GAS EXCHANGE AND ACCLIMATION OF RADISH IN REDUCED PRESSURE ENVIRONMENTS

By

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TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

LIST OF PARAMETERS

P	pressure (kPa)
VPD	vapor pressure deficit (kPa)
RH	relative humidity (%)
pCO_2	partial pressure of CO_2 (Pa)
pO_2	partial pressure of O_2 (Pa)
L	leak rate (% Vol/day)
t	time interval (h)
P_0	initial pressure (kPa)
Pi	end pressure (kPa)
Ca	CO_2 concentration outside leaf air space (Pa)
Ci	CO_2 concentration inside leaf air space (Pa)
C_A	CO_2 assimilation rate (µmol m^{-2} s^{-1})
DR	Dark respiration rate (µmol m^{-2} s^{-1})
T	transpiration (mmol m^{-2} s^{-1})
FW	fresh weight (g)
DW	dry weight (g)
WUE	water use efficiency (mol CO_2 mol H_2O^{-1})
k	the Boltzmann constant, ergs/°K
m	molecular weight (g mol^{-1})
σ_{AB}	collision diameter, cm
a	stomata length (cm)
b	stomata width (cm)
d	stomata depth (cm)
D	diffusivity (cm^2 s^{-1})
n	number of stomata
hs	stomatal conductance (cm s^{-1})
hb	boundary layer conductance (cm s^{-1})
K	thermal diffusivity (cm^2 s^{-1})
L	length (cm)
Kv	kinetic viscosity (cm^2 s^{-1})
u	air speed (cm s^{-1})
Re	Reynolds number
µ	viscosity (g cm^{-1} s^{-1})
ρ	density (g cm^{-3})
D	diffusivity (cm^2 s^{-1})
Sc	Schmidt number

Abstract of Dissertation Presented to the Graduate School
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Plants grown on long-term space missions will likely be grown in low pressure environments (i.e., hypobaria). In general, the fundamental growth and photosynthesis of plants grown in hypobaria are similar to plants grown at normal atmospheric conditions with some exceptions. For example, transpiration rates can be elevated in low pressure resulting in plant wilting if water is not readily available. Plants may also exhibit poor growth and germination if the partial pressures of critical gases are not maintained to certain levels (e.g., pO_2 for seed germination and pCO_2 for photosynthesis). Since the gas phase of the environment is so critical for successful growth of plants in both normal and low pressure environments, the effects of the gas phase composition, particularly CO_2, on the gas exchange and growth of radish (*Raphanus sativus* var. Cherry Bomb II) in hypobaria were studied. Low pressure growth chambers were built that could monitor the environmental parameters for these studies. The fresh weight (FW), leaf area, dry weight (DW), CO_2 assimilation rates (C_A), dark respiration rates (DR), and transpiration rates from 26 day-old radish plants that were grown for an additional seven days at different total pressures (33, 66 or 101 kPa)

and pCO_2 (40 Pa, 100 Pa and 180 Pa) were measured. In general, the dry weight of plants was enhanced with CO_2 enrichment and with decreased total pressure. In limited pCO_2 (40 Pa), the transpiration for plants grown at 33 kPa was over twice that of controls (101 kPa total pressure with 40 Pa pCO_2). Increasing the pCO_2 from 40 Pa to either 100 or 180 Pa reduced the transpiration rates for plants grown in hypobaria and at normal atmospheric pressures. Plants grown at lower total pressures (33 and 66 kPa total pressure) and super-elevated pCO_2 (180 Pa) had evidence of leaf damage. Taken together, radish growth can be enhanced and transpiration reduced in hypobaria by enriching the gas phase with CO_2 although at high levels of CO_2 leaf damage can occur.

Since the diffusivities of gas increases as the atmospheric pressure drops, it is expected that transpiration and CO_2 assimilation in plants would increase as plants grow in hypobaria. A mathematical relationship based on the principles of thermodynamics was developed for calculating the transpiration and photosynthesis for plants. Stomatal conductance is sensitive to total pressure. At 33 kPa total pressure, stomatal conductance increases with the boundary increasing by a factor of ~1.7, thus the boundary layer thickness conductance increases by 70%. Since the leaf conductance is a function of both stomatal conductance and the boundary layer conductance, the overall conductance will increase resulting in significantly higher levels of transpiration as the pressure drops. The conductance of gases is also regulated by stomatal aperture in an inverse relationship. Stomatal aperture is directly influenced by concentration of CO_2 inside the leaf space. The higher CO_2 concentration inside the leaf air space during low pressure treatments may result in stomata closing partially or fully which may reduce the excessive transpiration caused by increased diffusivity. Therefore, a reduced

pressure environment with high CO_2 may be an ideal scenario for minimizing transpiration and maximizing the plant biomass yield in BLSS. The application of this model to data for plants acclimated long term to hypobaria (33 kPa) and reference values (101kPa) suggest that for transpiration the model predicts transpiration for plants that are grown at normal pressure fairly well, but for those grown at 33kPa the model over predicts transpiration observed data by almost

To understand the mechanisms of plant adaptation to hypobaria, plants were transferred to low pressure at different stages of growth (0, 2, 14, and 26 days after germination). The growth, C_A, transpiration and stomatal density were compared between these plants. Plants exposed short term to hypobaria (< 2 days in hypobaria) responded differently than those exposed to long-term hypobaria (>2 days). For example, plants that were grown entirely in hypobaria had smaller and thicker leaves compared to plants that were exposed for 2 days or less to hypobaria. These long-term treated plants also had higher C_A ($p<0.1$) and transpiration rates ($p< 0.05$) even though their overall growth (FW and DW) was not significantly affected by hypobaria. The stomatal density of plants grown long term in hypobaria was not significantly different than plants grown short term to hypobaria. Therefore, it appears that plants may respond to enhanced gas exchange in hypobaria by reducing their leaf area. Further studies on the mechanisms of plant adaptation are required to identify other biological or physiological mechanisms of plant acclimation to hypobaria.

Some of the engineering constraints to grow plants in Martian plant growth facilities can be offset by growing plants at reduced atmospheric pressure. Characterization of hypobaria response at reduced pressure can provide data which

can help in explaining some of the response which plants may encounter on Martian facility. These studies are important for understanding mechanism by which plant control water relations.

CHAPTER 1

ATMOSPHERIC CONDITIONS USED FOR BIOLOGICAL SYSTEMS DURING SPACE MISSIONS: IMPLICATIONS FOR HYPOBARIC PLANT BIOLOGY

Advanced Life Support Systems (ALS)

Long-term, space exploration with humans will require an Advanced Life Support system (ALS) that provides air, water, and food to explorers in a sustainable manner. This system will consists of the latest technologies for atmosphere revitalization, water supply, and food and fiber production as well as the recycling of these valuable resources. These technologies may be based on physico-chemical (P-C) or biological approaches. The P-C approach uses mechanical or chemical mechanisms to provide an ALS whereas the biological approach, the Biological Life-Support-System (BLSS), uses biological systems (e.g., plants, algae or microbes) to supply the requirements for ALS. The efficiency of each approach has been compared and estimates of the time until the system has reached sustainability have been modeled. This time to reach sustainability is an important aspect for cost analysis and as an indicator to how long a space colony can be supported without re-supply from Earth. Unfortunately, the results of these studies are conflicting as to which method is more efficient for long-term space missions. For example, Alan Drysdale (Boeing Corporation; Drysdale, 2001) suggests that the BLSS may take three years for sustainability making it a viable option for ALS. In contrast, Harry Jones (NASA- Ames: Jones, 2007) suggests that the BLSS could take up to ten years to reach a sustainable state and therefore he supports a P-C approach. Barry Fingers (Dynamac Corp., Fingers et al., 1996) suggests the ideal scenario is the hybrid of both the approaches, where P-C approach is used for the initial one to two years until the BLSS can become sustainable. The P-C approach has

already been used on the International Space Station (ISS) with much success but the costs associated with transport of materials for P-C systems are considerably lower for the station that is ~375 kilometers above earth than for distant planets. Arguments for the P-C approach suggest that the advancements in water and air recovery and biomass processing make the P-C more cost effective and reliable than BLSS. Whereas, the supporters of the BLSS suggest that eventually a biological system for material recycling will be required for long-duration missions and for their benefits to explorers living with plants and having a regular supply of fresh food (Wheeler, 2004). As one of the major components of developing a BLSS, the environment, including the gas phase, temperature and pressure used to support the biological system must be carefully monitored and controlled. This chapter discusses the environments (i.e., gas phase composition and pressures) that have been used in the past to support life on space missions and describes the effects of these types of environments on plants as part of a BLSS.

Gas Phase for Biological Life Support

Here, a brief history of the environments that have been used for life support is described with emphasis on those systems that have been used for humans and plants. The first biological system to go up in space was a dog named Laika sent by the United States of Soviet Russia (USSR) on Sputnik 2 on October 4, 1957. The pressure and atmospheric composition of the vessel was controlled with an oxygen generator, CO_2 and vapor scrubbing system with pressures and gas concentrations (CO_2, N_2 and O_2) maintained to levels of Earth sea level (Table 1-1). Unfortunately, the inadequate temperature control system resulted in the heatstroke and the death of Laika within a few hours (Malashenkov, 2002). After Laika, the USSR sent several dogs and cats in to

space of which some returned safely. Shortly thereafter (1958-62), during the Mercury missions, the US sent monkeys into space with a total atmospheric pressures of ~34 kPa at 100% oxygen. The advantage of using the low pressure in this case was that it required less total gas to be transported from Earth to support life and thus increased mission duration. In addition, the low pressure required a much lighter hull for the craft since a smaller pressure difference between the inside of the craft and the vacuum of space was maintained. High levels of O_2, greater than 35 kPa, (Baker 1981), can be toxic to humans and animals, thus 34 kPa of total pressure of O_2 was chosen as the highest pressure. This atmospheric composition and pressure was used for the Mercury, Gemini, and Apollo space missions (Martin and McCormick, 1992).

The success of these first missions with animals led to more challenging human missions by the USSR and the USA. On April 12, 1961, the first human, Yuri Gagarin, was sent to space by the USSR in Vostok 1. This vessel had sea level atmospheric conditions (100 kPa total pressure, 0.04 to 0.2 Pa CO_2, and 21kPa O_2). The gas composition and high pressure of the Vostok missions required strong materials for the cabin construction and the necessity for prolonged de-nitrogenation before any extra-vehicular operation since suits on these missions were much lower than cabin pressure. As a benefit of this gas composition and pressure, the lower oxygen concentration of the atmosphere prevented fire hazards and allowed for a more earthlike environment for biological studies (Baker, 1981). The oxygen was provided by potassium superoxide, CO_2 and excess water was absorbed by lithium hydroxide. These atmospheric conditions were used throughout the USSR program up until Mir (Baker, 1981). Shortly after the USSR manned space mission on May 5, 1961, the US sent Alan Shepherd to

space on Mercury Freedom 3 in similar atmospheric conditions as were used for the monkeys in the past Mercury missions (~34 kPa at 100% oxygen). A total of six manned missions were performed during the Mercury period using this enriched oxygen, low-pressure environment.

The first two-person mission and the first mission with the cosmonauts without a spacesuit were in Voskhod 1 (Oct 1964). The Voskhod spacecraft were modified Vostok vessels that had a parachute system removed to allow for a second cosmonaut. The atmospheric conditions were similar to those of the Vostok missions (100 kPa, 0.04 to 0.2 Pa CO_2, and 21kPa O_2). Voskhod 2 (Mar 1965) was the first mission that included a human spacewalk by Alexei Leonov. This required for extra vehicular activities the cosmonaut had to reduce the pressure in his suit to either 40.6 kPa or 27.4 kPa total pressures before exiting the vehicle which put the cosmonaut in danger of getting the bends (Baker, 1981). There was some difficulty entering the airlock on the vessel due to the rigidity of his pressurized suit relative to the airlock. The suit was designed to provide 45 minutes of oxygen for breathing and cooling and allowed for venting of gas and vapor to space.

After the success with upper orbit (Earth) flights, missions to the Moon became the focus of space exploration. However, before the astronauts could land on the moon several manned Gemini missions (1965-66) were used to survey the Moon and test the capabilities of the rockets and for landing. As a part of Biostake series of experiments, corn and mustard seeds were carried by astronaut Ed White in his space suit (at 25.5kPa, 100% oxygen; Grimwood et al., 1969) during the first US extravehicular activity (EVA) on Gemini 4 (1967). Though seeds were germinated during subsequent

growth studies on Earth at ambient pressure, abnormality in the plants was observed likely due to heavy ion radiation (Paul and Ferl, 2006). After Gemini, the Apollo missions were developed to bring astronauts to the moon. Unfortunately, on January 27, 1967, the Apollo 1 mission ended in disaster with a fire that killed the three astronauts on board while on Earth (Edward White, Virgil Grissom, and Roger Chaffee). The fire was caused by the high flammability of a pure oxygen environment inside the capsule (at ~100 kPa; Apollo 204 Review Board Report, 1967). This high pressure was required during launch since the craft were not built to withstand high pressure differences between the environments inside and outside of the vessel. A similar incident occurred earlier in March 1961 in the USSR which claimed the life of Soviet cosmonaut trainee, Valentin Bondarenko, when a fire started in the pure oxygen atmosphere in the chamber he had been in, although this incident was concealed from the public for years (Oberg, 2009). As a result of the Apollo fire, NASA set new criteria for gas phase composition for space missions. These included that the gas phase would consist of 60% oxygen and 40% nitrogen at roughly sea-level pressure at launch, then the pressure was lowered by releasing gas to maintain about a 40kPa difference between the inside and outside of the vessel during ascent until the atmosphere reached approximately 34kPa and 100% oxygen during the first 24 hours in space. The astronauts were acclimated in space suits from the time of launch at 100% oxygen (34 kPa), approximately three hours before launch to prevent the bends during the depressurization and ascent. Once the craft reached the set point atmospheric conditions, the astronauts could remove their suits and move about the cabin. These Apollo missions ran from 1967-72. Unfortunately, the hazards of oxygen flammability

were realized again during the Apollo-13 mission, when, on the return flight to Earth the oxygen gas tank was damaged and ended up starting a fire. To address this, the Apollo 14 mission had several modifications to the gas tank systems. Interestingly, the Apollo 14 mission had one of the first long-term treatments of seed in space when astronaut Stuart Roosa, the flight commander, carried 500 seeds in his personal belongings and brought them back to Earth. The seeds were of several tree species including sycamore, pines, and fir. After the 9-day mission, these seeds were given to several educational institutes, including the University of Florida, the trees germinated from these seeds appear to have no signs that the mission to the moon had negatively affected their growth (Klein, 1981).

During the Biosatellite program (1966-1969) NASA sent the Bion series of satellites with specimens of fruit fly, frog eggs, bacteria and wheat seedlings to study the effects of weightlessness on living organism. Total pressure was maintained at approximately 100 kPa and oxygen partial pressure was 21 kPa. In the Biosatellite II, pepper plant experiment, a camera recorded the positions of plant leaves with respect to time to study the effect of microgravity on plant movement (Thimann, 1968).

In order to conduct long-term research on living systems in space as well as to establish a space laboratory for small animals, plants, microorganisms, and humans, the USSR launched Salyut (1971-82) and the USA launched Skylab (1973-79). Salyut was at approximately sea level pressure and atmosphere composition. Salyut carried the Oasis, the first of its kind plant growth system with a cinematic recording system (Neichitaleo and Mashinski, 1993). It was used to cultivate *Brassica capitata, Linum usitatissium* and *Allium porrum*. The biological experiments also included variety of

species including spiders, worms, fishes, and frogs. Many of the experiments were performed to study the effects of radiation and space environment on biological systems. In contrast to Salyut, the Skylab platform was operated at 34 kPa total pressure but the atmospheric composition was 70% oxygen and 30% nitrogen. Nitrogen helped reduce the risk of pure oxygen while still maintaining the health of the Astronauts from hypoxic conditions. One of the plant experiments (Skylab experiment ED-61/62) studied phototropism of tomato in low gravity. This was the first time the seeds were germinated in agar in space microgravity. These studies also compared various light intensities that are required to produce photosynthesis for plants grown in space (Cramer et al., 1984)

Interestingly, a combined Skylab-Salyut station was proposed by the US and the USSR. But, as noted above, each station had different gas mixes and pressures. Since each station would need to make modifications, it was proposed that a 55kPa total pressure environment, slightly enriched in oxygen would be used to compromise and minimize the modifications required for one station. However, this combined station was never realized.

To replace Salyut, the USSR built Mir (1980 -98). The Mir, the Russian word for world or peace, station had an atmosphere of 101 kPa total pressure and earth ambient concentration of gases (21 kPa O_2, 80 kPa N_2 and 0.04-0.2 kPa CO_2). Seed germination experiments on board Mir were done with variety of small, closed or ventilated/partially ventilated chambers, from simple a beaker containing moist soil (Kosmos 1129**;** Parfeenov and Arbanova, 1981) to a sophisticated greenhouse, called Svet, the Russian word for cosmos (Nechitailo and Mashinsky, 1993). During the early

experiments on seed germination, plants exhibited poor growth and stress (Kordyum et al., 1985). This was possibly due to the lack of natural convection of air movement in microgravity leading to formation of stagnant air layers around seedlings within the closed chamber (Musgrave et al., 1988). Another attempt to germinate seeds and complete the life cycle in space resulted in a failed experiment (Mashinsky et al, 1994). However, a complete plant life cycle was performed with wheat grown in the 'Svet' greenhouse which had a better control system for the gas phase (Svetlana et al., 2005). However, after these plants were returned to Earth (STS-81, 1997), it appeared that the wheat flowers had sterile seeds. Initially, microgravity was assumed to be the cause of the seed sterility but when researchers grew a dwarf wheat variety at the same levels of atmospheric composition that the super dwarf was exposed to in space, they found higher concentration of ethylene on the station to be the cause (Salisbury 1995). Plants tolerate up to 4-5 ppb concentration of ethylene, with anything higher resulting in reduced plant growth. Salisbury et al. (1995) found that the ethylene in the growth chamber was as high as 1-2 ppm, ~1000 times higher than the tolerable limit for plants. Since ethylene produced by plants was indicated as the source of ethylene and since convection is limited in microgravity, these researchers developed a dwarf variety of wheat (Apogee) that is insensitive to higher concentrations of ethylene. The Apogee wheat produced non-sterile flowers and viable seeds on board Mir and the ISS suggesting that ethylene and not microgravity was the cause for flower sterility found in previous missions (Levinskikh et al., 2000).

Due to the problems with high ethylene and CO_2 concentrations in the cabin of Mir, which were harmful to plant growth, it was decided that an independent,

controllable growth chamber was required to conduct specialized experiments which would protect plants from exposure to cabin level CO_2 and ethylene. Therefore, the Svet greenhouse (1990-2000) was built by Russia and was the first automated plant growth facility. The greenhouse was at 101 kPa total pressure and atmospheric composition similar to Earth sea level except CO_2 levels were up to 0.2 kPa. However, it had open type air ventilation system that had air in contact with the Mir cabin atmosphere (Svetlana et al., 2005). Improvements to the plant growth chambers were made to SVET in the late 1990s which included the ability to monitor the plant growth in real time by measurement of CO_2 and H_2O exchange rates, temperature, and relative humidity. This allowed the measurements of photosynthesis and transpiration rates based on the current plant CO_2, vapor, temperature and relative humidity levels. However, the open air system was not ideal for careful control of gas phase surrounding plants.

After the Biosatellite program was cancelled in 1968, it was not until 1982 during the third space flight program (STS-3) that the first Plant Growth Unit (PGU) was launched by the USA to perform seedling growth experiments (Cowles et al., 1984). PGU served for 15 years for use on plant growth studies before Astroculture was introduced**.** The Astroculture Growth Chamber (ASC-GC) was the first of its kind. It was a completely automated growth chamber developed in the early 1990's to provide support system for plant growth in closed environment. It was developed by the Wisconsin Center for Space Robotics and Automation at the University of Wisconsin, Madison. The CO_2 was maintained in the range of 300 – 2000 ppm and the ethylene concentration could be reduced to less than 50 ppb using a catalytic ethylene scrubber. The main objective of ASC-GC was to perform short- and long-term plant experiments

in microgravity to study seed-to-seed cycle in space with automatic control for up to 30 days. The ASC-GC hardware was efficiently used for studies on Arabidopsis (STS-68). In one study, the reproductive ability of pre-germinated *Arabidopsis* seedlings in various gas compositions and ventilation regimes were studied. At a CO_2 concentrations of 300-2000 ppm Arabidopsis had sterile pollen and embryos, and at very high CO_2 concentration (8000 ppm) plants exhibited early abortion of ovules and mature pollen, and the release of pollen from anthers was restricted preventing fertilization (Kuang et al., 1996). The Mir station was in operation for fifteen years until March 23, 2001, when it was deliberately de-orbited, breaking apart during atmospheric re-entry over the South Pacific Ocean.

The International Space Station (ISS)

The International Space Station (ISS) is a joint project of several space agencies across the world led by NASA. The ISS construction began in 1998 and has had continuous human presence since November 2000. The ISS platform runs at 101 kPa total pressure with Earth sea level concentration of the gases. Advanced Astroculture made its debut flight during the second ISS increment to study the effects of microgravity on seed to seed development of *Arabidopsis thaliana.* The main objective of this study was to grow a second generation of plants using the first generation of seeds and harvesting the living plant tissues for gene expression analysis (Fourth and Fifth increment; Link et al., 2003).

The completion of a complete life cycle by *Brassica rapa L* and wheat plants of Apogee variety in the Svet greenhouse on board Mir indicated that plants can be grown in consecutive generations in space **(**Levienskikh et al., 2000; Musgrave et al., 2002). During March 2003 to April 2005, a Russian group led by Sychev studied five

consecutive generations of genetically engineered dwarf green peas in a greenhouse called LADA in the Russian module of ISS. The LADA has mainly earthlike atmospheric pressure and gas concentrations of CO_2, N_2 and O_2; though CO_2 concentration can reach up to 0.1 kPa. They reported that pea plants grown over a complete ontogeny cycle on board ISS were similar to ground controls in terms of plant development and genetic characteristics (Sychev et al., 2007).

