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# The Biophysical Limitations in Physiological Transport and Exchange in Plants Grown in Microgravity

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## Abstract

This paper describes how changes in the physical behavior of fluids and gases in microgravity can limit the physiological transport and exchange in higher plants. These types of effects are termed indirect effects of microgravity because they are not due to gravity interacting with the mass of the plant body itself. The impact of limiting gravity-dependent transport phenomena has been analyzed by the use of mathematical modeling to simulate and compare biophysical transport in the 1*g* and spaceflight environments. These data clearly show that the microgravity environment induces significant limitations on basic physiological and biochemical processes within the aerial and rootzone portions of the plant. Furthermore, this mathematical model provides a solid foundation for explaining the physiological effects that have been noted in past spaceflight experiments.

**Key words:** Biophysics; Convection; Diffusion, Gas exchange; microgravity; Photosynthesis; Reproduction; Rootzone; hypoxia; Transpiration

## Introduction

How is life on earth dependent upon gravity? That is the question that research in space life sciences has been trying to answer for almost five decades. There are now two main areas of concentrations in this field. The first and most intensely studied area focuses on direct mechanical force and gravity sensing effects that are related to the mass of the organelle, cell, tissue, organ, or whole organism in the gravitational field. These types of effects are direct effects of gravity and are manifest by gravitropism, gravimorphogenesis, and direct mechanical loading in both plants and animals. The second area of weightlessness effects has been emphasized in laboratory and flight experimentation only over the last 10–15 years. This field studies how gravity affects the basic physical phenomena of all matter and how that, in turn, might affect a biological system. These indirect effects of microgravity emphasize the interactions between the physiology of the organism and the gravity-dependent behavior of matter that the organism interacts with.

Exchange (flux) of ions, gases, and water between plant tissues and the environment are partially dependent upon the physical behavior or matter outside of the plant tissues. Diffusion is based on the random Brownian motion of molecules and is not

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dependent upon gravity. Unless there is some induced flow or movement in the gases or fluids that surround the plant tissue, many aspects of mass transport and exchange between the plant and the environment can become diffusion limited. In the presence of gravity, buoyancy-driven thermal convection (BDTC) induces the flow of gaseous or fluid media around the plant, which physically reduces the size of the diffusion-limited boundary layer. There are two different sources or types of BDTC to consider and these have been termed free and forced convection (Nobel 1991). Free convection is the result of a temperature differential between the plant tissue and the outside environment. A temperature differential can result from the absorption of visible and infrared light by the leaves, or by metabolism in the roots. The air or water in contact with the tissue would be heated by conduction and that heated gas or fluid would become less dense than the surrounding media. The heated mass of less dense gas would then be subject to buoyancy driven upward movement, in the presence of gravity. In this manuscript, this will be termed "auto-convection" because it is driven by a temperature differential between the organism and the environment. Forced convection results from temperature differentials within the environment and will be referred to as "system convection" in this manuscript. On earth, the most significant form of system convection is wind.

What is the impact on plant growth when gravity is not present and all transport and exchange are diffusion limited? Perhaps we can learn something from the work conducted by physicists using a very simple system, a burning candle. In a 1g environment, buoyancy-driven thermal convection directs air movement around a burning flame (Figures 1A and 1B). When compared to a flame in microgravity (Figure 1C), it is obvious that the shape of the flame is actually sculpted by this gravity-dependent physical process. Without gravity-driven convective transport, the spherical flame will continue to burn for only 30–40 seconds (Ross and others 1991), since the availability of oxygen in this diffusion-limited environment is below that required for the process of combustion to continue. Similar limitations in mass transport and exchange would be expected to occur with biological systems in microgravity because we know that most, if not all, net biological transport and exchange exceed that which can be achieved by slow diffusion.

Plants, unlike animals, do not have mechanical systems to assist in gas exchange and fluid transport. In the aerial portions of a growing plant, diffusion-limited transport and exchange can affect transpiration, photosynthetic gas exchange, and $O_2$ metabolism during plant reproduction, and heat dissipation during photosynthesis. Similarly, in the rootzone, we could expect problems with metabolic gas exchange, mineral nutrient transport, and rhizosphere root exudation, as well as metabolic heat dissipation. The significance of diffusion limitations in microgravity should not be underestimated. For example, it is well known that protein crystallization is greatly enhanced in microgravity and this is partially due to the lack of gravity-dependent buoyancy-driven thermal convection in aqueous systems. Some calculations predict that, in aqueous solutions, convection is $10^6$ times more effective as a means of gas transport than diffusion (Denny 1993). The impact of diffusion limitations would be expected to be even higher in an aerial environment as the thermal conductivity and viscosity of the media are much lower.

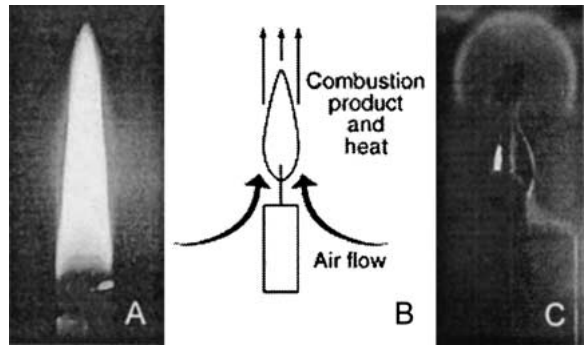


**Figure 1.** Image (**A**) and schematic depiction (**B**) of a simple candle flame in a 1*g* environment as compared with an image of a candle flame in microgravity (**C**) during the USML-1 experiment (Ross and others 1991). In microgravity, the shape of the flame is spherical due to the inhibition of BDTC directed airflow and has a much lower temperature. On average, such a flame will burn 30–40 seconds before going out due to the fact that oxygen transport is limited to diffusionary levels and this is not sufficient to maintain combustion.

