. Academic Press, New York, NY, U.S.A.

Kuznetsov, O.A., Brown, C.S., Salwo, M.M. and Hasenstein, K.H. (1997) Magnetograviphoretic studies show differences in starch metabolism of spaceflown soybean. *ASGSB Bull.* 11: 24.

Levine, H.G., Kann, R.P. and Krikorian, A.D. (1990) Plant development in space: observations on root formation and growth. *In* Fourth European Symposium on Life Sciences Research in Space. Edited by David, V. pp. 503–508. ESA Publ. Div. 307. Paris, France.

Merkys, A. (1990) Plant growth under microgravity conditions: experiments and problems. *In* Proceedings of the Fourth European Symposium on Life Sciences Research in Space. Edited by David, V. pp. 509–515. ESA Publ.

Div. 307. Paris, France.
Mortensen, L.M. (1987) Review: $CO_2$ enrichment in greenhouses, crop responses. *Scienta Horticul.* 33: 1–25.
Nedukha, E.M. (1996) Possible mechanisms of plant cell wall changes at microgravity. *Adv. Space Res.* 17: 37–45.
Nelson, B. (1987) The role of plant pathology in development of controlled ecological life support systems. *Plant Dis.* 71: 580–584.
Reuveni, J. and Bugbee, B. (1997) Very high $CO_2$ reduces photosynthesis, dark respiration and yield in wheat. *Ann. Bot.* 80: 539–546.
Schmitthenner, A.F. (1985) Problems and progress in control of Phytophthora root rot of soybean. *Plant Dis.* 69: 362–368.
Schmitthenner, A.F. (1989) Phytophthora rot. *In* Compendium of Soybean Diseases, 3rd edition. Edited by Sinclair, J.B. and Beckman, P.A. pp. 35–38. APS Press, Minneapolis, MN, U.S.A.
Schmitthenner, A.F., Hobe, M. and Bhat, R.G. (1994) *Phytophora sojae* races in Ohio over a 10-year interval. *Plant Dis.* 78: 269–276.
Sionit, N., Hellmers, H. and Strain, B. (1980) Growth and yield of wheat under $CO_2$-enrichment and water stress. *Crop Sci.* 20: 687–690.
Taylor, G.R. (1974) Space microbiology. *Annu. Rev. Microbiol.* 28: 121–137.
Taylor, G.R., Henney, M.R. and Ellis, W.L. (1973) Changes in the fungal autoflora of Apollo astronauts. *Appl. Microbiol.* 26: 804–813.
Visser, E., Nabben, R., Blom, C. and Voesenek, L. (1997) Elongation by primary lateral roots and adventitious roots during conditions of hypoxia and high ethylene concentrations. *Plant Cell Environ.* 20: 647–653.

(Received January 23, 2001; Accepted April 3, 2001)