Radish (*Raphanus sativa* L. cv. Cherry Bomb II) Growth, Net Carbon Exchange Rate, and Transpiration at Decreased Atmospheric Pressure and / or Oxygen

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ABSTRACT

To simplify engineering requirements for plant growth structures on the Moon or Mars, lower pressures are desirable to reduce mass and decrease atmospheric leakage. In order to establish the effect of reduced pressure and reduced oxygen on carbon assimilation, dark period respiration, transpiration, and plant growth, radishes (*Raphanus sativa* L. cv. Cherry Bomb II) were grown at 98 (ambient pressure), 66 (2/3 atm), 33 (1/3 atm), and 10 (1/10 atm) kPa total pressures with oxygen partial pressures of 20, 14, 7, and 2 kPa for 21 days. All plants were grown in rockwool using recirculating nutrient film technique hydroponics. Analysis of growth showed no significant difference among the 98, 66, 33, and 10 kPa total pressure environments when the oxygen partial pressure was ≥ 7 kPa, but a significant reduction was observed when the oxygen partial pressure was dropped to 2 kPa, regardless of the total pressure. Net carbon exchange rate (NCER) and transpiration showed a similar pattern, with no significant effect with pressure treatments. Only the reduced oxygen partial pressure treatment of 2 kPa resulted in significant reductions in NCER and transpiration. Results indicate that pressure has little effect on radish productivity as long as oxygen levels are maintained at or above 7 kPa.

Key words: Advanced Life Support; Atmospheric Control; Controlled Environment; Hypobaric; Hypoxia; Low Pressure; Plant Growth Chamber

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INTRODUCTION

Interest in manned space exploration has furthered research into human life support technologies. As mission durations increase, the ability to re-supply from Earth diminishes (Simpson and Young, 1998; Wheeler et al., 2001). It is likely that advanced life support systems for long-term space exploration and habitation will be comprised of some combination of physical-chemical and bioregenerative systems, and that plants (for food and oxygen production and carbon dioxide removal) will be an integral part of those systems (Wheeler et al., 2001; Ferl et al., 2002b; Spanarkel and Drew, 2002). The utilization of low pressure plant growth facilities would lessen force on the structure, decrease the requirement for start-up consumables (e.g., pressurizing gas), and reduce the amount of atmospheric leakage from the structure (Corey et al., 1996; Rygalov et al., 2001, 2002; Spanarkel and Drew, 2002). Past studies of pressure effects

on plants have typically used small to moderate scale growth chambers that allowed short-term experiments of small numbers of compact crops (Musgrave et al., 1988; Corey et al., 1996; Daunicht and Brinkjans, 1996; Iwabuchi et al., 1996; Massimino and André, 1999; Ferl et al., 2002a; Goto et al., 2002; Spanarkel and Drew, 2002; Richards et al., 2006; He et al., 2009). Many of these pioneer systems were built from chambers originally constructed for an alternate use, and often lacked the appropriate degree of environmental control (e.g., temperature, humidity, CO_2 partial pressure, O_2 partial pressure) (Daunicht and Brinkjans, 1996; Massimino and André, 1999). Additionally, deficiencies in available instrumentation and control systems limited the ability to produce consistent and reliable results (Daunicht and Brinkjans, 1996; Spanarkel and Drew, 2002).

The effects of reduced pressure and oxygen partial pressure on long-term plant growth and development have not been fully characterized. Due to increased diffusion coefficients at low atmospheric pressures, it has been hypothesized that there will be increased gas exchange between the plants and their environment thereby increasing photosynthesis by elevating the availability of carbon dioxide in the mesophyll (Gale, 1973; Rygalov et al., 2002). Past experiments have examined a variety of species including wheat (Massimino and André, 1999), tomato (Rule and Staby, 1981; Daunicht and Brinkjans, 1996), lettuce (Corey et al., 1996; Spanarkel and Drew, 2002; He et al., 2003; He et al., 2006, 2007), spinach (Iwabuchi et al., 1995), rice (Goto et al., 2002), and radish (Levine et al., 2008). But the results of these studies are difficult to compare if both total pressure and O_2 partial pressure (pO_2) changed (Richards et al., 2006; He et al., 2007, 2009) and if total exposure times varied (Iwabuchi and Kurata, 2003). Thus, depending on the study, the effects of pressure on plant growth have varied, but it is apparent that plants can withstand some degree of atmospheric alteration and that some acclimation likely occurs (Goto et al., 2002; Paul et al., 2004; Richards et al., 2006).

