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ORIGINAL ARTICLE

O. Monje · G. Stutte · D. Chapman

Microgravity does not alter plant stand gas exchange of wheat at moderate light levels and saturating CO_2 concentration

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Abstract Plant stand gas exchange was measured non-destructively in microgravity during the Photosynthesis Experiment Subsystem Testing and Operations experiment conducted onboard the International Space Station. Rates of evapotranspiration and photosynthesis measured in space were compared with ground controls to determine if microgravity directly affects whole-stand gas exchange of *Triticum aestivum*. During six 21-day experiment cycles, evapotranspiration was determined continuously from water addition rates to the nutrient delivery system, and photosynthesis was determined from the amount of CO_2 added to maintain the chamber CO_2 concentration setpoint. Plant stand evapotranspiration, net photosynthesis, and water use efficiency were not altered by microgravity. Although leaf area was significantly reduced in microgravity-grown plants compared to ground control plants, leaf area distribution was not affected enough to cause significant differences in the amounts of light absorbed by the flight and ground control plant stands. Microgravity also did not affect the response of evapotranspiration to changes in chamber vapor pressure difference of 12-day-old wheat plant stands. These results suggest that gravity naïve plants grown at moderate light levels (300 μmol m^{-2} s^{-1}) behave the same as ground control plants. This implies that future plant-based regenerative life support systems can be sized using 1 *g* data because water purification and food production rates operate at nearly the same rates as in 1 *g* at moderate light levels. However, it remains to be verified whether the present results are reproducible in plants grown under stronger light levels.

O. Monje (✉) · G. Stutte · D. Chapman
Mail Code: Dyn-3, Kennedy space center,
FL 32890, USA
E-mail: monjeoa@kscems.ksc.nasa.gov
Tel.: +1-321-8612935
Fax: +1-321-8612925

Keywords Microgravity · Photosynthesis · Vapor pressure difference · Water use efficiency · Wheat · Evapotranspiration

Abbreviations BPS: Biomass production system · DAP: Days after planting · ET: Plant stand evapotranspiration · HCS: Humidity control system · ISS: International Space Station · LAI: Leaf area index · NDS: nutrient delivery system · PESTO: Photosynthesis Experiment Subsystem Testing and Operations · PGC: plant growth chamber · P_{net}: plant stand photosynthesis · SE: standard error · VPD: vapor pressure difference

Introduction

The manned exploration of space will employ plant-based bioregenerative life support systems to reliably assimilate CO_2, produce O_2, provide food, and recycle water for human crews living in microgravity aboard spacecraft or in planetary bases (Olson et al. 1988; Wheeler et al. 2001). Thus, a fundamental understanding of how plants and ecosystems respond to prolonged exposures to reduced gravity forces (Earth—1 *g*, Mars—3/8 *g*, Moon—1/6 *g*, and spacecraft—microgravity) found in space, is needed (Monje et al. 2003). Experiments examining the responses of plant stand evapotranspiration (ET) and photosynthesis (P_{net}) to the absence of gravity may help us determine whether these key metabolic processes are gravity dependent, and also help identify what countermeasures can be adopted to ensure normal plant growth and development in space. Studying responses of ET and P_{net} to microgravity is important because changes in these rates impact the size of the growing area required for plant-based life support systems (Wheeler et al. 2001; Salisbury 1991), and may also impact species composition in spaceborne ecosystems (Morey-Holton 2003).

Presently, fundamental research exploring longterm plant growth and development in space can only be conducted aboard the International Space Station (ISS). However, mass, volume and power constraints of spaceflight experiments often limit the ability to control the environment in which the plants are grown, so that plant responses to environmental changes can not be easily separated from plant responses elicited solely by the absence of gravity (Cook and Croxdale 2003). In past spaceflight experiments, plant growth rates in microgravity have been generally observed to be less than ground controls (Halstead and Dutcher 1984; Porterfield 2002), but recent spaceflight plant studies suggest that differences between spaceflight-grown and 1 *g*-grown plants may be caused indirectly, through microgravity-mediated plant stresses. Plant stress in space may result from waterlogging due to moisture redistribution in the rootzone compared to 1 *g* (Bingham et al. 1996; Jones and Or 1998), or from inadequate supply of CO_2 and O_2 to plant organs caused by insufficient ventilation coupled with the absence of buoyancy driven convection (Musgrave et al. 1997; Porterfield et al. 1997; Ferl et al. 2002; Porterfield 2002). Other factors related to conducting plant experiments aboard spacecraft, such as exposure to high ethylene concentrations (Klassen and Bugbee 2002; Stutte 1999), growth at elevated CO_2 concentrations (Wheeler et al. 1999; Monje and Bugbee 1998), fluctuating environmental parameters of spacecraft cabin air (Levinskikh et al. 2000; Monje et al. 2000), and exposures to volatile organic compounds with biogenic activity (Stutte 1999; Stutte and Wheeler 1997) may also affect plant stand gas exchange rates indirectly through morphological changes in plant size and leaf area. Thus, differences in plant growth rates observed in space compared to 1 *g* controls cannot be solely attributed to direct effects of microgravity on metabolism when the flight hardware employed provides variable, suboptimal or uncontrolled environmental conditions for growth.

Recent advances in the design of spaceflight plant growth hardware have allowed tighter environmental control compared to previous experiments (Morrow and Crabb 2000; Musgrave 2002; Monje et al. 2003), thereby allowing longterm studies to examine what, if any, direct effects the lack of gravity may have on plant growth. In this study, it was hypothesized that gravity would have no direct impact on plant gas exchange rates (ET and P_{net}), because they are driven by gradients of partial pressure between leaves and the air surrounding them. This hypothesis was tested during the 73-day long PESTO experiment aboard the ISS. Several wheat crops were grown in three controlled environment chambers of the Biomass Production System spaceflight hardware during identical spaceflight and ground control experiments. Rates of plant stand ET and P_{net} were measured nondestructively using gas exchange methods in six replicate 21-day-long experiments and the rates from microgravity grown plants were compared with ground (1 *g*) controls.