## Gravity-Dependent Biophysical Flux In Aerial Plant Tissues

The inhibition of BDTC airflow in microgravity would potentially affect all basic physiological processes in the aerial tissue that are dependent upon gas exchange with the environment. These include transpiration, photosynthesis ($CO_2$ uptake/$O_2$ release), and respiration in sink tissues. The question is, what is the theoretical impact of this inhibition on the aerial parts of the plant, and does this explain the general problems of plant cultivation in mi-

crogravity? Here we do not need to consider auto-convection because system convection is much more important in moderating the size of the diffusional boundary layer. Even at very low wind speeds of 0.1 m $sec^{-1}$, system convection dominates auto-convection (Nobel 1991). System convection can, at best, be described as "chaotic." The best models of convection in large systems that have been produced involve equations of fluid dynamics that are only solvable using super computers. It is not possible here, or even necessary to directly model system convection within a plant growth chamber, as we can model the relationship between the velocity of convective airflow and the thickness of the unstirred diffusional boundary layer that ultimately limits all exchange processes. At the surface of the plant, an unstirred diffusion-limited boundary layer is present which results from shear stress between the surface and the air (for more information refer to Grace 1977; Schlichting 1979). These boundary layers are stable even in significant airflow. As we move from the bulk air, through the boundary layer to the leaf surface, the flow of air decreases to near zero. Because this boundary layer region is a gradient of varying levels of chaotic airflow, it is difficult to model. We can, however, calculate the effective boundary layer thickness ($BL_e$), given the leaf length in the direction of airflow and the velocity of airflow around the leaf surface. This is done using hydrodynamic theory that explains laminar flow adjacent to a flat surface. The theory was modified (Pearlman and others 1972) for application to plant biophysics and to better explain actual field data. The effective boundary layer (mm) at the leaf surface can be calculated using the following formula:

$$BL_e = 4.0\sqrt{1/v} \tag{1}$$

where $BL_e$ = boundary layer thickness (mm), 1 is the average leaf length in the direction of airflow (m), and $v$ is the velocity of airflow (m $sec^{-1}$). This formula predicts that the effective boundary layer will increase with leaf size. The factor 4.0 is the displacement coefficient and is in the terms of mm $sec^{-1/2}$. This value does diverge from hydrodynamic theory, which would predict a number closer to 6.0, but wind tunnel experiments conducted by Gates (1980) showed that a number closer to 4.0 is better and accounts for the general size and shape of leaves. Similarly, we can calculate the effective boundary layer thickness around a cylindrical structure (Nobel 1974) using the formula:

$$BL_e = 5.8\sqrt{d/v} \tag{2}$$

where $d$ is the diameter of the cylindrical structure (m).

The $BL_e$ represents the thickness of the rate-limiting diffusional transport boundary that will moderate the movement of $CO_2$ into the leaf, as well as the movement of $O_2$ and water out of the leaf. Diffusional transport can be calculated using Fick's law. For the flat planar leaf, the formula is:

$$J = D\frac{\Delta C}{\Delta x} \tag{3}$$

where $J$ = flux (moles $cm^{-2}$ $sec^{-1}$), $D$ = the diffusion coefficient for the particular species ($cm^2$ $sec^{-1}$), $\Delta C$ is the concentration differential across the boundary layer (moles $ml^{-1}$), and $\Delta x$ is the size of the diffusional layer (cm), or in this case, the $BL_e$. If we substitute the formula for $BL_e$ into the diffusion equation for $\Delta x$, then we can calculate the flux of gases into and out of the leaf surface boundary layer, given the amount of BDTC mediated system convection using the equation

$$J = D\frac{\Delta C}{4.0\sqrt{1/v}} \tag{4}$$

For a cylindrical structure, flux per unit length can be calculated using a modification of the Fick equation (Crank 1975):

$$J = \frac{2\pi D\Delta C}{In(R_1/R_2)} \tag{5}$$

where $R_1$ is the radius of a cylindrical structure such as the seed pod or silique, and $R_2$ is the distance from the outside edge of the $BL_e$ to the geometric center. If we combine Formulas 2 and 5 we get:

$$J = \frac{2\pi D\Delta C}{In(r/(r + 5.8\sqrt{d/v})} \tag{6}$$

where $r$ is the radius of the cylindrical structure.

The formulas (equations 1 and 2) have been used to illustrate the relationship between airflow speed and the effective boundary layer thickness (Figure 2) for both a small leaf (average length 1 cm in direction of airflow) and a cylindrical structure (average diameter of 2 mm). The local airflow velocity (wind speed) at the tissue surface is a function of the amount of system convection. We can relate the $BL_e$ values to the calculation of $CO_2$ flux into a small leaf (Figure 3) and $O_2$ flux into a cylindrical fruit (Figure 4) using formulas 4 and 6, respectively. To relate these $BL_e$ limited flux values to normal physiological conditions, we must have some estimates of the airflow levels that terrestrial plants are typically exposed to. Annual average airflow values

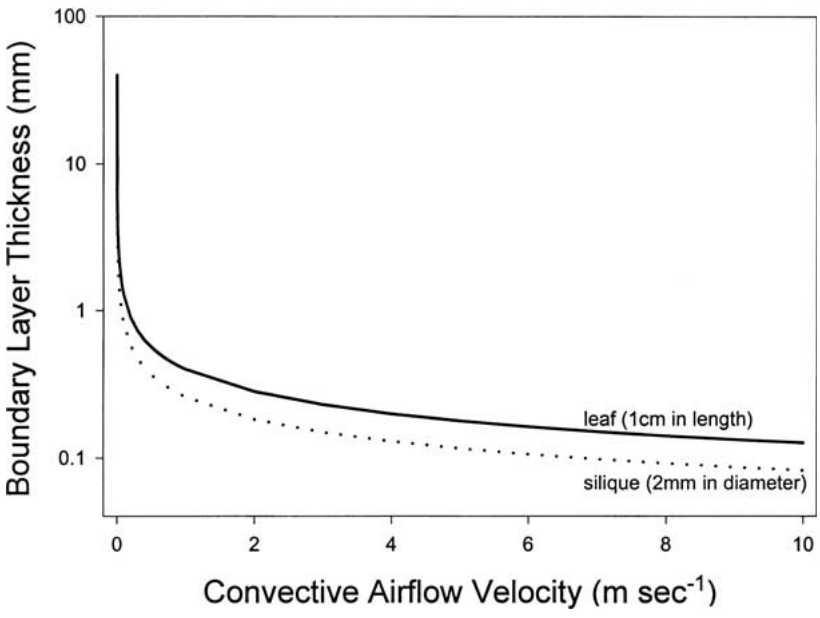


**Figure 2.** Plot showing the relationship between the thickness of the effective boundary layer ($BL_e$) and the velocity of the convective airflow for a flat planar leaf and a cylindrical structure like a stem or a fruit. These models were generated using equations 1 (Pearlman and others 1972; Gates 1980) and 2 (Nobel 1974), respectively. The thickness of this layer defines the diffusional boundary that limits the exchange of gasses between the plant tissue and the environment. This includes the influx of $CO_2$ and efflux of $O_2$ and $H_2O$ from photosynthetically active leaves and $O_2$ influx into sink tissues such as the reproductive silique. Note that the model predicts that the $BL_e$ will become larger as the size of the leaf or cylinder increases.