Many plant studies have shown the importance of atmospheric oxygen in seed development (Quebedeaux and Hardy, 1973, 1975; Musgrave and Strain, 1988; Kuang et al., 1998; Wehkamp et al., 2007) and during seed germination and early seedling establishment (Bewley and Black, 1994). As well, many plant species can tolerate some level of decreased oxygen partial pressure (hypoxia), such as deep in root tissue or during periods of high rates of cellular metabolism (Geigenberger, 2003; Bailey-Serres and Chang, 2005). Oxygen partial pressures are known to affect plant growth and development and the plant's responses are dependent on the cell or tissue types, developmental stage, genotype, severity and duration of hypoxia, light and temperature conditions (Fukao and Bailey-Serres, 2004). Low oxygen levels reduce respiration by limiting adenosine triphosphate (ATP) production by oxidative phosphorylation (Geigenberger, 2003; Fukao and Bailey-Serres, 2004; Bailey-Serres and Chang, 2005), and in C3 plants low oxygen can increase net carbon exchange rates (NCER) and reduce photorespiration by reducing the oxygenase activity of RuBisCO (Warburg effect). Recent studies with lettuce grown under different combinations of pressure and oxygen have shown that pressures down to 25 kPa do not adversely affect gas exchange or vegetative growth (He et al., 2006, 2007, 2009).

To study the effects of reduced pressure and oxygen partial pressure on the growth and development on plants further, the following study was conducted with radish plants, an edible root vegetable, which have storage organs (hypocotyls) partially below ground and hence might be especially sensitive to reduced pO_2 levels. In this experiment, all other environmental parameters were maintained at stable setpoints and the radish plants were grown from young seedlings in order to ascertain the effects of reduced total pressure and/or oxygen partial pressure throughout plant development. Radish was chosen as the test crop due to its rapid growth, high harvest index, desirable nutritional characteristics, and its common inclusion as a candidate crop for space-based life support systems (Salisbury and Clark, 1996).

MATERIALS AND METHODS

Hypobaric Chambers

Each of the four fully automated (Argus Controls, White Rock, British Columbia, Canada)

hypobaric chambers used in this investigation measured 1.0 x 1.8 x 2.5 meters (WxHxD), with a total volume of approximately 4500 liters and an internal plant growing area of 1.5 m^2 (Figure 1). All internal surfaces were made of 316 stainless steel, with the exception of the heat exchangers, which were made of brass with a Heresite baked enamel coating, and the glass roof panels (20.5 mm 2 layer laminate).

Figure 1. One of five hypobaric chambers in the Controlled Environment Systems Research Facility (CESRF) at the University of Guelph (left). Visible are the main door and closing mechanisms, lighting canopy, and nutrient system. Also shown is a 21 day old radish crop prior to harvest (right).

Control of temperature and vapour pressure deficit (VPD - the difference between the amount of moisture in the air and how much moisture the air can hold when it is saturated at a given temperature) was performed by recirculating chamber air with a variable speed blower through chilled water (5°C) and hot water (55°C) heat exchange coils located at the rear of each chamber. The cold exchange coil was used to control the VPD, while the hot exchange coil was used to reheat the cooled air to regulate the final desired temperature. In order to maintain adequate airflow through the plant canopy at all pressures, the blower speed control was coupled to monitored pressure values. Chamber temperature and relative humidity were measured using two combination T/RH sensors (Model 4139: Honeywell Inc., Mississauga, ON, Canada) per chamber. Temperature control on the hot and cold heat exchange coils utilized Argus TN2 temperature sensors (4 per chamber), as did the nutrient film technique (NFT) media temperature probes (2 per chamber). Control of temperature averaged +/- 0.5°C over the course of the experiments at all pressures. VPD remained within 0.05 kPa of setpoint for the duration of the

experiments, although there were slight fluctuations during transitions between day and night due to changes from the lamp heat load. As VPD is coupled to temperature, the changes were greater in the low pressure (33 and 10 kPa) treatments. Relative humidity was controlled within +/- 2.5% of setpoint.

Evapotranspiration was measured by collecting atmospheric condensate from the heat exchange coils using a tipping bucket (Model: WS-7048U, La Crosse Technology, La Cross, WI, USA). The resolution obtained using this method was approximately 4.2 mL. Condensate values were converted to liters of evapotranspiration per m^2 per day and corrected for direct evaporation using condensate production rates collected when seedlings were small and transpiration was negligible. This provided an estimate of actual plant canopy transpiration.

Pressure control utilized a large vacuum pump (Model NC0070.ABMG.000F, Busch Vacuum, Mississauga, ON, Canada), capable of reducing ambient pressure to less than 1 kPa. Chamber pressure was measured using a pressure sensor (MKS Baratron Type 627B, MKS Instruments, Ottawa, ON, Canada) and was continuously monitored by the control system. When the pressure was above the setpoint, the control system opened a solenoid valve (Model SS-9254-C-SI, Swagelok, Sarnia, ON, Canada) connected to the low pressure system. The available control range was +/- 0.1 kPa, however a larger range was used to minimize potential volume losses during heating/cooling cycles, which alter chamber pressure and could signal a requirement for air removal.