Materials and methods

Spaceflight hardware

The Biomass Production System (BPS; Orbitec, Madison, WI) is a shuttle middeck locker-sized plant growth unit. It provides four plant growth chambers (PGCs) composed of a light bank, chamber walls and a root module. Each PGC permits independent monitoring and control of air temperature, relative humidity, light level, CO_2 concentration, and rootzone matric potential. The plant chambers are removable for on-orbit access to plants for sampling and harvest. Light in each PGC is provided by cool white fluorescent lamps, and ethylene is removed by a photocatalytic TiO_2 scrubber. Each PGC has 0.0264 m^2 of ground area with 13-cm tall chamber walls and a 3-cm deep root module. The root module is separated from the aerial portion of the chamber by a foam cover and a manifold to circulate air. Water is supplied to the substrate via three porous tubes by a metered peristaltic pump in order to maintain a constant root zone matric potential. PESTO was conducted in PGCs 1–3 of the BPS. The 4th PGC was planted with *Brassicca rapa* as part of a separate hardware validation test (Morrow et al. 2001; Iverson et al. 2003).

Spaceflight activities

The BPS was loaded with live plants 4 days before launch. One day before launch the BPS was transferred to the middeck-locker of Shuttle Endeavour. BPS was launched from the Kennedy Space Center during the STS-110/8A mission on April 8, 2002. It remained on Endeavor until it was transferred to the ISS on April 12, 2002, and was installed onto EXPRESS Rack 4 of the Destiny module. The BPS was tended by Payload Engineer Dan Bursch, who was responsible for on-orbit operations including imbibing pre-planted root modules, tissue fixation, and plant harvests. Data and near real-time video from inside the growth chambers was transmitted from BPS to Earth via the Ames Telescience Center using the Communication and Data System (CDS v1.02). The BPS returned to Earth on June 19, 2002 on STS-111/UF-2 (Edwards Air Force Base landing). The total on-orbit duration for the BPS was 73 days.

Cultural conditions

Wheat (*Triticum aestivum* L., cv. USU Apogee) is a high yielding dwarf variety (40 cm tall) suited for growth in controlled environments because it is half the height of normal wheat (Bugbee and Koerner 1997). Apogee was chosen for the experiment because its gas exchange rates at high CO_2 concentrations had been characterized (Monje and Bugbee 1998), it grew rapidly, and did not

have a vernalization requirement for germination. Each PESTO root module was packed with ~500 g of Turface (calcined montmorillonite clay sifted to 1–2 mm) mixed with Osmocote (7 g l^{-1} or 12 g kg^{-1} of substrate) slow release fertilizer. The root modules contained 32 wheat plants seeded in four rows at a planting density of 1200 plants m^{-2} (Stutte et al. 2000). This corresponded to planting densities used in large-scale (20 m^2) controlled environment tests in the Biomass Production Chamber (BPC) at the Kennedy Space Center (Wheeler et al. 2003). Pre-planted root modules were removed from stowage, imbibed until saturated using a syringe (~540 ml), and placed in the BPS by the payload engineer. The root zone matric potential (suction) was −0.30 kPa, which equals a hydrostatic head of −3.0 cm measured at the center the root module. Light in each PGC was provided by a bank of cool white fluorescent lamps.

The PESTO experiment had three plantings: (1) plants germinated at 1-*g* and launched, (2) plants germinated and grown entirely in microgravity, and (3) plants germinated and grew in microgravity that landed. The root modules of the 1st planting were imbibed on the ground (1 *g*) and the remaining ones were imbibed in microgravity. The plants germinated and grew at a photosynthetic photon flux (PPF) of ~280 μmol m^{-2} s^{-1} (measured at the top of the chamber), and a photoperiod of 20 h day/4 h night. During the each 21-day planting, the relative humidity and air temperature setpoints were 75% and 24°C, respectively. Ethylene concentration was maintained at < 50 nmol mol^{-1} by a photocatalytic scrubber. Daytime CO_2 concentration was not allowed to fall below 1500 ± 50 μmol mol^{-1}, however, it took ~1–2 h to decrease from the night time value when the lights were turned on depending on plant age. This drawdown at the beginning of each day allowed a secondary measure of canopy gas exchange. The 3rd planting allowed measurements of leaf area and absorbed PPF from 23-day-old flight and ground control plants upon landing.

Ground control experiment

The ground control experiment was started on Earth two weeks after the launch in an identical BPS to that used during spaceflight. The environmental parameters (air temperature, relative humidity, and CO_2 concentration) of the ISS cabin observed during the flight were reproduced in the Orbital Environmental Simulator (OES) chamber at the Kennedy Space Center. The average chamber environmental values during the three PESTO plantings are shown below (Table 1). All the on-orbit operations performed during spaceflight were mimicked during the ground control experiment.

In addition to the ground control experiment, a second set of chamber control plants was grown in flight-like PGCs used in the PESTO experiment at 1 *g*. These PGCs were grown under identical environmental conditions in a conventional walk-in plant growth chamber (Environmental Growth Chambers, Model EGC M-48, Chagrin Falls, OH). The differences between the flight and ground control plants were exposure to microgravity and the spaceflight operations (launch, ISS transfer, and landing). The difference between the ground and chamber control plants was that humidity and temperature control was provided by the plant growth chamber. Root zone moisture control was provided by a stand-pipe system, which provided a constant matric potential (−0.3 kPa) (Monje et al. 2001). Environmentally, the ground control plants had an average root temperature of 27°C and the chamber control plants had a root temperature of 24°C, while all other environmental parameters were identical.

Plant stand evapotranspiration

The amount of water used by each flight and ground control PGC was monitored continuously during each experiment cycle for the entire mission. Evapotranspiration moistens the air in the PGC and the Humidity Control System (HCS) removes any excess moisture via a porous plate dehumidifier. Moisture condensed from the PGC atmosphere by the HCS is metered as it is pumped into a water reservoir and the rate of condensate removal is proportional to ET. In the nutrient delivery system (NDS), a pressure sensor in the root module maintains a constant matric potential in the root zone by supplying metered amounts of water from the water reservoir by a pump. This 'on demand' addition of water is also proportional to ET of the stand (Fig. 1). The daily water additions for each chamber were summed and used to determine the daily rates of water ET.