Advanced Plant Studies on the ISS

The ISS now has more sophisticated plant growth chambers such as the European Modular Cultivation System (EMCS). The EMCS was launched in February 2007; as one of the European Space Agency (ESA) contributions to the ISS. It is the part of European Columbus Lab section of the ISS. It has two centrifuge rotors, which can provide different gravitational accelerations from 0.001 to 2*g* (Brinckmann, 2005). The advantage of EMCS is that the hardware can be experiment specific. The incubation chamber controls relative humidity down to 30 %, oxygen at 0-21 % and CO_2 from 200- 2000 ppm. The EMCS also has an ethylene scrubber which prevents ethylene accumulation. The EMCS was originally designed for plant experiments but due to the recent budget cuts to the space program, it has also been used for other organisms such as fruit flies (Brinckmann, 2005).

The US and the USSR have chosen different levels of gas phase composition and total pressures to maintain environments for life support. In the future, it is likely that life support systems will be maintained at lower pressures for the cost savings in transporting valuable gases and reduction in the cost of materials to maintain structural integrity of chambers used to house living systems. The effects of low pressure on plant growth are described in the next section.

Hypobaric Plant Biology

Plants will be an integral part of BLSS for human space exploration since they can provide a source of food, can revitalize air and water for human use, and offer psychological benefits to space travelers during long-term missions away from Earth (Figure 5-2). During many of these missions, plants will likely be grown at reduced atmospheric pressure in environmentally controlled chambers (Paul and Ferl, 2006). The low pressure environment will minimize the amount of atmospheric gases that need to be transported from Earth and the low pressure will require less structural mass of the chambers to withstand the low pressure/vacuum environments found in space or on many planets or moons. The study of plants in low pressure is termed hypobaric plant biology and, on Earth, it includes the study of plants at high altitudes. The existence of flora and fauna at very high altitudes, up to 5000 m (total atmospheric pressure of 55 kPa) with warmer temperatures suggests that plants have existing mechanisms for growth at low pressure at least down to approximately ½ of an atmosphere (Korner, 2004). Because plants will likely be grown in low pressure environments on the Moon or Mars, a brief history of hypobaric plant biology as it relates to space biology is described.

The Effects of Hypobaria on Seed Germination and Plant Growth

Successful crop growth in hypobaria depends on a variety of interacting factors such as the relative gas composition (particularly O_2 and CO_2), absolute total pressure, vapor pressure deficit (VPD) of the atmosphere, and the temperature, among other factors. Many studies have been performed to identify the effects of low pressure on germination, plant growth and development and many of these studies have recently been reviewed (Paul and Ferl, 2006) but are described here briefly and summarized in

Table 1-2. In one of the first experiments on germination in hypobaria, rye seeds that were grown in approximately 3 kPa total pressure with Mars level carbon dioxide partial pressure (0.45.kPa) without oxygen had no seed germination (Siegel, 1963). However, at 3kPa of pure oxygen the seeds germinated. Other studies also report seed germination at partial pressure of oxygen of 5-8 kPa at various total pressures (Musgrave et al., 1988; Schwartzkopf and Mancinelli; 1991). These studies indicate that for many species the lower limit for germination in a pure oxygen environment is at approximately 5kPa.

Although seed germination appears to be restricted to a small window of partial pressure of oxygen, the results on the effects of these low pressures on plant growth are conflicting. For example, tomato plants had reduced growth in 40 kPa compared to control (93 kPa) in one study (Daunicht and Brinkjans, 1992), but showed enhanced growth at 33kPa compared to control in another (Rule and Staby, 1981). Mungbean growth was also enhanced in hypobaria at 22 kPa total pressure (Musgrave et al., 1988). These differences in responses were attributed to the differences in composition of the atmosphere with respect to CO_2 and O_2. Reduced growth may have been due to the lower partial pressure of oxygen (pO_2at ~8.4 kPa) which can induce hypoxia stress in plants and result in poor growth (Musgrave et al., 1988). In general, for short-term studies in hypobaria, plants have increased growth if enough oxygen and water are supplied. This increased growth is partially attributed to enhanced photosynthetic rates of the plants as a result of reduced pO_2 or alternatively, due to the increases in diffusion of CO_2 **(**Iwabuchi et al., 1996; Corey et al., 1996, 2002; Goto et al., 1996; Richards et al., 2006**)**. Lower pO_2 inhibits photorespiration since CO_2 has less competition with O_2

for the enzyme Ribulose Bisphosphate Carboxylase/Oxygenase (RUBISCO), the first step in carbon fixation (Drake et al., 1997). The increased photosynthesis at low pressure and low pO_2 was decreased by injecting O_2 into the environment suggesting that indeed the ratio of CO_2 to O_2 was the cause of the increased growth in some studies (Corey et al., 2002). Even under normal pressures (101 kPa), Arabidopsis had increased photosynthetic rates when plants were grown in hypoxic conditions (2.1 kPa ppO_2; Richards et al., 2006). Further studies with Arabidopsis with greater range of pressures showed less of a difference in carbon dioxide assimilation (C_A) after 16 hours in hypobaria relative to controls at 100 kPa compared to plants that were exposed to hypobaria for only 1 hour, this suggests a possible adaptive response after 16h in hypobaria (Richards et al., 2006). In rice, the negative effects of hypoxia on growth at 25kPa total pressure were alleviated by maintaining the pO_2 at 10 kPa (Goto et al., 2002). Therefore, the benefits of low pO_2 for increased photosynthesis in hypobaria need to offset the negative effects of hypoxia on plant growth. Others suggest that the enhanced diffusion of CO_2 in hypobaria to the leaf allows for improved CO_2 fixation and this may account for the increased growth for plants grown in hypobaria **(**Goto et al., 1995; Daunicht and Brinkjans, 1996; Massimino and André, 1999**)**. In contrast, some long-term experiments reported that plants had similar rates of photosynthesis in hypobaria as those grown in normal atmospheric conditions and had no increase in growth (Iwabuchi and Kurata, 1996; Spanarkel and Drew, 2002). This may be a result of reduced stomata apertures in acclimated leaves during the long-term hypobaria treatments thus reducing CO_2 diffusion into the leaf (Iwabuchi and Kurata, 1996).

Further studies on the dynamics of CO_2 diffusion to the leaf and its effects on plant growth are required to understand this response.

Other gases in the hypobaria environment also diffuse at a greater rate relative to high pressures environments and these include ethylene and other plant volatiles. Ethylene is known to cause a variety of responses in plants including enhanced leaf senescence and reduced overall growth (Finlayson et al., 2004). To study the effects of ethylene in hypobaria, He et al. (2007and 2009) exposed lettuce and wheat to 25 and 101 kPa total pressure at various oxygen concentrations and monitored ethylene. Their results suggested that hypobaria does not affect the plant growth and that ethylene concentration was the same in both the hypobaric conditions and in the normal pressure controls. However, this low level of ethylene may be a result of the reduced oxygen partial pressure since this can reduce ethylene synthesis in plants (Burg 2004).

The effects of hypobaria on gene regulation were studied in Arabidopsis plants grown at 10 and 101 kPa total pressure and at 2 and 21 kPa pO_2 (Paul et al., 2004). This study identified genes that were regulated in low pressure but were not caused by low levels of oxygen, i.e., not due to hypoxia. These genes that were specifically regulated by low pressure included genes involved in desiccation-related pathways such as dehydrins, ABA–related proteins, and cold-responsive (COR)-related proteins (Paul et al., 2004). This suggests that plants do undergo stress in hypobaria, particularly water stress. Future studies on the gene expression of plant grown in hypobaria are required to identify the downstream pathways of this response and the effects of long term acclimation on gene regulation.

Recent studies on plant growth for radish that were grown entirely in hypobaric conditions (Levine et al., 2008; Wehkamp, 2009) suggest that plants reduce their excessive water loss over time in hypobaria by reducing their transpiration and CO_2 assimilation rates to levels similar to plants grown at ambient pressure. In spinach, the transpiration rates were slightly higher during the short-term exposure (1 day) to hypobaria but after 10 days in hypobaria, both the CO_2 assimilation and transpiration rates were similar to those of plants grown in ambient pressure (Iwabuchi and Kurata, 2003). However, it is still not clear how plants acclimate to long-term exposure. Ground-based studies have also been performed to test the fruit and nutritional quality of radish grown at low pressures (33, 66 kPa) and these studies found that the biochemical composition of the radish was not significantly different from plants grown at ambient levels for the compounds tested (Levine et al., 2008).

Summary

Maintaining life in space is a challenge. Throughout the history of the space program, a careful balance of keeping the gas composition at pressures that allow life to thrive at economical levels while minimizing the dangers of these atmospheres to life has been a primary goal of engineers. As we advance to the next step of bringing life to distant planets, further modifications in the atmospheric conditions to support life are likely. Due to the cost savings for growing plants in reduced pressures, low pressure growth chambers are likely to be used. Although the exact level of total atmospheric pressure has not been set, it is suggested that plants will be grown at approximately 55kPa in a Moon or Mars greenhouse (personal communication, Ray Wheeler, NASA). Studies on seed germination, CO_2 enrichment, ethylene evolution, vegetative growth and even gene expression profiles in low pressure on Earth and in space suggest that

plants can be grown at low pressures and be a viable food source for explorers. These studies also give ample evidence that the fundamental biological processes of photosynthesis and growth are essentially the same in low pressure and Earth sea level pressures. The use of plants in a biological life support system (BLSS) seems to be a promising approach, although it is far from mature. Since the inception of the concept 'salad machine', extensive research has been performed to study the plant response to various environmental factors which have contributed to the development of the hardware that is currently used in space for plant growth. However, there are several technological and operational issues associated with ALS which will need to be addressed before humans colonize space. For example, how does a low pressure environment affect plants over several generations? The genetic studies suggest that hypobaria may result in a metabolic drain on plants and therefore they may not be physiologically healthy and over generations these may result in poor growth and reproductive health. Comprehensive studies on the interacting effects of all environmental parameters in hypobaria on plant growth and development has not been performed. As we plan for the future to grow plants on the Moon or Mars, the proper selection of pressure and environmental parameters is required to ensure a sustainable plant growth system. The range of gas compositions that are likely to be selected will depend on not only the species but also the stage of plant development since O_2 requirements are different in the vegetative and reproductive periods (Wheeler 2004). In addition, gas composition may be dictated by the available resources on each planet. For example, CO_2 may be used to pressurize plant growth chambers on Mars due to its

availability. The task of optimizing gas phase composition and pressures for plant growth for space exploration will require many more studies.

During the last decade, our understanding of the effects of hypobaria on plant growth from the molecular level to whole plant level has improved (Paul et al., 2004; Wehkamp 2009). Studies have shown that seed germination, seedling growth and plant development can occur in hypobaria when pO_2, pCO_2, and $pH_2O$are at levels that support growth. Gas exchange studies on plants grown in low pressure suggest that plants may have increased CO_2 assimilation and transpiration but this may depend on the duration of exposure to hypobaria (Rule and Staby, 1981; Andre and Massimino, 1992; Daunicht and Brinkjans, 1996; Corey et al., 1997; Iwabuchi and Kurata, 2003). On Mars, CO_2 is readily available and can be used to pressurize the chambers for plant growth but the effects of very high CO_2 concentration on plant growth in hypobaria are not known. Also, only a few experiments have studied the effects of long-term hypobaria on plant growth, thus the acclimation mechanisms that plants utilize to adapt to hypobaria are unclear. Therefore, for this dissertation, the gas exchange rates of radish in hypobaria in various levels of CO_2 are studied and the effects of long-term hypobaria on plant acclimation are explored. Radish was chosen as the model plant for these studies since it is selected by NASA as one of the salad crops (Wheeler, 2001), it has been used in several hypobaria studies (Wilkerson, 2005; Levine et al., 2008; Wehkamp 2009), and the anatomy of radish lends itself to perform transpiration studies. The radish roots and leaves are also edible and provide excellent nutritional value to future space explorers (Levine et al., 2008). To study the gas exchange and acclimation of radish in hypobaria, the following four objectives were addressed:

1. Design and build four, mid-size, low pressure growth chambers (LPGC) that can monitor temperature, pressure, humidity, CO_2, O_2, light and plant weight.
2. Predict the effects of pressure on the diffusivity of H_2O and CO_2 and the implication of these on plant growth.
3. Study the interacting effects of hypobaria and pCO_2 on plant growth, photosynthesis, and transpiration.
4. Compare growth, transpiration, photosynthesis and stomata between long- and short-term acclimated plants in hypobaria.

Structure of the Dissertation

This dissertation is organized into topical research areas that address the above objectives. Chapter 2 describes the construction and testing of low pressure growth chambers (LPGCs) that can monitor environmental parameters important for plant growth. Chapter 2 also describes the procedures for sensor calibration and data management using data acquisition. The prediction of the effects of reduced pressure on the diffusivity of H_2O and CO_2 and the implication of these on plant growth is described in Chapter 3. Chapter 4 describes the interacting effects of total pressure (33, 66 and 101 kPa) and partial pressure of CO_2 (0.04, 0.1 and 0.18 kPa) on growth, photosynthesis and transpiration of radish plants. Chapter 5, describes studies on the effects of long-term (1-4 weeks in hypobaria) and short-term (2 days in hypobaria) acclimation to hypobaria on growth, photosynthesis and transpiration of radish plants. In Chapter 6, the transpiration model described in Chapter 3 is applied to predict transpiration rate at various reduced pressures, vapor pressure deficits (VPD) and stomatal widths. Finally, in Chapter 7, the general conclusions and recommendations for future studies are stated. The program for sensor and parameter control is included in the appendices.

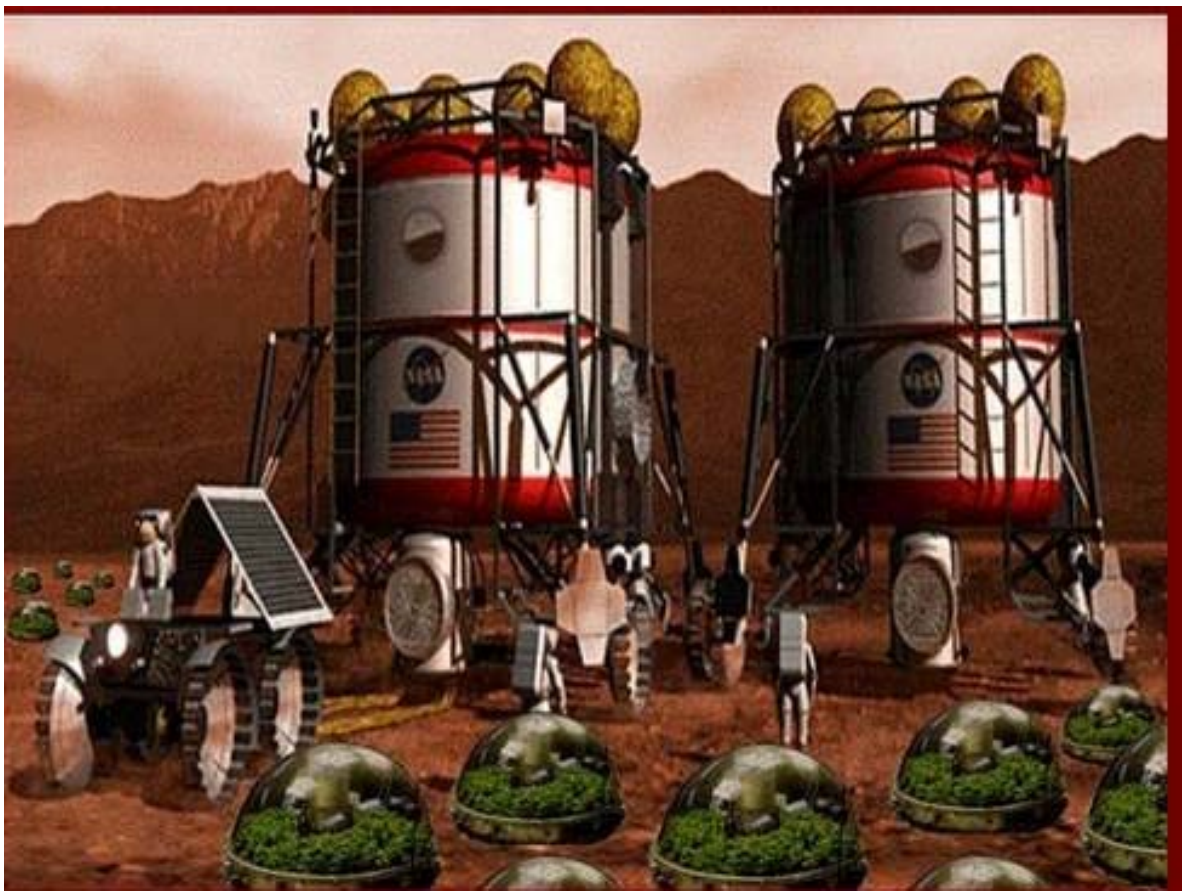
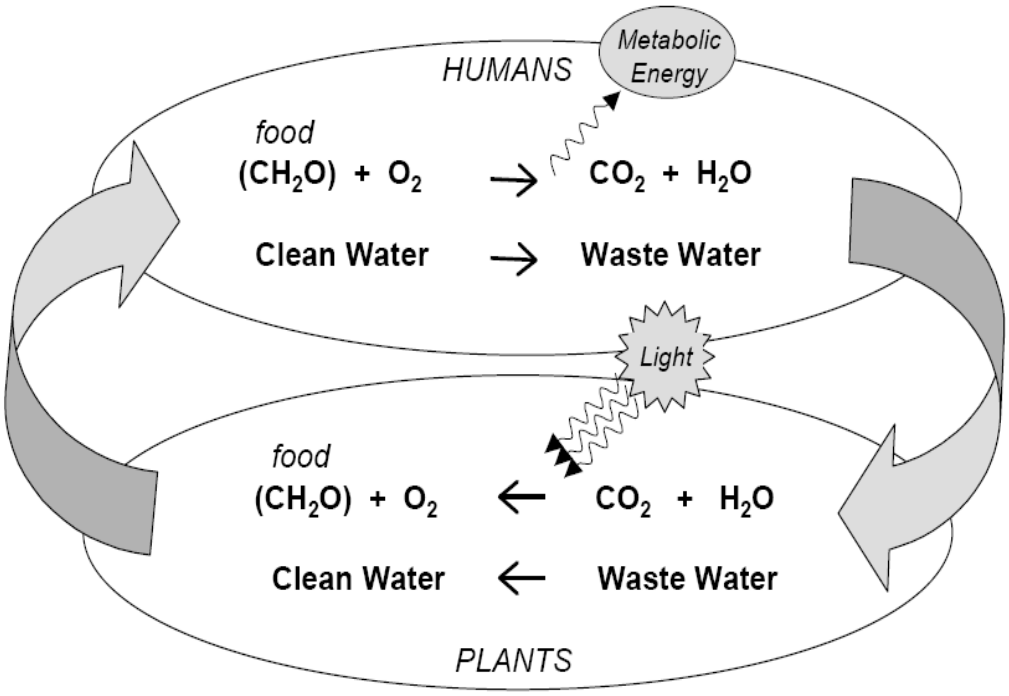


Figure 1-1. Theoretical depiction of a future Martian farm. Courtesy: nasa.gov



(Adapted from Wheeler et al., 2004)

Figure 1-2. Simplified equations showing human respiration (top) and plant photosynthesis (bottom). The products of photosynthesis are oxygen (O_2), which can be used by explorers and carbohydrate (CH_2O), which can be used for food. Through transpiration, plants can be used to purify wastewater, i.e., the transpired water can be condensed as clean water.

Table 1-1. Total pressure and gas composition used to support life in various space missions.

Year	Atmospheric composition	Comment
1957, Sputnik	Sea level gas concentration and total pressure (101 kPa)	Dog (Laika) was the first to enter into space: died of heat stroke (Maleshankov, 2002)
1961-63, Vostok	Sea level gas concentration and total pressure (101 kPa)	First human Yuri Gagarin entered into space (Baker, 1981)
1958-63, Mercury	Total pressure 34 kPa, Pure O_2	Monkeys and Chimpanzees (Martin and McCormick, 1992)
1964-65, Voslhod	Sea level gas concentration and total pressure (101 kPa)	Space walk by cosmonauts (Baker, 1981)
1965-66, Gemini	Total pressure 34 kPa, Pure O_2	Corn (Baker, 1981)
1968, Biosatellite	Sea level gas concentration and total pressure (101 kPa)	First complete plant growth system by USA, Gravitropism studies on pepper plants (Baker, 1981)
1967-72, Apollo	Total pressure 34 kPa, Pure O_2	First Lunar Landing: seed material exposed to lunar atmosphere (Klein, 1981)
1973-79, Skylab	Total pressure 34 kPa, 70 % O_2, 30 % N_2	First US Space Station with Astronauts (Klein, 1981)
1971-83 Salyut 1	Sea level gas concentration and total pressure (101 kPa)	Orchids for psychological benefit and peas, Onion cultivated on board (Smolders, 1973)
1980-98, Mir-I	Sea level gas concentration and total pressure (101 kPa)	Plant complete life cycle on several plant species (Salisbury 1995)
1998- ISS	Sea level gas concentration and total pressure (101 kPa)	Advanced plant level studies (eg. Gene expression),(Brinckmann, 2005)

Table 1-2. Hypobaria studies at various total pressures and gas composition.