The carbon dioxide/oxygen sampling system was based on repressurization of hypobaric chamber air. Air was continuously removed by a vacuum pump (Model UN820.3 FTP, KNF Neuberger Inc., Trenton, NJ, USA) and repressurized in a sampling loop controlled by a non-bleed precision pressure regulator (Model 35503020, Parker Hannifin Instrumentation Products Division, Cleveland, OH, USA) and needle valve (Model H-300-SS-L-R-1/4-A: HAM-LET Valves and Fittings, Mississauga, ON, Canada) for precise control. A pressure gauge (Model 25-210-3psi: Noshok, Berea, OH, USA) pH levels were maintained through manual adjustment daily to +/- 0.1 pH units. Acid, base, was used to monitor and manually set the sampling stream to 1.4 kPa at chamber pressures from 90 to 10 kPa. Ambient pressure treatments did not require pressurization for sampling. Prior to introduction of the gas stream to the CO_2/O_2 analyzer (Model 200: California Analytical Instruments, Inc., Orange, CA, USA for 0-6000 μmol mol^{-1}, LI-COR LI-820, Lincoln, NE, USA for 0-20,000 μmol mol^{-1}), the air stream was chilled to remove water. Condensate and the sampled air stream were returned to the chamber to ensure full system gas loop closure.

Chamber gas composition was controlled by analyzer feedback to the control system that operated separate mass flow controllers (Model 810S: Sierra Instruments, Inc., Monterey, CA, USA) for pure oxygen, carbon dioxide, and nitrogen gases. Pure gases were supplied by external K-size cylinders (BOC Gas Supply, Ltd., Guelph, ON, Canada). The available carbon dioxide control range was between 0 and 20,000 μmol mol^{-1}, while oxygen could be controlled between 0 and 100%. Nitrogen was used to make up the balance of the gas composition.

Chambers were outfitted with six 1000 Watt HPS lamps (P.L. Light Systems Inc., Beamsville, ON, Canada). The externally mounted lighting canopy was cooled with a chilled water heat exchanger coupled to a blower that circulated chilled air across the chamber glass roof panels. Two LI-190SA PAR sensors (LI-COR Inc., Lincoln, NE, USA) continuously monitored irradiation from the light source.

The nutrient solution delivery system utilized a nutrient film technique design. Water was stored in a 200 liter temperature controlled external stainless steel tank. A pump (Model PKG-UOG: International Pump Technology Inc., Fergus, ON, Canada) provided sufficient pressure for in-tank circulation through a sensor loop and chamber trough delivery. Return of water from the chamber was by gravity. All external storage tanks for stock nutrients, acid, and base were maintained at chamber pressure through a series of pressure compensation lines. Electrical conductivity (EC) sensors (Argus Control Systems, Inc., White Rock, BC, Canada) were used to measure nutrient concentration and EC control with a setpoint of 1200 μS was +/- 10 μS. and nutrient stock solutions were added using gravity feed from stainless steel reservoirs

controlled with air actuated valves (Model SS-92S4-C-S1, Swagelok, Sarnia, ON, Canada).

Assessment of Growth and Productivity

Plant growth and productivity measurements for the series of 21-day pressure studies with radish included dry mass of roots (swollen hypocotyls) and leaves as well as leaf area (LA). Values were obtained using an electronic balance (Sartorius, Gottingen, Germany) and a leaf area meter (LI-3000, LI-COR Inc., Lincoln, NE, USA). Data were collected on a per plant basis for the entire chamber and harvest index (HI) and specific leaf area (SLA) calculated from the resultant data. No attempts were made to retrieve fibrous roots from the rockwool media, but previous hydroponic studies have shown these comprise only about 5% of the total plant biomass (Mackowiak et al., 1994).

Carbon Assimilation and Dark Respiration

Whole stand NCER measurements were calculated from the slope of the daily carbon dioxide injection profile. Dark period respiration rates were calculated from the slope of the carbon dioxide evolved over the night period. As it was impossible to access the plants during the experiment, the photosynthesis, respiration, and transpiration data could not be reported on a dry weight or leaf area basis and was therefore expressed on the available growing area. Hence these rates are closely correlated with changing canopy cover (and light interception) as the plants grow. The growth period examined was from 3-17 days after planting (DAP). Carbon assimilation and dark respiration data for 18-21 DAP were not available due to subsequent tests to determine light and carbon dioxide compensation points, which are not reported here.