Plant stand transpiration could be estimated from ET by subtracting the rate of root module evaporation. Although evaporation was not measured in this study, it

Table 1 Summary of environmental parameters: light intensity (PPF), air temperature (Tair), relative humidity (RH), chamber CO_2 concentration ($[CO_2]$), and root temperature (Troot)

	PPF (μmol m^{-2} s^{-1})	Tair (°C)	RH (%)	$[CO_2]^a$ (μmol mol^{-1})	Troot[b] (°C)
Flight	267 ± 23	24.0 ± 0.2	76.6 ± 6	4364 ± 1788	27.9 ± 0.5
Ground	275 ± 24	24.2 ± 0.2	76.3 ± 1	3322 ± 1379	27.1 ± 1.0

Each value is the average chamber environment from the three growth cycles (mean ± SD)
[a] CO_2 was only maintained at 1,500 μmol mol^{-1} during the 20 h light cycles
[b] Root temperature was not controlled

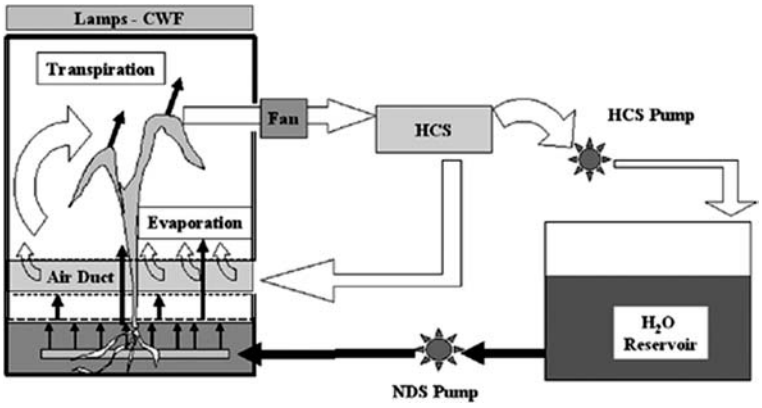

Fig. 1 Air and water fluxes of the humidity control (*HCS*) and nutrient delivery (*NDS*) subsystems of the Biomass Production System. Water supplied to the root module evaporated from the substrate and was transpired by leaves. Water vapor condensed in the HCS was supplied to the water reservoir

was assumed to equal the rate of ET in PGC 4 (~14 ml/day or ~0.35 mmol m^{-2} s^{-1}). The Brassica plants in PGC4 grew very slowly, relative to wheat, so most of the ET in that chamber was due to evaporation from the root module.

Plant stand photosynthesis

The rate of net canopy carbon assimilation in the chamber (P_{net}) was obtained using the semi-closed gas exchange system of the BPS. P_{net} was estimated daily from the rate of CO_2 additions used to maintain a constant CO_2 setpoint in the BPS chambers during the light period. Photosynthesis measurements were possible only after the plants in the chambers were large enough to bring down the chamber CO_2 concentration below the 1500 μmol mol^{-1} setpoint. The rate of CO_2 additions was corrected for chamber leak rate and for chamber "cross-talk" associated with multiplexing of sample gas lines to a common detector (Morrow et al. 2001; Stutte et al. 2000).

Light distribution

Light distribution measurements were made with chamber control plants during ground studies to characterize how light intensity diminishes with height within each BPS chamber. A gallium arsenide (GaAs) photodiode (G1116; Hamamatsu), which was calibrated against a quantum sensor (LI-190, LiCor, Lincoln, NE) under cool white fluorescent lamps, was used to measure light intensity at various heights above the root module in an empty chamber (Fig 2a). Similar measurements were made with 15, 18 and 22-day-old wheat plants in the chamber control PGCs in order to characterize how leaves attenuate incident radiation as leaf area increases (Fig. 2b).

Absorbed PPF and leaf area

Leaf area and absorbed PPF from the 3rd planting of flight and ground control plants were measured at 23 days after planting (DAP). The flight plants were harvested within 2 h after landing. Similarly, leaf area and absorbed PPF from chamber control plants were measured at 15, 18, and 21 DAP. Absorbed PPF was measured prior to harvest according to the method described in Monje and Bugbee (1998). The amount of PPF absorbed by a plant stand is determined from four components: incident PPF, reflected incident PPF, transmitted PPF, and reflected transmitted PPF. Whole-plant leaf area was measured destructively using a portable leaf area meter (Model LI-3000A, LiCor, Lincoln,

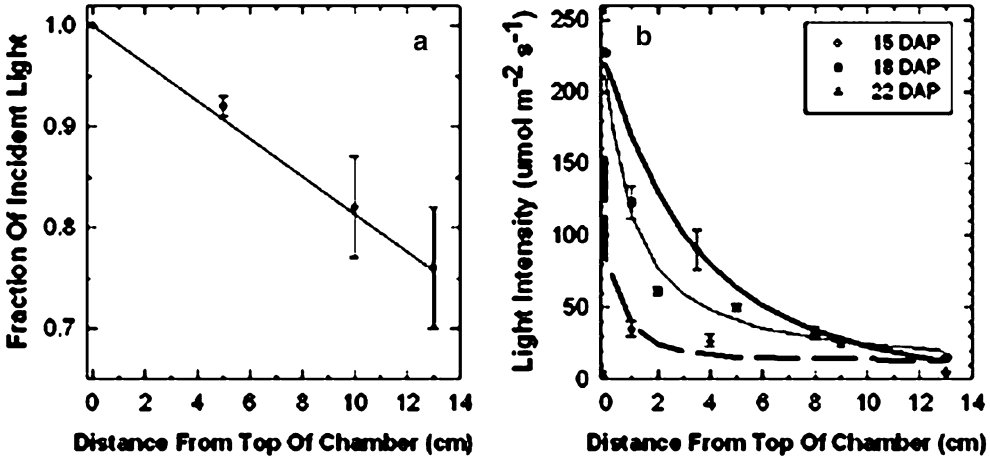

Fig. 2 Light distribution measured in chamber control plants at various distances from the top of the chamber. **a** In an empty chamber, light intensity decreased linearly and light distribution was more variable with depth. **b** Light intensity decreased exponentially with depth when plants were present in the chamber. Older plant stands (18 and 22 DAP) attenuated the light level more rapidly than younger ones (15 DAP) because of a higher leaf area index

NE). Leaf area index (LAI, meter square leaf area per meter square of ground area) was estimated using measurements of leaf area of single plants. LAI was calculated by multiplying the single plant leaf area (cm^2 / plant) by 32 plants per PGC and dividing by the ground area of the chamber (264 cm^2).

Leaf area distribution

Apogee wheat can rapidly fill the PGCs with leaves during vegetative growth since its height at 21 DAP (22–24 cm) exceeds the height of the BPS chambers (13 cm). Leaf area distribution was estimated using chamber control plants to ensure that the leaf area in the chamber did not cause excessive self-shading that might reduce gas exchange rates.

