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ORIGINAL ARTICLE

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Microgravity effects on thylakoid, single leaf, and whole canopy photosynthesis of dwarf wheat

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Abstract The concept of using higher plants to maintain a sustainable life support system for humans during long-duration space missions is dependent upon photosynthesis. The effects of extended exposure to microgravity on the development and functioning of photosynthesis at the leaf and stand levels were examined onboard the International Space Station (ISS). The PESTO (Photosynthesis Experiment Systems Testing and Operations) experiment was the first long-term replicated test to obtain direct measurements of canopy photosynthesis from space under well-controlled conditions. The PESTO experiment consisted of a series of 21–24 day growth cycles of *Triticum aestivum* L. cv. USU Apogee onboard ISS. Single leaf measurements showed no differences in photosynthetic activity at the moderate (up to 600 μmol m^{-2} s^{-1}) light levels, but reductions in whole chain electron transport, PSII, and PSI activities were measured under saturating light (> 2,000 μmol m^{-2} s^{-1}) and CO_2 (4000 μmol mol^{-1}) conditions in the microgravity-grown plants. Canopy level photosynthetic rates of plants developing in microgravity at ~280 μmol m^{-2} s^{-1} were not different from ground controls. The wheat canopy had apparently adapted to the microgravity environment since the CO_2 compensation (121 vs. 118 μmol mol^{-1}) and PPF compensation (85 vs. 81 μmol m^{-2} s^{-1}) of the flight and ground treatments were similar. The reduction in whole chain electron transport (13%), PSII (13%), and PSI (16%) activities observed under saturating light conditions suggests that microgravity-induced responses at the canopy level may occur at higher PPF intensity.

Keywords Bioregeneration · Bioregenerative Life Support · Photosystem II · Photosystem I · Space · *Triticum aestivum L*

Abbreviations BLSS: Bioregenerative Life Support System · BPS: Biomass Production System · BRIC: Biological Research in Canisters · CDS: Communication and Data System · DAI: Days After Imbibition · ISS: International Space Station · KSC: Kennedy Space Center · LN2: Liquid Nitrogen · NASA: National Aeronautics and Space Association · OES: Orbiter Environment Simulator · P_{net}: Net Photosynthesis rate · PAR: Photosynthetically Active Radiation · PESTO: Photosynthesis Experiment System Testing and Operation · PGC: Plant Growth Chamber · PPF: Photosynthetic Photon Flux · PSI: Photosystem I · PSII: Photosystem II · QY: quantum yield · STS: Space Transport System · WCE: Whole Chain Electron transport

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Introduction

Using higher plants as the basis for a biological life support system (BLSS) that regenerates the atmosphere, purifies water and produces food during long-duration space missions has been researched for over 40 years (Myers 1954; Eley and Myers 1964; Miller and Ward 1966; Halstead and Durcher 1987; Wheeler et al. 2001). The photosynthetic rate of higher plants is the critical component of plant-based atmospheric regeneration systems being proposed for long-duration space missions since it is the fundamental biological process driving atmospheric regeneration and food production

processes (Gitelson and Okladnikov 1994; Wheeler et al. 2001). Because of that criticality, it is essential to determine the impacts of the microgravity (μg) environment on the development of the photosynthetic apparatus and its functioning in space. It is only through direct measurement of photosynthesis under adequate light and controlled CO_2 in μg that an informed decision can be made on the suitability and design of biologically based subsystems for use in a BLSS.

The limited direct information on photosynthesis from spaceflight experiments suggests that μg has negative effects on the development of the photosynthetic apparatus and the efficiency of photosynthesis of developing tissue (Brown et al. 1996). This conclusion significantly impacts the feasibility of using higher plants for atmospheric regeneration under μg conditions (Wheeler et al. 2001; Drysdale et al. 2003) since the total mass and volume of a BLSS would have to be increased by an appropriate factor to meet the minimum crew requirements for a long-duration mission. The associated increase in size, mass, and cost ultimately, has far reaching implications in the overall design and feasibility of a BLSS. However, none of the data on photosynthetic rates in μg being used to make these assessments, either direct or indirect, has been obtained in the relevant environmental conditions (moderate light levels, elevated CO_2, temperature, and RH control) anticipated for growing plants on long-duration missions (Goins et al. 2003).

Although NASA's interest in plant biology is over 40-years-old and extensive research has been conducted to understand the basic physiological responses of plants in space, there is a paucity of information on photosynthesis per se. Long-term experiments to determine biological adaptations of higher plants are not possible with drop towers (1–2 s μg), parabolic flights (20–35 s μg) or space shuttle (5–16 day μg) experiments. In addition, limited power for supplying adequate levels of photosynthetic photon flux (PPF), coupled with a lack of on-orbit instrumentation for measuring photosynthesis during spaceflight experiments, has hindered our ability to obtain real-time, non-destructive data. As a result, most of the information on photosynthetic responses of plants in μg has been derived from post-flight analysis of tissue upon landing or from clinostat experiments (Brown et al. 1995; Tripathy et al. 1996; Jiao 1999).

The first attempt to measure photosynthesis directly in space was conducted on algae flown on the OV1–4 Satellite in 1966 (Ward et al. 1970). No differences in photosynthesis or respiration between ground controls and space-grown *Chlorella sorokiniana* or *Spirodella polyrhiza* were found, but technical issues during the experiment made the interpretation complex (Halstead and Dulcher 1987). During STS-73, a 16-day spaceflight experiment using *Solanum tuberosum* leaf explants, photosynthesis and respiration were observed in the CO_2 data streams, but rates of photosynthesis could not be resolved (Brown et al. 1997) because chamber leak rates and canopy area were not known. Canopy gas exchange measurements of *Triticum aestivum* L. cv Super Dwarf were made over a 12-day period during the SVET Greenhouse IIB experiment onboard the Orbital Station Mir, but fluctuating environmental conditions on Mir and unknown contributions from respiration of decaying plant material precluded calculations of canopy photosynthesis (Monje et al. 2000). In addition, those measurements lacked a high-fidelity ground control from which to make comparisons of gas exchange rates.