Plant Material

Radish (*Raphanus sativa* L. cv. Cherry Bomb II) was grown from seed in 1.4 x 0.4 m stainless steel troughs with stainless steel covers to minimize evaporation and algal growth on the growth medium. Each cover had 4 cm diameter holes placed at 9-10 cm centers within the rows and between troughs. There were five troughs per chamber and 24 plants per trough for a total of 120 plants per chamber. Rockwool slabs (Grodan, Hedehusene, Denmark) were used as the growth medium and a channel was cut from the underside of the slab to facilitate nutrient solution flow. Prior to planting, the rockwool was rinsed twice with deionized water to remove any fabrication residues or particulates. Nutrient solution was a modified, half-strength Hoagland's solution (Wheeler et al., 1999) and the solution was maintained at an electrical conductivity of 1200 $\mu S\ cm^{-1}$ using concentrated stock solutions. pH was manually adjusted daily to 5.8 with 0.5 M nitric acid (HNO_3) or 0.5 M potassium hydroxide (KOH). Prior to the experiment, the nutrient reservoirs, feed lines, and troughs were rinsed with >10 ppm aqueous ozone, and the nutrient stock solutions were autoclaved to reduce microbial contamination for a concurrent bacterial study not reported here.

Radish seeds were planted three per position and allowed to germinate under ambient pressure (98 kPa) for 72 hours. Seedlings were then thinned to one per position and the defined pressure/oxygen treatment imposed. All plants were harvested at 21 days after planting (DAP).

Experimental Conditions

Temperature was isothermal and held at 22ºC with a 16/8 day/night photoperiod. A light intensity of 300 $\mu mol\ m^{-2}\ s^{-1}$ PPF at the hydroponic trough level was provided by dimming the lamps with neutral density screening. Carbon dioxide and the VPD were maintained at partial pressures of 0.12 kPa (equivalent to 1200 $\mu mol\ mol^{-1}$ at ambient pressure) and 0.9 kPa (equivalent to 65% RH at 22ºC), respectively. Treatments included four total pressures (10, 33, 66, or 98 kPa) and four oxygen partial pressures (2, 7, 14, or 20 kPa) with 98/20 kPa combination acting as the ambient control. The average atmospheric pressure in Guelph, Ontario, Canada (334 meters above sea level) averages approximately 98 kPa.

Statistical Analysis

Experimental design for the 21-day plant tests was a randomized block design with each of four chambers being considered a replicate and each treatment was replicated four times. Replication was achieved over time and replicates were cycled through the four chambers to minimize chamber effects.

Regression analysis was performed in S-PLUS version 7.0/8.0 for Windows (Insightful Corporation, Seattle, WA, USA) and ANOVA analysis was performed using SAS version 9.1.3 for Windows (SAS Institute Inc., Cary, NC, USA).

RESULTS

Growth

Visually, there was little difference observed in the quality of the radish plants harvested from the control and the reduced pressure treatments (Figure 2) and there was no significant difference in leaf dry mass, root dry mass, specific leaf area (SLA), or harvest index (HI) across the different pressures when the oxygen partial pressure was at 7, 14, or 20 kPa (Table 1). There was, however, a visual decrease in plant size with plants grown at 2 kPa of oxygen being clearly stressed and stunted. Similarly, there were significant decreases leaf and root dry mass, SLA, and HI at an oxygen partial pressure of 2 kPa regardless of the total pressure (Table 1).

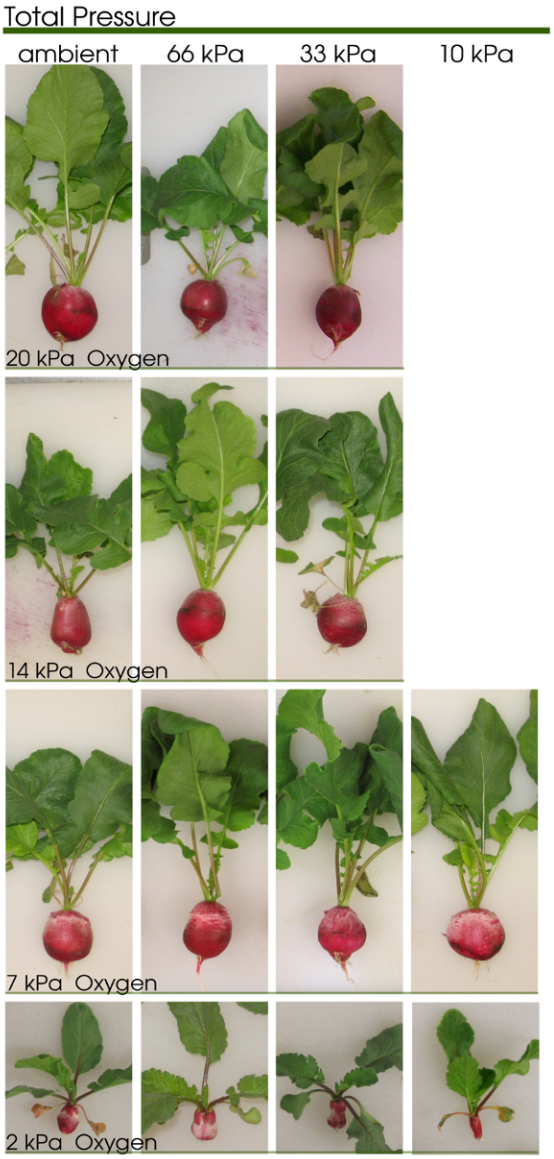

Figure 2. Visual analysis of radish (*Raphanus sativa* L. cv. Cherry Bomb II) grown at ambient, 66, 33, or 10 kPa total pressures (from left to right) and oxygen partial pressures (from top to bottom). Pictures were taken during harvest which was 21 days after planting.