LAI and absorbed PPF were used to determine the canopy extinction coefficient, k, which combines many plant and canopy characteristics into a single property, including leaf size, shape, thickness, degree of vertical leaf area distribution, and the proportions of direct and diffuse radiation (Monteith and Unsworth 1990). k was calculated from the following relation: Absorbed PPF = $[1-\exp(-k \times \mathrm{LAI})]$ (Campbell and Norman 1998). Once k was known, leaf area distribution in the chambers was derived from light attenuation measurements using the Monsi-Saeki Equation: $I = \mathrm{Io} \times \exp(-k \times \mathrm{LAI})$, where Io is the incident light intensity, I is the intensity at the bottom of the canopy (I at 13 cm below the light bank). This approach calculates the LAI of successive horizontal layers of the plant canopy from light intensity measurements (Io and I) made at different heights below the top of the chamber. The leaf area distribution determined using this method is only an approximation to the actual leaf distribution in the PGCs because absorbed PPF, which is used to derive k, was determined by removing the canopy from the PGC.

Plant stand ET responses to VPD

Plant response to vapor pressure difference (VPD) was measured during the 2nd planting of PESTO using 12-day-old wheat flight and ground control plants. ET was varied by changing the vapor pressure gradient (0.35–1.3 kPa) of the chamber through pre-programmed changes in air temperature (20°C, 24°C, 28°C) and relative humidity (65%, 75%, 85%). ET was measured every 2 min, which is equivalent to 60 data points/hr. Each temperature and humidity setpoint combination was run for 3 h to ensure that steady state conditions were reached. A 2 h period of 24°C and 75% RH was run between each of these setpoint combinations. Plant stand water use was determined at each VPD level. ET rates were derived from the rate of water removed from the chamber into the HCS system and from the average rate of water addition to the root module through the NDS system during each three-hour period.

Water use efficiency

Plant stand water use efficiency (WUE; μmol CO_2 mmol H_2O^{-1} or g CO_2 (kg H_2O)$^{-1}$) was calculated daily from the ratio of P_{net} to ET for flight and ground control plants.

Statistical analysis

The statistical model was established so that the three plantings (launched, stowed, and landed) were the main plots and the days after planting (6–20 DAP)were the split plots. Daily gas exchange rates (ET, P_{net}, and WUE) from six chambers were used for the analysis. The gas exchange rates were averaged within plantings across chambers.Using plantings as the main plots, rather than chambers, is justified by assuming that the chambers are all identical and the random variation in the experimental units is in the plantings and not in the chambers (D. Poritz, personal communication).

The PESTO experiment had six plantings (main plots), three for flight and three for ground. Status (flight or ground) is the treatment factor applied to the main plots.This means that there is 1 degree of freedom (df) for status, 4 df's for error from main plots, and thus 5 df's for the corrected total. The average gas exchange rate of three plantings was used at each DAP for each treatment. The split plots are the 15 DAPs from day 6 to day 20, and time in days is the treatment factor applied to the split plots.The DAPs are not, strictly speaking, split plots because time cannot be assigned in random order, but they are repeated measurements within the main plots. The covariance among gas exchange rates over DAPs was estimated to be zero.The statistical power of this analysis was computed, which is the probability of rejecting the null hypothesis when an alternative hypothesis is true. This power computation was based on the t-distribution with 4 df.

Results

Leaf area

Leaf area, absorbed PPF (I_{abs}), and LAI in the chamber control plants increased as the plants grew from 15 DAP to 21 DAP (Table 2). The extinction coefficient decreased as the canopy intercepted more radiation and plant stand height remained constant after 18 DAP. The flight and ground control plants grown in the BPS were different than the chamber control plants: lower leaf area, lower absorbed PPF, lower LAI and ~30 cm taller (data obtained from the 3rd planting upon landing). The leaf area reduction was such that the 23-day-old flight and ground control plants had nearly the same leaf area of the 18-day-old chamber control plants (Table 2). These differences indicate that the growing environment within the BPS was markedly different than the

Table 2 Leaf area per plant, absorbed PPF (I_{abs}), leaf area index (*LAI*), canopy extinction coefficient (*k*), and plant height at harvest in ground control (23 DAP), flight (23 DAP), and chamber control plants (15, 18, 21 DAP). Data represent mean ± SD

	DAP	Area (cm^2)	I_{abs}	LAI	k	Height (cm)
Chamber control	15	26 ± 1^{a}	81 ± 1	3.1	0.54	278 ± 4^{a}
	18	41 ± 2^{b}	82 ± 3	4.2	0.41	299 ± 3^{b}
	21	52 ± 6^{d}	85 ± 2	5.7	0.34	298 ± 8^{b}
Ground control	23	$41 \pm 4^{c,b}$	82 ± 2	3.9	0.44	330 ± 3^{c}
Flight	23	$31 \pm 5^{a,b}$	80 ± 3	2.8	0.56	334 ± 9^{c}

The different letters indicate significant difference at $P < 0.05$

environment in a conventional plant growth chamber set at the same environmental conditions.

Leaf area distribution

In an empty chamber, light intensity decreased linearly with depth (Fig. 2a). The light intensity at the top of the root modules (~13 cm below the top of the chamber) was 75% of the intensity at the top. In contrast, light intensity within chamber control plant stands declines exponentially with depth, and the rate of decline depends on the age of the plant (Fig. 2b). Light intensity in the PGCs becomes low (< 100 µmol m^{-2} s^{-1}) after only 4 cm of depth at 15 DAP, and this decreases further (< 50 µmol m^{-2} s^{-1}) at 22 DAP as self-shading becomes accentuated. Younger plants have less leaf area to intercept incident light (Table 2), thereby allowing light to penetrate deeper into the chamber at 15 DAP than at 18 and 22 DAP.

The light attenuation data in Fig. 2b and the extinction coefficient, *k* (Table 2) were used to estimate leaf area distribution within the PGCs (Fig. 3). In the chamber control plants (solid lines), the leaf area index found at the top of the chamber at 15 DAP was much less than at 18 DAP. For comparison, the leaf area distribution from the flight (dashed line) and ground control (thin line) plants was calculated using the measured k values and the light profile from an 18-day-old chamber control plant stand, because they had similar leaf areas (Table 2). Although leaf area distribution was not measured on ISS, this comparison shows that even though the flight plants had significantly less leaf area than the ground control plants, their leaf area distribution was similar, so that a large difference in absorbed PPF would not be expected.