Post-flight analysis of wheat cv. Super Dwarf germinated on Earth and grown for 14 days during STS-63 by Tripathy et al. (1996) indicated a 27% reduction in wheat cv. Super Dwarf leaf disc O_2 evolution rates at saturating light and CO_2 conditions. This result was consistent with the 25% reduction in fresh weight of the space-grown plants observed in that study. Microgravity apparently increased leaf respiration rates and disrupted electron transport through PSI and PSII (Tripathy et al. 1996) in these studies with whole chain electron (WCE) transport being reduced 28%. It is important to note that plants used in these experiments were grown under low light (50–60 μmol m^{-2} s^{-1} PPF), not much greater than the light compensation point (10–20 μmol m^{-2} s^{-1} PPF) and the electron transport rates were subsequently monitored at saturating light intensities (2,000 μmol m^{-2} s^{-1} PPF). Similarly, Jiao (1999) observed a 30% decrease in PSI electron transport rate in *Brassica rapa* cotyledons that had been exposed to 14 day of μg during STS-87. Jiao (1999) also reported a 16–35% reduction in the abundance of PSII proteins in the flight leaf tissue. As with Tripathy (1996), these cotyledons were grown at relatively low light levels (50–60 μmol m^{-2} s^{-1} PPF) and the PSI and II was then characterized at saturating light conditions. The plant growth chambers used in both these experiments provided limited environmental control, and the plants were exposed to high relative humidity (RH) (85–100%), fluctuating temperature (23–28°C), and fluctuating CO_2 (1,500 to 8,000 μmol mol^{-1}) conditions, making differentiation of effects between μg and environmental stress difficult.

Photosynthetic measurements of clinorotated plants (i.e., altered gravity conditions) also suggested an effect of gravity on photosynthesis. Brown et al. (1996) reported that *Pisum sativum* grown on a horizontal clinostat had greater CO_2 assimilation rates than vertically rotated or stationary control plants, and no apparent differences in respiration rate between the treatments were observed. In contrast, horizontal rotation had no effect on photosynthesis of maize plants, but resulted in a decrease in respiration rate. These results are in partial agreement with the findings of Ward and King (1978), who found both increased photosynthetic and respiratory gas exchange rates in horizontally rotated marigold plants and contrary to the findings of Delolph and co-workers (1966, 1967), who reported significant increases in respiratory gas exchange in clinorotated oat seedlings.

A NASA "Tiger team" report (Goins et al. 2003) identified some of the environmental constraints of growing plants on long-duration space missions and recommended that 25°C, 300 μmol m^{-2} s^{-1} PPF, and 1,500 μmol mol^{-1} CO_2 be considered "baseline" for plant chambers with active CO_2 control. Recent advances in the design of plant growth chambers (PGCs) for use in space have overcome many of the technical limitations of growing plants in space (Morrow and Crabb 2000; Musgrave et al. 2000; Monje et al. 2003) and allow stress-free plant growth under well-controlled environments. These advances allow mitigation or control of environmental factors impacting plant growth in space such as elevated ethylene (Klassen and Bugbee 2002; Stutte 1999; Wheeler et al. 2004), super-elevated CO_2 (Wheeler et al. 1999), fluctuating atmospheric parameters in the cabin atmosphere (Levinksikh et al. 2000; Monje et al. 2002) and exposure to biologically active volatile organic compounds (Stutte 1999; Stutte and Wheeler 1997; Stutte et al. 2004) that may also affect photosynthetic rates.

Although direct measurement of photosynthesis under *μg* conditions is not available, indirect evidence suggests an effect of *μg* on photosynthesis and subsequent carbon assimilation. The objective of this spaceflight experiment was to test the hypothesis that canopy photosynthesis is reduced under the stress of the *μg* environment. This hypothesis was tested by direct, non-destructive measurement and characterization of carbon assimilation rates of wheat canopies developed entirely in *μg* and post-flight analysis of PSI, PSII, and WCE transport.

Materials and methods

Spaceflight hardware

The Biomass Production System (BPS; Orbitec, Madison, WI, USA) is a space shuttle double mid-deck locker-sized plant growth unit. It provides four PGCs composed of a light bank, chamber walls, and a root module. Each PGC permits independent control of air temperature, relative humidity, light level, CO_2 concentration, and root zone matric potential. The plant chambers are removable for on-orbit access to plants for sampling and manipulation such as harvest or pollination. Light in each PGC is provided by cool white fluorescent lamps, and ethylene is removed by a photo-catalytic TiO_2 scrubber. Each PGC has 0.0264 m^2 of plant growth area with 13 cm tall chamber walls and a 3 cm deep root tray. The root tray 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. The PESTO experiment was conducted in PGCs 1–3 of the BPS. The fourth PGC was planted with *B. rapa* as part of a separate hardware validation test (Morrow and Crabb 2000; Iverson et al. 2003).

Spaceflight activities

The BPS was loaded with imbibed root modules at 3 days before launch. The root modules contained plants that were either 8 (PGC2), 2 (PGC3) or 0 (PGC1) days after imbibition (DAI). One day before the launch the BPS was transferred to the mid-deck locker of Shuttle Endeavor. BPS was launched from the Kennedy Space Center (KSC) during the STS-110/8A mission on April 8, 2002. The BPS remained on Endeavor until it was transferred to the ISS on April 12, 2002. The BPS was installed onto EXPRESS Rack 4 of the Destiny module of ISS (Fig. 1a). The PESTO experiment was maintained by Payload Engineer Dan Bursch, who was responsible for on-orbit operations including tissue fixation, plant harvests, and imbibing pre-planted root modules. Data (Fig. 1b) and near real-time video (Fig. 1c) from inside the growth chambers were transmitted from the PGC 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.

Plants were harvested in orbit at 21–24 DAI by removing the appropriate PGC from the BPS chamber, taking photographs, selecting two plants for fixation, and then excising each individual plant (Fig. 1d). The freshly harvested leaf tissue was wrapped in aluminum foil and placed at −25°C for storage in the ARTIC freezer onboard the ISS. Ten days before landing, the foil sample packets were transferred to Biological Research in Canisters (BRICs) that had been stowed at −176°C in the KSC Liquid N_2 (LN2) freezer, and then placed in LN2 stowage. The LN2 freezer was transferred to the mid-deck of the Space Shuttle Endeavor 5 days prior to landing.