Table 1. The effect of atmospheric pressure (10-98 kPa) and oxygen partial pressure (2-20 kPa) on the growth of radish (*Raphanus sativa* L. cv. Cherry Bomb II) over a 21-day period. The partial pressure of carbon dioxide was maintained at 120 Pa.

Oxygen (kPa)	Pressure (kPa)	Leaf dry mass (g plant^{-1})	Root dry mass (g plant^{-1})	Specific Leaf area (cm^2 g^{-1})	Harvest Index (%)
20	98	0.596[1] (0.0080)a[2]	1.19 (0.019)a	286.9 (1.64)a	65.80 (0.253)a
	66	0.619 (0.0086)a	1.17 (0.018)a	255.5 (5.05)a	64.64 (0.296)a
	33	0.593 (0.0086)a	1.11 (0.017)a	265.9 (6.29)a	64.79 (0.278)a
14	98	0.585 (0.0070)a	1.16 (0.017)a	284.2 (2.03)a	65.80 (0.227)a
	66	0.569 (0.0085)a	1.07 (0.016)a	264.2 (1.60)a	64.83 (0.242)a
	33	0.568 (0.0080)a	1.00 (0.016)a	254.7 (1.56)a	63.18 (0.232)a
7	98	0.567 (0.0100)a	1.17 (0.015)a	288.3 (2.93)a	67.24 (0.301)a
	66	0.552 (0.0070)a	1.06 (0.015)a	274.9 (2.01)a	65.46 (0.284)a
	33	0.554 (0.0073)a	0.96 (0.013)a	256.0 (1.69)a	62.97 (0.257)a
	10	0.553 (0.0096)a	0.76 (0.014)a	243.6 (1.95)a	57.77 (0.369)a
2	98	0.383 (0.0065)b	0.14 (0.004)b	118.2 (1.47)b	26.20 (0.399)b
	66	0.470 (0.0080)b	0.17 (0.005)b	112.9 (1.76)b	25.17 (0.473)b
	33	0.443 (0.0077)b	0.13 (0.006)b	111.5 (1.53)b	22.58 (0.323)b
	10	0.363 (0.0064)b	0.07 (0.002)b	97.9 (2.10)a	15.43 (0.283)b

[1]The means reflect the average of single plants harvested from four replications of the experiment. The standard errors of the means are contained in brackets.
[2]Means comparison performed with PROC Mixed lsmeans ($p \leq 0.05$). Means with the same letter within the same column are not significantly different.

Carbon Assimilation and Dark Respiration

There was little change observed in whole canopy net carbon exchange rates over the range of pressures from 33-98 kPa and oxygen partial pressures from 7-20 kPa (Figure 3). At 10 kPa total pressure and 7 kPa of oxygen there was a slight but significant decrease in the rate of canopy carbon assimilation, and at 2 kPa oxygen there was a further decline (approximately 50%) (Figure 2, Table 1). Similarly, dark period canopy respiration showed no significant change over the entire 10-98 kPa range of pressures and oxygen levels down to 7 kPa. As with canopy carbon assimilation, respiration levels were decreased by approximately 50% at an oxygen level of 2kPa (Figure 3).

Transpiration

The response of canopy transpiration to reduced pressures and oxygen showed results similar to those observed with carbon assimilation. There was little effect in response to pressures from 33-98 kPa and oxygen partial pressures of 7-20 kPa, with all the rates near 4 L m^{-2} day^{-1} at 17 DAP (Figure 4). Transpiration at 10 kPa of total pressure and at oxygen partial pressures of 7 and 2 kPa were significantly lower than the other treatments, and barely measurable at the 10/2 kPa treatment level.