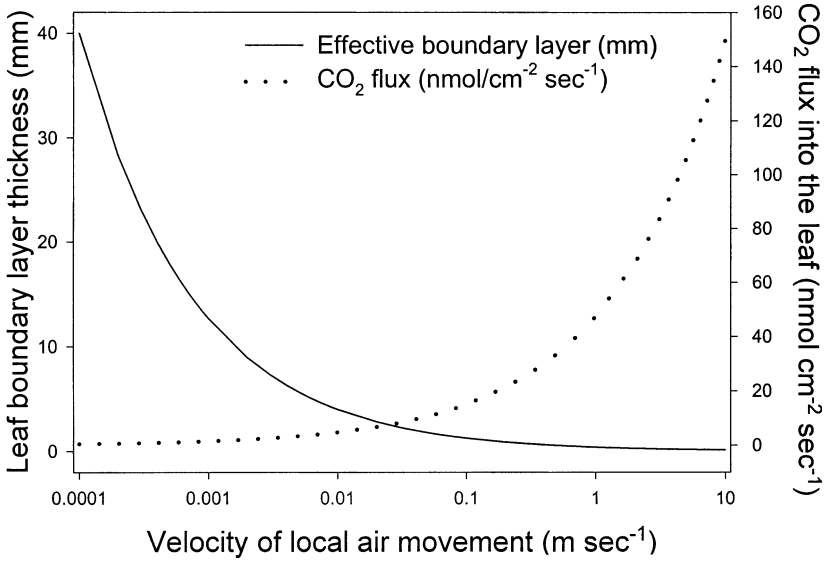


**Figure 3.** Plot depicting the effect that the changes in the effective boundary layer ($BL_e$) have on $CO_2$ flux into a 1 $cm^2$ leaf. This was calculated using a modified version of the Fick equation (equation 4) that accounts for the $BL_e$. For this model, the flux was presumed to be across the abaxial stomata-containing surface only and the stomata were assumed to be fully open. The diffusion coefficient for $CO_2$ in air at 1 atmosphere of pressure at 25°C is 0.16 $cm^2$ $sec^{-1}$ (Lide 2000). The differential $CO_2$ concentration across the boundary layer was calculated based on atmospheric $CO_2$ being 360 ppm and $CO_2$ inside the leaf being at 70 ppm, which is the median $CO_2$ compensation point for a C3 plant (Nobel 1992). Note that the model predicts that the flux will decrease as the leaf size increases.

vary based on geography and range from 7 m $sec^{-1}$ in coastal regions to 1 m $sec^{-1}$ in topologically flat inland areas (Grace 1977). Endogenous movements of the plant organ are very small compared with wind. If a leaf or stem were to move as much as a meter over a period of 1 day that would only translate into a wind velocity of approximately 11 μm $sec^{-1}$. Therefore, we can conclude that endogenous plant movements do not contribute significantly to modification of the $BL_e$ when compared with wind. Plants grown in environmental plant growth chambers, with airflow below 0.5 m $sec^{-1}$, typically exhibit morphological signs of poor health (Jaffe 1980). While many of these morphological malformations have been attributed to hormonal signals associated with thigmotropism (Jaffe 1985), we should not ignore the biophysical limitations in physiological transport that would result from the size of the diffusion-limited boundary layer when the airflow speed is 0.5 m $sec^{-1}$ or less.

Both equations 1 and 2 demonstrate that the thickness of the $BL_e$ is inversely proportional to the square root of the airflow velocity. Therefore, if we decrease the airflow velocity by a factor of 2 then the $BL_e$ would be expected to increase by a factor of 1.4. Such an increase in the thickness of the $BL_e$ represents a significant limitation to biophysical transport into and out of the leaf. For $CO_2$ transport,

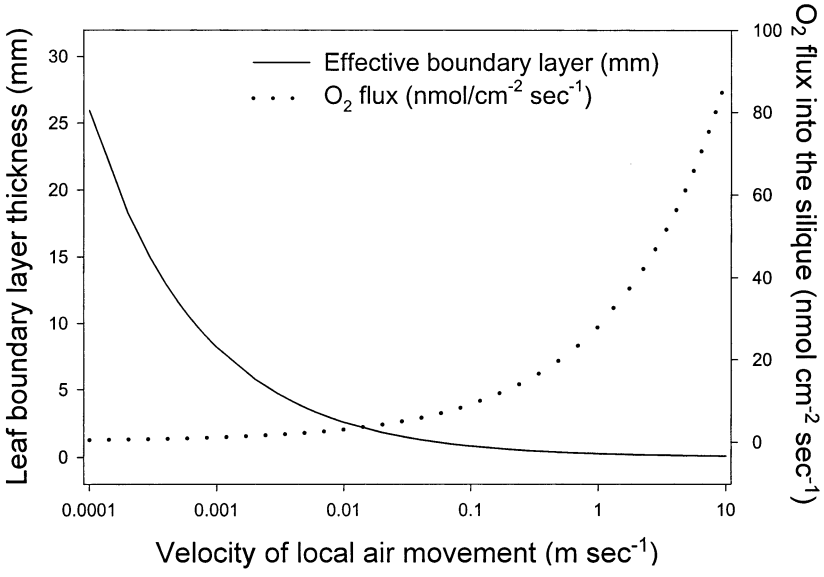


**Figure 4.** Plot depicting the effect that the change in the effective boundary layer ($BL_e$) has on $O_2$ flux into a small fruit. The size of this model fruit (diameter=1 mm, length = 1 cm) was chosen to simulate an *Arabidopsis* silique, as it has been the most commonly studied plant species used for spaceflight reproduction experiments. The values were calculated using a modified version of the Fick equation (Equation 6) that accounts for the $BL_e$. The diffusion coefficient for $O_2$ in air at 1 atmosphere of pressure at 25°C is 2.087 $cm^2$ $sec^{-1}$ (Lide 2000). The differential $O_2$ concentration across the boundary layer was calculated based on atmospheric $O_2$ being 21% and $O_2$ inside the silique being at 7.49% as was measured using an oxygen microelectrode (Porterfield and others 2000). Note that the model predicts the flux will decrease as the cylinder size increases.

the flux would decrease approximately 30%. Likewise, the flux of oxygen out of the leaf would be retarded (data not shown) and the resulting change in the $CO_2/O_2$ concentration ratio could lead to elevated levels of photorespiration in the leaf. This explains the biochemical differences in the status of plants grown in inadequate growth chambers. In a zero gravity environment, BDTC would be completely inhibited and the system convection-driven airflow velocity would be zero. Under these conditions, the $BL_e$ becomes large and transport becomes diffusion limited.