Cultural conditions

Triticum aestivum L. cv. USU Apogee was used for this experiment. USU Apogee is a dwarf wheat variety that has been selected for growth in controlled environments expected for spaceflight (Bugbee and Koerner 1997). It was selected for the experiment because vernalization is not required for germination, it grows rapidly following germination, and gas exchange rates at high CO_2 concentrations have been characterized (Monje and Bugbee 1998). 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 0.012 g g^{-1} of substrate) slow release fertilizer. The root modules contained 32 wheat plants seeded in four rows at a planting density of 1,200 plants m^{-2} (Stutte et al. 2000). This density corresponds to planting densities

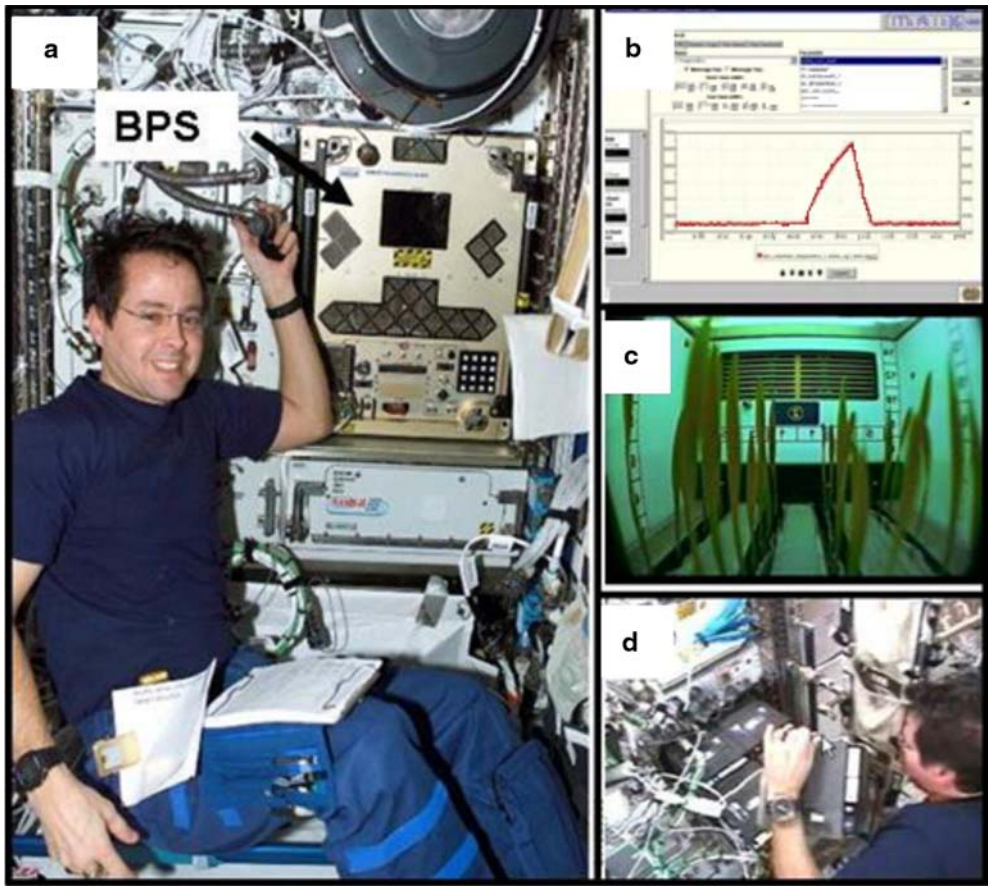

Fig. 1 **a** The PESTO experiment was conducted onboard the International Space Station in the Biomass Production System (BPS) that was installed in EXPRESS rack 4. Payload Engineer Col. Daniel Burch was responsible for experiment oversight during the mission. **b** Continuous experiment monitoring was achieved by remote access to the environmental data that were being archived by the CDS v.1.1 system. **c** Video cameras allowed frame grabs to be obtained at 2 h intervals for the remote monitoring of growth. **d** Each of the BPS PGCs could be independently removed for harvest and planting operations without impacting the environmental control of the remaining plant chambers

used during large-scale (20 m^2 growing area) BLSS testing of wheat (Wheeler et al. 2003). The root zone matric potential (suction) was −0.30 kPa, which equals a hydrostatic head of −3.0 cm measured at the center of the root module. The root modules were removed from stowage, imbibed until saturation using a syringe and placed in the BPS by the payload engineer.

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 in microgravity that landed. The first root modules of the first planting were imbibed on the ground (1*g*) and the remaining ones were imbibed in *μg*. The plants germinated and grew at a light level of ~280 μmol m^{-2} s^{-1} (measured at the top of the chamber), and a photoperiod of 20 h light/4 h dark. During each 21-day planting, the relative humidity and air temperature set-points were 75% and 24°C, respectively. Ethylene concentration was maintained at < 50 nmol-mol^{-1} by a photocatalytic scrubber. CO_2 concentration during the light cycle was not allowed to fall below 1,500 ± 50 μmol mol^{-1}. CO_2 was not controlled during the dark cycle.

Ground control experiment

The ground control experiment was started on Earth 2 weeks after the launch in a BPS chamber identical to the flight unit. 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 (KSC). There were no statistical differences between the flight and ground treatments for PPF (260 ± 23 vs. 270 ± 24 μmol m^{-2} s^{-1}), air temperature (24.1 ± 0.2 vs. 24.1 ± 0.2°C), relative humidity (79.4 ± 6 vs. 76.5 ± 1%), or CO_2 concentration (2,538 ± 870 vs. 2,600 ± 1,100 μmol mol^{-1}). It should be noted that CO_2 concentration was controlled to the 1,500 μmol mol^{-1} setpoint only during the 20 h light cycle, and not during the 4 h dark cycle when CO_2 increased in the chambers due to canopy respiration. All the on-orbit operations performed during spaceflight were mimicked during the ground control experiment.

Plant stand photosynthesis

Canopy level photosynthesis was measured to determine whether *μg* affected the growth of the wheat. This was possible since there was no difference observed in the germination and early seedling establishment between the flight and ground treatments (Stutte et al. 2003). The rate of canopy photosynthesis (P_{net}) was obtained using the semiclosed 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 concentration in the BPS

chambers during the light cycle. Photosynthesis measurements were possible only after the plants in the chambers were large enough to bring down the chamber CO_2 concentration to the 1,500 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 (Stutte et al. 2000; Morrow et al. 2001).