DISCUSSION

There have been few reports describing plant growth, NCER, and transpiration at reduced atmospheric pressure and reduced partial pressure of oxygen at a canopy scale. In this study, hypobaria (10, 33, or 66 kPa) had no significant effect on biomass productivity. Canopy photosynthesis, dark respiration, and transpiration were unaffected by pressure in all but the 10 kPa total pressure treatment. Similarly, oxygen partial pressures of 7 kPa or greater

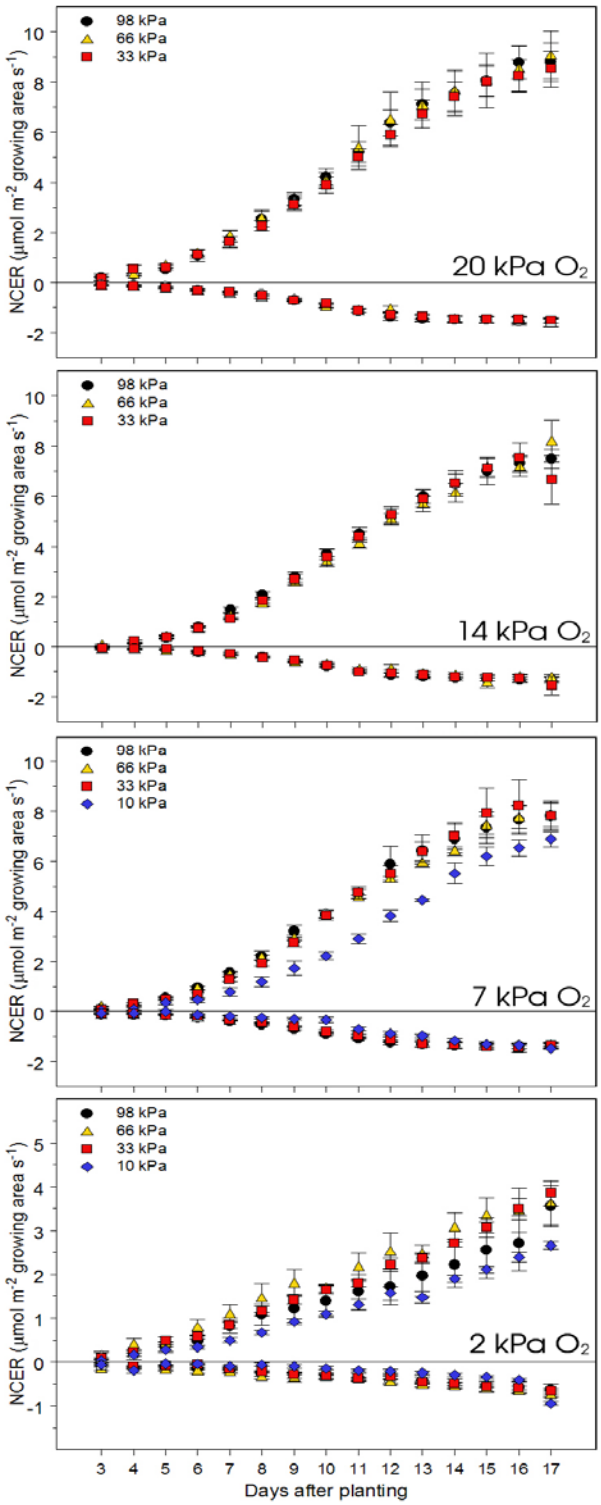

Figure 3. Whole canopy carbon assimilation and respiration rates (µmol m-2 growing area sec-1) for radish (*Raphanus sativa* L. cv. Cherry Bomb II) grown with atmospheric pressures of 98, 66, 33, or 10 kPa and oxygen partial pressures of 2, 7, 14, or 10 kPa over a 21-day period. Data shown (3-17 DAP) represent the period from chamber closure until a series of secondary tests performed at 18-20 DAP. The pCO_2 was maintained at 120 Pa. The error bars represent the standard error of the means.