In the spaceflight environment of low earth orbit, BDTC would be inhibited in relation to the effective gravitational force. If we assume that the relationship between effective gravity and system convection is linear, then airflow would be $10^{-4}$ the airflow of a terrestrial plant. Based on this assumption, we can compare the flux of $CO_2$ into a small leaf (1 $cm^2$) in the terrestrial environment where system convection-driven airflow is 1 m $sec^{-1}$ with the spaceflight environment where the airflow would be $10^{-4}$ m $sec^{-1}$. The $CO_2$ flux values calculated for the earth-grown plants (47 nmoles $cm^{-2}$ $sec^{-1}$) are 100-fold higher than for microgravity plants (0.47 nmoles $cm^{-2}$ $sec^{-1}$). However, such a significant change in airflow is not necessary in order to have a drastic impact on biophysical flux. If BDTC airflow were decreased by only 100-fold in microgravity (that is, from 1 to 0.01 m $sec^{-1}$), then the flux of $CO_2$ into the leaf would drop down to 10% of the normal level. If we calculate the changes in oxygen flux into a cylindrical fruit under the same conditions, then we would see that the oxygen flux into the sink tissue would be 11% of the terrestrial value.

Transpiration would also be affected by the biophysical diffusion limitations described above. The relationship between the flux of water from a hypothetical leaf and convective airflow (Figure 5) was calculated using Equation 4. The relative impact of the restrictions associated with the microgravity environment is identical to that described above for the flux of $CO_2$ into the leaf. The consequences of this condition are changes in the water potential of the aerial plant tissues as well as disruption of bulk transport of ions and hormones from the roots. So, in addition to local biochemical inhibitory effects on the leaf, these biophysical limitations would exert a negative effect on the whole plant physiology by reducing the transpiration-driven xylem transport.

The biophysical considerations presented above explain much of the metabolic and developmental problems that have been documented in spaceflight experimentation (Musgrave and others 1988). Biochemical evidence of decreased levels of carbon fixation has been noted throughout the entire history of microgravity plant flight experimentation, although the connection has not always been made. Analysis of pea plants grown on the Salyut 7 space station revealed that the stunted plants almost entirely lacked any starch reserves (Abilov and others 1986; Aliyev and others 1987). Experiments on pepper plants grown aboard the Biosatellite II experiment also contained significantly less starch reserves (Johnson and Tibbitts 1968). There has been work conducted using *Arabidopsis* which also indi-

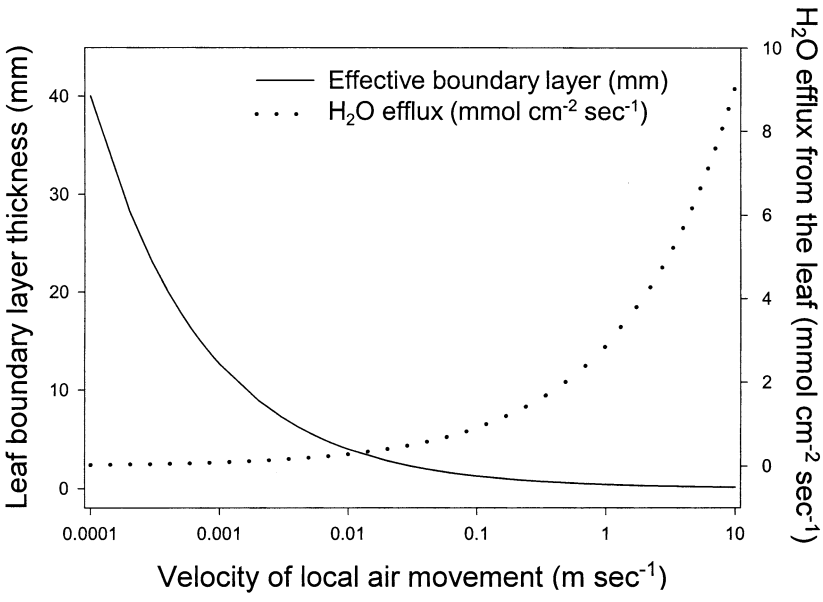


**Figure 5.** Plot depicting the effect that the change in the effective boundary layer ($BL_e$) has on $H_2O$ flux out of a 1 $cm^2$ leaf. This was calculated by a modified version of the Fick equation (Equation 4) that accounts for the $BL_e$. For this model, the flux was presumed to be across the abaxial stomata-containing surface only and the stomata were assumed to be fully open. The diffusion coefficient for $H_2O$ in air at 1 atmosphere of pressure at 25°C is 2.42 $cm^2$ $sec^{-1}$ (Lide 2000). The differential concentration across the boundary layer was calculated based on atmospheric $H_2O$ being at 50% relative humidity and at a temperature of 20°C. If the temperature inside the leaf is 20°C and the water potential of the mesophyll tissue is −1.0 m Pa. then the relative humidity would be 99.3% (NobeL 1992). These relative humidity values correlate with absolute water vapor concentration values of 0.48 mol $m^{-3}$ for the air outside of the $BL_e$ and 0.95 mol $m^{-3}$ on the inside of the leaf (Nobel 1992). Note that the model predicts that the flux will decrease as the leaf size increases.

cated that the starch content was lower in microgravity (Guisinger and Kiss 1999; Laurinavicius and others 1988; Musgrave and others 1998). Musgrave and others (1998) measured total foliar carbohydrates in *Arabidopsis* and found that these levels were reduced to 40% of the ground control levels. All of these experiments were conducted in hardware that lacked gas exchange and/or a mechanical convection system and, therefore, would have been dependent upon gravity-driven airflow for gas exchange.