Measurements of canopy response to CO_2 concentration and photosynthetic photon flux levels were made at 15 and 20 DAI using closed system gas exchange techniques (Pearcy et al. 1991; Wheeler 1992; Stutte et al. 2000, 2001) that were possible due to the low chamber leakage rates and CO_2 monitoring capabilities of the BPS. Light and CO_2 response curves were measured to determine if *μg* reduced the efficiencies of CO_2 fixation or light capture. By determining whether the CO_2 compensation point changed for the canopy, *μg* effects on canopy respiration could be inferred (Pearcy et al. 1991). The closed system techniques are referred to as "drawdowns" since, in the absence of active CO_2 control in the plant chamber, the canopy removes CO_2 through photosynthesis and "draws down" the CO_2 concentration in the chamber. Changes in the CO_2 in the chamber during these closed system tests were also corrected for leakage to the ISS cabin air. In preparation for these tests, the CO_2 in the PGC was increased to 2,500 μmol mol^{-1} and allowed to stabilize for 30 min, the CO_2 control was disabled for 45 min and the CO_2 drawdown in the chamber measured every 2 min. At the end of the drawdown cycle, the CO_2 was increased to an elevated level (~2,500 μmol mol^{-1}), the PPF reduced to 75% PPF, and the cycle repeated. The CO_2 response curves were obtained at PPF's corresponding to 100, 75, 50, 25 and 0% of full power (280, 215, 123, 76, and 0 μmol m^{-2} s^{-1}). The change in CO_2 concentration in the chamber over 10 min was used to calculate the P_{net} of the canopy. A 5-point (10 min) running average was used to calculate the mean CO_2 concentration observed during that 10 min drawdown period. The resulting P_{net} was then determined for each CO_2 concentration and the P_{net} versus [CO_2] response determined. The mean P_{net} between 1,500 and 2,000 μmol mol^{-1} CO_2 was used to determine the CO_2-saturated P_{net} at each PPF level. Canopy P_{net} was expressed on a ground area basis.

Characterization of chlorophyll, PSI, PSII, and WCE transport of live plants upon landing

Non-destructive measurements of chlorophyll content from single leaves were obtained with a portable chlorophyll detector (Minolta SPAD 502, Spectrum technologies) on 14- and 24-day-old leaves approximately 9 h after returning to Earth in order to determine the light harvesting potential of the leaf. Chlorophyll fluorescence 'yield' (Fv/Fm) was obtained using an Opti-Sciences OS-5 modulating fluorometer to determine if *μg* reduced the light absorption efficiency of the plants. Measurements of PSI, PSII, and WCE transport on isolated chloroplast membranes were obtained using a Hansatech Clark Electrode following techniques of Tripathy et al. (1996) to identify whether *μg* induced changes in the light harvesting apparatus of the 14- and 24-day-old plants that had been returned to Earth. PPF response curves were obtained from single leaves of the 14- and 24-day-old plants using a LiCor 6400 Leaf Photosynthesis meter (Licor, Lincoln, NE) and on isolated leaf disks using the Hansatech Clark Electrode. Lighting for both the single leaf and leaf disks measurements was provided by red LED's.

Dry mass determination

Accumulation of dry mass was calculated from the canopy level CO_2 fixation rates in order to determine how closely changes in P_{net} were correlated with actual dry mass. The daily integral of P_{net} was calculated using the daily "drawdown" data and the total mass flow data into the chambers. The P_{net} was converted to dry mass using a carbon conversion efficiency of 0.69 that was empirically determined through proximate analysis of leaves. Root dry mass was estimated using the techniques of Monje (1993).

Results

Canopy photosynthetic rates were monitored non-destructively for the entire 21-day cycle using a semi-closed gas exchange system technique (Fig. 2). Closed system gas exchange techniques were used to measure canopy light and CO_2 responses at two stages of development corresponding to emergence of two leaves at

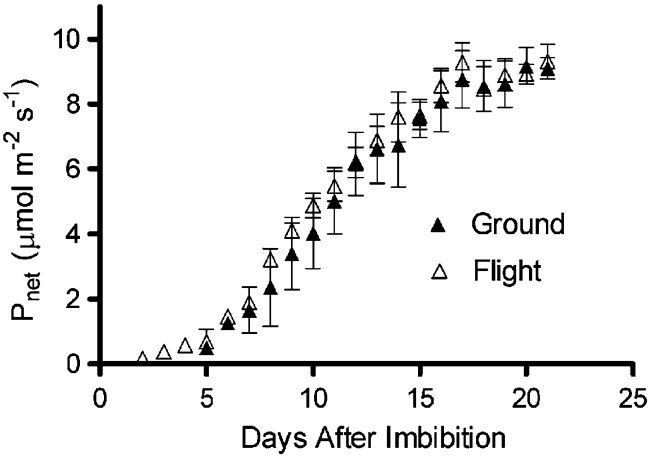

Fig. 2 Canopy net photosynthetic rate (P_{net}) of *T. aestivum* cv. USU Apogee over 21 days of development in *μg*(-△-) or 1*g*(-▲-) ($n = 4$ chambers). Data derived using semiclosed system analysis of daily CO_2 additions to maintain CO_2 setpoint in the chamber. Data represent mean ± standard deviation

~15 DAI and emergence of five or more leaves at ~20 DAI (Stutte et al. 2000). The response of a 15 DAP canopy to changes in CO_2 and PPF are shown in Fig. 3a. The chamber was allowed to equilibrate with CO_2-saturated canopy photosynthesis at 300 µmol m^{-2} s^{-1} PPF (~2500 µmol mol^{-1} CO_2); CO_2 control into the chamber was disabled allowing the canopy to "drawdown" CO_2 to the compensation point Fig. 3b. This allowed measurement of CO_2 response curves at each light level (Fig. 3c).