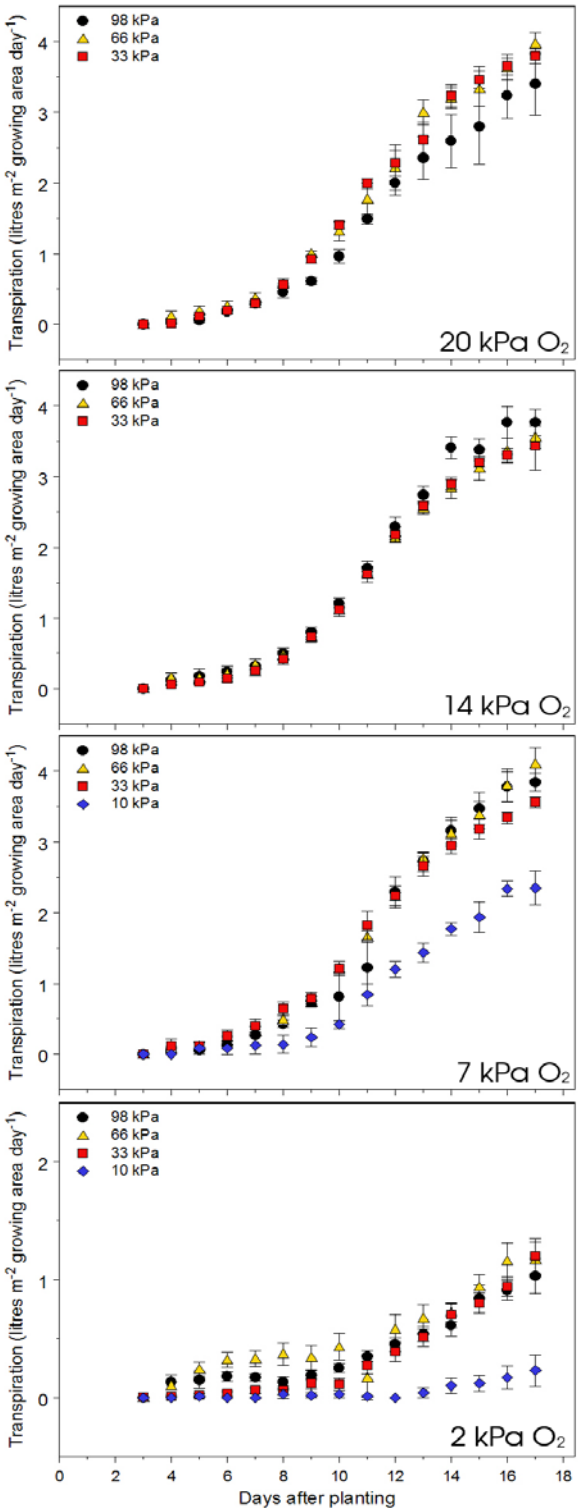

Figure 4. Transpiration (liters m^{-2} growing area day^{-1}) for radish (*Raphanus sativa* L. cv. Cherry Bomb II) grown at atmospheric pressures of 98, 66, 33, or 10 kPa and oxygen partial pressures of 2, 7 14, 20 kPa. The pCO_2 was maintained at 120 Pa. Data shown (3-17 DAP) represent the period from closure until a series of secondary tests were performed at 18-20 DAP. Error bars represent the standard error of the means. Evaporation values (total evapotranspiration at 3 DAP) were subtracted from total daily evapotranspiration to provide an estimate of transpiration.

(7, 14, or 20 kPa) showed no significant effect on reported parameters. Only when the partial pressure of oxygen was reduced to 2 kPa was growth significantly reduced. These results agreed with previously published findings that demonstrate that plants can be grown for extended periods under hypobaric conditions with no detrimental effect on net productivity (Dixon et al., 2005; He et al., 2007).

Growth

Across all treatments from 10-98 kPa of pressure and 7-20 kPa of oxygen, radish growth parameters showed only minor decreases, which were similar to that observed by Levine et al. (2008), but these differences were not statistically significant. Hypobaric studies with lettuce (Spanarkel and Drew, 2002; Dixon et al., 2005; He et al., 2006, 2007, 2009) and spinach (Iwabuchi et al., 1995) also suggested that long-term growth at reduced pressure was comparable to ambient levels. Thicker leaves, as indicated by the decrease in SLA, were observed at reduced pressures. From this observation, one might expect a reduction in growth as the thicker leaves reduce the LA available to capture incident light. At an oxygen partial pressure of 2 kPa, the suppression of radish growth was most significant and would not be suitable in life support conditions where the crops would be the main source of nourishment for the crew and act as the air regeneration system. Although the low oxygen should have favored photosynthesis through the inhibition of the oxygenase activity of RuBisCO (i.e., photorespiration), the elevated CO_2 partial pressure (0.12 kPa) across all treatments should have eliminated photorespiration. It is likely the 2 kPa O_2 limited essential aerobic metabolism in the roots and throughout the plants during the dark cycles, resulting in decreased growth, and the adverse effects of the low oxygen partial pressures on radish are consistent with studies of lettuce, which showed reduced growth at 6 kPa O_2, irrespective of total pressure (He et al., 2007)

Carbon Assimilation and Dark Respiration

We detected no significant differences in the rate of carbon assimilation at reduced atmospheric pressure when oxygen was kept at or above 7 kPa and total pressure was at or above 33 kPa. At 10 kPa total pressure and 7 kPa oxygen however, carbon assimilation was reduced when compared to the higher oxygen and pressure treatments, which is consistent with gas exchange measurements with lettuce under hypoxic condition and saturating CO_2 (He et al., 2007, 2009). This contrasted with the growth results, which showed no significant difference in dry mass accumulation in this treatment compared to the others. With all other variables (O_2, VPD, T) being equal within each pressure series, this response is likely due to hypobaria alone. The plant response to hypobaria is complex, as demonstrated by the altered regulation of more than 200 genes (Paul et al., 2004). It is probable that long-term changes and acclimation are occurring, as noted by the decreased variation in pressure treatments observed by Richards et al. (2006) after only 16 hours of acclimation. The higher pressure treatment (33, 66, or 98 kPa) results were contrary to previous short-term studies in wheat (Massimino and André, 1999) and tomato (Rule and Staby, 1981), but were consistent with the long-term results with spinach (Iwabuchi et al., 1995) and lettuce (He et al., 2007). Although enhanced photosynthesis at lower pressures was expected due to the increased diffusion coefficients at low atmospheric pressures (Gale, 1973; Rygalov et al., 2002), it is not clear whether the observed lack of improvement was due to physical, biochemical, or combined adaptations, i.e., plant acclimation.