It is unlikely that the reduction in carbon fixation that these data suggest is the result of some microgravity-induced problem with photosynthesis, as has been suggested by Tripathy and others (1996). Although they measured a general decline in photosynthetic functions in wheat, these measurements were made after the plants had been returned to earth, not in the microgravity environment. It is also important to note here that the spaceflight plants in the aforementioned study showed a general decline in overall health, as indicated by a 25% decrease in fresh weight. The reduced fresh weight could be explained by biophysical limitations in the plant growth system that lacked mechanical airflow and gas exchange. At best, these measurements of photosynthetic functions can only be considered to be a symptom of the general decline in overall plant status, and not the cause of the decrease in plant growth and carbon fixation in microgravity. This interpretation is supported by work conducted on etiolated soybean seedlings. The soybean seedling is a unique system because, during early germination, the etiolated cotyledons can fix carbon in the dark through the Calvin cycle and store it as starch (Brown and Huebner 1987, 1988). This process allows the plant to convert metabolic stores of protein and lipid into carbohydrates. Experiments conducted using this system revealed that total starch content is reduced in the microgravity-exposed plants (Brown and others 1995, 1999). Furthermore, the $CO_2$ levels in the headspace of the experimental canisters were found to be double that of ground control plants (Brown and others 1998), suggesting that biophysical transport limitations, not disrupted photosynthesis, explain the reduced growth and lower carbon reserves in microgravity.

Plant reproduction has also been an area of intense research. Attempts at supporting reproductive development in flowering plants were first done aboard the Soviet Salyut space stations (Halstead and Dutcher 1987; Musgrave and others 1997; Neichitailo and Mashinski 1993). Despite numerous attempts, it was not until the experiments on Salyut 7 that Merkys and Laurinavicius (1983) were able to report the occurrence of flowering and seed production in *Arabidopsis*. However, the seeds that were produced suffered low viability, and the plants showed significant delays in development as the life cycle of the plants were approximately double that of ground controls.

Are problems with plant reproduction due to biophysical transport limitations? This question has been answered by a series of three flight experi-

ments conducted aboard the space shuttle in the 1990's. These experiments utilized the same plant growth hardware and were conducted using *Arabidopsis* plants that had been grown to the first part of the reproductive cycle on earth. In the first experiment, the plants were flown for 6 days in microgravity in plant growth chambers that were closed and lacked mechanically induced airflow. Under spaceflight conditions, both male and female reproductive structures suffered complete failure, whereas the controls showed normal development (Kuang and others 1995). The spaceflight materials revealed significant evidence of general reproductive malfunction, such as shrunken and collapsed pistils, empty ovules, and a complete absence of viable pollen. The next experiment that was conducted used the same experimental design but the investigators sought to alleviate the hypothesized biophysical limitations in photosynthetic carbon fixation (Musgrave and others 1997, 1998) by increasing the concentration of $CO_2$ in the chamber headspace to 8000 ppm and by supplementing the root medium with sucrose. When this was done, development of reproductive structures in the spaceflight-exposed plants was normal (Kuang and others 1996a). Although the pollen and female gametophytes were determined to be viable, fertilization and reproduction still failed. The siliques contained empty shrunken ovules and the anthers, in most cases, were indehiscent. Fertilization and seed development were normal in the ground controls. The third experiment in this series utilized a hardware modification that allowed for mechanically driven convective airflow to exchange the plant chamber atmosphere with the air inside the shuttle orbiter. The use of this air exchange system allowed reproduction to proceed normally. Furthermore, the (immature) seeds were identical to those produced in ground control experiments (Musgrave and others 1997).

The most direct measurements of gravity-mediated biophysical effects in aerial tissue were made by Kitaya and others (*in press*). Using infrared thermal imaging and a chamber that included an infrared gas analyzer, heat/gas exchange between plant leaves and the ambient air were measured. In the parabolic aircraft flight experiment, the leaf temperatures and net photosynthetic rates of plant leaves were evaluated at 0.01,1.0,1.5, and 2.0 g. The leaves of the plants were illuminated during the experiment at a level of 0.5 mmol $m^{-2}$ $s^{-1}$. The measurements showed that in barley, the mean leaf temperature increased by 1°C and the net photosynthetic rate decreased by 13% during the 20-second period when the gravity levels were decreased from 1.0 to 0.01 g. The leaf temperature decreased by 0.5 °C and the net photosynthetic rate increased by 7% when the gravity levels increased from 1.0 to 2.0 *g* for 20 seconds. Similar results were reported in experiments using sweet potato leaves. These data show direct, real-time correlation between gravity and BDTC-dependent biophysical exchange between a plant leaf and the environment.

## Gravity-Dependent Biophysical Convection in the Rootzone

Bulk flow of air into and out of the soil can be induced by pressure differences between the soil air and the atmosphere. This can be driven by changes in barometric pressure and temperature gradients within the soil, as well as wind gusts. It is important to point out that all of these mechanisms are gravity dependent. Additionally, gravity drives the penetration of water down into the soil, which can induce bulk movements of soil air, as do fluctuations in the water table. The question is, to what degree will these processes contribute to maintaining soil atmosphere oxygenation? Although most early soil scientists tended to assume that diffusion was the primary mechanism, recent work has provided both direct and theoretical evidence supporting the convection model of soil air transport (Farrell and others 1966; Grable 1966; Kimball and Lemon 1971, 1972; Rolston 1986; Scotter and Raats 1968; Vomocil and Flocker 1961).

How do we know there is BDTC in soil? The answer is in two ways. First, we can ask what we would expect the concentration profile of oxygen to be in the soil if it were diffusion limited and compare that to real world data. Jaynes and others (1984) developed a one-dimensional model of what the oxygen profile would be in the soil system if transport and flux were a diffusion-limited process. This model predicts that the oxygen concentration in the soil would decrease very rapidly with increased depth. However, high oxygen concentrations can be measured far too deep inside a soil matrix (Erickson and others 1985; Jaynes and others 1983) to be explained by that model. The second way we can address this question is to use mathematical models to estimate what levels of convective flow would be possible given a particular soil and temperature gradient. Guo and others (1994) analyzed complex soil media and provided both theoretical and direct evidence showing that significant BDTC-mediated oxygen transport does occur in a soil system given a temperature gradient of only 0.1°C. Naturally, temperature gradients do exist

both horizontality and vertically within the soil due to uneven heating, evaporation at the soil surface, and geothermal processes.