The results suggest that no difference in canopy P_{net} exists between *µg* and 1*g* plants grown under moderate light (daily integral of 21.16 M PAR day^{-1}) and saturating CO_2 (1,500 µmol mol^{-1}) conditions. Maximum canopy photosynthetic rates (7.8 µmol m^{-2} s^{-1}) and the CO_2 compensation points (121 and 118 µmol mol^{-1}) were not significantly different between the *µg* and 1*g*-grown plants at 15 DAI (Table 1). Similar results were obtained from the canopy P_{net} measurements of 20 DAI plants that had rates of 7.4 and 7.2 µmol m^{-2} s^{-1} in the flight and ground treatments, respectively (Monje et al. 2005).

It was also possible to determine the response of canopy P_{net} to PPF from the CO_2 drawdowns conducted during each cycle. The resulting response curves to PPF showed that P_{net} increases linearly with increasing PPF up to 280 µmol m^{-2} s^{-1} (maximum output of the BPS fluorescent lamp canopy) at saturating CO_2 concentrations (1200–2000 µmol mol^{-1}) in both the *µg* and 1*g* treatments (Fig. 3c). Similar results were obtained from the canopy gas exchange measurements of 20 DAI plants (data not shown).

The PPF compensation point (85 µmol m^{-2} s^{-1}) and quantum yield (QY) of canopy photosynthesis from 15 DAI plants were also not statistically different between *µg* and 1*g*-grown plants (Table 1). The canopy QY of 0.036 for plants at 15 DAI on orbit and 0.043 for 20 DAI plants were typical of those observed in wheat grown in 20 m^2 (Wheeler et al. 2003), 1 m^2, (Monje et al. 1998), and 0.0264 m^2 (Stutte et al. 2000, 2001) planting areas in closed systems at comparable CO_2 concentrations and PPF.

Dry mass per plant was not significantly different between the flight (0.113 g) and ground (0.122 g) treatments (Table 1). This is consistent with canopy P_{net} being the same in flight and ground treatments. When the steady-state measurements of photosynthesis were used to estimate chamber dry mass, the results underestimated the actual dry mass by 7–9% (Table 2). This is most likely due to the inability of the semiclosed gas exchange technique to account for the re-assimilation of respiratory CO_2 produced during initial seed germination and establishment. Carbon uptake could not be measured because the PGCs are closed, the CO_2 accumulated in the chambers, and no additions were made until the plants were able to reduce CO_2 to the setpoint (typically 5–7 DAI). In contrast, the closed gas exchange technique resulted in estimates within 4% of the actual dry mass for both the flight and ground control plants

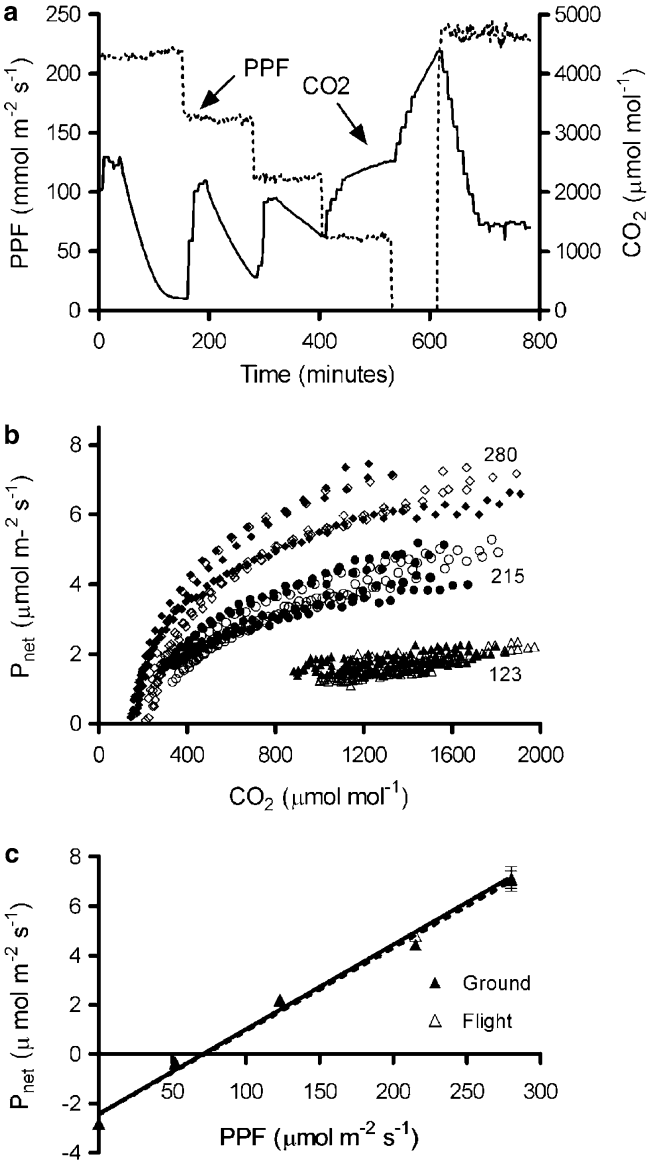

Fig. 3 Derivation of carbon dioxide and light effects on 15-day-old *T. aestivum* cv. USU Apogee canopy in microgravity. **a** Typical data downloaded from the BPS during showing the change in CO_2 (—) in the PGC in response to differing PPF (—-) levels used to characterize canopy photosynthetic responses at 100, 75, 50, 25, and 0% power to the lamps. **b** Canopy CO_2 response curves obtained under *µg* (open) and 1*g*(closed) conditions at 15 DAI ($n=4$) at three light levels: 280 (◇, ◆), 215 (○, ●), and 125 (△, ▲) µmol m^{-2} s^{-1}. There was no significant difference ($P>0.05$) between the CO_2 response curves for the light levels tested. **c** The mean P_{net} between 1,300 and 1,700 µmol mol^{-1} CO_2 was obtained using the PPF response curve of canopy photosynthesis at saturating CO_2. Data derived from drawdowns for both *µg* (△) and 1*g*(▲) control ($n=4$ chambers) at five light levels: 280, 215, 123, 52 and 0 µmol m^{-2} s^{-1} PPF was used. Data represent the mean and standard error for each measurement ($n=$ 32–58)

(Table 2). The growth of plants in each chamber was remotely monitored using downlink of images from the BPS at 2 h intervals. There were no visual effects on plant growth, such as chlorosis, epinasty, or disorientation, observed during the experiments (Fig. 4a-l). Non-

Table 1 Plant dry mass, canopy photosynthesis, CO_2 compensation point, PPF compensation point and quantum efficiency from μg and 1*g*-grown *T. aestivum* cv. USU Apogee plants.