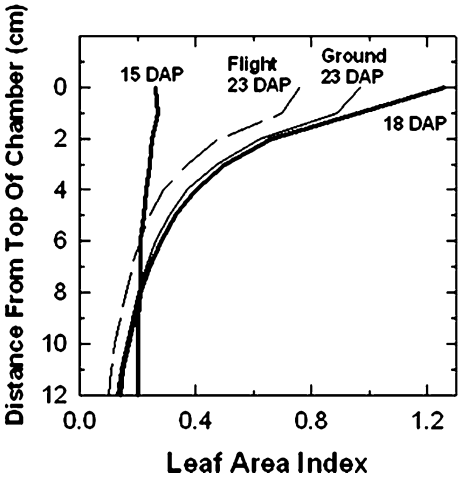

Fig. 3 The leaf area at various heights was estimated from measured canopy extinction coefficients (Table 2) and light profile data (Fig. 2) using Eq. 1. The highest leaf areas were found in the uppermost 2–3 cm of the chamber by 18 DAP in chamber control plants, which suggests that the top layers of leaves intercept most of the incident light. The leaf area distribution of 23-day-old flight plants (*thin line*) was similar to that of ground control (*dashed line*) plants. A large difference in absorbed PPF would not be expected between the flight and ground control plants because they had a similar leaf area distribution

Evapotranspiration and photosynthesis

The cumulative amount of water used by flight and ground plant stands was not significantly different in microgravity (Fig. 4a). Using the customary values of statistical size (5%) and statistical power (80%), the experimental setup used can detect a difference in ET greater than ± 0.29 mmol m^{-2} s^{-1}. There was a significant decrease in ET for flight compared to ground, since the mean ET in flight was 0.37 mmol m^{-2} s^{-1} smaller than the ground control (standard error (SE) of 0.07). The effect of DAPs on ET was also significant because plants grew as they aged, but the interaction of status with DAPs was not significant. A difference of -0.37 mmol m^{-2} s^{-1} is too small to be considered physiologically significant, however, it may reflect the reduced leaf area in the flight plants compared to the ground control plants (Table 2).

Plant stand photosynthesis measurements began as soon as the plants in, the chambers were large enough to reduce the chamber CO_2 concentration below the 1,500 µmol mol^{-1} setpoint (~4–5 DAP). Photosynthesis increased daily as the plants grew and accumulated leaf area (Fig. 5a). P_{net} increased until ~16 DAP when the plant canopy became closed e.g., when all incident light was absorbed. Figs. 2b and 3 suggest that P_{net} is mostly due to the uppermost leaf layers after 15 DAP. The lack of gravity had no effect on plant stand P_{net} during the 21 days that it was measured. The power computation determined that the experimental setup could detect a difference in P_{net} greater than ± 1.35 µmol m^{-2} s^{-1}. The difference in

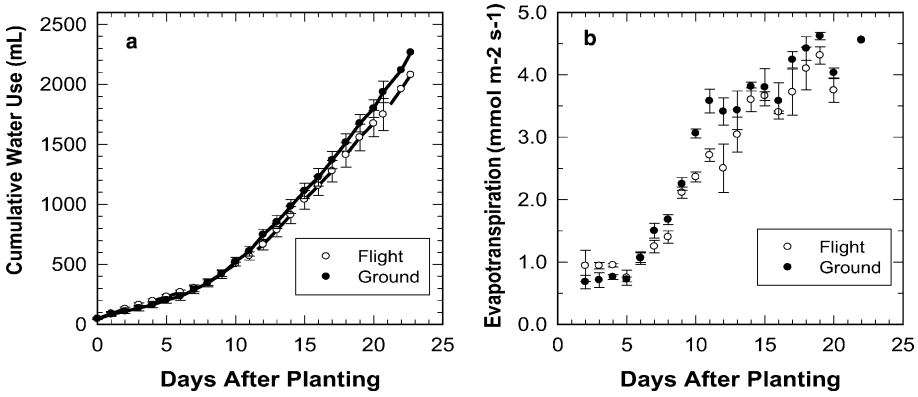

Fig. 4 **a** Evapotranspiration (*ET*), measured as the cumulative amount of water supplied to the root module, increased as the plants grew during the experiment. The flight and ground control plants had similar dry masses at harvest and similar ET, indicating they grew at similar growth rates. **b** ET increased during the life cycle reaching a plateau of 4.5 mmol m^{-2} s^{-1} after 17 DAP. There was no significant physiological difference in plant stand ET between flight and ground control plants. Each data point is the average daily water use or ET from the three plantings (launched, stowed, and landed)

P_{net} for flight minus ground was −0.20 μmol m^{-2} s^{-1} with a SE of 0.37. The effect of DAPs on P_{net} was significant, but the interaction of status with DAPs was not. An examination of the least squares means for DAPs shows clear leveling off of P_{net} with time starting with day 16 (data not shown).

Water use efficiency

Water use efficiency increased from 1.3 μmol $mmol^{-1}$ (3.5 gCO_2 $(kgH_2O)^{-1}$) at 5 DAP to 2.7 μmol $mmol^{-1}$ (6.5 gCO_2 $(kgH_2O)^{-1}$) by 15 DAP (Fig. 5b). After 15 DAP, WUE leveled off as the chamber became filled with leaves (Fig. 3). The flight plants (thin line) had a slightly higher WUE than the ground control plants (thick line; Fig. 5b), however, this difference was not statistically significant. The ground control plants had a lower WUE because they had a slightly higher ET than the flight plants (Fig. 4b). The power computation determined that the experimental setup used can detect a difference inWUE greater than ±0.34 μmol $mmol^{-1}$. The difference in WUE for flight minus ground was 0.13 μmol $mmol^{-1}$ with an SE of 0.12.

Plant stand ET and VPD

The response of ET to chamber VPD was examined by measuring the amount of water removed by the HCS system at each VPD. Chamber VPD increased when chamber temperature was increased or humidity was lowered. The relation between ET measured by HCS condensate removal and chamber VPD was linear until it saturated after 0.8 kPa (Fig. 6). The response of ET to VPD measured by water added to the NDS system did not saturate after 0.8 kPa (data not shown) and remained linear up to 1.35 kPa. There was no significant effect of microgravity on the response of ET to VPD, as evidenced by similar slopes between ET and VPD in flight (slope = 2.88, $r^2 = 0.989$) and ground control plants (slope = 3.04, $r^2 = 0.959$).