Gravity-dependent movement of water will also drive gas exchange between the soil air and the atmosphere, thereby maintaining root system oxygenation. When water is introduced into a soil system by rain or irrigation, it will move downward. This movement can be modeled using Darcy's law for water flow. Likewise, it is also possible to modify this formula to model air movement through the soil. Another source of water movement in the soil that plays a significant role in mediating soil oxygen transport to the root is transpiration-dependent soil water uptake. Assuming that the $O_2$ consumption rate of a plant species is 0.2 ml $h^{-1}$ $g^{-1}$, the rooting density is 0.1 $g^{-1}$ $cm^{-3}$, the water extraction rate is 7.5 mm $day^{-1}$, and the bulk soil density is constant, then the process of soil water extraction alone can provide up to 70% of the oxygen required for the root system (Vomocil and Flocken 1960). Remember that soil water extraction is dependent upon transpiration and would, therefore, be sensitive to inhibition of water flux from the leaves in microgravity (Figure 5).

Does inhibition of gravity-mediated oxygen transport in the soil affect plant growth in microgravity? Specific aberrations induced by microgravity suggest that there are changes in metabolism associated with reduced oxygen availability. At the ultrastructural level, modifications in mitochondrial shape and size were noted by others (Kordyum 1994; Laurinavicius and others 1994; Moore 1990; Podlutsky 1992; Rasmussen and others 1992; Rubin and others 1980; Slocum and others 1984; Tairbekov and others 1980), along with a general decrease in amyloplast starch reserves (Guisinger and Kiss 1999; Kordyum 1994; Laurinavicius and others 1994; Moore 1990; Podlutsky 1992; Rasmussen and others 1992; Volkman and others 1986). Both changes in mitochondrial ultrastructure (Oliveira 1977) and decreases in tissue starch reserves (Hanson and Jacobsen 1984; Hurng and Kao 1993; Guglielminetti and others 1995) are known to indicate exposure of roots to low oxygen (hypoxic) environments.

We can monitor root system metabolism for changes in oxygenation if we know how plants deal with hypoxic stress. This has been well documented and involves the induction of an array of ''anaerobic polypeptides'' (ANPs) that includes enzymes that allow for anerobic fermentative metabolism (Drew and Stolzy 1996). Fermentation of lactate and/or ethanol is thought to contribute to cell survival in the short term by allowing the continuation of substrate level phosphorylation by the glycolytic pathway. Although fermentative metabolism can lead to lactate production, ethanol is thought to be preferred because it does not contribute to cytoplasmic acidosis (Drew l997).

Recent work has shown that spaceflight exposure induces a metabolic response in the roots of *Arabidopsis* plants (Porterfield and others 1997b) that is consistent with the hypoxia. In two separate experiments, plants were exposed to microgravity during 6 or 11 days of spaceflight. Post-flight analysis included measurement of root alcohol dehydrogenase (ADH) activity, localization, and expression. During the first experiment, ADH activity was 90% higher in the spaceflight roots as compared with ground controls. Analysis of the root materials from the second experiment, using a ribonuclease protection assay, revealed a 136% increase in ADH mRNA, associated with an 89% increase in enzyme activity in spaceflight materials. Similar responses have been observed in two other plant species. Dwarf wheat (*Triticum aestivum* L. cv. Yecora Rojo) and *Brassica rapa* L. (CrGC#1-33) were exposed to microgravity for 8 days during a shuttle spaceflight experiment (Morrow and others 1995; Porterfield and others 2000a). In both dwarf wheat and *Brassica rapa,* ADH activity increased significantly as a result of exposure to the spaceflight environment. For dwarf wheat, when compared with the ground control roots, ADH increased 3-fold based on soluble protein and 2.5-fold based on fresh weight. For *Brassica rapa,* ADH activities from spaceflight samples increased almost 6-fold based on soluble protein and 334% based on fresh weight, when compared with controls.

A more detailed analysis of the metabolism of spaceflight-exposed roots was conducted during the Collaborative Ukrainian Experiment (Stout and others 2001). In this experiment, there were two different treatments allowed for the analysis of plant responses at different stages of development. *Brassica rapa* plants, that were germinated in orbit, were between 8 and 15 days old at harvest, and another group of *Brassica* plants were pre-grown for 13 days and were actively flowering during the period of spaceflight exposure. In addition to ADH, PDC and glucose-6-phosphate dehydrogenase (G6PDH) activities were measured. In plants that were germinated in orbit, a 47% increase in ADH activity and 9% increase in PDC activity were measured, with no change in G6PDH. In plants that were 13 days old at the time of launch, a 489% and 149% increase in ADH and PDC activities were measured, respectively. Like in the younger plants, there were no significant differences in G6PDH

**Table 1.** Summary of Spaceflight Experiments that Have Quantified Hypoxic Metabolism in the Root System.

| Experiment | Plant species | Nutrient delivery technique | Observations |
|---|---|---|---|
| Chromex-03 (Porterfield and others 1997b) | *Arabidopsis thalina* (flowering) | Agar solidified gel | 90% increase in ADH activity |
| Chromex-05 (Porterfield and others 1997b) | *Arabidopsis thalina* (flowering) | Agar solidified gel | 90% increase in ADH activity, 140% increase in ADH mRNA, no change in ADH localization |
| Astroculture-05 (Porterfield and others 2000a) | *Triticum aestivum* cv Yecora Rojo (vegetative) | Zeolite and embedded porous tubes | 2.5–3 fold increase in ADH activity, no change in ADH localization |
| Astroculture-05 (Porterfield and others 2000a) | *Brassica rapa* (flowering) | Zeolite and embedded porous tubes | 3–6 fold increases in ADH activity |
| CUE (Stout and others 2001) | *Brassica rapa* germinated on orbit (vegetative) | Phenolic foam | 50% increase in ADH activity, 10% increase in PDC activity, no change in G6PDH activity |
| CUE (Stout and others 2001) | *Brassica rapa* grown 13 days before launch (flowering) | Phenolic foam | Almost 5-fold increase in ADH activity, 1.5-fold increase in PDC activity, no change in G6PDH activity |

activity. Presumably older plants, that had more developed root systems and were actively flowering, exerted a greater demand on the nutrient delivery system for both mineral nutrients and oxygen.