Reference	Crop	CO_2 (Pa)	O_2 (kPa)	Pressure kPa	Comment
Seigel et al., **1963**	rye	0.24 %	0.1 %	3, 10, 50, 100	Seeds fail to germinate at 3 kPa total pressure
Mansell et al., **1968**	turnip	40	21	50, 93	Transpiration rate increased at 50 kPa
Rule and Staby, **1981**	tomato	14,28,40	3,7,10	17, 34, 51	Hypobaria increased C_A and biomass
Musgrave et al., **1988**	mungbean	40	2,5,8,21	21-23	No adverse effects of 22 kPa on growth
Goto et al., **1995**	spinach	50, 100	2,8,15,21	50, 75, 101	No difference in growth at 50, 75 & 100 kPa
Corey et al.,**1996**	lettuce	40, 80	10, 21	50, 101	At 50 kPa Pn and biomass increased by 25 %
Daunicht, Brinkjans,**1996**	tomato	40	40	40, 70, 100	At 40 kPa tomato plant had reduced growth
Iwabuchi et al., **1996**	spinach	40	21	25, 101	Growth was same in hypobaria and 101 kPa
Corey et al.,**1997a**	wheat	120	14,21	70	Pn rates slightly increased (15%) at 70 kPa
Massimino and Andre **1999**	wheat	65, 100	8, 18	10, 20	dry mass increased by 75 % at 10 kPa
Goto et al., **2002**	rice	50, 100	21	50,70, 101	Growth normal at 50 kPa, reduced at 34 kPa
Sparnakel and Drew, **2002**	lettuce	70	21	70, 100	Slightly enhanced growth at 70 kPa
He et al., **2003**	lettuce,Wheat	100	6, 12, 21	30	Ethylene synthesis down by 65% at 30 kPa
Iwabuchi and Kurata, **2003**	spinach	40	20	25	At 25 kPa stomata pore size, aperture reduced
Paul et al., **2004**	Arabidopsis	100	2,21	10, 100	over expression of drought related genes
Wilkerson, **2005**	radish	100	21	33, 100	Transpiration enhances at reduced pressure
Paul and Ferl, **2006**	Arabidopsis	100	2, 21	25, 100	Expression of ADH gene under hypoxia
Richards et al., **2006**	Arabidopsis	40, 100	5,10,15,21	25,50,75,100	Pn rates and growth increased at hypobaria
He et al., **2007**	lettuce	100	6, 12, 21	25	Oxygen below 12 kPa reduced plant growth
Levine at al., **2008**	radish	100	21	33, 66, 101	Little effects of hypobaria on nutritional quality
He et al., **2009**	lettuce	100	12, 21	25, 100	Ethylene reduces growth under hypobaria
Wehkamp, **2009**	radish	100	2,6,12,21	10,33,66,101	Long-term hypobaria reduced transpiration loss
Rajapakse et al., **2009**	lettuce	100	6, 10,21	25, 100	Hypoxia and hypobaria increases phyto-chemical

CHAPTER 2

DESIGN AND CONSTRUCTION OF LOW PRESSURE GROWTH CHAMBERS (LPGCS)

In order to grow plants at low pressures, a chamber capable of maintaining low pressure for long periods and a system to control and monitor environmental parameters such as temperature, light, relative humidity (RH) and gas composition is required. The objective of this chapter is to describe the design and construction of four, medium-scale; low pressure plant growth chambers (LPGCs) that were used for the experiments that are described in Chapters 4 and 5 of this dissertation.

Maintaining a plant growth facility on Mars or the Moon at reduced atmospheric pressure (hypobaria) compared to Earth sea level pressure will reduce the cost of launching and transporting the components to build the facility since a smaller pressure difference between the inside and the outside of the facility would require materials with less structural mass. This low pressure facility would also need less gas to pressurize the chamber thus reducing the amount of valuable gases that would be supplied from Earth. These lighter structural materials will promote ease of construction but they must also withstand the harsh conditions of a distant planet or moon. Such a LPGC should be small enough to be efficient in modulating the thermal heat transfer and promote mixing of the gases but minimize the re-supply of gases such as CO_2 for maintaining plant growth. In contrast, the chamber should be large enough to allow for plant growth without hindrance to leaf and plant expansion. According to one estimate, the required area to grow a crop to fulfill 100 % of the food requirements for one person is 50 m^2 (Wheeler et al., 2001). Since building of such a large system is costly, the objective of this project was to build smaller-scale, research chambers (0.09 m^3) that could

accommodate three plants while being able to fit all three chambers into a larger environmentally controlled incubator at UF (Environmental Growth Chambers, Chagrin Falls, Ohio). Sensors in the LPGC were designed to monitor O_2, CO_2, relative humidity, temperature, pressure, light and accommodate three load cells for monitoring transpiration and plant growth.

Other LPGCs have been built in the past to study plant growth but unfortunately, for most of these, there is little documentation or details as to the design and construction of the systems (Purswell, 2002). Most of the LPGCs had limitations in terms of the range of experiments that could perform either due to the small size or due to other drawbacks in their construction. For example**,** Corey et al. (1996) used a system which had very high leak rates thus the plant growth chamber could not be operated below 70 kPa. Daunicht and Brinkjans (1996) used a mass flow controller to regulate the air flow and gas composition and mechanical precision vacuum controller to maintain low pressure; however specifics about the chamber were not given. In their system, excess CO_2 and ethylene was removed by constantly ventilating by bringing in the outside air at normal pressure. Schwarzkopf et al. (1991) designed a LPGC which could be operated down to 1 kPa with leak rates of 1% chamber volume/h but the type of sensors used to measure various parameters was not reported. Simpson and Young (1998) devised a growth chamber to simulate the Martian atmosphere which could operate down to ~0.133 kPa but the data collection system was not described. Recent reviews on the design and construction of other LPGCs are described elsewhere (Mu 2005, Wilkerson 2005, Hublitz, 2006).

The low pressure chambers that were built for this project are based on the designs of previous LPGCs by Wilkerson (2005) and Hublitz (2006) with minor modifications. The largest of these systems used for hypobaria studies was constructed to fit inside a large stainless steel vacuum chamber (1.2 m X 1.2 m X 1.0 m) that simulated the Martian atmosphere of 0.6 kPa (Hublitz, 2006). The LPGC was constructed with a polycarbonate hemispherical dome (1 m diameter) that served as the top of a Mars greenhouse and an aluminum flat plate as a base. This chamber was housed within the larger vacuum chamber. The primary purpose of this LPGC was to study the heat and mass transfer in two of the main Martian conditions, i.e., reduced pressure and extremely low temperature. An industrial freezer (~ 2m X 2m X 2 m) brought the temperature of the vacuum chambers to as low as -22 °C. Hublitz (2006) and used mass flow controllers and solenoid valves to regulate the gases and pressure and grew lettuce plants for approximately seven days in these chambers. A visual interface (LabView software, National Instruments, TX) was used to collect the data and control gas and pressure (Hublitz, 2006). However, localized temperature gradients within the chamber and difficulties in collecting condensate resulted in large ice blocks that accumulated in the lower portion of the chamber. To date, this is the only LPGC that simulated the actual pressure conditions that would be used in a LPGC on Mars, i.e., the chamber at higher pressures than the surrounding environment but still much lower than Earth sea level.

Wilkerson (2005) used a simplified bell jar system which had an aluminum base to house wiring, sensors, a cooling coil and a humidifier with a glass bell-jar top to house the plants. This chamber was 22 cm in diameter and 38 cm high. These

chambers were mainly used to study plant growth and transpiration in low pressure. Although they had a relatively small area for plant growth and were awkward to instrument, the major advantage of these systems were the flexibility in their design with many ports and the o-ring seal for the bell jar was leak proof. The top was also easy to replaced before and after experiments. These chambers were used to grow radish at various pressures for one day. The design of LPGCs described here are larger versions (i.e., 6 times the volume) of the chambers of Wilkerson (2005).

System Description

The chambers designed for these studies (Figure 2-1) consist of two main components, a transparent Plexiglas tube for the cylindrical wall of the cover (1.2 cm thick, at 20 cm in diameter and 60 cm tall) that was topped with a circular (3 cm thick, 20 cm diameter) acrylic disk (Professional Plastics, Fullerton, CA). The top disc had a groove cut out that had an O-ring (2.57 mm diameter) which provided a gas tight fit with the tube. The top of an aluminum cube (25 cm X 25 cm X 25 cm) was welded to a circular aluminum disk (46 cm diameter) that had a square (25 cm X 25 cm) cut out to open the upper chamber to the lower cube. A circular groove (1.9 cm length) in the aluminum disk housed a rubber gasket (Gainesville rubber & Co. Inc., FL) and provided a gas-tight seal when the Plexiglas tube was placed on top and placed under low pressure. The transparent cover makes it easy to monitor plant growth and allows for light transmission. These chambers can be run at pressures as low as ~2 kPa. The chamber is large enough to contain three (7.4 cm X 7.4 cm) pots and all the various sensors to monitor the environment. The aluminum base houses the cooling fan, wires and circuitry for the many sensors. Four LPGCs (internal volume of 0.09 m^3) were

assembled; one for testing and calibrating of the sensors and three for experimental treatments (Figure 2-1). Six, feed-thru (type K) ports in the sides of LPGC allowed wires to pass from the inside to the outside of the chamber (PFT2NPT-4CU, Omega Inc, Stamford, CT). In the studies reported here, only three of the ports were utilized. The chambers have sensors for temperature (DS-75, DS10 Dallas Semiconductors, Dallas, TX), pressure (MPXH6115AC6U, ASCX15AN, Sensym ICT, Milpitas, CA), CO_2 (T-6004, OEM ultrasonic, 6004, Goleta, CA), O_2 (MAX-250, Matec Co., Salt Lake, UT), relative humidity (HH-4602-L-CPREF, Honeywell, Morristown, NJ), light (LI-190, Li-Cor, Lincoln, NB) and three load cell (LPS-0.6 kg, Celtron Tech. Inc. Colvina, CA). A vacuum pump (373 watts) was used to remove gases from the chambers to maintain the low pressure environment.

Gas Leakage Tests

One of the objectives for the LPGC was to minimize the amount of gas leakage. Leaks in the chambers result in the loss of gasses and difficulty in maintaining set points for experimental procedures. The leak rate will also determine how often the vacuum pump must be operated to keep pressures at the set levels. Leaks were prevented by applying vacuum grease to the gaskets that was in the aluminum plate of the base, and through the use of Teflon^{TM} tape around all screw threads. The use of plastic tubing instead of copper tubing that was previously used in other chambers (Wilkerson, 2005) also improved this chamber to make an almost airtight system. These LPGCs are able to hold a pressure as low as 1.3 kPa pressure with the leak rate of 0.03 kPa/h. This low level of leakage required minimal use of the vacuum pump during the experimental procedures (often only once a week). Solenoid valves (F-822-G-4 24VDC, Gulf Controls

Company LCC, Gainesville, FL) were fitted in the vacuum line for air-tight closing of the system. The following formula was used to calculate the leak rate,

$$L = \frac{(Pi - Po) * 100 * 1440}{Pi * t} \quad (2\text{-}1)$$

Where,

L = Leak rate (% Vol/day)

Pi = Initial pressure (kPa)

Po = Final Pressure (kPa)

t = Time interval (hr)

1 Day = 1440 minutes

Data Acquisition and Control System

Wilkerson et al. (2005) used Opto 22 interface hardware (SNAP ultimate brain, Opto22, Temecula, CA) to monitor plant growth and collect data. Although the hardware is very effective for monitoring and controlling sensors, it is bulky and is not easy to modify. The present research used a CR10 data logging system (Campbell Scientific Inc, Logan, USA). The CR10 is a centralized control system which requires fewer components to interface with sensors, it is easy to use and scalable. The data logger is a small computer combined with a sensitive voltmeter that records the voltages (raw analog signals) from the sensors (Figure 2-2) and can be connected with a PC via a cable interface. The PC used software PC208 (Campbell Scientific, Logan, Utah) to convert the voltage data into sensor readings. For example, a 0 to 5 volt signal range can represent either 0 -100 % relative humidity or 0 – 100 % oxygen. It provides the input voltage of environmental parameters at small time interval (programmable) and data can be saved and logged either on the data logger or on the PC. When an

excitation voltage is applied to a sensor, the sensor transmits a voltage signal to the CR10 based on the environmental condition. The PC208W software (Campbell Scientific Inc, Logan, Utah) converted voltage reading into a final reading that is appropriate for the parameter being measure (e.g., relative humidity in %). The software assisted with creating the program, monitoring real-time measurements, and retrieving stored data.

The standard CR10X has 12 single ended inputs which can also be used as 6 differential inputs. It can measure DC voltage up to 2.5 volts. For each chamber, there were two differential type of sensors (a load cell and light sensor) and four, single-ended channels for CO_2, O_2, RH and pressure sensors. The additional channels were not required for the environmental parameters measured in the experiments presented in this dissertation. A single-ended input measures the difference of a single conductor relative to ground; whereas a differential input measures the voltage difference between two conductors. The CR10 requires a nominal 12 volt DC power supply, which was provided by a 12 volt battery. The battery was continuously charged using a 12 volt battery charger.

Sensors and Their Calibrations

Pressure Sensor

The pressure sensors are small integrated circuit sensors. The pressure sensors (ASCX15AN, Sensym ICT) were calibrated against a precision pressure gauge (Digiquartz T60 series, Paroscientific Inc. Redmond, WA) following a method used by Wilkerson (2005). They were calibrated for a range from 0 kPa to 101 kPa. All the sensors were calibrated by linear regression analysis. The response of the sensor was linear with respect to voltage and an example of this calibration for one of the four

sensors is shown in Figure 2-3. The program for controlling pressure ±2 kPa via solenoid valve is contained in Appendix A.

Relative Humidity Sensor

Relative humidity values are not dependent on total pressure, thus calibration at normal pressure was conducted. Relative humidity sensors (HH-4602-L-CPREF, Honeywell, Morristown, NJ) were calibrated in a closed container using saturated salts (Greenspan, 1977). Five containers each had a 500 mL beaker containing saturated salt solutions to maintain specific level of relative humidity at steady state and were incubated for 24-48 h. They were saturated solutions of $MgCl_2$ (32.8% RH), NaBr (57.6 % RH), NaCl (75.3 % RH), KBr (81.8% RH) and K_2SO_4 (97.3% RH). One sensor at a time was calibrated. The sensor was kept inside the container for a period of time until the reading stabilized. Sensors were connected to the CR10 for recording the signals and then the voltage reading of a sensor was recorded. The known relative humidity vs. voltage reading was plotted for each chamber. A slope equation gives an offset values and a multiplier values which were then input into the PC208 program. During the trials all four RH sensors varied <1% of the range compared to each other. Figure 2-4 is an example of one of the RH sensor calibration plots.

Oxygen Sensor

Oxygen concentration was measured by galvanic cell type oxygen sensors (MAX-250, Maxtec, Salt lack city, UT). The Maxtec MAX-250 senses between 0 and 100% oxygen. Due to the pressure differences that were used in this experiment, the factory calibration data was not valid for the low pressure studies used here. The sensor was calibrated following a method used by Wilkerson (2005) and Mu (2005). At the start of the calibration, the chambers were purged twice using nitrogen gas to remove any

residual oxygen. The sensors were calibrated using different mixtures of oxygen and nitrogen. For example, for a 10% oxygen concentration a chamber would be filled to 90 kPa with nitrogen, and an addition 10kPa of oxygen for a total pressure of 100 kPa. This was repeated for other total pressure and oxygen levels and is shown in Figure 2-5A. The slopes and intercepts were then plotted against pressure and regression curves were performed using Excel (Microsoft, Seattle, WA 2003). The data from calibration of one of the three oxygen sensor is this is shown in Figure 2-5B. These lines show the slope and intercept as a function of pressure over time, and can be applied to the sensor readings to determine the oxygen percent at any given signal and pressure using equation 2-2. Where slope is defined by equation 2-3 and intercept is defined by equation 2-4.

$$O_2\,[\%] = \text{Slope}\,(P)*\text{mvolts} + \text{Intercept}\,(P) \quad (2\text{-}2)$$

$$\text{Slope}\,(P) = 367.15*P^{-1.1475} \quad (2\text{-}3)$$

$$\text{Intercept}\,(P) = 0.0012*P^{2} - 0.1581*P + 3.4498 \quad (2\text{-}4)$$

Carbon Dioxide Sensor

The CO_2 sensors used for these studies were the OEM-6004 module (T-6004, OEM ultrasonic, 6004, Goleta, CA). These cost effective modules had a range of 0-2000 ppm. Because these sensors are pressure sensitive, they had to be calibrated at different pressures following a method used by Wilkerson (2005). At the start of calibration, chambers were purged twice using nitrogen gas to get rid of any residual CO_2. The sensor was calibrated using different mixtures of nitrogen (ranging 20 to 100 kPa) and carbon dioxide ranging 0.04 to 0.15 kPa CO_2. Since the carbon dioxide portion was so small, it did not affect the total pressure. The CO_2 gas was added using known

amount of syringe volume (based on the total volume of chamber). Data at these percentages were collected at different total pressures, ranging from 20 kPa to 100 kPa, and plotted against the pressure in units of millivolts. These data were compared on the basis of pressure and a linear regression of each pressure at different percentages was found. These results are shown in Figure 2-6A. The slopes and intercepts were then plotted against pressure and regression curves were performed using Excel (Microsoft, Seattle, WA). The data from an example calibration of one of the three CO_2 sensor are shown in Figure 2-6B. These lines show the slope and intercept as a function of pressure over time, and can be applied to the sensor readings to determine the carbon dioxide percentage at any given signal and pressure using equation 2-5. Where the slope is defined by equation (2-6) and intercept is defined by equation (2-7). Later CO_2 sensor readings were compared against the known syringe volume of CO_2 gas in to chamber (Figure 2-7)

$$CO_2\ [\%] = \text{Slope (P)}*\text{mvolts} + \text{Intercept (P)} \quad (2\text{-}5)$$

$$\text{Slope (P)} = y = 225.51*P^{-1.2942} \quad (2\text{-}6)$$

$$\text{Intercept (P)} = y = -0.0845*P^{2} + 16.533*P - 928.34 \quad (2\text{-}7)$$

Load Cell

The voltage output from the load cells used in these experiments to record the weight of the plants and the flasks did not depend on the total pressure of the environment, thus, the load cells were calibrated at normal pressure following a method used by Wilkerson (2005). A total of nine load cells (LPS-0.6 kg, Celtron Tech. Inc. Colvina, CA)), three per each chamber, were used for the experimental chambers. Each sensor was factory calibrated in terms of FSO (Full Scale Output), which is mV/V output. Since the excitation voltage of CR10 was 5 volts, FSO was multiplied by 5. For

example, a load cell with FSO sensitivity of 1.0 mV/V has full scale = 5.1 mv. The multiplier was calculated by dividing 600g (full scale weight) with FSO. For this example, it was 600/5.1 = 115.88 grams. The offset value is a reading by the sensor when nothing is on the load cell. Load cell readings were tested for accuracy with standard weights (Figure 2-8).

Light Sensor Calibration

The light sensors (LI-190SA, Li-Cor Inc, Lincoln, NE) were insensitive to variation in temperature, pressure and gas composition. Thus, the factory calibration was used for the light sensor following a method used by Wilkerson (2005). Each sensor had certified values of an offset and multiplier. Since the sensor output is in the millivolt range, the signal was amplified in order to be read correctly. A LI-COR millivolt adapter was used to amplify the mvolts into voltage data. The light readings were compared against the reading by a hand held light meter (LI-250A, Li-Cor Inc, Lincoln, NE) from the same company that had been calibrated at the factory. The plot of the hand held vs. connected sensor is given in Figure 2-9.

Temperature Sensors

The temperature sensor (DS10 Dallas Semiconductors, Dallas, TX) is factory calibrated and does not require further calibration even at very low pressures. The temperature sensors were shielded with aluminum foil as they are sensitive to ambient radiation. Leaf temperature was measured by fine gauge- type-K thermocouples (OS36SM-K-140F, Omega; Stamford, CT). The performance of temperature sensor at constant temperature in all three experimental chambers is given in Figure 2-10.

Summary and Future Improvements

Four, LPGCs were designed and met the specifications required. The chambers designed for these studies are suited for studying physiological aspects of plant growth and development in low pressures, including transpiration and the effects of the gas phase on plant growth and development. The LPGSs were able to maintain pressure, O_2 concentration, air temperature and relative humidity to given set points while measuring the plant weight and CO_2 uptake rates. Vacuum feed through ports successfully prevented air leak in to the chambers.

There were a few limitations to this system. For example, nutrient and water supply was not controlled. A circulating hydroponics system would be a better alternative to the flask system that was used here. In addition, water that was transpired, collected on the walls of the Plexiglas top and at the bottom of the metal base for initial studies and this was improved with salt saturated solutions that absorbed excess water. Future improvements would include a mechanism for collecting condensate using a cooled coil or other mechanisms. Humidity control was not available for values below 85%. Future improvements would include a humidity control system to study the interacting effects of humidity and low pressure on plant growth. For these studies, the the light levels were at 250 µmol m^{-2} s^{-1} using cool-white, florescent lamps to avoid hazards in environmental chambers associated with other lamps. However, on the surface of planets the light levels may be very high at or above 400-600 µmol m^{-2} s^{-1} so improvements to the lighting system may be required. Although the CR 10 data logging system is easy to use, it requires battery power supply which needs to be charged at regular intervals. The limited number of ports on the CR10 prevented the addition of more sensors. A centralized data logging system will be more effective as it

requires less wiring and is less complex and could readily be adapted to connect to user friendly software for programming such as LabView (National Instruments, Austin, TX) and scale up of the instrumentation would be relatively easy. An efficient way to maintain and control concentration of nitrogen, oxygen and carbon dioxide is required and a mass flow controller which automatically controls the gas levels is needed. Ethylene can be scrubbed by circulating a chamber air through stainless steel tube containing potassium permanganate (He et al., 2009). Although, in the present study temperature control within a chamber was not required, it will be necessary to have temperature control for self-contained chambers that would be used on the Moon or Mars.