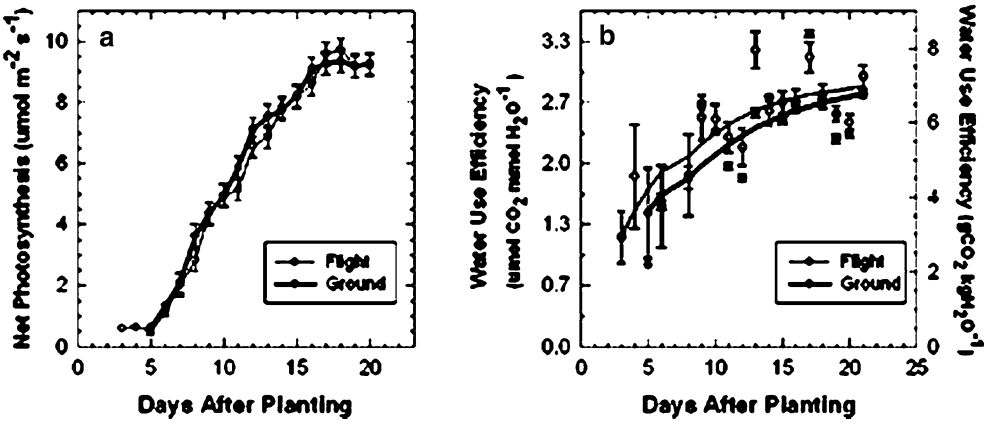

Fig. 5 **a** Daily net photosynthesis (P_{net}) in flight plants (*closed*) was not significantly different from photosynthetic rates measured in the ground control plants (*open*). The flight and ground control plant stands had similar germination rates and dry masses at harvest, indicating they grew at similar growth rates. **b** Rates of water use efficiency (*WUE*), calculated from (P_{net}/ET), were not

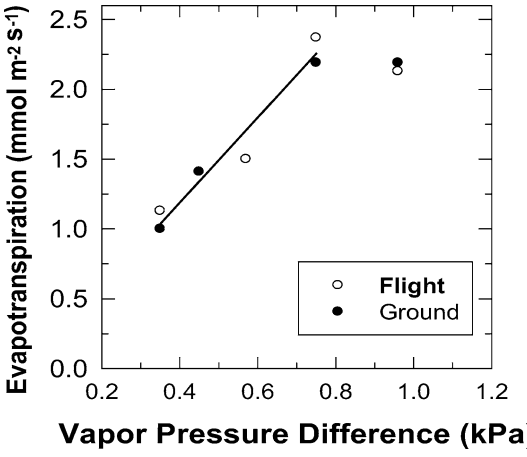

Fig. 6 Evapotranspiration (*ET*) obtained using the humidity control system (*HCS*) water removal data of flight and ground control plants had a similar response (same linear slope) to vapor pressure difference. ET from HCS water removal data saturated after 0.8 kPa, but ET estimated from nutrient delivery system (*NDS*) water addition data was linear up to 1.3 kPa (data not shown). Each value is the average chamber ET measured in 12 DAP wheat during the 2nd planting

Discussion

The effects of weightlessness on growth and development of plants are of primary importance for developing the appropriate technologies and cultural conditions ensuring stress-free plant growth in microgravity (Monje et al. 2003), for understanding the basic mechanisms responsible for gravitropic responses (Chen et al. 1999; Kern et al. 2001; Laurinavicius et al. 2001), and for evaluating the use of plants in regenerative life support systems (Miller and Ward 1966; Olson et al. 1988; Wheeler et al. 2001). An objective of the ISS PESTO experiment was to determine the effect of microgravity on plant stand gas exchange. Plant transpiration and photosynthesis are controlled by physiological (e.g. stomatal conductance), morphological (e.g. leaf area distribution) and environmental (e.g. PPF, VPD, $[CO_2]$) factors (Monteith and Unsworth 1990). In the PESTO experiment, the experimental design allowed the manipulation of environmental controls (VPD), as well as the capacity to detect changes in plant morphology because live plants grown in space were harvested on Earth. The hypothesis that gas exchange in space is governed by the gradients between leaves and the air surrounding them was studied by comparing replicated measurements of plant evapotranspiration and photosynthetic rates in flight and ground control plant stands.

PESTO utilized the BPS, a unique and versatile plant growth system that provided a tightly controlled, replicated environment for rapid plant growth in microgravity. The BPS was deployed on ISS and several plant stands were grown for 21-day cycles at constant light level, air temperature, relative humidity, $[CO_2]$, ethylene concentration, and root zone matric potential. The BPS accommodated high rates of ET, while still maintaining adequate air temperature and humidity control. It also permitted the nondestructive measurement of canopy gas exchange rates (ET and P_{net}) at moderate light levels (~300 μmol m^{-2} s^{-1}) and elevated CO_2 (1500 μmol mol^{-1}). The BPS PGCs provided a growing volume of ~3400 cm^3 (264 cm^2 of ground area by 13 cm of height), which is small for growing wheat for a full life cycle. USU Apogee, a semi-dwarf wheat cultivar reaching a height of 30–40 cm at maturity, was grown for only 21 DAP, therefore the leaves grow towards the light until 13–14 DAP when they reach the top of the chamber (Stutte et al. 2003). After 18 DAP, the canopy becomes closed as the leaves start bending, leaf area increases along the top of the chamber, and self-shading becomes more important (Fig. 3). In each PGC, ET was measured by the amount of water condensed by the HCS system and P_{net} was measured by the amount of CO_2 added to maintain a constant chamber $[CO_2]$ set-point. The gas exchange rates were measured continuously during 21-day cycles and were corrected for chamber leak rates and chamber-to-chamber cross-talk (Stutte et al. 2000). A comparison between chamber control and ground control plants revealed that there are large morphological differences (taller plants with less leaf area) between BPS-grown plants and plants growing in conventional controlled environments. Potential environmental factors responsible for these morphological differences may be the atmospheric closure in the BPS compared to a conventional growth chamber or increased root zone temperature (~3°C higher) in the BPS.

ET ranged from 1.5 mmol m^{-2} s^{-1} at 10 DAP to ~4.5 mmol m^{-2} s^{-1} (or 6.8 L m^{-2} d^{-1}) at 21 DAP (Fig. 4b). The rates of ET reported here are lower than ET of 20–21 DAP wheat (4–5.8 mmol m^{-2} s^{-1}) measured at 2.3 times the light level (650 μmol m^{-2} s^{-1}) and comparable $[CO_2]$ (Wheeler 1992; Cornett et al. 1994). However, rates of ET in the BPS are higher than ET rates measured in the SVET greenhouse aboard Space Station Mir (2–2.5 mmol m^{-2} s^{-1}) at the same light level (Monje et al. 2000). Morphological differences in leaf area may have been responsible for these comparatively higher transpiration rates measured in BPS. The plants in SVET were exposed to cabin air containing 0.6–1 μmol mol^{-1} of ethylene (Levinskikh et al. 2000), which has been reported to cause reduced leaf area and stunted growth in wheat (Klassen and Bugbee 2002).