Recent work has sought to gain insight into this question by using a transgenic *Arabidopsis* plant in a specialized experimental design to monitor the plant for qualitative changes in ADH expression. This plant was transformed with a DNA construct composed of the β-Glucuronidase (GUS) gene driven by the *Arabidopsis* ADH promoter. This approach is equivalent to the use of cytochemical stains, to reveal ADH activity localization used in previous flight experiments (Porterfield and others 1997b; Porterfield and others 2000a; Stout and others 2001). The transgenic *Arabidopsis* plants were used in a flight experiment and showed that, unlike control plants, the ADH gene was activated in the root tips of the plant during the experiment (Paul and others 2001). The flight plants were also compared with a terrestrial hypoxic control. The terrestrial plants that were exposed to rootzone hypoxia expressed ADH in the root tips and the shoot apex. This is consistent with work originally done by Dolferus and others (1994) who showed this pattern of expression in *Arabidopsis* using the ADH-promoted GUS gene. Therefore, the spaceflight and ground hypoxia ADH expression controls differed in the qualitative expression pattern and in the lack of expression in the shoot apex. This result indicates that either the signal transduction between the roots and shoots is disrupted in microgravity, or that ADH induction in the roots is an anomaly and not indicative of root system hypoxia. The first interpretation is plausible since there was no mechanical gas exchange or induced system convection in the experimental plant chamber. Under these conditions, water flux from the leaves would be severely inhibited (Figure 5) and would also inhibit transpiration-dependent signal transduction through the xylem (for discussion see previous section).

In considering these ADH results, it is also important that in addition to the response to hypoxia, ADH can be induced by other forms of environmental stress including salt, temperature, and soil drying (Dolferus and others 1994; Jarillo and others 1993; Naidoo and others 1992; Russell and Sachs 1992). In all of the aforementioned spaceflight experiments, the control treatment plants were grown by identical procedures and exposed to the same regimen of temperature, light, and outside gas composition as was experienced during spaceflight. Therefore, none of these known stress factors would explain the changes in ADH activity observed in these spaceflight experiments. The fact that increases in ADH activity can be elicited by other types of environmental stress does support the interpretation that ADH increases may be the result of a nonspecific stress response (Paul and others 2001), possibly related to the plant's inability to detect a gravity stimulus. This alternative hypothesis is not supported by the trends that are evident in all of these experiments (Table 1). If the ADH response were non-specific and anomalous, there would not

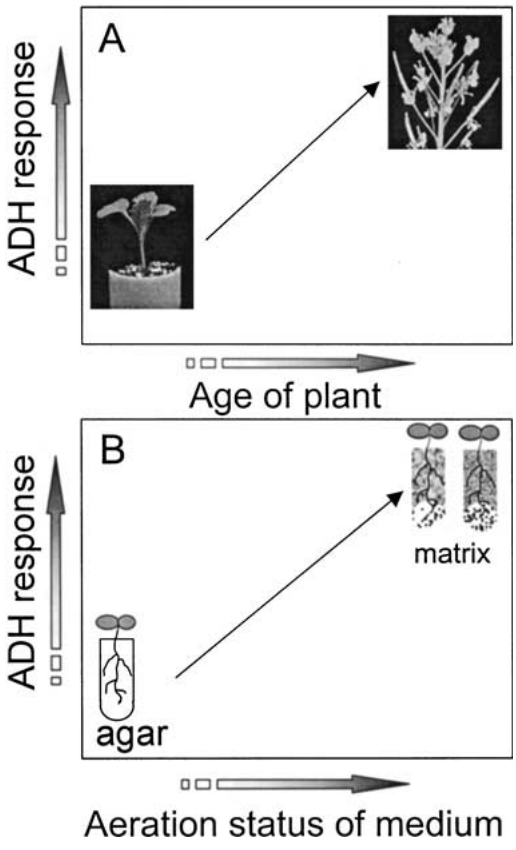


**Figure 6.** Important trends in observed spaceflight responses, as summarized from the data in Table 1. Both plant age and the intrinsic oxygenation of the root media were, shown to alter the measured ADH response of plants grown in microgravity. Plants grown in media that was intrinsically low in oxygen, like agar, exhibited less of a response than plants grown in media that contained more oxygen (Porterfield and others 1997a, b). Also, younger plants that had less biomass and exerted less of a load on the media for oxygen showed a smaller response to microgravity than did older plants with larger root systems. Plant shown in panel A is *Brassica rapa*.

be variations in the response that correlate to media type and plant age (Figure 6).

The biophysical oxygen limitation hypothesis was also recently tested during a KC-135 paraboliclight experiment using a recently developed sensor technology (Porterfield and others 2000c). The objective was to make direct physical measurements of the changes in oxygen bioavailability that may occur in a root matrix in microgravity. Given the fact that the absolute oxygen concentration of the atmosphere is not directly influenced by gravity, a standard oxygen concentration sensor could not be used, as it would not detect changes in non-diffusionary transport. Instead, a sensor was constructed that simulates the relative oxygen consumption activity and geometry of a growing root tip. Because of these design criteria, we would expect that this root oxygen bioavailability (ROB) sensor would be sensitive to changes in convective oxygen transport, as would an actual root. The results of an experiment aboard NASA's KC-135 test aircraft show that oxygen bioavailability is directly modulated in phase with the microgravity/gravity profiles measured by the accelerometer on the KC-135 (Monje and others 2000; Porterfield and others 2000c). These direct physical measurements show that oxygen bioavailability is lowered in reduced gravity levels. In short, these observations support the concept of gravity-dependent convective soil oxygen transport.

Inhibition of gravity-mediated oxygen transport can also impact tropic curvature patterns in space-grown roots. The role of gravity in mediating tropic responses in roots is well documented, and root responses in the absence of gravity have been described as being random by investigators studying gravity sensing during spacefiight (Cowles and others 1984; Dutcher and others 1994; Johnsson and others 1996; Kiss and others 1999; Merkys and others 1981; Merkys and others 1984; Perbal and Driss-Ecole l993, 1994; Perbal and others 1986; Schulze and others 1992; Slocum and others 1984). During the investigations focusing on root metabolism and ADH expression (Porterfield and others 1997a; 1997b), metabolic changes, consistent with hypoxia, were measured in the roots that also exhibited the typical altered root growth patterns, (data not shown). The metabolic data combined with the observations of altered root growth patterns suggested that root orientation, in the absence of gravity, might not have been random but rather directed toward oxygen. Another more poignant way to phrase this is: "oxygen-mediated tropic responses (oxytropism) explain why the roots would grow away from water and nutrients and towards light in microgravity"? Oxytropism was tested in wild type and agravitropic pea plants using a specialized apparatus for creating an oxygen gradient in an experimental rootzone (Porterfield and Musgrave 1998). The results showed that the roots did respond positively to the presence of an oxygen gradient and reoriented their growth toward the higher oxygen concentration. In the spaceflight environment, oxytropism may be exaggerated by a combination of direct and indirect effects. Specifically, where gravity is not present, gravitropism would be inhibited. Disruption of convective oxygen transfer, combined with consumption of oxygen by the roots themselves, will produce highly exaggerated and stable diffusion gradients of oxygen. With the only source of oxygen being the air outside of the root medium,

the resulting root growth would be directed straight out of the root medium towards the air. This is just another way that biophysical limitations can impact root growth in microgravity.