Treatment	Dry mass (g plant^{-1})	P_{net} (μmol m^{-2} s^{-1})	CO_2 Comp (μmol mol^{-1})	PPF Comp (μmol m^{-2} s^{-1})	QY ($\frac{\mu mol\ PPF\ m^{-1}s^{-1}}{\mu mol\ CO_2\ m^{-1}s^{-1}}$)
Flight (*μg*)	0.113	7.8	121	85	0.036
Ground (1*g*)	0.122	7.8	118	81	0.035
$P > 0.05$	NS	NS	NS	NS	NS

P_{net} Maximum canopy photosynthetic rate at 280 μmol m^{-2} s^{-1} PPF and saturating (1,500–2,000 mmol mol^{-1}) CO_2; CO_2 comp = CO_2 compensation point; PPF_{comp} PPF compensation point, QY quantum yield of canopy photosynthesis. Dry mass values obtained from lyophilized 21-day-old plant tissue. Mean separation by *t*-test. *NS* non-significant.

Table 2 Dry mass accumulation of *T. aestivum* cv. USU Apogee grown in microgravity vs. ground and comparison of steady-state and non-steady-state techniques for measuring canopy level P_{net} used to estimate dry mass accumulation

Measurement	Flight (g dm PGC^{-1})	Ground (g dm PGC^{-1})	$P > 0.05$
Dry Mass: direct	4.76	4.86	NS
Steady state: indirect	4.45	4.43	NS
(% direct)	(93)	(91)	–
Non-Steady State: indirect	4.98	5.06	NS
(% direct)	(104)	(104)	–

Dry Mass: directly determined from lyophilized tissue harvested on orbit. *PGC* Plant growth chamber, each with 30–32 plants per chamber. Mean separation by i-test. *NS* non-significant, * significant $P > 0.05$.

destructive measurements of leaf chlorophyll content of the flight and ground tissue of the 14-day-old (162 vs. 152 μg mm^{-1}) and 24-day-old (126 vs 135 μg mm^{-1}) living tissue returned to Earth showed no significant differences in chlorophyll content (Table 3). There was slight, but statistically significant, decrease in the Fv/Fm ratio (0.77 vs. 0.79) observed in the 24-day-old flight tissue that was returned to Earth (Table 3; Fig. 4).

Light response curves were also obtained from isolated leaf disks of plants from PGCs 1 and 3 developed in *μg* that had been returned to Earth using a Clark O_2 electrode (Tripathy et al. 1996). There was no difference in light response curve of single leaves up to 800 μmol m^{-2} s^{-1} PAR for either the 14 or 24 DAI plants. At PPF intensities greater than 800 μmol m^{-2} s^{-1} there was a decrease in P_{net} from 7 to 12% in the *μg* versus the 1*g* tissues. The activities of PSI, PSII, and WCE were obtained on isolated chloroplast membranes isolated from 14 and 24 DAI plants under saturating PPF and CO_2 conditions within 9 h of their return to Earth. The PSII activity of the isolated chloroplasts was 6–8% lower in the *μg* than 1*g* treatments for plants that were 14 DAI and 13% lower in plants that were 24 DAI under these CO_2 and PPF saturating conditions (Fig. 5). There was a slight decrease in the PSI and WCE activities of the 14-DAI-old flight tissue (3.4 and 2.4 %, respectively). The reduction in PSI and WCE activities (13.8% and 15.6%, respectively) of the 24-DAI plants was comparable to that observed with PSII.

Table 3 Chlorophyll content, and chlorophyll fluorescence of 14- and 21-day-old *T. aestivum* cv. USU Apogee grown in microgravity and analyzed 9 h after return to Earth

	Chlorophyll (*μ* g mm^{-1})		Fv/Fm	
Treatment	14D	24D	14D	24D
Flight (*μg*)	162	126	0.80	0.77
Ground (1*g*)	152	135	0.79	0.79
$P > 0.05$	NS	NS	NS	*

Mean separation by *t*-test. *NS* non-significant, * significant $P > 0.05$

Discussion

These experiments represent the first direct, replicated measurements of stand level photosynthesis under *μg* conditions. The data strongly suggest the hypothesis that canopy photosynthesis rates are reduced by the stress of *μg* is not correct and that photosynthetic carbon assimilation operates at the same rate as in 1*g*. The data also suggest that the function of the photosynthetic apparatus per se is not negatively affected under moderate light levels and saturating CO_2 conditions.

P_{net} of plants initially germinated under 1*g* conditions and transferred to *μg* were identical to P_{net} from gravity naïve plants. These results contrast from those reported by Kordyum (1997) and Tripathy et al. (1996), who showed a reduction of 20–25% in dry mass as well as PSI and/or PSII efficiency. It is probable that indirect effects caused by lack of control of the secondary environmental effects such as the lack of buoyancy-driven convection, fluctuating CO_2 conditions, and lack of RH control in the PGC used in the earlier experiments resulted in reduction in photosynthesis (Porterfield 2002; Monje et al. 2003). The upward, turbulent air flow through the BPS PGC would have been sufficient to counter the microgravity-induced increases in the leaf boundary layer (Monje and Stutte, unpublished). Further, the high fidelity environmental control of the BPS chambers and the replication of the growth cycles in microgravity, provides strong support to the absence of the *μg* effect under these conditions. Especially signifi-