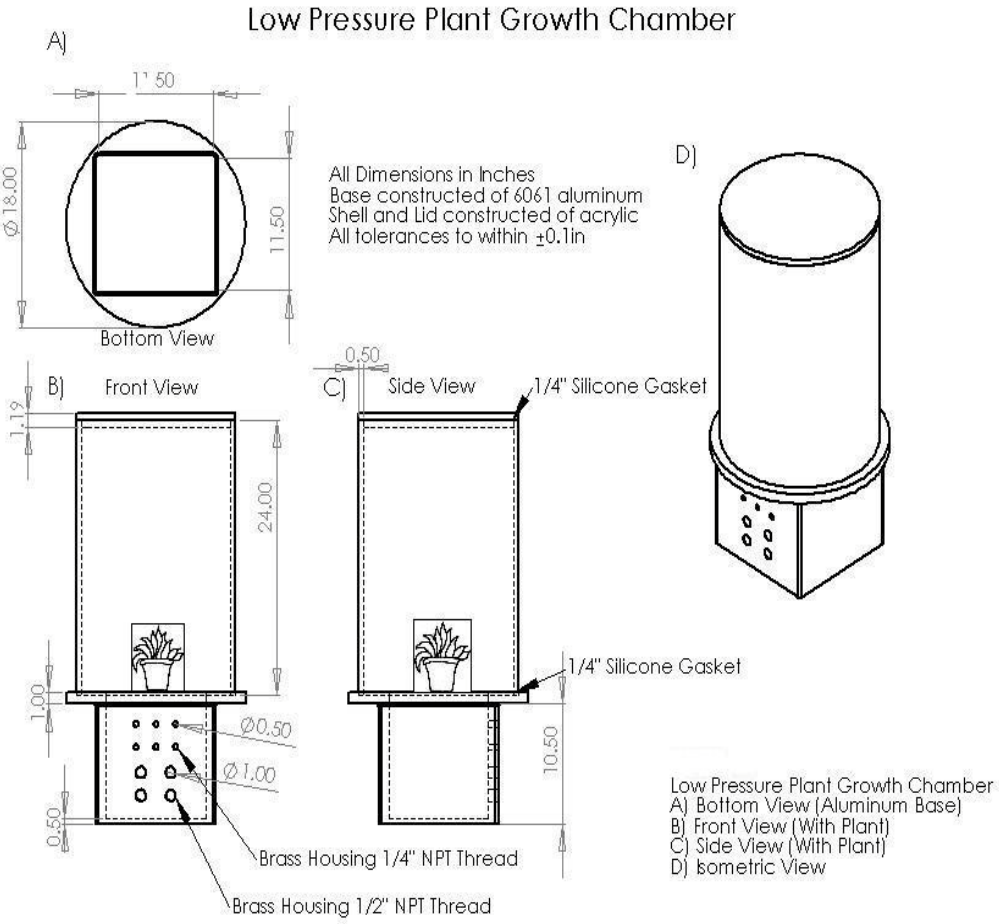


Figure 2-1. Low Pressure Growth Chamber (LPGC)

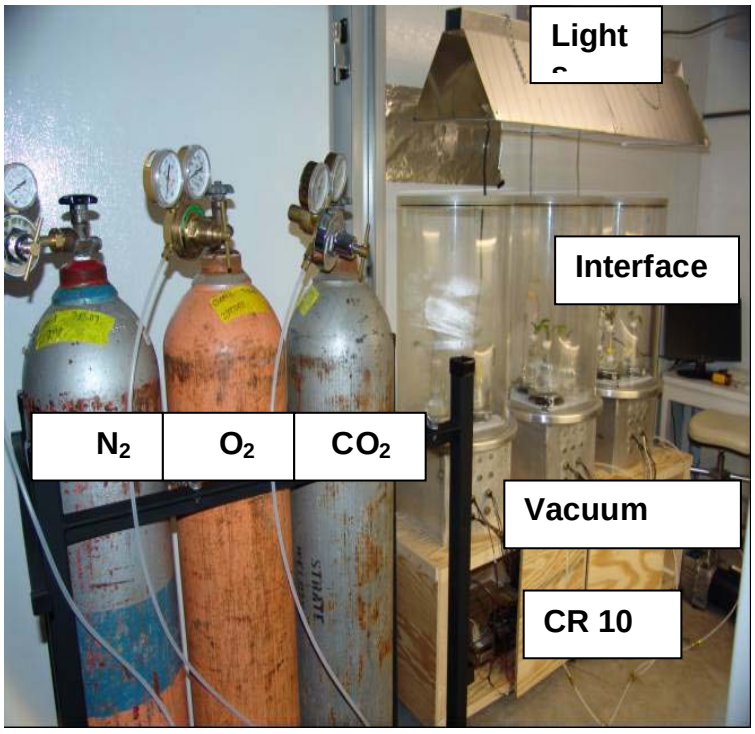


Figure 2-2. Low Pressure Growth Chambers developed for this objective with gas tanks, datalogger (CR10) and PC interface.

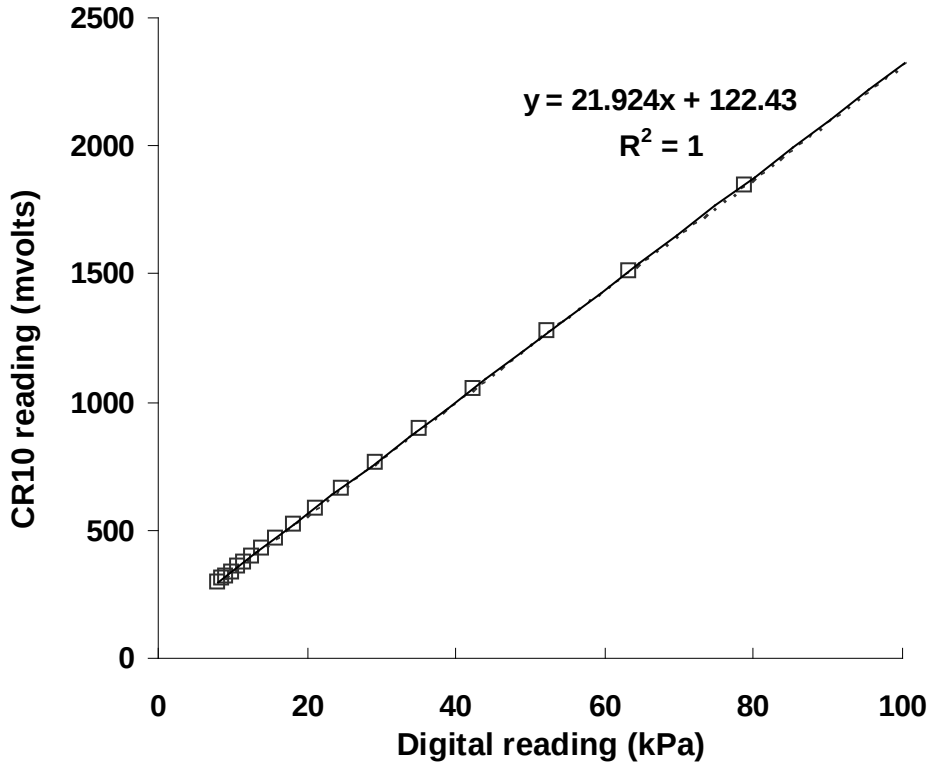


Figure 2-3. Calibration of the pressure sensor comparing pressure gauge reading against millivolts readings.

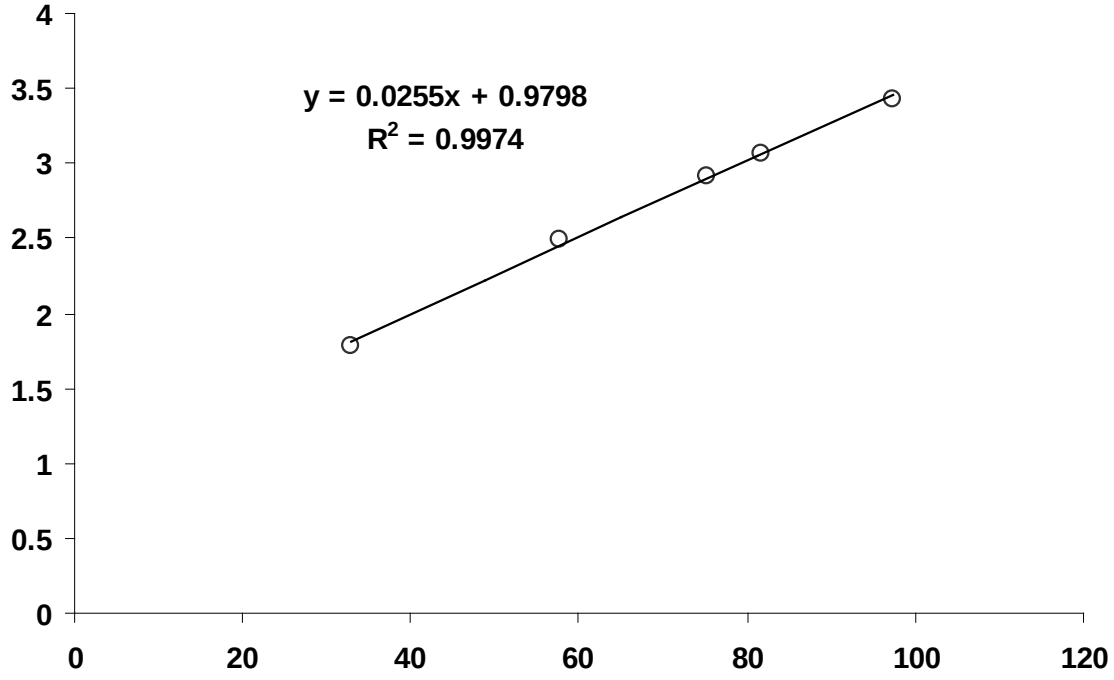


Figure 2-4. Calibration of the relative humidity sensor against known saturated salt solutions.

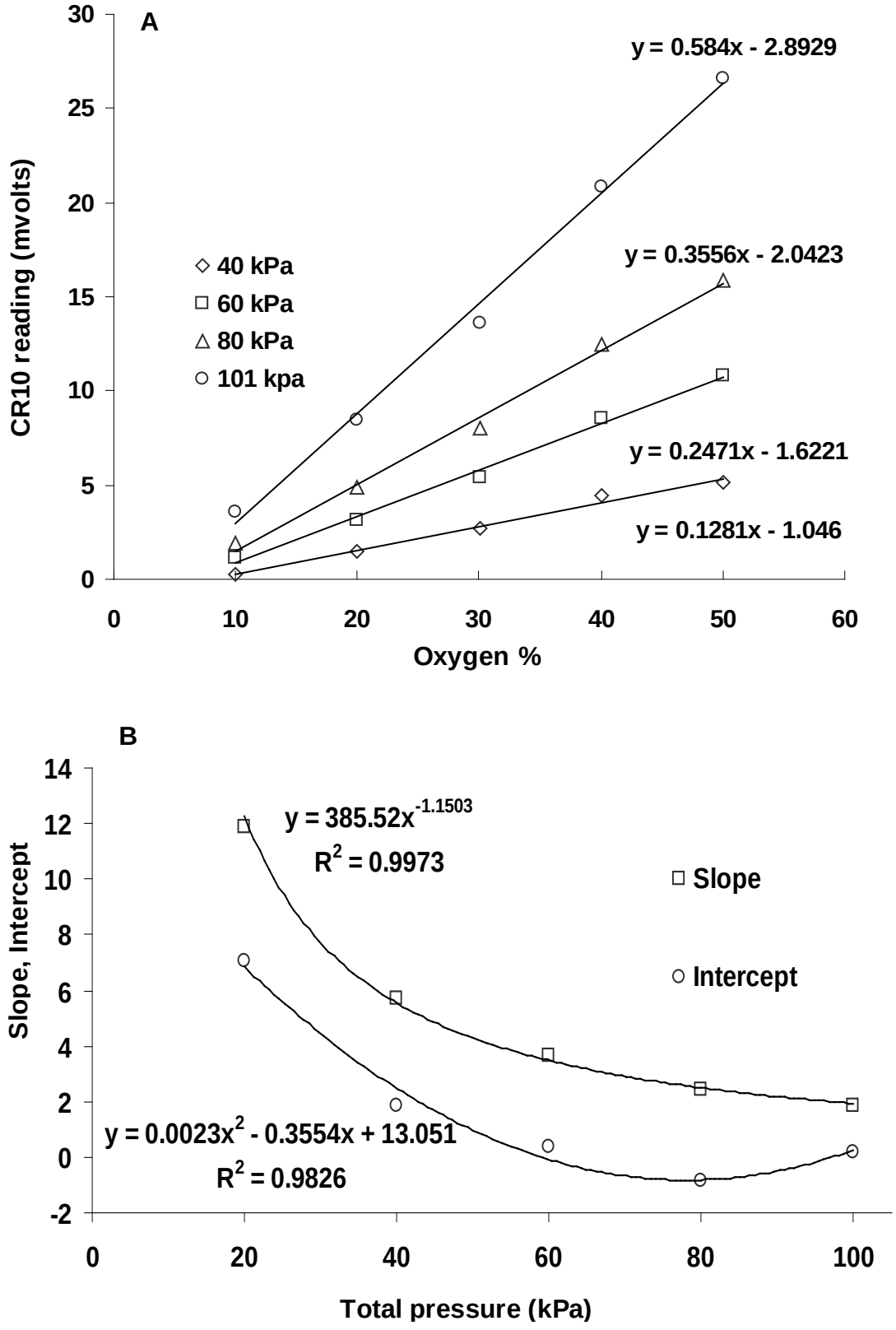


Figure 2-5. Calibration curves of an oxygen sensor at different pressures (5A) and curves of slope and intercept (5B).

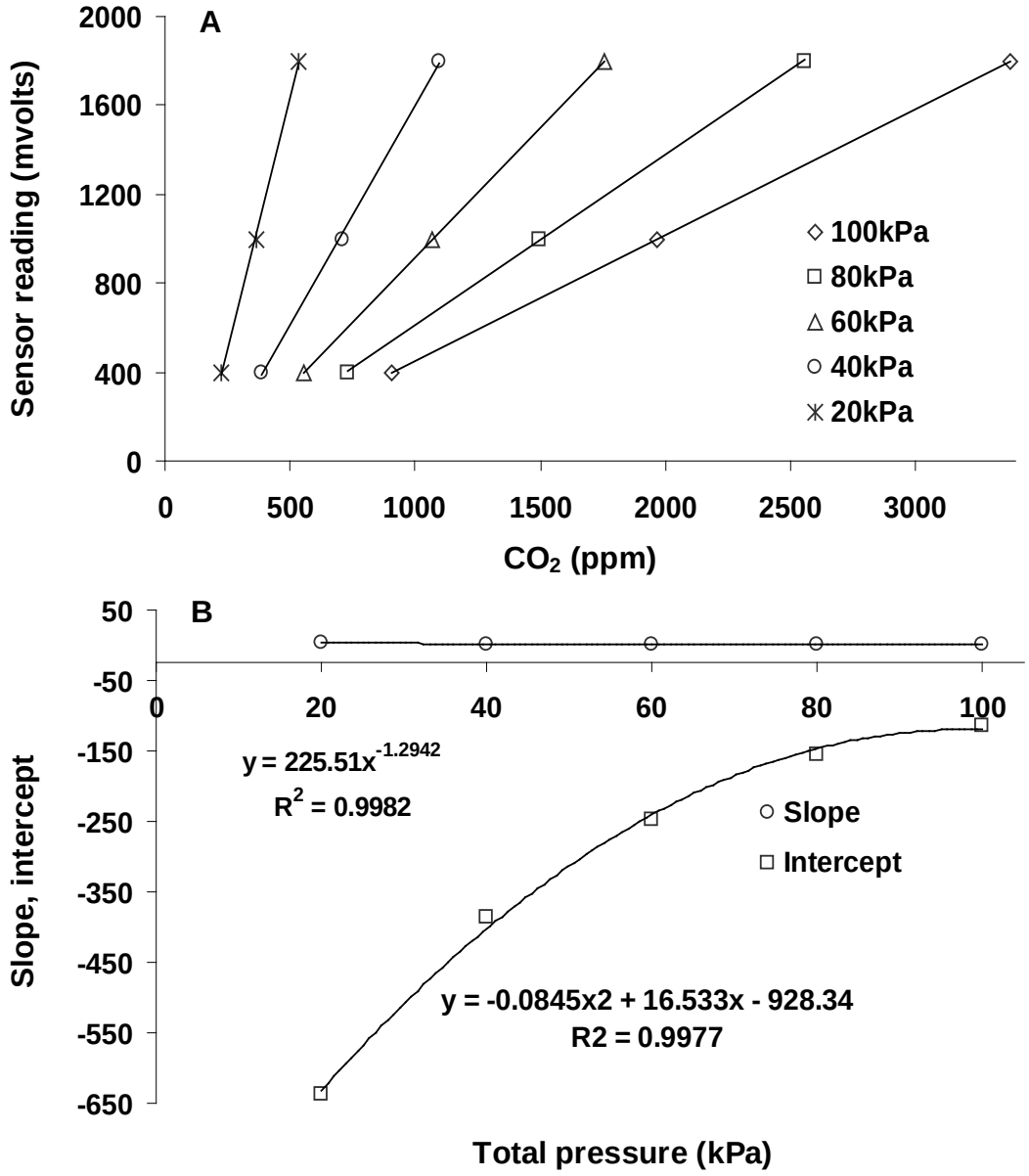


Figure 2-6. Calibration curves for a CO_2 sensor at different pressures (A) and curve of slope and intercept (B).

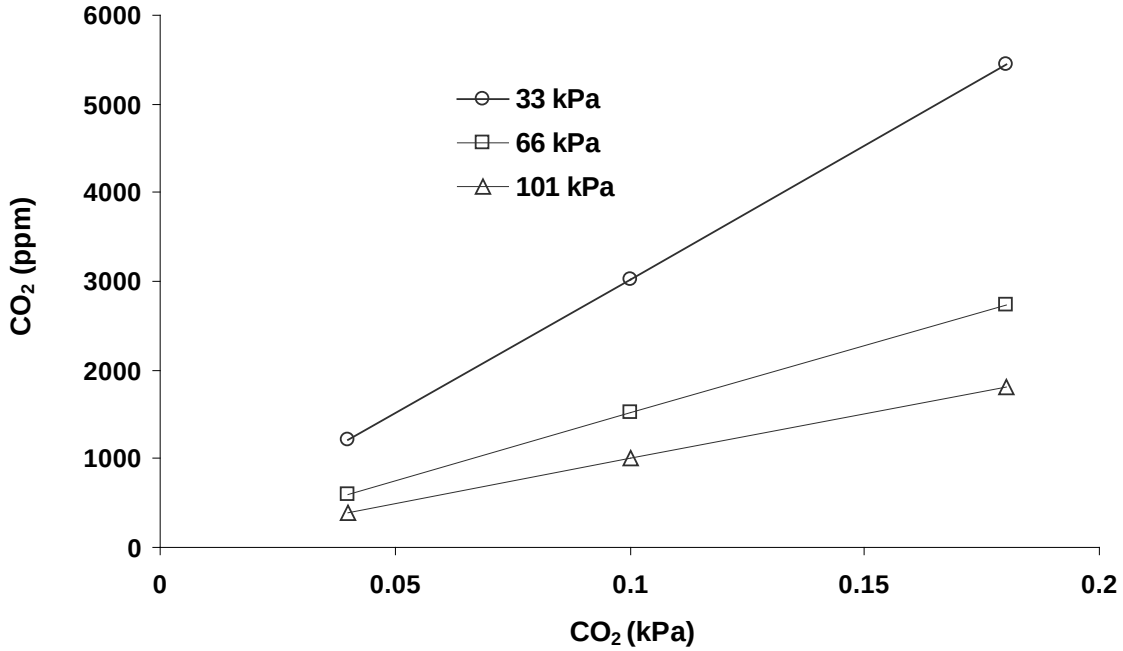


Figure 2-7. CO_2 sensor reading against the equivalent syringe volume

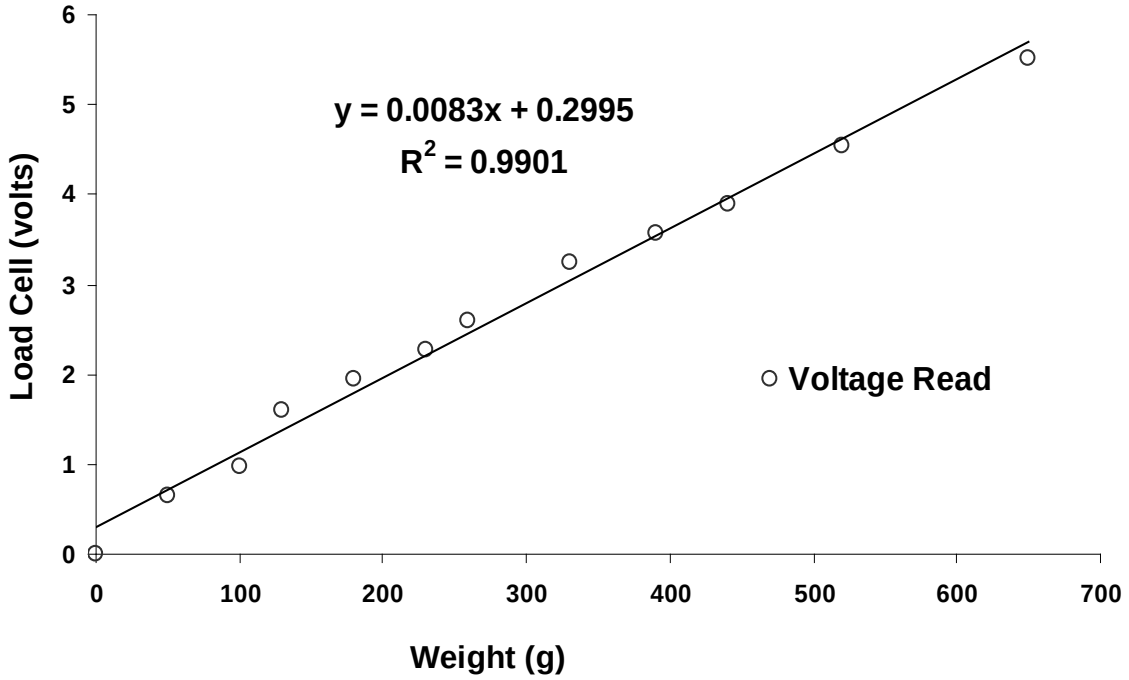


Figure 2-8. Calibration for a load cell used to measure the weight of the flask containing plants.