## Summary and Conclusions

In the weightless environment, inhibition of gas exchange in leaves will effectively lower the concentrations of $CO_2$ down to the compensation point and increase the $O_2$ concentrations in the leaf tissue. Based on the enzymatic properties of Rubisco, higher $O_2$ is likely to promote photorespiration and severely decrease the productivity of the plant. Reduced productivity may lead to a general decline in the status of the plant and could explain the slower development and reduced accumulation of biomass in microgravity experiments that lack forced mechanical ventilation. Symptoms of inhibition of photosynthesis could include changes in biochemical composition, enzyme expression, altered morphology, as well as a decline in general vigor.

The reported problems with reproductive viability are most likely caused by a combination of the general reduction in the availability of photosynthate for reproductive tissues as well as by direct boundary layer limitations at the surface of the reproductive structures themselves. Flowers and fruits are sink tissues in the plant and therefore would be expected to require oxygen from the environment to fully utilize the carbon being translocated to its tissues. This concept was studied in both *Arabidopsis* and *Brassica* using oxygen microelectrodes (Porterfield and others 1999). These studies showed that, although the reproductive tissues are photosynthetic, they depend upon oxygen from the atmosphere to support the burst of metabolic activity that starts with pollination (Porterfield and others 1999). The impact of lowering atmospheric oxygen levels, which has also been studied in ground experiments, could also be caused by the biophysical limitations in oxygen flux into the sink tissue (Kuang and others 1998). These studies report that many of the spaceflight aberrations could be reproduced in ground experiments where the oxygen concentrations were reduced.

Although limiting the bioavailability of oxygen in the root zone has a direct impact on the metabolism and physiological function of the root system, the most serious effect in terms of whole plant physiology and productivity is the inhibition of ion nutrient uptake and transport to the shoots (Drew and others 1988). Plants with roots deprived of oxygen, have dramatic decreases in nitrogen, potassium, and phosphorus in the leaf tissue (Hopkins and others 1950; Letey and others 1961, 1965; Leyshon and Sheard 1975; Porterfield and others 1997a, 2000b; Trought and Drew 1980c). The reduction in minerals is thought to result from a decreased capacity for ATP-dependent transport of ions in hypoxic roots (Drew and Siswaro 1979; Trought and Drew 1980a, 1980b). The resulting nutrient deficiencies would also contribute to a general decline in plant growth in microgravity. In spaceflight experiments, inhibition of gravity-dependent convection might also directly inhibit ion transfer to the root surface, further compounding nutritional effects. Furthermore, the inhibition of transpirational transport is likely to limit the translocation of absorbed ions, as well as hormones to the shoot from the root system.

Understanding the biophysical limitations of transport and exchange in plants will ultimately allow us to develop and construct technologies that will overcome these problems. Inhibitory effects associated with microgravity that occur in the aerial portions of the plant can be easily controlled by providing mechanically induced convection in the plant growth chamber. Past problems associated with reproductive failure in *Arabidopsis* were corrected by using an air exchange system that induced moderate airflow in the chamber (Musgrave and others 1997). Controlling and alleviating the potential effects of biophysical limitations in the rootzone will require a more sophisticated approach. The problems in the root system are compounded by the need to provide both nutrient solution and oxygen to the roots. The quality of the soil atmosphere could be further compromised in microgravity by redistribution and channeling of the nutrient solution within a soil matrix. One possible solution would be to include gas exchange systems when developing nutrient delivery system technologies, or to modify existing technologies to include such a subsystem.

The porous tube plant nutrient delivery system (Dreschel and others 1989, 1994) utilizes a porous ceramic tube to deliver water and mineral nutrients to the roots, which are growing directly upon the surface of the tube. The roots are maintained in a gas-filled region between the surface of the porous ceramic tube and an outer housing that protects the roots from light and desiccation. This system could easily be modified to include a gas exchange system that would induce airflow over the surface of the roots and exchange the rootzone atmosphere to replenish oxygen used by respiration. Another approach to microgravity nutrient delivery technologies is to embed a porous stainless steel tube in a matrix of clay particles (Morrow and others 1995;

Porterfield and others 2000a). The Russians have also used this type of system in the Svetoblock plant growth hardware used on board the spacestation Mir (Neichitailo and Mashinski 1993). These systems utilize a series of soil moisture sensors to maintain the rootzone water potential at a given set point. Because of homogeneous distribution of water in the clay particle matrix, water channeling, and the practice of maintaining constant water potential within the matrix, it is possible that there is very little effective gas exchange in the matrix atmosphere. Instead, an ebb-flow approach that is common in terrestrial hydroponics systems might be adopted. This would involve completely flooding the soil and displacing the soil atmosphere for a few minutes, followed by the complete drainage of most of the nutrient solution into a reservoir and reintroduction of a replenished matrix atmosphere. This could be done numerous times a day to maintain both the rootzone atmosphere and the nutrient solution in the clay matrix.

The foundation of future manned space exploration will be the development of human life support technologies that will include high performance crop production systems. As we work towards this goal, we further our understanding of the relationship between plant life and gravity on earth. Past progress in this area has been impeded by a lack of emphasis on work to document biophysical limitations in microgravity, as well as the use of flight experiment hardware that does not provide adequate control for these potentially devastating artifacts. The impacts of biophysical limitations on whole plant physiology are profound. The emphasis of future studies to be conducted in microgravity flight experiments on the space shuttle and space station should include the understanding of indirect effects of microgravity. Such experiments are likely to lead to the development of complete plant growth hardware that compensates for these indirect effects. Such technology would make it possible to better use the microgravity environment to study other aspects of direct effects of microgravity on plants.

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