Growth in microgravity did not change the amount of water transpired and evaporated from the root modules (Fig. 4a). This direct comparison between flight and ground control plants was only possible since there were no differences in germination rates between the flight and ground root modules (Stutte et al. 2003), and each root module had a similar number of plants. Similarly, long-term exposure to microgravity did not result in significant differences in gas exchange rates: ET (Fig. 4b) or P_{net} (Fig. 5a). Altogether these results indicate that microgravity does not alter plant stand growth rates. A more important finding from these gas

exchange measurements was that there were no significant differences among the three PESTO plantings (launched, stowed, and landed), which means that plants grown at 1 *g* at moderate light levels (300 μmol m^{-2} s^{-1}) and exposed to spaceflight behave the same as gravity naïve plants.

The flight plants had significantly lower leaf area (~22%; Table 2) than the ground control plants, however, this difference did not translate into a significant reduction in absorbed PPF (Table 2). The analysis in Fig. 3 indicates that the reduced leaf area probably did not alter leaf area distribution enough to cause corresponding reductions in absorbed PPF. Therefore, the observed morphological changes did not result in significant changes in gas exchange rates, except for a small, albeit statistically significant reduction in ET in flight compared with ground control plants (Fig. 4b). A corresponding decrease in P_{net} would not be expected unless a large change in absorbed PPF was observed because it is determined by the amount of illuminated leaf area at the top of the chamber, and the contribution of the underlying shaded leaf area to canopy photosynthetic rates is small.

The hypothesis that gas exchange in space is governed by the gradients between leaves and the air surrounding them was further tested by studying the response of ET to changes in chamber VPD in microgravity. No direct effect of microgravity on gas exchange is expected as long as wind speed is high enough to prevent diffusion-limited transport because microgravity may reduce the leaf boundary layer conductances at low wind speeds due to the absence of buoyancy driven convection (Kitaya et al. 2003). Porterfield (2002) concluded that significant reductions in photosynthesis in and other physiological processes may occur when gravity-dependent transport phenomena no longer operate. ET responses to VPD were measured using 12-day-old plants in a well-ventilated PGC. In wheat growing at constant VPD and elevated [CO_2], a linear relation between canopy stomatal conductance and transpiration was observed (Monje and Bugbee 1996), and canopy stomatal conductance was less responsive to increased VPD at elevated [CO_2] (Monje 1998; Bunce 1998; Bunce 2000). Exposure to dry air was expected to increase ET in proportion to the increased VPD between the leaves and air, unless the drier air reduced stomatal conductance. ET increased linearly with increasing chamber VPD in both flight and ground control plants up to a VPD of 0.8 kPa (Fig. 6), which suggests that stomatal conductance became constant at high VPD. Microgravity did not affect the response of ET to VPD since the slope of the response in the flight plants was the same as in the ground control plants (similar slopes; Fig. 6).

Canopy water use efficiency is a parameter used in field situations to measure a plant stand's ability to cope with water stress, and it is also an indicator of CO_2/H_2O vapor exchange, which is ultimately regulated by stomatal conductance (Farquhar and Richards 1984). Canopy WUE was unaffected by microgravity. In the BPS chambers, canopy WUE of wheat was ~2.7 μmol $mmol^{-1}$ (6.6 gCO_2 $(kgH_2O)^{-1}$), which is greater than typical values of WUE for C_3 plants in the field (0.4–1.2 μmol $mmol^{-1}$ or 1–3 gCO_2 $(kgH_2O)^{-1}$; Nobel 1999). WUE in a controlled environment plant chamber is expected to be higher than in the field because stomata are less open at lower light levels, and growth at elevated CO_2 closes stomata further.

In conclusion, microgravity did not affect gas exchange rates (ET and P_{net}) of wheat plant stands growing at moderate light levels (~300 μmol m^{-2} s^{-1}) and saturating CO_2 concentrations. These findings suggest that plant stand water purification and food production rates will not change in space because the underlying biological processes operate at the same rates as in 1 *g* at moderate light levels. This finding implies that future plant-based regenerative life support systems operating at similar light levels can be sized using 1 *g* data. However, it remains to be verified whether the present results are reproducible in plants grown under stronger light levels. In previous studies, a significant (~17%) reduction in whole-chain electron transport was observed in chloroplast extracts from plants raised in microgravity at high light levels (Tripathy et al. 1996). In this study, decreased leaf area was not large enough to cause significant differences in plant stand gas exchange rates or growth rates, but a slight reduction in ET and a small increase in WUE were discerned in the plant stands growing in microgravity. Decreased leaf area may explain the lower ET, but the reason for the reduction in leaf area cannot be ascribed to differences in environmental parameters between the flight and ground control plants. Future work will be needed to assess the underlying cause of the decreased leaf area observed in microgravity-grown plants.

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References

Bingham GE, Jones SB, Podolski I, Yendler B (1996) Porous substrate water relations observed during the greenhouse in flight experiment (MIR Space Station–1995). SAE Technical Paper Series No. 961547

Bugbee B, Koerner G (1997) Yield comparisons and unique characteristics of the dwarf wheat cultivar "USU-Apogee". Adv Space Res 20:1891–1894

Bunce JA (1998) Effects of environment during growth on the sensitivity of leaf conductance to changes in humidity. Global Change Biol 4:269–274

Bunce JA (2000) Responses of stomatal conductance to light, humidity and temperature in winter wheat and barley grown at three concentrations of carbon dioxide in the field. Global Change Biol 6:371–382

Campbell GS, Norman JM (1998) An Introduction to Environmental Biophysics, 2nd edn. Springer-Berlin Heodeberg, New York

Chen R, Rosen E, Masson PH (1999) Gravitropism in higher plants. Plant Physiol 120:343–350

Cook ME, Croxdale JL (2003) Ultrastructure of potato tubers formed in microgravity under controlled environmental conditions. J Exp Bot 54:2157–2164

Cornett JD, Hendrix JE, Wheeler RM, Ross CW, Sadeh WZ (1994) Modeling gas exchange in a closed plant growth chamber. Advances in Space Research 14:337–341