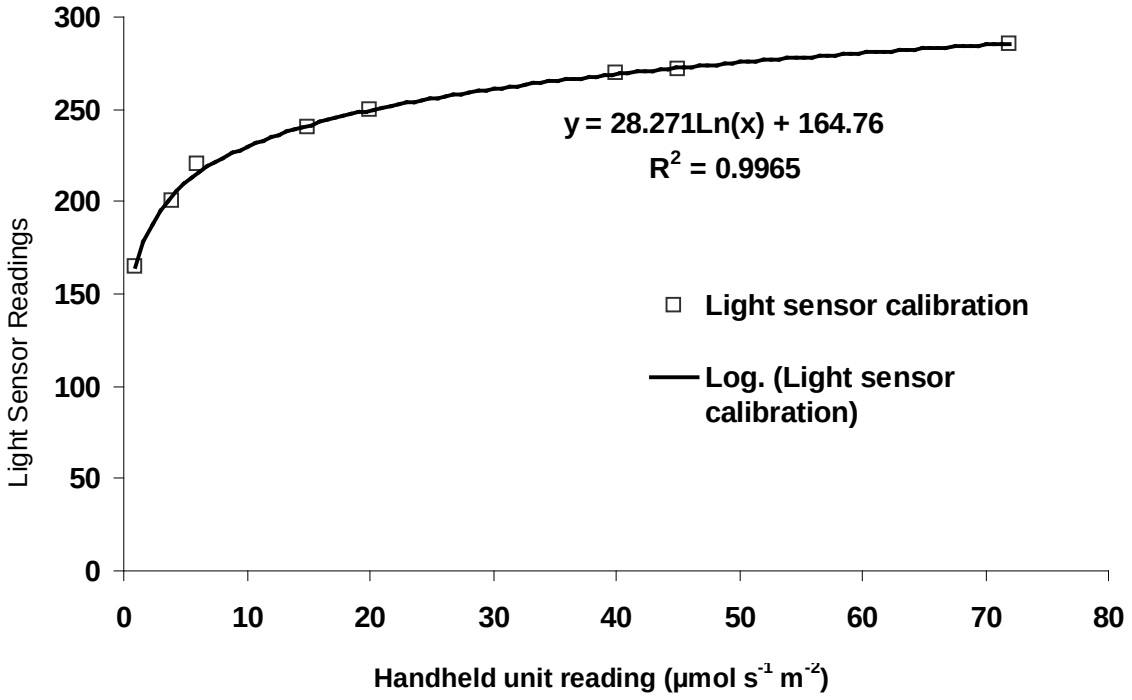


Figure 2-9. Calibration curve for light sensor readings against handheld light meter

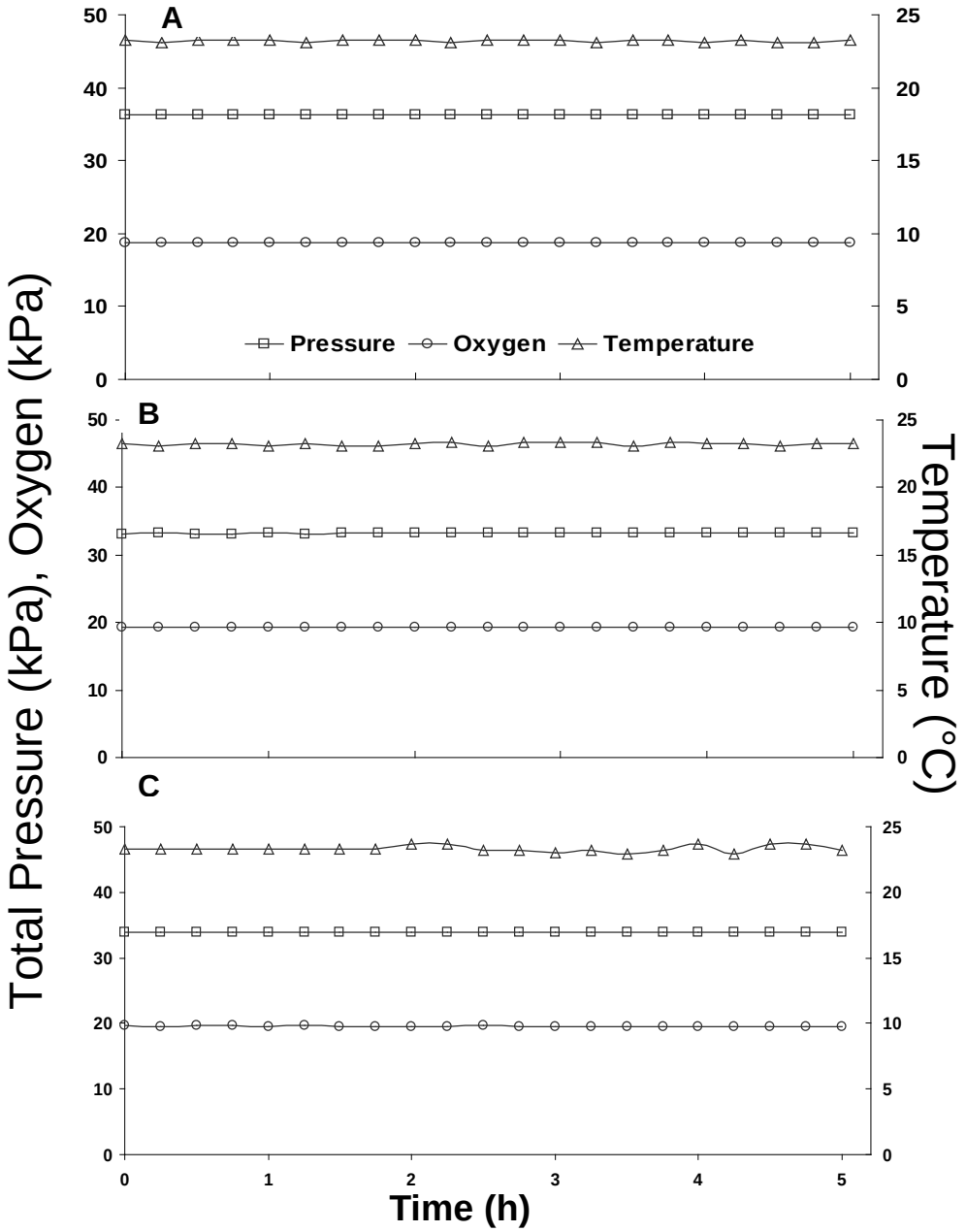


Figure 2-10. Pressure (~33 kPa), O_2 (~20 kPa) and temperature (~23 °C) recordings for five hour for chamber A (top panel), chamber B (middle panel) and chamber C (bottom panel).

CHAPTER 3

MODELING GAS DIFFUSIVITY, PHOTOSYNTHESIS AND TRANSPIRATION UNDER HYPOBARIA

As an integral part of Bioregenerative Life Support System (BLSS) for long-term space missions, plants will likely be grown at reduced pressure. At reduced pressures, the diffusivity of gases increases. This will affect the rates at which CO_2 is assimilated and water is transpired through stomata. To understand the effects of reduced pressure on plant growth, the diffusivities of CO_2 and H_2O at various total pressures (101, 66, 33, 22, 11 kPa) and CO_2 concentrations (0.04, 0.1 and 0.18 kPa) were calculated. The diffusivity is inversely proportional to total pressure and shows dramatic increase at pressures below 33 kPa (1/3 atm.). A mathematical relationship based on the principles of thermodynamics was developed for calculating the transpiration and photosynthesis for plants. Stomatal conductance is sensitive to total pressure. At 33 kPa total pressure, stomatal conductance increases with the boundary increasing by a factor of ~1.7, thus the boundary layer thickness conductance increases by 70%. Since the leaf conductance is a function of both stomatal conductance and the boundary layer the overall conductance will increase resulting in significantly higher levels of transpiration as the pressure drops. The conductance of gases is also regulated by stomatal aperture in an inverse relationship. Stomatal aperture is directly influenced by concentration of CO_2 inside the leaf space. The higher CO_2 concentration inside the leaf air space during low pressure treatments may result in stomata closing partially or fully which may reduce the excessive transpiration caused by increased diffusivity. Therefore, a reduced pressure environment with high CO_2 may be an ideal scenario for minimizing transpiration and maximizing the plant biomass yield in BLSS.

Plants in Hypobaria

Long-term missions to Moon and Mars will require a well-studied and predictable Advanced Life Support System (ALS) for space explorers. Among other components such as like waste treatment and energy production, bio-regeneration (BLSS) through the use of plants has been identified as an integral part of ALS. Plants will be utilized for CO_2 removal from the air that is respired from explorers, for oxygen regeneration and water purification systems, and as a source of food for human use (NASA, 2003). There is no atmospheric pressure on the Moon with very little of an atmosphere on Mars (less than 1 kPa total pressure). Chambers used to grow plants will likely be maintained at reduced total pressure (i.e., hypobaria). Operating the chamber at reduced pressure will mitigate the negative effects caused by large pressure differences between the inside of the chamber and the vacuum/low pressure environments of the Moon or Mars (Bucklin et al., 2004). The reduced pressure will also reduce consumable and structural components of ALS, thus launch cost. Another advantage of lower pressure would be reduced leakage of valuable gases requiring less total gas to pressurize the chamber. Richards et al. (2006) and Paul and Ferl (2006) reviewed the advantages of low pressure environments for plant growth on space missions. One of the fundamental differences in hypobaria compared to normal atmosphere is the diffusivity of gases (Rygalov et al., 2004), which may influence rates of water vapor loss and carbon dioxide (CO_2) assimilation by plants. The effects of diffusivity on the plant transpiration at normal atmosphere have been studied (Mott and Parkhurst, 1991) but there are very few studies (Gale, 1972; Goto et al., 1996) on the effects of diffusivity of CO_2 and water on plant growth at hypobaria. This article reviews the physical relationships that

influence the diffusivity of H_2O and CO_2 in hypobaria, and the consequent implications for plant growth.

Binary Diffusion of Gases

The general relationships defining gaseous diffusion in physical, chemical and biological systems have been developed, although not applied for understanding gas exchange by plants in hypobaria. Gas flux density, according to Fick's law is directly proportional to the driving force, i.e. partial pressure gradient, and the conductance of the gas in the gas mixture. Gas conductance is dependent on the mass diffusivity of the binary gas system (D_{AB}) as a function of temperature, pressure and the gas composition. In a binary gas system, D_{AB} is proportional to temperature and inversely proportional to pressure. It is the sensitivity of D_{AB} to pressure that is of special interest in growing plants in hypobaria chambers. The earliest attempts to derive the mathematical relationship to calculate binary gas diffusivity at reduced pressure used a Stephan-Maxwell hard-sphere model (Hirshfelder et al., 1948; Bird et al., 1954). They presented the mathematical expression of binary gas diffusion based on the kinetic theory describing constant motion of atoms, ions and molecules at temperature above absolute zero. The diffusion coefficient of the gases at reduced pressure can be estimated from the intermolecular forces of the fluxes (Slattery and Bird, 1958). To improve upon the calculations based on theoretical properties and get more accurate gas phase co-efficient, Bird et al. (1960) simplified the derivation of Slattery and Bird (1958) by using the Chapman-Enskog model (Equation 4-1; Chapman and Cowling, 1952), which defines the binary diffusion coefficient of gas A in gas B (D_{AB}, $cm^2\ s^{-1}$) in terms of potential energy of interaction between a pair of molecules in the gas. They presented the theoretical diffusion over the range of pressures from 10 to 100 kPa and

compared it with experimental data. Though, the predicted values were within 5% of the calculated values, it required considerable calculations.

$$D_{AB} = \frac{3}{32 n \sigma_{AB}^2} \left[\frac{8kT}{\pi} \left(\frac{1}{m_A} + \frac{1}{m_B} \right) \right]^{1/2} \quad (3\text{-}1)$$

where n = total concentration of both species, mol cm^{-3}
T = temperature, °K
k = the Boltzmann constant, ergs/°K
m_A, m_B = molecular mass, g mol^{-1}
σ_{AB} = collision diameter, separation between molecular centers of unlike pairs upon collision, cm

In deriving Equation 3-1, the following assumptions were made,

The gases are non reactive.
The gases as a whole are assumed to be at rest but the molecular motion is taken into account.
All molecules have velocities, representing the region of last collision.
Temperature profile of gas corresponds to the Fourier's law of heat conductance.
Thermal conductivity for polyatomic gases is calculated at low density.

Equation 3-1 has two main limitations because it is based on the hard-sphere model (Fuller et al., 1966).

There is a difference between theory and experimental observations of the values of σ because the increased temperature softens the molecule which reduces the σ.

Very few values of σ are available in literature, and they are applicable for only a narrow temperature range because of the temperature dependence of σ.

Arnold's (1930) original suggestions to overcome these limitations were implemented by Fuller et al. (1966) by,

- Replacing the temperature dependence with Sutherland temperature corrections to improve the temperature dependence.
- Replacing σ with cube root of sums of Le-Bas atomic volume parameter which is an easily measured property of a diffusing substance.

An empirical analysis done by Fuller et al. (1966) described D_{AB} by the following general equation.

$$D_{AB} = \frac{c\ T^{b}\left(\frac{1}{m_A} + \frac{1}{m_B}\right)^{1/2}}{p\left[(\Sigma V_A)^{\alpha 1} + (\Sigma V_B)^{\alpha 2}\right]^{\alpha 3}} \qquad (3\text{-}2)$$

where c = an empirical constant
p = pressure, Pa
b = empirical temperature power dependence
V_i = special diffusion parameters to be summed over atoms of diffusing species at normal boiling point, $m^3\ kmol^{-1}$ here, V_{air} =20.1, V_{H2O} = 12.7, V_{CO2} = 26.7
α_1, α_2, α_3 = empirical exponents to the diffusion volumes.

Fuller et al. (1966) used results from about 300 experimental values to obtain the empirical coefficients for various gas mixtures by using a non-linear least square analysis. Optimization to obtain the smallest standard deviation resulted in the following empirical relationship.

$$D_{AB} = \frac{10^{-3} T^{1.75}\left(\frac{1}{m_A} + \frac{1}{m_B}\right)^{1/2}}{p\left[(\Sigma V_A)^{1/3} + (\Sigma V_B)^{1/3}\right]^{2}} \qquad (3\text{-}3)$$

The Effects of Background Gases on Overall Diffusivity

Generally, air is composed of multi-gas components and can be considered one single gas if the concentration of individual gases does not change. However, in a system where total pressure and the partial pressures of the individual gases are varying, considering air as a single gas may not give correct values of overall diffusivity of CO_2 and H_2O. In complex biological systems such as alveolar regions of lungs, closed plant/animal growth chambers, fermentation vessels and plant leaf stomata, the air cannot be represented by binary gas constituents (Johnson, 1999). The partial

pressure of the background gases directly affects the overall diffusivity of a gas. For example, diffusivity of oxygen in a mixture of nitrogen will not be the same as in a mixture of helium, even though the total pressure and pO_2 remains the same (Mott and Parkhurst, 1991). Higher values of mass diffusivity mean larger amounts of mass transfer.

Fuller et al. (1966) proposed a method to calculate the individual constituent mass diffusivity (or overall diffusivity of particular gas) in a multi-component gas at different mole fractions. The calculation of mass diffusivity of a constituent gas (for e.g. CO_2 in air composed of N_2, CO_2, O_2 and H_2O) requires first the calculation of binary diffusivity of that gas (CO_2) in all gas components (Equation 3-3).

$$D_{\mathrm{A}} = \left[\sum_{j=1}^{N} \frac{\mu_j}{D_{AB}} \right]^{-1} \tag{3-4}$$

where, D_A = mass diffusivity of a constituent *A* in multi gas ($m^2\ s^{-1}$).
μ_j = the mole fraction of gaseous component
D_{AB} = Binary mass diffusivity of constituent *A* in a gas *B* ($m^2\ s^{-1}$).
Eq. 5 can be applied to our case to define D_{CO2} in a chamber composed of CO_2, O_2, N_2 and H_2O.

$$D_{CO_2} = \left(\frac{\mu_{CO_2}}{D_{CO_2 - CO_2}} + \frac{\mu_{H_2O}}{D_{CO_2 - H_2O}} + \frac{\mu_{O_2}}{D_{CO_2 - O_2}} + \frac{\mu_{N_2}}{D_{CO_2 - N_2}} \right)^{-1} \tag{3-5}$$

Diffusivity of CO_2 and H_2O in Hypobaria

The earlier studies on various aspects of plant growth under reduced pressure have suggested that plant growth is possible down to 10 kPa (Musgrave et al., 1988). Also, high partial pressures of CO_2 up to 200 Pa can be beneficial to plant growth. Therefore, for clarity, the diffusivity of H_2O and CO_2 in binary gas systems was calculated at various total pressures and pCO_2 using equation (Equation 3-4). The

pH_2O and temperature were held constant for all calculations at 2.64 kPa and 22 °C, respectively.

As shown in Figure 3-1, the diffusivity of both H_2O and CO_2 is highly sensitive to pressure. Diffusivity of the gases increases as pressure decreases, reflecting the diminished interaction among gas molecules. The increase in diffusivity is not linear but shows a dramatic increase at low pressures of below 20 kPa. Increasing the CO_2 partial pressure in air does not significantly change the diffusivity from physiological levels. For example, at 33 kPa total pressure, when CO_2 is increased from 40 to 180 Pa, the mole fraction increases from 0.001 to 0.005 (mol $kmol^{-1}$), which is much less when compared to the sum of mole fraction of remaining gases (pO_2 at 21 kPa = 0.606 mol $kmol^{-1}$ and pN_2 at 12 kPa = 0.313 mol $kmol^{-1}$).

The ratio of the diffusivity of H_2O to CO_2 is interesting because of the impact of the exchange of gas by plants for these two gases. The diffusivity of H_2O under 101 kPa total pressure is generally about 1.6 times higher than CO_2 due to two main reasons (i) the difference in the molecular weight of water (18 g mol^{-1}) and CO_2 (44 g mol^{-1}) and (ii) the atomic volume of water (12.7 g mol^{-1}) and CO_2 (26.7 g mol^{-1}). Since these two factors are not sensitive to pressure, the ratio of diffusivity of the two gases is virtually insensitive to pressure (inset Figure 3-1). At standard temperature and pressure, the ratio of gaseous phase resistance of water and CO_2 in air is 1.7 (Bieurhuizen and Slayer, 1964a). The calculation of the ratio based on assumptions by Gale et al. (1972) also agrees on value of the diffusive resistance around of 1.7 though their assumptions for the diffusive resistance ratio equation did not include the low pressure.

Gas Diffusion in Leaves

The growth and health of plants is dependent on their ability to transpire H_2O and assimilate CO_2. Both of these gas exchange processes by leaves is dependent on the sensitivity of gas diffusivity. This can be shown by examining the basic equations defining leaf gas flux density. The fundamental description of mass flux density for a leaf is the Onsager expression for coupled non-equilibrium flow as a result of water vapor and temperature gradients (Katchalsky and Curran, 1967).

$$J = L_v \Delta\mu_v + L_{vT} \Delta T \tag{3-6}$$

where, J = Vapor flux density (mol cm^{-2} s^{-1})
L_v= Onsager coefficient of vapor
L_{VT} = Onsager vapor-temp coupling coefficient
$\Delta\mu_v$ = vapor chemical potential difference across the diffusion path. (J mol^{-1})
ΔT = Temperature difference across the diffusion path (°K)

Since in most plant systems there is a small temperature gradient and a comparatively small coupling coefficient, Equation 3-6 reduces to the familiar simplified expression of flux density of individual gas species under steady state conditions. In Equation 3-7 the Onsager coefficient is expressly defined by

$$J = m\rho_v \left(-\frac{\partial\mu}{\partial \mathrm{X}} \right) \tag{3-7}$$

where m = mobility co-efficient, cm^2 mol J^{-1} s^{-1}
ρ_v = vapor density, mol cm^{-3}
μ = chemical potential of vapor, J mol^{-1}
x = distance, cm

For the short distances of vapor flux between leaves and the bulk atmosphere, the chemical potential is defined by the vapor pressure gradient (J cm^{-3}). When the atmosphere adjacent to the liquid surface is saturated with vapor (P_v*)

$$J = -\frac{m}{\Delta x}\left(P_v * - P_v\right) \tag{3-8}$$

The mobility coefficient can be explicitly defined as a function of gas diffusivity.

$$m = D\frac{\rho_a}{P_a} \tag{3-9}$$

where, P_a = Pressure of air, J cm^{-3}
ρ_a = density of air, mol cm^{-3}

On the molar basis, the evaporation rate can be written simply as

$$E = \frac{D}{\Delta x}\frac{\rho_a}{P_a}\left(P_v^* - P_a\right) \tag{3-10}$$

The D/Δx defines the conductance (cm s^{-1}) for vapor flux from a surface at saturated vapor pressure.

Leaf Transpiration

Transpiration is more complicated than simple diffusion because the surface for evaporation is buried inside a leaf at the cell walls. Transpiration requires the diffusion along the pathway from the cell walls through stomatal pores and then through the leaf boundary layer. The conductance limitation to vapor in this pathway from inside leaves is predominantly stomatal (Rand, 1977) at about 90 % or more of the limitation (Noble, 1990). Therefore, the conductance for transpiration must account for gas conductance through stomata pores. The stomatal conductance (h_s, cm s^{-1}) is directly dependent on gas diffusivity as well as pore aperture and pore depth (Parlange and Wagoner, 1970). Equation 4-11 describes this relationship.

$$h_s = \frac{\pi \cdot a \cdot b \cdot D \cdot n}{d + \frac{b}{2}\ln\left(\frac{4a}{b}\right)} \tag{3-11}$$

where *a, b* = length of major and minor axis of pore aperture, respectively, cm
d = pore depth, cm
D = diffusivity of water vapor (cm^2 s^{-1})
n = stomata density (number cm^{-2})

The value 'b' (pore width) is dependent on conditions in the leaf and commonly varies from 0 to $4*10^{-4}$ cm (Sinclair, 1980). Environmental factors such as light, CO_2 concentration and vapor pressure deficit can have large influences on pore width.

Equation 3-11 also clearly demonstrates that stomata conductance is dependent on gas diffusivity. As shown previously, the value of D is a function of a total pressure, and hence total pressure will have direct influence on stomatal conductance. Under low pressure situations Figure 3-1 shows that D is increased, which according to Equation 3-11 means stomata conductance is increased. For example, for plants grown at one third of the standard earth atmospheric pressure stomatal conductance will increase by the ratio of mass diffusivity at 0.3 atm to that of at 1 atm (Figure 3-1), thus increasing in stomatal conductance by approximately a factor of 3. Thus, transpiration rate and CO_2 assimilation rates would be substantially increased under hypobaria conditions.

The conductance of the boundary layer surrounding the leaf is also dependent on gas diffusivity, although in a complex manner because of the convective movement of air. The theoretical considerations used for the flat plate evaporative loss through boundary layer can be applied for the plant leaf (Sinclair, 1980). The effects of boundary layer on a flat plate of an infinite length (Gebhart, 1961) can be estimated by the following equation.

$$h_{bl} = 0.664 \frac{K}{L} \mathrm{Re}^{1/2}\, Sc^{1/3} \qquad (3\text{-}12)$$

where, h_{bl} = vapor boundary layer conductance, cm s^{-1}
K = fluid thermal diffusivity (k/ ρ*Cp), 0.215 cm^2 s^{-1} at 20 C
Where,
k = thermal conductivity ($W.m^{-1}.K^{-1}$), C_p = specific heat (J kg^{-1} K^{-1})
ρ= the fluid density (kg m^{-3})
Re = Reynolds number
Sc = Schmidt number

The fluid thermal diffusivity is a function of 1/ ρ whereas the thermal conductivity and specific heat will remain almost constant for constant temperature and low pressure ($p < 2$ atm; Salazar, 2003). For example, at 33 kPa the K will be ~3 times the value of K at 101 kPa.

Re = Reynolds number (uL/K_v)

where L = length of flat surface, cm
u = air speed, cm s^{-1}
Kv = kinematic viscosity at 0.15 cm^2 s^{-1} at 20 °C.

The *Re* is inversely of related to kinematic viscosity (μ/ρ). Thus, the Reynolds number is significantly affected by pressure. The dynamic viscosity does not significantly change with pressure. For example at 33 kPa, the Re will decrease by a factor of 3 compared to normal atmospheric pressure since the density of gases is 1/3 of the normal pressure.