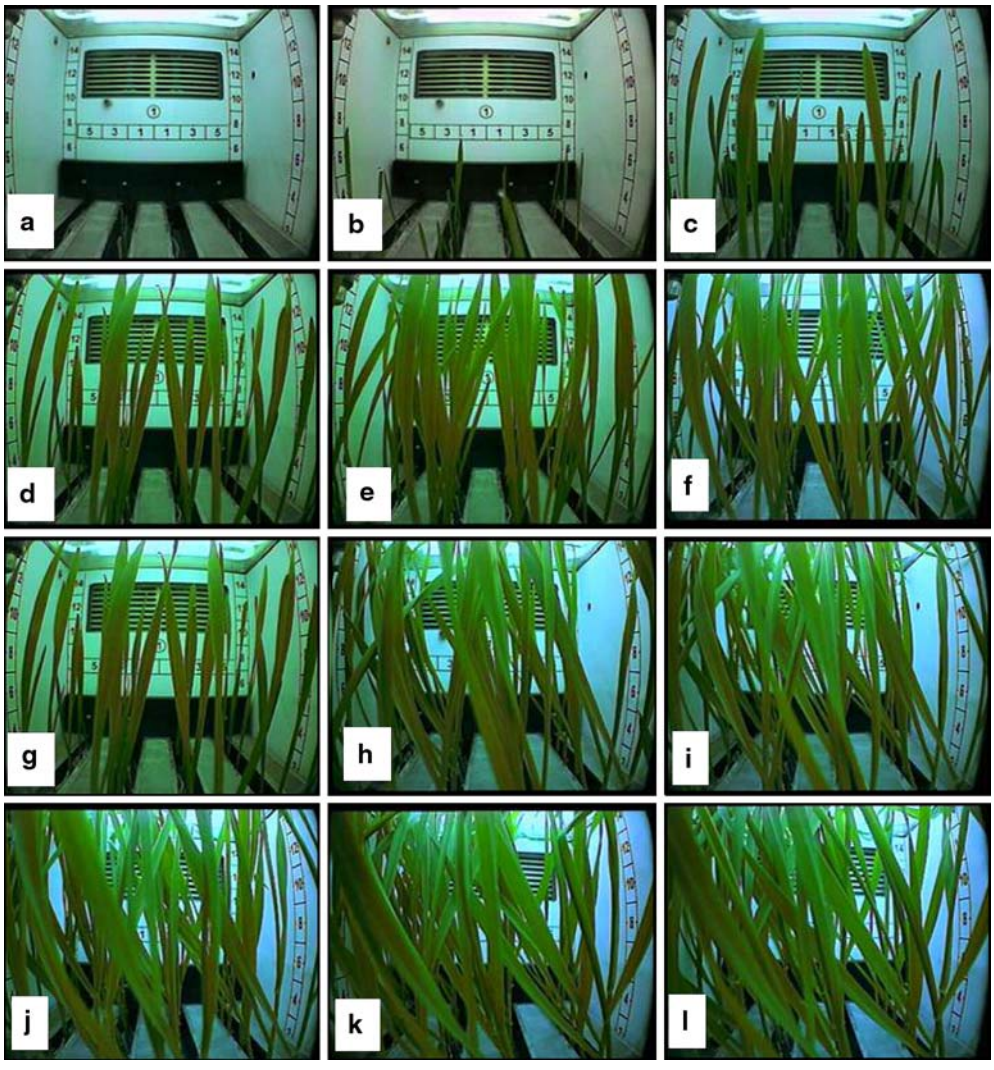

Fig. 4 Growth of *T. aestivum* cv. USU Apogee in microgravity from 4 to 15 DAI (**a–l**). The images were obtained from camera installed in the Biomass Production System at 2 h intervals and downloaded from ISS daily during mission . There were no obvious alterations in crop development (e.g., shoot orientation, epinasty) at any stage of development

cant would be the presence of adequate environmental control to overcome secondary events related to lack of convective current and CO_2 effects in chambers used in earlier plant growth experiments. The chambers used in experiments of Tripathy et al. (1996) lacked active air circulation and RH control, and as such, experimental artifacts associated with increased boundary layer around leaf surfaces may have reduced intracellular CO_2 resulting from diffusion-limited gas exchange, and high relative humidity in the chambers may have altered results. A second difference is that the results obtained from plants grown between the flight and ground were measured at saturating PAR and CO_2 concentrations on plants that were grown under very low PAR and super-elevated CO_2 concentrations. The post-flight analysis of PSI, PSII, and WCE transport of leaves grown during PESTO suggested that PSII was reduced at saturating light (2,500 μmol m^{-2} s^{-1}) but not at lower light intensities (< 800 μmol m^{-2} s^{-1}). The decrease in PSII and PSI was not as great as reported in the previous flight experiments (Tripathy et al. 1996; Jiao 1999) and may reflect adaptation of the plants to the higher (300 μmol m^{-2} s^{-1}) PPF levels. However, this decrease in photosynthetic efficiency under saturating light did not appear to result from alteration in the chlorophyll–pigment complexes as evaluated by low-temperature chlorophyll fluorescence (data not shown).

There was a slight reduction in the PSII (7.4%), WCE (3.4%), and PSI (2.3%) activities of the 14 DAI plants that were returned to Earth. As the plants aged, the effect of *μg* on the photosynthetic apparatus had increased by ~13% for all components. This effect was manifested under saturating light and CO_2 conditions, and it is difficult to determine what the primary effect of *μg* on the photosynthetic apparatus is. There were no apparent differences in the structure of the thylakoid membranes, although preliminary microscopy evidence indicates that grana stacking were not so dense in chloroplasts developed under flight conditions (data not shown).

The CO_2 compensation point of 120 μmol mol^{-1} for both the flight and ground treatments suggests that *μg* conditions are not affecting the basal respiration rates of the canopy (Pearcy et al. 1989). These values were ob-