Farquhar GD, Richards RA (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Aust J Plant Physiol 11:539–552

Ferl RJ, Wheeler R, Levine HG, Paul AL (2002) Plants in space. Current Opinion in Plant Biol 5:258–63

Halstead TW, Dutcher FR (1984) Status and prospects: experiments on plants in space. Ann of Bot Suppl 54:317–345

Iverson JT, Crabb TM, Morrow RC, Lee MC (2003) Biomass Production System hardware performance. SAE Technical Paper 2003-01-2484

Jones SB, Or D (1998) Microgravity effects on water flow and distribution in unsaturated porous media: analyses of flight experiments. Water Resources Res 35:929–942

Kern VD, Smith JD, Schwuchow JM, Sack FD (2001) Amyloplasts that sediment in protonemata of the moss Ceratodon purpureus are nonrandomly distributed in microgravity. Plant Physiol 125:2085–2094

Kitaya Y, M Kawai, J Tsuruyama, H Takahashi, A Tani, E Goto, T Saito, Kiyota M (2003) The effect of gravity on surface temperatures of plant leaves. Plant Cell Environ 26:497–503

Klassen S, Bugbee B (2002) Sensitivity of wheat and rice to low levels of atmospheric ethylene. Crop Sci 42:746–753

Laurinavicius R, Svegzdiene D, Gaina V (2001) Force sensitivity of plant gravisensing. Adv Space Res 27:899–906

Levinskikh MA, Sychev VN, Derendyaeva TA, Signalova OB, Salisbury FB, Campbell WF, Bingham GE, Bubenheim DL, Jahns G (2000) Analysis of the spaceflight effects on growth and development of Super Dwarf wheat on the Space Station Mir. J Plant Physiology 156:522–529

Miller RL, Ward CH (1966) Algal bioregenerative systems. In: Kammermeyer K (ed) Atmosphere in space cabins and closed environments. Appleton-Century-Croft, New York

Monje O (1998) Predicting canopy transpiration rates of hydroponically-grown plant canopies in controlled environments. PhD Dissertation, Utah State University

Monje O, Bugbee B (1996) Characterizing photosynthesis and transpiration of plant communities in controlled environments. Acta Horticulturae ISHS 440:123–128

Monje O, Bugbee B (1998) Adaptation to high CO_2 concentration in an optimal environment: radiation capture, canopy quantum yield and carbon use efficiency. Plant Cell Environ 21:315–324

Monje O, Bingham GE, Salisbury FB, Campbell WF, Carman JG, Eames BK, Sychev V, Levinskikh MA, Podolsky I (2000) Canopy photosynthesis and transpiration in microgravity: Gas exchange measurements aboard Mir. Adv Space Res 26:303–306

Monje O, Wang HT, Kelly C, Stutte GW (2001) Nutrient delivery system water pressures affect growth rate by changes in leaf area, not single leaf photosynthesis. SAE Technical Paper 2001-02-2277

Monje O, Stutte GW, Goins GD, Porterfield DM, Bingham GE (2003) Farming in space: environmental and biophysical concerns. Adv Space Res 31:151–167

Monteith JL, Unsworth MH (1990) Principles of environmental physics. Chapman and Hall Inc., New York

Morey-Holton ER (2003) The impact of gravity on life. In: Rothschild L, Lister A (eds) Evolution on planet earth: the impact of the physical environment. Academic, New York,

Morrow RC, Crabb TM (2000) Biomass production system (BPS) plant growth unit. Adv Space Res 26:289–298

Morrow RC, Crabb TM, Iverson JT, Frank JG (2001) Science accommodations in the Biomass Production System. SAE Technical Paper 2001-01-2231

Musgrave ME (2002) Seeds in Space. Seed Science Res 12:1–16

Musgrave ME, Kuang A, Matthews SW (1997) Plant reproduction during spaceflight: importance of the gaseous environment. Planta 203:S177-S184

Nobel PS (1999) Physiochemical and environmental plant physiology, 2nd edn. Academic, San Diego

Olson RL, Oleson MW, Slavin TJ (1988) CELSS for advanced manned mission. HortScience 23:275–286

Porterfield DM (2002) The biophysical limitations in physiological transport and exchange in plants grown in microgravity. J Plant Growth Regul 21:177–190

Porterfield DM, Matthews SW, Daugherty CJ, Musgrave ME (1997) Spaceflight exposure effects on transcription, activity, and alcohol dehydrogenase in the roots of Arabidopsis thaliana. Plant Physiol 113:685–693

Salisbury FB (1991) Lunar farming: achieving maximum yield for the exploration of space. HortScience 26:827–833

Stutte GW (1999) Phytochemicals: Implications for long duration space missions. In: Cutler H, Cutler S (eds) Biologically active natural products: agrochemicals and pharmaceuticals. CRC Press, Boca Raton, pp 275–286

Stutte GW, Wheeler RM (1997) Accumulation and effect of volatile organic compounds in closed life support systems. Adv Space Res 20:1913–1922

Stutte GW, Monje O, Goins GD, Ruffe L (2000) Evapotranspiration and photosynthesis characteristics of two wheat cultivars grown in the biomass production system. SAE Technical Paper 2001–0202180

Stutte GW, Monje O, Anderson S (2003) Wheat growth on board the International Space Station: Germination and early development. Proc Plant Growth Reg Soc Am 30:66–71

Tripathy BC, Brown CS, Levine HG, Krikorian AD (1996) Growth and photosynthetic responses of wheat plants grown in space. Plant Physiol 10:801–806

Wheeler RM (1992) Gas-exchange measurements using a large, closed, plant growth chamber. HortScience 27:777–780

Wheeler RM, Mackowiak CL, Yorio NC, Sager JC (1999) Effects of CO_2 on stomatal conductance: do stomata open at very high CO2 concentrations. Ann Botany 83:243–251

Wheeler RM, Stutte GW, Subbarao GV, Yorio NC (2001) Plant growth and human life support for space travel. In: Pessarakli M (ed) Handbook of plant and crop physiology, 2nd edn. Marcel Dekker Inc., New York, pp 925–941

Wheeler RM, Sager JC, Prince RP, Knott WM, Mackowiak CL, Stutte GW, Yorio NC, Ruffe LM, Peterson BV, Goins GD, Hinkle CR, Berry WC (2003) Crop production for advanced life support systems: observations from the Kennedy space center breadboard project. NASA TM-2003–211184