Sc = Schmidt number ($\mu/\rho D$)

where μ = fluid viscosity (N s m^{-2})
D = mass diffusivity, m^2 s^{-1}.

The *Sc* is a function of fluid density and diffusivity. At 33 kPa, the diffusivity of gases increases by three times compared to normal atmospheric pressure but this value is offset by the decrease in the density of air. The value of *Sc* at 101 kPa as well as at 33 kPa (20 °C) equals approximately 0.63 for water vapor**.** Overall, at 33 kPa the value of h_{bl} will be increased by a factor of ~1.7 compared to normal atmospheric pressure (101kPa).

Combining the constants at 20 °C, the boundary layer conductance (h_{bl}) for both sides of leaf in a laminar flow can be predicted from Equation 3-13. Thus, the diffusion coefficient also influences the boundary layer conductance through the Schmidt

number. However, the fact that the Schmidt number is taken to the cube root and the boundary layer conductance offers little limitation to gas flux density, the following simplified equation is generally appropriate (Sinclair, 1980).

$$h_{bl} = 0.63\sqrt{\frac{u}{L}} \tag{3-13}$$

where L is the length (cm) of the leaf
u is the wind velocity ($cm^2\ s^{-1}$).

At 33 kPa, the co-efficient value will be 1.096, which is 1.74 times the co-efficient for 101 kPa.

The combined conductance (stomatal and boundary layer) can be designated as h ($cm\ s^{-1}$).

$$h = \frac{h_s * h_{bl}}{h_s + h_{bl}} \tag{3-14}$$

This equation suggests that the combined conductance will increase as pressure decreases. For example for h at 33 kPa, the values would be 3 and about 1.7 times the normal atmospheric pressure values, for h_s and h_{bl}, respectively. This is assuming similar stomatal open geometries and air velocities between 33kPa and 101kPa.

Inserting the stomatal and boundary layer conductance into an equation in the general form of Equation 3-15, the transpiration (T, units) can be defined by the following equation (Sinclair, 1980).

$$T = h*(P_L - Pv) \tag{3-15}$$

where, P_L = saturated vapor pressure at leaf temperature, Pa
Pv = vapor pressure outside the leaf air space, Pa

It should be noted that as transpiration rates increase in lower pressure, the surface leaf temperature will drop due to latent heat of vaporization. This drop in

temperature will result in a lower vapor pressure deficit in low pressure compared to higher pressures. This would decrease transpiration rate below the initial estimate.

Leaf Photosynthesis

Photosynthesis is a function of the partial pressure carbon dioxide gradient but the direction of diffusion is opposite of that of transpiration. Similar to *T*, photosynthesis is a function of the CO_2 partial pressure (Pa) difference between the atmosphere and the inside of the leaf, and total gas conductance (Equation 3-16; Sinclair 1980).

$$A = 44 * h * (P_{CO2a} - P_{CO2i}) \quad (3\text{-}16)$$

A is the photosynthetic rates and 44 is the molecular weight of CO_2. The P_{CO2a} and P_{CO2i} are the leaf outer and internal leaf partial pressures (Pa) of CO_2, respectively. The 'h' is the total conductance for CO_2 which was described for transpiration and would increase as pressure drops. Stomatal conductance is a function of the stomata aperture, which in turn is dependent on the diffusion coefficient of CO_2 (Montieth and Unsworth, 1990). Because the mass diffusivity is a function of a pressure and the molecular weight, the effects of reduced pressures should be reflected in its effects on plant growth (i.e., the CO_2 assimilation and the transpiration rates). At reduced pressure the diffusion limitation to CO_2 and water transfer through stomata should be reduced proportionately (Mott and Parkhurst, 1991). Thus, resulting in enhanced photosynthesis and reduced transpiration.

Conclusion

There is a strong correlation between the total pressure and the diffusivity of CO_2 and H_2O (Figure 3-1). At reduced pressure, the diffusivity of individual gases increases. The increased diffusion should result in increased number of CO_2 molecules transferred

through stomata thus increasing the plant biomass. Higher CO_2 levels in conjunction with reduced pressure can enhance the plant growth. The CO_2 assimilation via photosynthesis (A) is also subject to the stomatal regulation which implies that there is a threshold limit of CO_2 beyond which stomatal aperture will tend to close preventing further A and it is expected that this value be lower at lower pressures compared to higher pressures. Similarly, due to increased diffusion more water molecules should move out of the stomatal pore as less resistance for water in liquid phase to reach the equilibrated gas phase water. Thus, transpiration rates will also increase. High CO_2 partial pressure combined with reduced pressure can decrease transpiration through stomatal closure. Further studies in low pressures with various gas compositions is required to identify the threshold level of each gas to enhance the plant growth and reduce transpiration rates without adverse effects.

This analysis of the sensitivity of the diffusivity of CO_2 and H_2O to pressure shows that engineers designing ALS systems have two variables to consider. Transpiration and photosynthesis are both sensitive to the partial pressure of each gas in the bulk atmosphere around the plants and the total pressure of the atmosphere. Due to the high sensitivity of the diffusion coefficients at hypobaria pressure, and hence gas flux, plant growth and water loss can be influenced by the pressure of the growth chamber. If water flux, for example, is a critical aspect of a water purification system, transpiration rate of the plants can be increased or decreased by simply adjusting the plant chamber pressure. Similarly, plant growth will be responsive to the pressure changes as enhanced CO_2 assimilation is predicted.

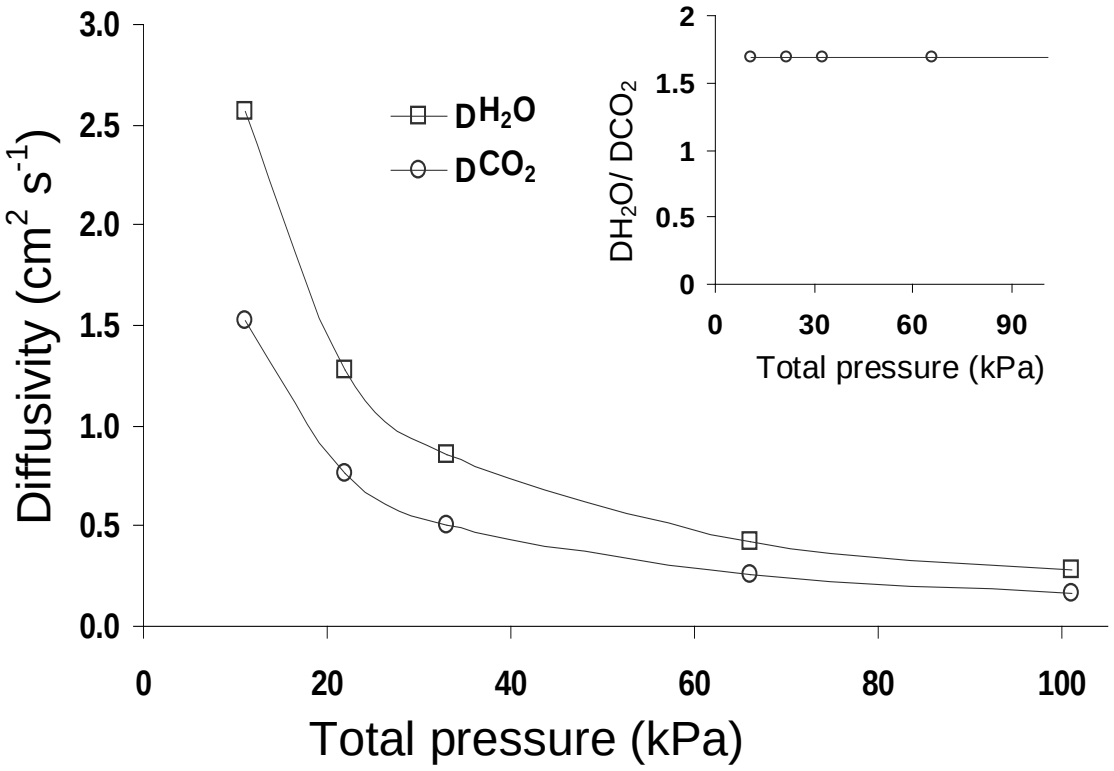


Figure 3-1. The individual mass diffusivity of CO_2 and H_20 calculated from the binary mass diffusivity using the empirical formula given by Fuller et al. (1966). Inset: The ratio of diffusivity of H_2O over CO_2.

CHAPTER 4

THE INTERACTING EFFECTS OF CO_2 AND HYPOBARIA ON GROWTH AND TRANSPIRATION OF RADISH (*RAPHANUS SATIVUS*)

Plants grown on long-term space missions will likely be grown in low pressure environments (i.e., hypobaria). However, transpiration rates can be elevated in low pressure resulting in plant wilting or stress. It is possible to reduce transpiration by increasing the partial pressure of CO_2 (pCO_2), but the effects of altering CO_2 to super-elevated levels on plant growth and transpiration in hypobaria are not known. Here, the interacting effects of pCO_2 and total atmospheric pressure on the growth and transpiration of radish (*Raphanus sativus* var. Cherry Bomb II) were studied. This material also appears in Gohil et al., (2010). The fresh weight (FW), leaf area, dry weight (DW), CO_2 assimilation rates (C_A), dark respiration rates (DR), and transpiration rates from 26 day-old radish plants that were grown for an additional seven days at different total pressures (33, 66 or 101 kPa) and pCO_2 (40 Pa, 100 Pa and 180 Pa) were measured. In general, the dry weight of plants was enhanced with CO_2 enrichment and with decreased total pressure. In limited pCO_2 (40 Pa), the transpiration for plants grown at 33 kPa was over twice that of controls (101 kPa total pressure with 40 Pa pCO_2). Increasing the pCO_2 from 40 Pa to either 100 or 180 Pa reduced the transpirations rates for plants grown in hypobaria and at normal atmospheric pressures. However, plants grown at lower total pressures (33 and 66 kPa total pressure) and super-elevated pCO_2 (180 Pa) had evidence of leaf damage. Radish growth can be enhanced and transpiration reduced in hypobaria by enriching the gas phase with CO_2 although at high levels of CO_2 leaf damage can occur.

Introduction

Plants will be an integral part of an Advanced Life Support system for long-term, human space exploration. Plants will be utilized for CO_2 removal from the air that is respired from explorers, they will act as oxygen regeneration and water purification systems, and they will be a source of food and fiber for human use (NASA, 2002). On planets with little to no atmospheres, the chambers that will be used to grow plants will likely be maintained at reduced total pressure (i.e., hypobaria). The low pressure inside the chamber will mitigate the negative effects caused by large pressure differences between the inside of the chamber and the vacuum/low pressure environments of the planet or moon (Bucklin et al., 2004). Maintaining the chambers at low pressure will also reduce costs associated with transporting heavy materials used to build chambers that could withstand large pressure differences and the lower pressure would reduce leakage of valuable gases and require less total gas to pressurize the chamber. Since transport costs associated with supplies are expected to be one of the major limitations in long-term space missions, any mechanisms to reduce these costs should be explored. For review on the advantages of low pressure environments for plant growth on space missions see Richards et al., (2006) and Paul and Ferl (2006).

On Earth, many species of plants grow at high altitudes with low total pressure (~55 kPa). This suggests that plants can adapt to hypobaria. Since pressures for growing plants on the Moon or Mars will be even lower (i.e., ~33 kPa, Paul and Ferl 2006) than that found on Earth, studies on the effects of these lower pressures on plant growth have been performed. The results of these studies have shown that seed germination (Musgrave et al., 1988), plant growth and development (Iwabuchi and

Kurata 1996; Goto et al., 2002; Spanarkel and Drew, 2002; Richards et al., 2006; He et al., 2007), and fruit ripening (Burg and Burg, 1966) are possible in hypobaric conditions for a broad range of species with species surviving in pressures as low as 10 kPa provided there is enough pO_2, pCO_2, and pH_2O (Massimino and Andre, 1999; Goto et al., 2002). Although it is well known that plants can grow and develop in hypobaria, the effects of low pressure on the photosynthesis, biomass accumulation, and transpiration rates of plants are unclear. For example, some studies report increased biomass, photosynthesis or transpiration in hypobaria (Rule and Staby, 1981; Andre and Massimino, 1992; Daunicht and Brinkjans, 1996; Corey et al., 1997; Iwabuchi and Kurata, 2003) while others report reduced growth or no change in either plant biomass, photosynthesis or transpiration in hypobaric conditions (Goto et al., 1995; 1996; Iwabuchi et al., 1996; Spanarkel and Drew, 2002; Iwabuchi and Kurata, 2003; Richards et al., 2006; He et al., 2007). The differences in the experimental procedures between these studies make it hard to interpret the effects of hypobaria on plant growth and development. For example, the plant species, pCO_2, pO_2, pH_2O, and the duration of the experiments all varied between experiments and likely account for the differences in plant responses to hypobaria. In order to develop decision support tools for growing plants on the Moon or Mars, further studies on the effects of low pressure environment on plant growth responses are required.

As total atmospheric pressure decreases, the diffusivity of water vapor and gases increase (Gale, 1971). The enhanced transport of water from the leaf surface and CO_2 toward the plant from the surrounding gas phase may account for the enhanced evapotranspiration/transpiration and photosynthesis that was found in some studies for

plants grown in hypobaria (Iwabuchi et al., 1996; Goto et al., 1996; Daunicht and Brinkjans, 1992, 1996; Corey et al., 1996, 2002; Wilkerson, 2005). Enhanced transpiration in hypobaria can have significant negative effects on plants if the water is not readily replaced. For radish, the increased evapotranspiration in hypobaria resulted in severe wilting of radish within 30 minutes of transfer from high (101k Pa) to lower pressure (25k Pa, Wilkerson, 2005). This water stress in hypobaria appears to cause the differential expression of drought-related genes (Paul et al., 2004). These changes in gene expression may trigger downstream responses in plants to induce stomata closure to reduce water loss. For example, stomata apertures from spinach grown in hypobaria long-term (10 days) were smaller with similar transpiration rates as plants grown at normal atmospheric pressure (Iwabuchi and Kurata, 2003). Increasing the pCO_2 can also reduce stomata aperture and thus reduce transpiration rates even in hypobaria. For example, a twenty percent decrease in transpiration rates for tomato grown in hypobaria was reported when CO_2 was increased from 40 to 100 Pa (Daunicht and Brinkjans, 1996). The CO_2 levels for Advanced Life Support systems may be at super-elevated levels (~0.6 kPa or higher, Wheeler et al., 1999) and CO_2 may be used as a pressurizing gas for growing plants on Mars which would result in very high pCO_2 (Wheeler, 2001). Unfortunately, the interacting effects of high levels of pCO_2 and pressure on plant growth, transpiration and photosynthesis are unclear. Therefore, the objectives of the present study were to study the interacting effects of CO_2 and pressure on growth (dry weight, DW, and fresh weight, FW), CO_2 assimilation (C_A), and transpiration rates of radish. These studies provide insight into the detrimental effects of

high levels of CO_2 on plant development at low pressures and identify methods for reducing transpiration and improving growth in hypobaria with CO_2 enrichment.

Materials and Methods

Plant Material

Seeds of radish *(Raphanus sativus* L. cv. Cherry Bomb) were germinated and grown in pots containing soil (MetroMix 300, Sun Grow Horticulture, Bellevue, WA) with nutrient solution of 1/6 strength MS (Murashige and Skoog, 1962) medium applied three-times-a-week. Plants were grown for 20 days at 22°C with a 12h photoperiod with lighting at 150 μmol m^{-2} s^{-1} from cool-white-florescent lambs in environmentally controlled chambers at 85% relative humidity. After growth in pots, plants were rinsed in ddH_2O and transferred to 250 mL flasks containing ~300 mL of 1/6 strength MS medium, pH adjusted to 6.8 with HCl (1 mM) or NaOH (1 M). The volume of flask was large enough for the short-term studies that the level of nutrient solution at the end of the experiment was well above the majority of roots. The roots were well-developed and reached far into the flask at this growth stage. Plants were placed in the holes of the stoppers and surrounded by putty (Plumbers Putty, P/N 43048, Ace Hardware, IL) to minimize water loss during experiments. Plants were grown in flasks under similar conditions of pots for 4 to 5 days before being transferred to experimental growth chambers for acclimation. The fresh weight (FW) of plants was measured on the acclimation date at 4.0 ± 0.9 g. The nutrient level was then filled to ~300 mL prior to acclimation. Plants were then acclimated in low-pressure chambers at normal atmospheric pressure (101 kPa with 40, 100 or 180 Pa pCO_2 and 21 kPa pO_2 and 2.2 kPa pH_2O) for one day prior to experimental treatments. Plants were grown for an

additional seven days under pressure and gas treatments then removed for plant harvest.

Growth Chambers and Environmental Control

The experiments were performed in three chambers (Figure 4-1) of total volume of 0.09 m^3 with a fan (Delta Model, P/N BFB0512M, Silicon Valley Compucycle, San Jose, CA). The chambers had sensors for temperature (DS10 Dallas Semiconductors, Dallas, TX), pressure (P/N ASCX15AN, Sensym ICT, Milpitas, CA), CO_2 (OEM ultrasonic, 6004, Goleta, CA), O_2 (MAX-250, Maxtec Co., Salt Lake, UT), relative humidity (HH-4602-L-CPREF, Honeywell, Morristown, NJ), light (LI-190, Li-Cor, Lincoln, NB) and three balances for weight determination per chamber (LPS-0.6 kg, Celtron Tech. Inc. Colvina, CA). Calibration of O_2 and CO_2 sensors was performed according to Richards et al., (2006). CO_2 and O_2 levels were adjusted daily at 4 hours into the photoperiod. CO_2 assimilation studies were started at the same time in the photoperiod for all tests and were repeated daily. After the acclimation period, the gas and pressure composition were brought to set points daily (Table 4-1). Environmental conditions for the one day acclimation in the growth chamber were 22 ± 2 °C, 12 hour photoperiod, at 250 μmol m^{-2} s^{-1} using cool-white, florescent lamps and 85% relative humidity. The pO_2 was maintained at ~21 kPa with other environmental conditions described (Table 4-1).

Each chamber contained three individual plants. Pressure was maintained with a vacuum pump (1/2 HP, JB Industries, Chicago, IL) and chambers had leakage rates of ~0.03% (chamber volume/h). Data collection and controls were performed using a CR10 datalogger (Campbell Scientific, Logan, UT) that was connected to a PC. Experiments were performed in triplicate.

Plant Harvest

After the experimental treatments in the chambers, plants were removed from the chamber blotted dry and analyzed for fresh weight, leaf area, and dry weight. Dry weight analysis was performed by placing plants on pre-weighed pans at 60°C for 48h and reweighing the contents. Leaf area was measured on day 7 with digital images using Image Pro Plus software (ver. 6.0 Media Cybernetics, Bethesda, MD).

Statistical Analysis

For CO_2 and pressure treatments, the analysis was treated as a two-factor analysis of variance (ANOVA) using SAS (SAS institute ®, NC, USA) and the mean of three individual plants of independent experiments (here, n=3).

Results

Plant Growth

Plants grown with 40 or 100 Pa pCO_2 appeared healthy at all total pressure treatments (33, 66, or 101 kPa). However, at 180 Pa pCO_2, leaves from plants grown at 33 and 66 kPa pressures were yellow, had red speckles and leaf tip burn, and appeared to have reduced chlorophyll content. This “leaf damage” was not observed for plants grown at 180 Pa pCO_2 and 101 kPa pressure (Figure 4-2). Despite this apparent leaf damage, plant dry weight was the greatest in the 180 Pa pCO_2 treatments for all pressures (33, 66, or 101 kPa, Table 4-2). The pressure and pCO_2 both significantly affected the dry weight ($p < 0.01$) but the interaction between these two parameters was not significant ($p > 0.05$). The dry weight of plants was maximal at 66 kPa total pressure and 180 Pa pCO_2 with ~0.65 g per plant and lowest at 101 kPa total pressure and 40 Pa pCO_2 at ~0.42 g per plant (Table 4-2). For all pressure treatments, increases in pCO_2 resulted in increases in dry weight (Table 4-2). Radish fresh weight and % water content

were not significantly affected by total pressure, pCO_2, or the interaction between the two treatments (Table 4-2*).* Leaf area decreased as pCO_2 levels increased for plants grown at 33 kPa total pressures (Figure 4-3). This reduction in leaf area with increasing pCO_2 levels was also observed for plants grown at 66kPa and 101 kPa pressures but to a much lesser extent (Figure 4-3). The reduction in leaf area may have been in part due to the senescence of the hypocotyl leaves since this was accelerated in higher pCO_2. Plants grown in low pCO_2 (40 Pa) and low pressure (33 kPa) had the largest leaf area (~240 cm^2 per plant) compared to other pCO_2 and pressure treatments (Figure 4-3).

CO_2 Assimilation

The CO_2 assimilation rates (C_A) for plants remained approximately constant from day 1 to day 7 for 40 and 100 Pa pCO_2 treatments for all pressures however, at 180 Pa pCO_2, the C_A decreased with each passing day (data not shown). This reduction in C_A was greater at 33 and 66 kPa than for plants grown at 101 kPa. The pressure and the pCO_2 treatments as well as their interaction significantly affected C_A with the greatest C_A at 100 Pa pCO_2 and 66 kPa at ~12.42 $\mu mol\ m^{-2}\ s^{-1}$ and the lowest at 180 Pa pCO_2 and 101 kPa at ~3.44 $\mu mol\ m^{-2}\ s^{-1}$(Table 4-2). The CO_2 uptake rates at low pCO_2 (40 Pa) and 33 kPa total pressure was greater than the uptake rates from plants grown at similar pCO_2 levels with 66 kPa or 101 kPa total pressure (Figure 4-4A). This corresponded to a greater leaf area in the low pCO_2, low pressure treatments compared to plants from other pressure and CO_2 combinations (Figure 4-3). At 100 Pa pCO_2, plants grown at 66 kPa had the greatest CO_2 uptake (Figure 4-4B) which corresponded to the maximal C_A (based on leaf area) and the greatest dry weight of all experimental treatments (Table 4-2). For plants grown at 101 kPa, the C_A (based on leaf area) was

similar for all CO_2 treatments (Table 4-2) despite having an increased slope in CO_2 uptake as pCO_2 levels increased (Figure 4-4). At super elevated levels of CO_2 (180 Pa), the CO_2 uptake rate was the lowest for plants grown at 33 kPa total pressure compared to plants from other treatment combinations (Figure 4-4C).