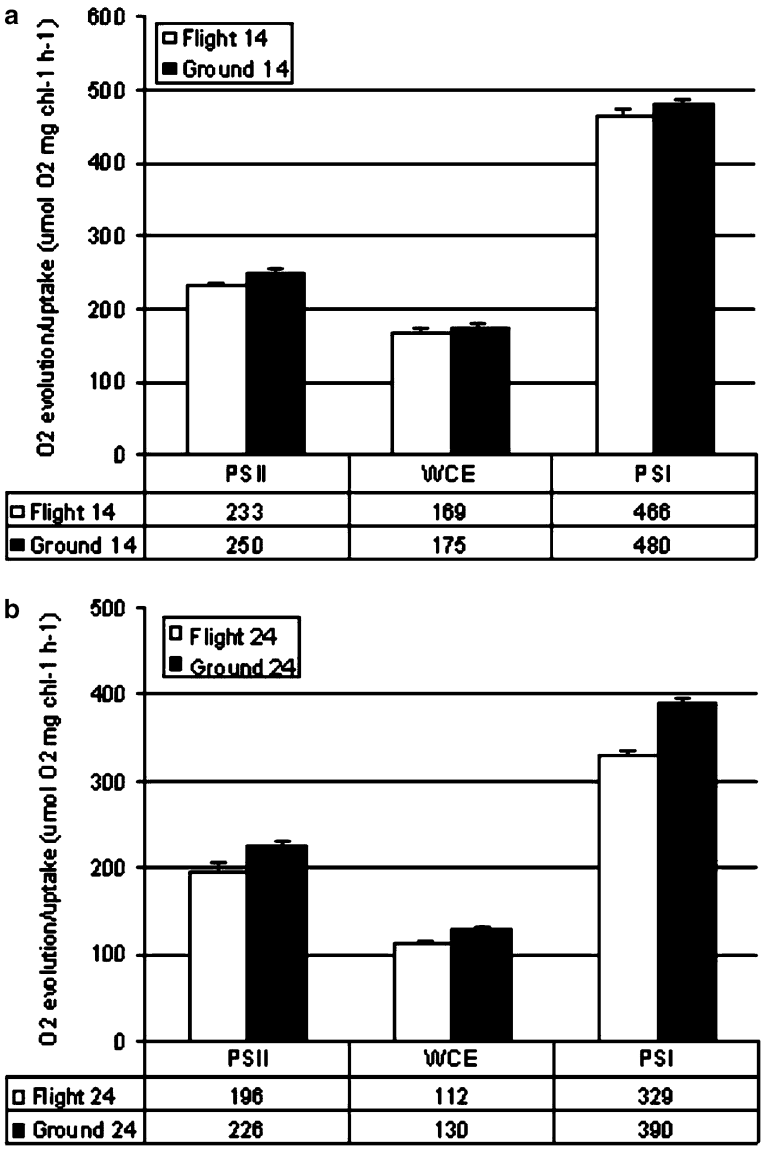

Fig. 5 Measurements of Photosystem I (PSI), whole chain electron (WCE) transport, and Photosystem II (PSII) were made with a Clark-electrode on 14 days (**a**) and 24 days (**b**) *T. aestivum* cv. USU Apogee that were grown in microgravity and returned to Earth. Measurements were made on isolated membranes under saturating CO_2 (5,000 µmol m^{-1}) and PPF (2,500 µmol m^{-2} s^{-1}) conditions within 9 h of landing

tained from multiple plantings (four) that had the same number of plants, growth rates (Stutte et al. 2003), and P_{net} (Monje et al. 2005) throughout development. The CO_2 compensation point, as expected, was independent of PPF (Pearcy et al. 1989).

Light saturation of P_{net} was not achieved in this experiment due to the limited number of lamps and power limitations imposed on the BPS (Iverson et al. 2003). However, the canopy level PPF obtained was the target PPF recommended by the NASA "Tiger team" for baseline studies on ISS (Goins et al. 2003). The PPF compensation point and the QY for wheat canopies were identical in the flight and ground treatments from 0 to 280 µmol m^{-2} s^{-1}. Similarly, the PPF compensation point and QY of single leaf measurements were identical from 0 to 800 µmol m^{-2} s^{-1} PAR. At PPF levels greater than 800 µmol m^{-2} s^{-1} the maximum P_{net} of single leaves was ~20% higher in the ground treatments. This effect is less than, but consistent with, the results reported by Tripathy et al. (1996). It is postulated that since the plants grown on ISS were at PPF levels ~4 × that of Tripathy et al. (1996) (280 vs. 60 µmol m^{-2} s^{-1}) the photosynthetic apparatus (PSII, WCE, PSI) was better adapted to perform at the higher light levels. This apparent decrease in photosynthetic efficiency does not occur at PPFs anticipated for flight systems (< 800 µmol m^{-2} s^{-1}). However, *µg* testing is necessary to confirm this hypothesis.

The use of non-steady-state gas exchange measurements to estimate dry mass provided a good correlation of total biomass in the chambers. The use of steady-state measurements tended to underestimate total biomass since the re-assimilation of respiratory CO_2 released during early seed germination was not accounted for. However, these data do indicate that the use of gas exchange measurements, coupled with remote imaging of plant development, is an effective tool for monitoring the growth and development of crops on spacecraft.

In conclusion, the CO_2 saturation, CO_2 compensation point, PPF compensation point, and QY of canopy P_{net} of wheat were not negatively affected by μg during the first 24 days of canopy development. The data suggest that μg per se is not a significant environmental stress affecting canopy photosynthesis and subsequent dry mass accumulation. There are indications that the μg environment reduces the activities of the PSI, WCE, and PSII under high (>800 μmol m^{-2} s^{-1} PAR) light conditions. If indeed the results are verified in subsequent spaceflight experiments, then these experiments underscore the importance of providing high-fidelity environmental control to counter secondary environmental influences of microgravity on plant growth and development (Musgrave et. al. 1997; Monje et al. 2003).

The ability of Apogee wheat to assimilate carbon in μg at the same rate as in 1g in ISS environments (24°C, 280 μmol m^{-2} s^{-1} PPF, and 1,500 μmol m^{-2} s^{-1} CO_2) has important implications for long-duration space exploration missions, such as 3 year missions to Mars, and even longer colonization missions. These environmental conditions are very similar to the ambient environment anticipated for long-duration transit and/or surface missions, and thus suggest that ground-based measurements of canopy P_{net} and dry mass production can be used to predict rates of biomass accumulation and O_2 evolution. These predictions are needed to design and model future facilities to support space exploration missions and should be applicable as long as a moderate light level and saturating CO_2 are provided. The data further suggest that under higher light intensities ($>$ 800 μmol m^{-2} s^{-1} PPF) μg-induced changes in PSI, PSI, and WCE may result in a lower rate of photosynthesis than projected from ground experiments. However, this hypothesis requires validation under μg conditions, since it is also possible that the reduction in PSI, WCE, and PSII activities observed in PESTO is due to testing the tissue returned to earth at PPF intensities $\sim 10\times$ than that greater under which they were grown.

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