Enhanced photosynthesis in response to low pressure environments previously observed by others was likely due to the inhibition of the oxygenase activity of RuBisCO, often seen when plants are subjected to reduced oxygen partial pressures (Warburg effect), particularly if the pCO_2 dropped with pressure, and as a secondary effect of decreasing the atmospheric pressure (Zelitch, 1983; Musgrave and Strain, 1988). Our studies used enriched carbon dioxide levels (0.12 kPa) across all treatments, which likely suppressed photorespiration even at the highest oxygen partial pressure used in our tests (Maleszewski et al., 1988; Drake et al., 1996).

In this study with radish, canopy dark respiration was unaffected by either hypobaria or hypoxia at oxygen partial pressures as low as 7 kPa. This differed from the results of He et al. (2009) who found a reduction in dark respiration in lettuce with the atmospheric pressure reduced

to 25 kPa in lettuce. When coupled with the decreased overall NCER (Figure 3) and no observable difference in vegetative growth (Table 1) between this and the other treatments, it is likely there was an increase in overall net carbon uptake induced by the reduced pressure. Gene expression in *Arabidopsis* has been shown to be different between hypoxia and with hypobaria (Paul et al., 2004), suggesting that the observed decrease in dark respiration was not a result of the lower oxygen partial pressure but is in fact unique to hypobaria.

Because the chambers could not be opened or accessed without violating total pressure and gas partial pressure control, all gas exchange measurements could only be monitored for the entire canopy. The reduced growth and reduced canopy cover at the lowest oxygen partial pressure would have resulted in less light interception, which would clearly affect NCER. This makes it difficult to assess whether there were direct effects of the reduced pO_2 on a per unit leaf area basis. This might be addressed in future studies by having real-time measurements of canopy cover and normalizing the data for an actual canopy cover. Yet this would still be complicated by overlapping leaves within the canopy.

Transpiration

Given that the VPD for any given temperature is not affected by pressure one might conclude that transpiration would remain stable, but enhanced transpiration at reduced atmospheric pressure has been postulated due to the corresponding decreased aerodynamic resistance and increased diffusion coefficients (Gale, 1973; Iwabuchi and Kurata, 2003). However, as with the carbon exchange rates in this study, little effect was noted in transpiration among treatments from 33-98 kPa total pressure and 7-20 kPa of oxygen. The transpiration trends observed here are similar to the results of Iwabuchi and Kurata (2003), who noted no difference in the transpiration of spinach after acclimation by the plants to a low pressure environment; yet the findings contrast with those of Richards et al. (2006) where transpriation of *Arabidopsis* increased as much as of 50% at reduced pressures. Terashima et al. (1995) suggested that increased transpiration would be observed at reduced pressures even if the VPD remained constant (as in this study) due to the flux driven by the increased diffusion coefficient. Iwabuchi et al. (1995) explained their lack of increased transpiration rates as a secondary response in which the increased diffusion coefficient initially accelerated transpiration, which lowered the leaf temperatures. The plants apparently acclimated to the pressure environment and maintained similar transpiration rates. Migge et al. (1999) suggested that the thicker leaves used to dissipate radiant energy would result in decreased water loss. Our results suggest that radishes under the conditions of this study may have acclimated to their pressure environments and used an adaptive response to maintain constant transpiration rates, similar to the results with spinach by Iwabuchi and Kurata (2003). Modifications in stomatal aperture, pore length or numbers, leaf thickness, and leaf temperature may have all contributed to the transpiration rates observed.

This study demonstrated the ability of radish plants to withstand the effects of reduced pressures from seedling to harvest. Contrary to some previous observations, NCER and transpiration with radish in our studies were not greatly enhanced and dark respiration was not suppressed by reduced pressure (Corey et al., 1996; Daunicht and Brinkjans, 1996; Massimino and André, 1999; Musgrave et al., 1988; Spanarkel and Drew, 2002). Clearly, results may vary among species and there might be a number of complex factors involved that cannot be assessed using past methods of excised plant tissues, or through short-term investigation using plants first established at ambient conditions, as the short-term gains in photosynthetic capacity can be offset by adaptive measures in the long-term (Usuda, 2006). These results represent a comprehensive analysis of plant growth from seedlings under hypobaric and reduced oxygen partial pressure with respect to the range of atmospheric alteration and control. Further research into the biochemical and morphological adaptations created by reduced atmospheric pressure is warranted and consideration should be given to the age and stage of development of the plants studied.

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