Dark respiration rates (DR) were significantly ($p < 0.01$) affected by total pressure but not by the CO_2 or interactions between the two (Table 4-2). In general, as total pressure was reduced from 101 kPa to 33 kPa, the DR increased for plants grown at all pCO_2 levels. The exception to this was at 100 Pa pCO_2 and 66 kPa total pressures where the DR was maximal at ~1.91 μmol m^{-2} s^{-1}. This corresponded with plants with the greatest dry weight (Table 4-2). In contrast, the lowest DR was found with plants grown at 180 Pa pCO_2 and 101 kPa total pressure at ~0.6 μmol m^{-2} s^{-1} that corresponded to the lowest C_A (Table 4-2).

Transpiration Rates

Plants grown at reduced pressures (33 or 66 kPa) had greater transpiration rates than plants grown at 101 kPa, regardless of the CO_2 treatment (Table 4-2). In fact, plants grown at 33 kPa total and 40 Pa pCO_2 had over twice the transpiration rate (~580 mL m^{-2} day^{-1}) as plants grown at 101 kPa total and 40 Pa pCO_2 (~210 mL m^{-2} day^{-1}, Table 4-2). Transpiration rates were reduced by increasing pCO_2 from 40 to 100 Pa for all pressure treatments but a further increase in pCO_2 to 180 Pa only reduced transpiration rates for plants grown at 101 kPa (Table 4-2, Figure 4-5). The cumulative water loss per plant was greatest in low pCO_2 (40 Pa) and low pressure (33 kPa) with ~50 mL of water lost per plant over the seven days in the chambers (1 day acclimation, 7 day treatment) compared to plants grown at 101 kPa total pressure with 180 Pa pCO_2

which lost the least amount of water for any treatments over the experiment at ~12 mL per plant (Figure 4-5). Transpiration was minimal at night as indicated by the plateaus on the curves of cumulative water loss for all pressure and pCO_2 treatments (Figure 4-5).

Discussion

Radish plants appeared healthy with no signs of water stress or leaf damage for most of the pressure and pCO_2 combinations that were used in these experiments. The exception to this was for plants grown at lower pressures (66 or 33 kPa) and super-elevated pCO_2 (180 Pa, Figure 4-2). These plants had yellow leaves with red speckles, and leaf tip damage. Previous studies with radish in hypobaria did not report any leaf damage at similar pressures (33, 66, and 96 kPa) even after 21 days in treatments with 120 Pa pCO_2 (Levine et al., 2008). The differences in leaf appearance between this and the previous study may be that the 180 Pa pCO_2 used here was at a high enough concentration to induce leaf damage at low pressure. Low pressures enhance diffusion rates of CO_2 (Gale, 1971) therefore, the detrimental effects of elevated CO_2 may occur at lower concentrations in hypobaria. Since plants appeared healthy at 101 kPa and 180 Pa pCO_2, it suggests that this level of pCO_2 is not detrimental to growth at normal atmospheric pressures. Previous studies at super-elevated levels of pCO_2 (500 and 1000 Pa) reported leaf bleaching in potatoes, wheat and soybeans at normal atmospheric pressure (Tisserat et al., 1997; Wheeler et al., 1999). In addition, high pCO_2 can cause accelerated leaf senescence (Usuda and Shimogawara, 1998). It is also possible that ethylene buildup in the system could have caused the leaf damage in our studies since ethylene was not scrubbed from the gas phase. Ethylene, which can

accumulate in closed systems with plants, can reduce chlorophyll levels in leaves and promote leaf senescence (He et al., 2009). The much larger chambers that were used in previous studies with radish in hypobaria (Levine et al., 2008) may have prevented high concentrations of ethylene from accumulating near the plant thus preventing leaf damage. Further studies with the removal of ethylene from the chambers may clarify the roles of ethylene in inducing leaf damage at super-elevated pCO_2 in hypobaria.

Despite the leaf damage in hypobaria with super-elevated pCO_2, plants from these treatments had enhanced dry weights compared to plants grown at lower pCO_2 levels (40 or 100 Pa pCO_2, Table 4-2). Super-elevated pCO_2 can greatly enhance starch accumulation in leaves (Levine et al., 2009) which may account for the increased dry weights that were observed. In general, CO_2 enrichment (100 or 180 Pa) increased the dry weight of plants for all pressure treatments compared to plants grown at limiting pCO_2 (40 Pa, Table 4-2). Many experiments have demonstrated that CO_2 enrichment at normal pressures causes significant increases in photosynthetic uptake of CO_2 and increases in biomass for a variety of plant species including radish (Usuda and Shimogawara, 1998; Long et al., 2004).

Radish had an increase in biomass (dry weight) in hypobaria compared to plants grown in normal atmospheric pressures (Table 4-2). While some studies report similar increases in biomass from plants grown in hypobaria (Andre and Massimino 1992; Corey et al., 1996; Spanarkel and Drew, 2002; Goto et al., 2002), others report no change or a decrease in biomass (Daunicht and Brinkjans, 1992; Iwabuchi et al., 1994; Goto et al., 1995; He et al., 2007, 2009). Not surprisingly, the photosynthetic rates reported for plants grown hypobaria also conflicts, with some studies reporting

enhanced photosynthetic rates and others reporting no change (Iwabuchi et al., 1994; Goto et al., 2002; Corey et al., 1997; Spanarkel and Drew, 2002; Iwabuchi and Kurata, 2003; Richards et al., 2006). For Arabidopsis, photosynthetic rates were increased with decreasing pressure for limiting pCO_2 (40 Pa) but were unchanged in response to hypobaria at non-limiting pCO_2 (70-100 Pa, Richards et al., 2006). In this case, the pO_2 was dropped as pressure was lowered thus resulting in lower pO_2/pCO_2 ratio. A lower pO_2/pCO_2 ratio can result in enhanced photosynthesis. The pO_2 was maintained in our experiments at ambient levels (21 kPa) yet plants had enhanced photosynthetic rates in hypobaria even in non-limiting CO_2 (Table 4-2, Figure 4-4). A previous study with radish also reported both an increase in shoot biomass and an increase in photosynthesis for plants grown at low pressure (33 kPa) with normal atmospheric levels of pO_2 (21 kPa; Levine et al., 2008). Therefore, it appears that for radish the biomass and photosynthetic rates are enhanced in hypobaria when the pO_2 is maintained at normal atmospheric levels.

The dark respiration rates (DR) were enhanced for radish in hypobaria (Table 4-2) which correlates with results found for Arabidopsis and lettuce (Spanarkel and Drew 2002; Richards et al., 2006). In contrast, others report a lower DR for lettuce that was grown longer-term in hypobaria (He et al., 2007). Since plants may adapt to low pressure it may be possible that plants adapted and reduced their DR in response to hypobaria.

Transpiration rates of radish were higher in hypobaria regardless of CO_2 levels compared to plants grown in normal atmospheric pressures (Table 4-2, Figure 4-5). Enhanced evapotranspiration or transpiration rates have been reported in hypobaria for

a few species (Daunicht and Brinkjans, 1996; Iwabuchi and Kurata, 2003; Wilkerson, 2005; Richards et al., 2006; Levine et al., 2008). The increased diffusivity of gases in low pressure can result in rapid water removal from leaves and promote wilting of plants that are transferred from high pressure to low pressure environments (Rygalov et al., 2004; Wilkerson, 2005). Drought related genes are differentially regulated in response to hypobaria suggesting that plants do respond to the enhanced water loss in hypobaria. Approximately 200 drought-stress-related genes were differentially regulated in Arabidopsis in response to hypobaria and included genes involved in desiccation-related pathways such as dehydrins and ABA–related proteins (Paul et al., 2004). Similar to the results of photosynthetic rates in hypobaria, the evapotranspiration rates in Arabidopsis studies were increased in hypobaria for low pCO_2 treatments but were similar to plants grown at normal atmospheric pressures when pCO_2 levels were non-limiting (Richards et al., 2006). For radish, enhanced transpiration rates in hypobaria (33 or 66 kPa) occurred even in non-limiting CO_2 (Table 4-2, Figure 4-5). These differences may be accounted for the different mechanisms of acclimation to hypobaria for different species with Arabidopsis responding quicker to the hypobaria or the differences in the measured parameter evapotranspiration versus transpiration. However, for spinach grown in non-limiting CO_2 (100 Pa) and short-term in hypobaria the transpiration rates were enhanced compared to plants grown at normal atmospheric pressure but after ten days acclimation in hypobaria plants had similar rates of transpiration as plants grown at normal atmospheric pressure (Iwabuchi and Kurata, 2003). The stomata apertures from the spinach that were acclimated long-term to hypobaria were smaller than those that were grown at higher pressure. It may be that the radish had not yet acclimated to

hypobaria even after seven days (Table 4-2, Figure 4-5). Transpiration rates were reduced by increasing pCO_2 from 40 to 100 Pa for all pressures (Table 4-2, Figure 4-5). However, an increase in pCO_2 from 100 to 180 Pa only reduced transpiration for plants grown in normal atmospheric pressure (101 kPa) and not for plants grown in hypobaria (33 or 66 kPa; Figure 4-5). Many studies have shown that at normal atmospheric pressure, stomata closure occurs in plants as pCO_2 is increased from 40 to 100 Pa but above 100 Pa there is little further decrease in the stomata aperture (Jarvis 1976; Stanghellini and Bunce, 1993; Assmann, 1999; Wheeler et al., 1999). This may explain why a further increase in pCO_2 from 100 to 180 Pa did not reduce transpiration for plants grown in hypobaria. Taken together, it appears that plants acclimate to hypobaria and the enhanced transpiration by closing stomata when CO_2 is not limited. However, in limited CO_2, stomata remain open in hypobaria provided that water is non-limiting. Further studies on the development and response of stomata in hypobaria may lead to identifying the mechanisms of adaptation for plants to the low pressure environment.

Summary

Radish plants grown in hypobaria had increased biomass (DW), CO_2 assimilation, dark respiration (DR), and transpiration compared to plants grown in ambient pressures. Transpiration was reduced and growth enhanced by enrichment with CO_2 for all pressure treatments. Plant transpiration rates remained constant over seven days of hypobaria treatments suggesting that plants did not acclimate to hypobaria by reducing stomata aperture during this period. Very high pCO_2 (180 Pa) when combined with hypobaria (33 or 66 kPa) induced leaf damage. The leaf damage was not found at lower pCO_2 (100 Pa) treatments suggesting that the threshold for pCO_2 uptake had been reached at 180 Pa pCO_2. Longer-term studies on the interaction of CO_2 and pressure

on plant growth in hypobaria will provide further insight into the mechanisms of plant adaptation to hypobaria that can be used to develop decision support tools for growing plants on future space exploration.

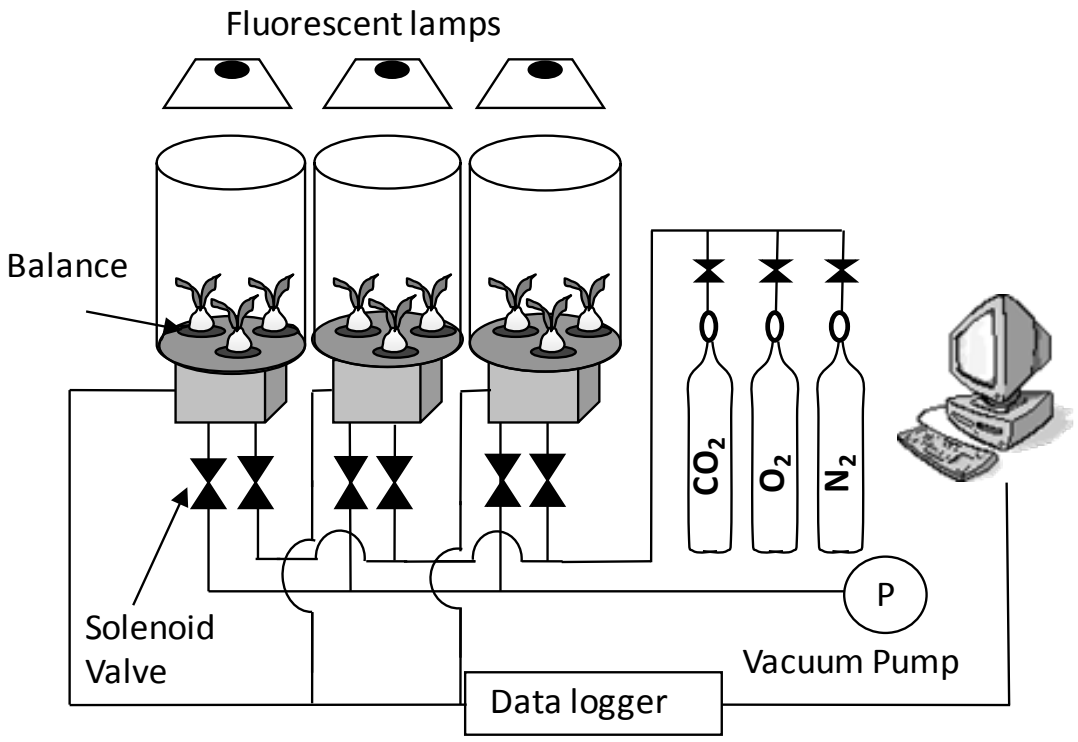


Figure 4-1. Schematic of the low pressure growth chambers used for experiments. Each chamber has 0.09 m3 total internal volume. The data logger (CR10) recorded electrical signals from temperature, CO2, O2, balances (n=3), pressure, relative humidity, and light sensors for each chamber.

Figure 4-2. Radish (26 day-old) grown for six days in super elevated CO_2 (180 Pa) at 101 (first row), 66 (second row), or 33 (third row) kPa total pressure. Side view is shown on left side of figure with corresponding top view in the right panel. All plants were acclimated in the chamber for one day prior to experiments.

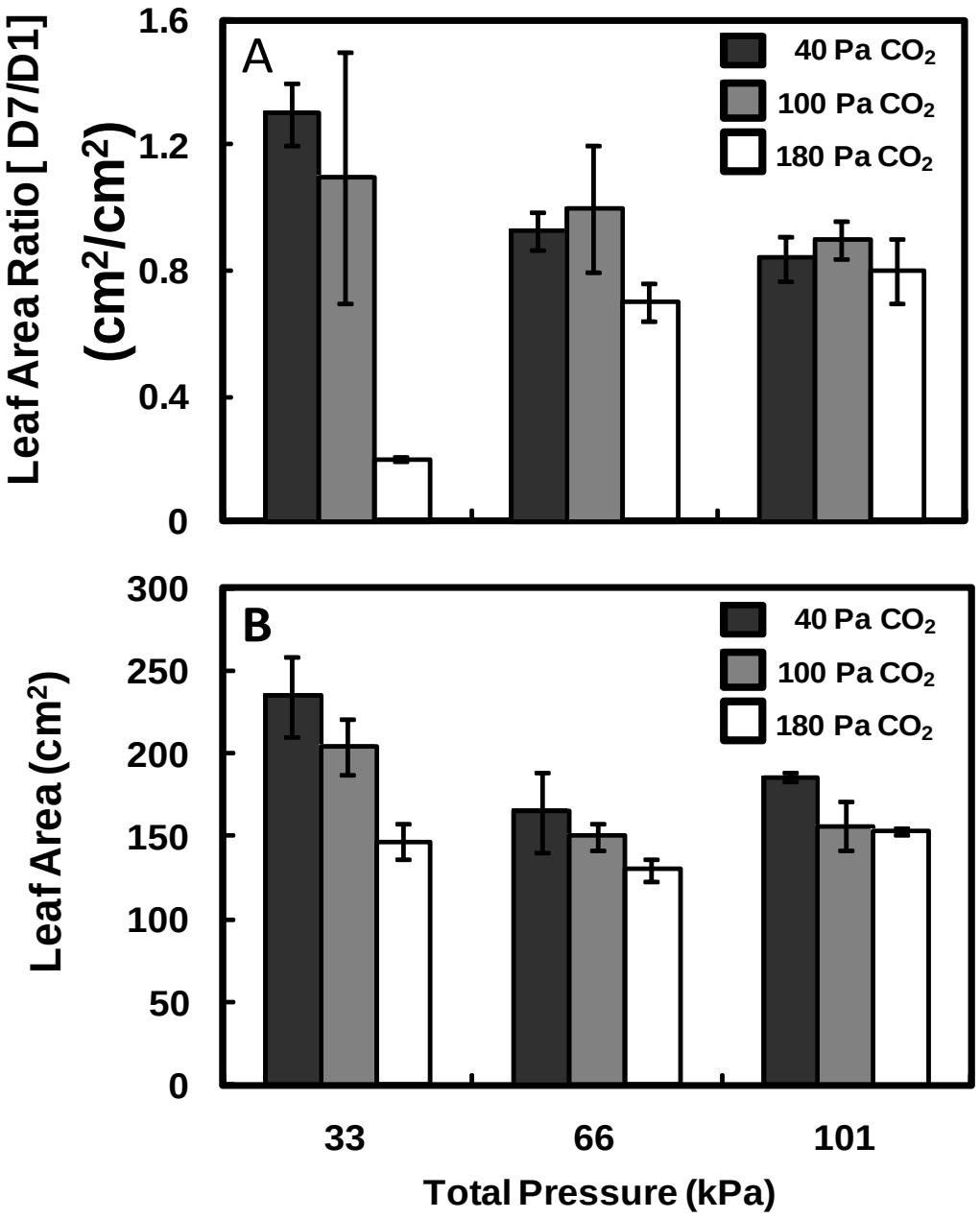


Figure 4-3. Total leaf area of radish grown at various total pressures and CO_2 levels. (A) Ratio of leaf area from the last day of the experiment, at day 7 (D7) to the first day (D1) for plants grown at 40, 100 or 180 Pa CO2 and at 33, 66 or 101 kPa total pressure. (B) Leaf surface area at D7 of treatment for plants grown for six days at 40,100 or 180 pCO2 and at 33, 66 or 101 kPa total pressure. Bars represent the mean ±STDEV from three experiments (n = 3) with each experiment having three plants.

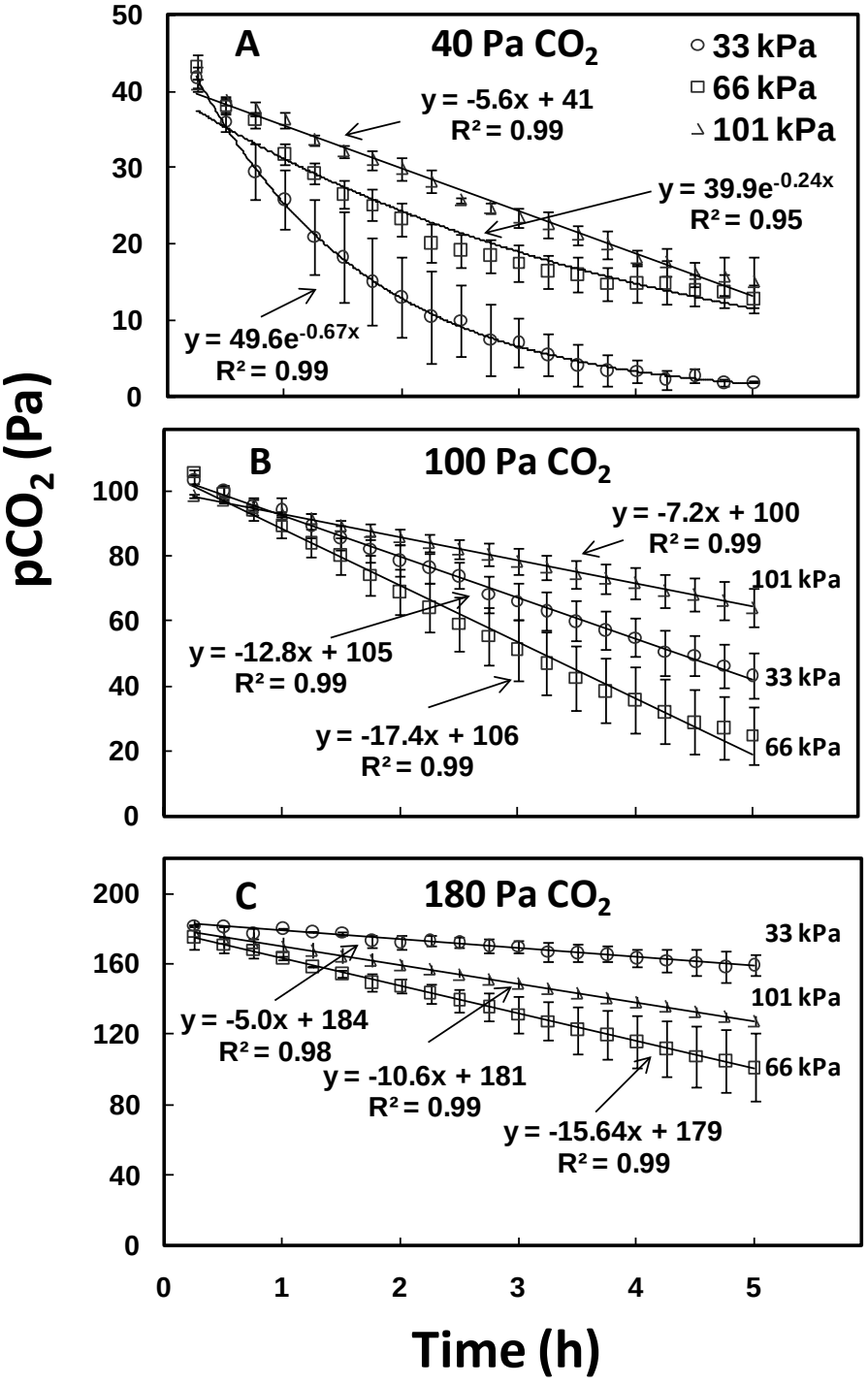


Figure 4-4. CO_2 drawdown curves on the final day of the experiment of 26 day-old radish grown for six days at 33, 66, or 101 kPa. The CO_2 levels (partial pressure) were either low (40 Pa; A); high (100 Pa; B) or super elevated (180 Pa, C). Bars represent the mean ±STDEV from three experiments (n =3) with each experiment having three plants.

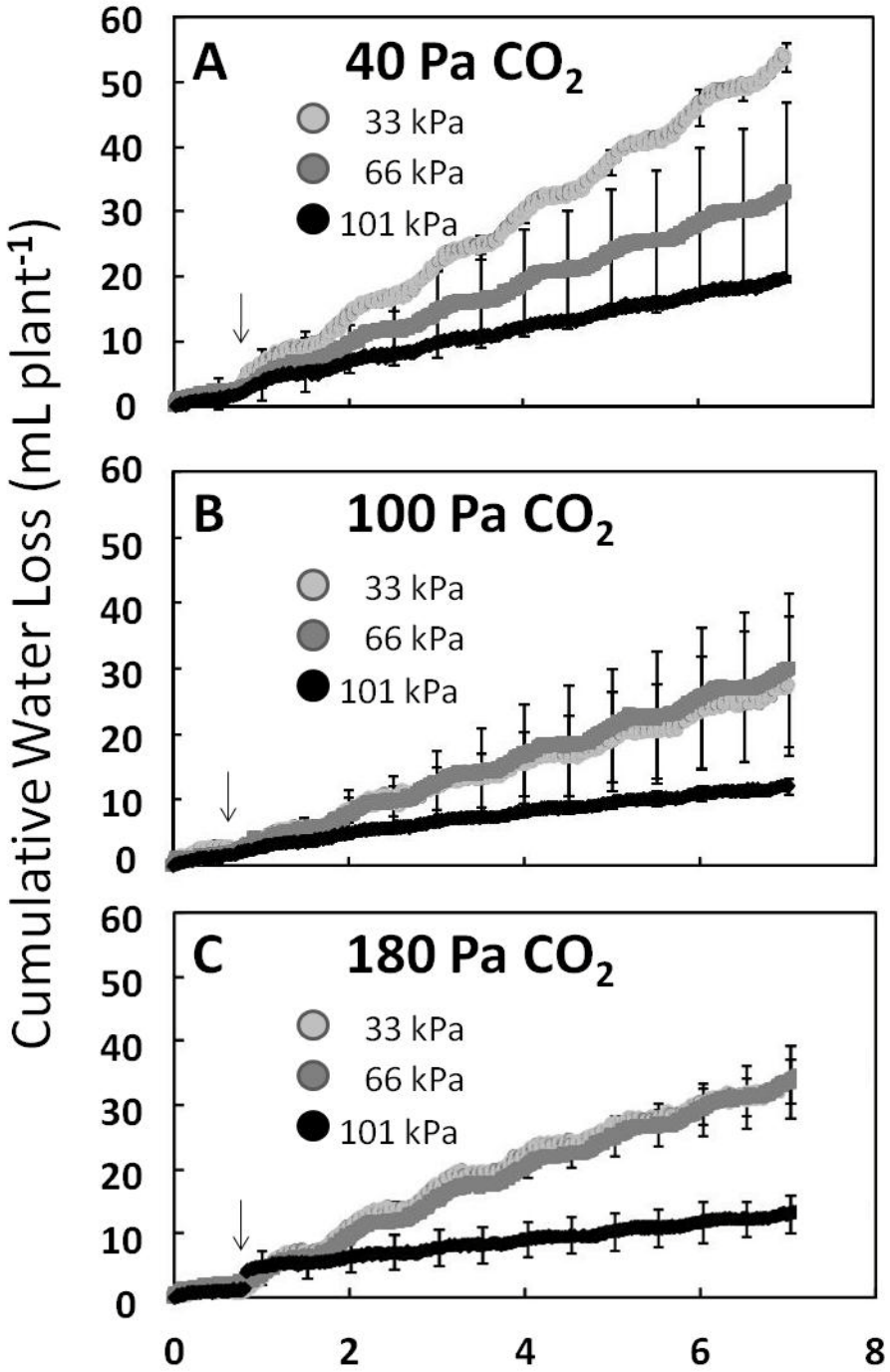


Figure 4-5. Water loss due to transpiration of 26 day-old radish grown for 7 days for low (40 Pa; A), high (100 Pa; B) or super-elevated CO2 (180 Pa; C) at 33, 66, or 101 kPa total pressure. Values represent the mean of three experiments (n = 3) with each experiment having three plants. Standard deviation bars have been included only for every ½ hour for clarity. Arrows indicate the start of pressure treatments.

Table 4-1. Set points for the environmental conditions

Parameter	Set point
Air Temperatures	22/20 ± 1°C day/night
Relative Humidity	>85%
PAR	250 µmol m^{-2} s^{-1}
Total Pressure	33± 2, 66 ± 2 or 101kPa
pO_2	21 ± 1 kPa
CO_2	40, 100, and 180 Pa

Table 4-2. The effect of pressure and CO_2 on plant growth (dry and fresh weight), water content, C_A and dark respiration (DR) and transpiration rates of 26-day-old radish plants grown for six days at the given pressure treatment. All values except C_A and DR are based on mean of three experiments with three plants per experiment (n = 3 ±STDEV). C_A and DR are based on three replicated chambers.

Treatment		Parameters					
Press	pCO_2	Fresh Wt.	Dry Wt.	Water	C_A	DR	Transpiration
(kPa)	(Pa)	(g plant^{-1})	(g plant^{-1})	(%)	(µmole m^{-2}s^{-1})	(mol m^{2}s^{-1})	(mLm^{-2}day^{-1})
33	40	3.78 ± 0.11	0.49 ± 0.07	88 ± 2	7.24 ± 1.44	1.71 ± 0.43	584± 39
	100	3.26 ± 1.08	0.57 ± 0.07	81 ± 4	6.84 ± 1.10	1.31 ± 0.17	339 ± 51
	180	3.26 ± 0.01	0.62 ± 0.21	80 ± 4	3.58 ± 1.69	1.66 ± 0.76	344 ± 59
66	40	3.36 ± 0.17	0.45 ± 0.10	88 ± 3	4.28 ± 0.99	1.61 ± 0.12	488 ± 112
	100	3.31 ± 0.16	0.61 ± 0.25	85 ± 8	12.42 ± 2.86	1.91 ± 0.61	337 ± 106
	180	4.65 ± 1.16	0.65 ± 0.16	87 ± 1	6.48 ± 3.20	1.19 ± 0.33	298 ± 50
101	40	3.00 ±.0.85	0.42 ± 0.05	85 ± 5	4.32 ± 0.65	1.15 ± 0.20	209 ± 27
	100	2.82 ± 0.41	0.47 ± 0.12	86 ± 6	4.43 ± 1.59	0.84 ± 0.23	134 ± 12
	180	3.66 ± 0.30	0.50 ± 0.04	86 ± 1	3.44 ± 1.04	0.60 ± 0.15	89 ± 23
P		N.S.	**	N.S.	**	**	**
CO_2		N.S.	**	N.S.	**	N.S.	N.S.
P X CO_2		N.S.	N.S.	N.S.	**	N.S.	N.S.

Significance was determined by two-way ANOVA
*p < 0.05, ** p < 0.01

CHAPTER 5

THE EFFECTS OF SHORT-TERM AND LONG-TERM ACCLIMATION OF RADISH TO HYPOBARIA

Introduction

Water is an extremely important resource for any agricultural system. Therefore, it is not surprising that plants adjust their growth in response to the differing amounts of water that is available to optimize their growth and survival. In water-limiting conditions, plants adjust their growth to promote water uptake and minimize water loss. This adjustment in growth depends on the duration and the extent of the water stress. For example, short-term responses to limited water availability include the closure of stomata (Ehleringer and Cooper, 1992), increased gene regulation of stress pathways (Knight and Knight, 2001), osmotic adjustment in the roots (Rodriguez et al., 1995), changes in signal transport (Morgan, 1984) and inhibition of growth (Schulze, 1986). For longer-term responses to water stress, plants may increase root growth relative to shoot growth (Rodriguez et al., 1995), reduce the transpiration area (Jackson et al., 2000), and have osmotic adjustments to their root systems (Morgan, 1984).

For long-term space missions, the conservation and recycling of water by plants that are used as part of an Advanced Life Support system will be important for the success of any mission to distant planets or moons. In these missions, plants will likely be grown at reduced pressures relative to Earth sea level pressure which can result in enhanced transpiration/evapotranspiration and photosynthesis (Iwabuchi et al., 1996; Goto et al., 1996; Daunicht and Brinkjans, 1992, 1996; Corey et al., 1996, 2002; Wilkerson, 2005). This enhanced transpiration can result in a variety of stress responses if water is not readily available to the plant. For example, radish grown at

ambient total pressure immediately wilted when transferred to hypobaria at 25 kPa total pressure (Wilkerson 2005). These plants recovered within several hours after being transferred to the low pressure environment although at reduced transpiration rates prior to the transplant suggesting that plants partially closed their stomata. In other cases, radish did not wilt after being transferred to low pressure (33kPa; Wehkamp 2009; Gohil et al., 2010). These plants likely had more water available to them since they were grown in hydroponic systems compared to the wilted plants that were grown in soil pots. Arabidopsis plants that were exposed to hypobaria also showed no sign of wilting when transferred to low pressure but they had over 200+ genes that were differentially regulated compared to plants that were maintained in ambient pressures (Paul et al.,, 2004; Paul and Ferl, 2006). These genes included several that are involved in water-stress-related biochemical pathways such as abscicic acid. In many cases, plants acclimated to hypobaria by adjusting their transpiration over time to levels that were similar to plants grown in ambient pressure (Iwabuchi and Kurata, 2003**;** Wehkamp 2009). The early adjustment or acclimation to hypobaria appears to involve a decrease in stomatal aperture reducing transpiration and the longer-term adaptation mechanisms including decreases in stomatal size (Iwabuchi and Kurata, 2003). Young leaves exposed to hypobaria or emerging in hypobaria are likely to go through some other physiological changes that older leaves do not go through but this response is not well studied. Taken together, it appears that plants respond to hypobaria and enhanced transpiration similar to a water stress but the extent of this is unclear and may depend on the type of substrate used to grow the plant. To understand the adaptation mechanisms used by plants to hypobaria, the CO_2 assimilation, transpiration, dry

weight, plant growth, leaf area and stomatal density were compared for plants that were grown both in long and short term hypobaria.

Materials and Methods

Plant Material

Seeds of radish (*Raphanus sativus* L. cv. Cherry Bomb) were germinated and grown in 350 mL flasks filled with about 300 mL of nutrient solution of 1/4 strength MS (Murashige and Skoog, 1962), pH adjusted to 6.8 with HCl (1 mM) or NaOH (1 M). Rockwool stoppers (35 cm diameter x 40 cm height; Grodan Delta, Aurora, CO) and absorption paper rolls 2 cm X 10 cm (Anchor paper, St. Paul, MN) served as a wicking system. Plants were grown for total of 28 days. The volume of flask was large enough for the 4 week experimental studies that the level of nutrient solution at the end of the experiment was well above the majority of roots. The roots were well-developed and reached far into the flask at this growth stage. Each stopper was soaked in a nutrient solution of 1/4 strength MS for 24 hours prior to the experiment setup and the pH was maintained around 6.8 through the use of 1 mM of HCl or 1 M NaOH. Each seed was rolled inside absorption paper, placed inside the center of the rockwool, and then placed so that the wick was ~ 7 cm within the nutrient solution. In order to avoid as much evaporation of the solution, the top of the rockwoo1 was covered in plastic wrap. Each flask was then wrapped in foil to reduce light exposure to the nutrient solution. For the five experimental treatments (Treatment A-E; Table 5-1), seeds were germinated and grown at 101 kPa or 33 kPa and then transferred to 33 kPa (0.1 kPa pp CO_2, 21 kPa pp O_2) on days 7, 14, 21 or 26 (Table 5-1). During the hypobaric studies the environmental conditions are described in Table 5-2.

Growth Chambers and Environmental Control

The experiments were performed in three chambers (Figure 5-1) of total volume of 0.09 m^3 each with a fan for air circulation (Delta Model, P/N BFB0512M, Silicon Valley Compucycle, San Jose, CA). The chambers had sensors for temperature (DS10 Dallas Semiconductors, Dallas, TX), pressure (P/N ASCX15AN, Sensym ICT, Milpitas, CA), CO_2 (OEM ultrasonic, 6004, Goleta, CA), O_2 (MAX-250, Maxtec Co., Salt Lake, UT), relative humidity (HH-4602-L-CPREF, Honeywell, Morristown, NJ), light (LI-190, Li-Cor, Lincoln, NB) and three balances for weight determination per chamber (LPS-0.6 kg, Celtron Tech. Inc. Colvina, CA). Calibration of O_2 and CO_2 sensors was performed according to Richards et al., (2006). CO_2 and O_2 levels were adjusted daily at 4 hours into the photoperiod. CO_2 assimilation studies were started at the same time in the photoperiod for all tests and were repeated daily. After the acclimation period, the gas and pressure composition were brought to set points daily (Table 5-2). Environmental conditions for the one day acclimation in the growth chamber were 22 ± 2 °C, 12 hour photoperiod, at 250 μmol m^{-2} s^{-1} using cool-white, florescent lamps and 85% relative humidity. The pO_2 was maintained at ~21 kPa with other environmental conditions described (Table 5-2).

Each chamber contained one to two individual plants. Pressure was maintained with a vacuum pump (1/2 HP, JB Industries, Chicago, IL) and chambers had leakage rates of ~0.03% (chamber volume/h). Data collection and controls were performed using a CR10 data logger (Campbell Scientific, Logan, UT) that was connected to a PC. Experiments were performed in triplicate.

Gas Exchange Rates

The CO_2 assimilation rates (C_A) were calculated by performing draw down curves for 5 hours period once CO_2 levels were adjusted at a set point (0.12 kPa) at noon. The slope of the regression equation represented the C_A rate per hour which was converted into µmol m^{-2} s^{-1}. The range of CO2 over a 24h period was (100 - 22). The transpiration rates were calculated by performing draw down curves of load cell values for 5 hours period once CO_2 levels were adjusted at set point (0.12 kPa) at noon. The slope of the regression equation represented the transpiration rate per hour which was converted into µmol m^{-2} s^{-1}.

Plant Biomass and Leaf Area

After the experimental treatments in the chambers, plants were removed from the chamber blotted dry and analyzed for fresh weight, leaf area, and dry weight. Dry weight analysis was performed by placing plants on pre-weighed pans at 60°C for 48h and reweighing the contents. Leaf area was measured on the last day of experiment with scanned digital images using Image Pro Plus software (ver. 6.0 Media Cybernetics, Bethesda, MD).

Scanning Electron Microscopy

Radish leaves were fixed in 2.5% glutaraldehyde in 0.1M sodium cacodylate, pH 7.24 and stored overnight at 4 °C. The fixed leaves were processed with the aid of a Pelco BioWave laboratory microwave (Ted Pella, Redding, CA, USA). Samples were washed in 0.1M sodium cacodylate pH 7.24, post fixed with 2% buffered osmium tetroxide, water washed and dehydrated in a graded ethanol series 25, 50, 75, 95, and 100% and critical point dried (Bal-Tec CPD030, Leica Microsystems, Bannockburn, IL, USA). Samples were mounted on carbon adhesive tabs on aluminum specimen mount,

Au/Pd sputter coated (DeskII, Denton Vacuum, Moorestown, NJ, USA) examined and high resolution digital micrographs acquired with field-emission scanning electron microscope (S-4000, Hitachi High Technologies America, Inc. Schaumburg, IL, USA).

Leaf Stomatal Density and Stomatal Index

The surface electron microscopy images were used to calculate leaf stomatal density, i.e., the total number of stomata per unit leaf area (Radoglou and Jarvis, 1990). Fully grown, young leaves from the plants used for measuring gas exchange rates were selected. Leaf discs of 8 mm diameter from the area about 1 cm from the mid rib were punched out from an attached leaf using a plunger and immediately transferred to the buffer solution. Three samples, two samples of hypobaria treatment and one sample for control (101 kPa), were analyzed at the electron microscopy facility at the University of Florida. The number of stomata (s) and epidermis cells (e) were counted from the scanned images. The leaf stomatal index (LSI) was calculated using the formula [s / (e + s)] X 100. The stomatal size was calculated by averaging the total length (µm) of primary axis of all the guard cells of the scanned images.

Results

Seed Germination and Plant Growth in Hypobaria

The germination of radish was significantly affected by the partial pressure of oxygen (pO_2) in hypobaria (33 kPa total pressure) with no germination at 1.5 kPa pO_2 and only 45% of the seeds germinating at 4 kPa pO_2. This suggests that the minimum pO_2 required to induce seed germination with the flask system at 33kPa total pressure is between 1.5-4 kPa (Figure 5-1). Interestingly, as pO_2 increased to 6 and 10 kPa, germination increased to 62 and 82%, respectively. In fact, at 10 kPa pO_2, the

germination was not statistically different from pO_2 of 21 kPa (at either 33 kPa or 101 kPa total pressures; Figure 5-1).

To study the effects of the age of the plants at the time of transfer to hypobaria on plant growth and acclimation, five treatments were performed. These treatments consisted of transferring seedlings after 0, 7, 14, 21, or 26 days of normal atmospheric pressures into hypobaria at 33kPa total pressure (Table 5-1; Treatments A-E). Seedlings appeared healthy except in the case for the seedlings that were transferred to hypobaria (33 kPa) after one week where the plants all had stunted growth or died (Treatment B; Figure 5-2). This suggests that the developmental stage of growth during which plants are transferred to low pressure is important for health and survival of a crop in hypobaria. Due to poor growth and in several cases the death of plants from the one–week-old stage treatment (Treatment B, Table 5-1), no further analyses were performed on these plants.

As far as the growth of the plants during the 28 day experiments, plants from the four treatments (A, C, D, and E) all had similar fresh and dry weights and root to shoot ratios (Table 5-3). The longer the plants were exposed to hypobaria, the smaller the leaf area of the plants and the thicker the leaves at the end of the experiment (Figure 5-3C; Table 5-4). The greatest leaf area was observed from plants grown with 2 days exposure to hypobaria (at 82 cm^{-2}) followed by leaves from plants grown at normal pressure (at 78 cm^{-2}) and the lowest leaf area was observed for plants grown completely in hypobaria (at 58 cm^{-2}; Figure 5-3C; Table 5-4).

CO_2 Assimilation Rates (C_A)

In general, the longer the time that plant remained in hypobaria, the greater the carbon dioxide assimilation rates (C_A) on the last day of the experiment (Fig. 5-3A).

However, the increase was significant only at $p< 0.1$ and not at $p<0.05$ value. The highest C_A was observed for Treatment A (28 days in hypobaria) at 23 μmol m^{-2} s^{-1} and lowest for Treatment D (2 days in hypobaria) at 14 μmol m^{-2} s^{-1} (Figure 5-3A). This suggests that plants have a rapid adjustment in C_A during the initial stages of plant transfer to hypobaria indicated by the slight drop in C_A for the two days treatment in hypobaria (Treatment D). Interestingly, when comparing C_A over the course of the experiment, there was very little difference in C_A between plants that were grown entirely in hypobaria and those grown in normal atmospheric pressure (Figure 5-4A). This may be a result of the limited amount of light used in these studies (250 μmol m^{-2} s^{-1}) and not by CO_2 availability since CO_2 diffuses at a faster rate in hypobaria than normal pressures and C_A would be expected to be higher in the hypobaria treatments. Further studies that compare C_A in higher light conditions in hypobaria and normal pressure are required to determine if that is the case.

Transpiration Rates (T)

Plants grown entirely in hypobaria (Treatment A) had significantly higher ($p< 0.05$) transpiration rates on the last day of the experiment compared to those plants exposed to just two days of hypobaria (Treatment D; Figure 5-3B). The highest transpiration rates were observed for these plants grown entirely in hypobaria at 2.6±0.5 mmol m^{-2} s^{-1} followed by the next longest treatment in hypobaria (Treatment C) at 2.3±0.6 mmol m^{-2} s^{-1}. The lowest transpiration rates were found for plants exposed to two days in hypobaria (Treatment D) at 1.7 ± 0.4 mmol m^{-2} s^{-1} (Figure 5-3B). Plants that were grown entirely in hypobaria had higher transpiration rates than those grown entirely in normal pressure (Treatment E) for the entire experiment except for day 21 suggesting that

plants did not acclimate to the enhanced water loss in the hypobaria treatments at least in the first two weeks of growth (Figure 5-4B).

Water Use Efficiency (WUE)

Water use efficiency (WUE) of plants grown in hypobaria was calculated as a ratio of transpiration rate over C_A, i.e., amount of water transpired per amount of CO_2 assimilated. The WUE were calculated on days 7, 14, 21 and 28 (last day) of the experiment (Figure 5-4C). The WUE of a plants grown in hypobaria was similar to WUE of plants grown at normal pressure at 21 and 28 days of growth but lower for days 7 and 14 (Figure 5-4C). Since C_A between these two treatments were similar (Figure 5-4C), this suggests that the increase in transpiration (T) on these days (Figure 5-4B) and not C_A (Figure 5-4A) was the cause for this lower WUE since in both cases, the WUE increased over time until a maximum rate was reached after 21 days with approximately $8.6x10^{-3}$ μmol of CO_2 assimilated per μmol H_2O transpired per leaf area (Figure 5-4C). The WUE of 24 days old spinach was ~ $4x10^{-3}$ μmol of CO_2 assimilated per μmol H_2O grown in hypobaria at 25 kPa and was similar to that of plants grown at normal pressure (Iwabuchi and Kurata, 2003).

Stomata Development

The stomatal density (i.e. number of stomata per cm^2 of leaf) on the abaxial side of leaf was similar between plants grown entirely in hypobaria (Treatment A) compared to plants grown entirely at normal pressure (Treatment E; Table 5-4). The average stomatal frequency of 1.42 X 10^5 per cm^{-2} was found for plants grown in hypobaria compared to 1.28 X 10^5 per cm^{-2} for plants grown at normal pressure. Other parameters

such as epidermal cell density, stomatal index, and length of stomata pore were not significantly different between the two treatments (Table 5-4).

Discussion

Seed Germination

In one of the earliest hypobaria studies, rye seeds did not germinate at 3 kPa total pressure with Mars level pO_2 (~0.3 kPa) and pCO_2 (~0.7 kPa; Siegel et al., 1963). Here, radish seeds did not germinate in hypobaria at 1.5 kPa pO_2 (33 kPa total pressure) and only had 40% germination at 4 kPa pO_2 (Figure 5-1). Mustard seeds did not germinate below 5 kPa pO_2 (Musgrave et. al., 1988). In other recent studies with the same cultivar of radish, only 2-5 % radish seeds germinated at 2 kPa pO_2 in hypobaria (25 and 50 kPa total pressures; Wehkamp, 2009). However, 30% of the seeds germinated at 5 kPa pO_2 in hypobaria (25 kPa total pressures). The lack of oxygen availability in low oxygen environments inhibits respiration in the seeds which prevents germination (Bewley and Black, 1994). The composition of the seed coat and availability of nutrient reserves within seeds could also affect seed germination and thus the level of oxygen required for germination will likely be species dependent. Al-Ani et al. (1985) reported that no germination of radish occurred at 2 kPa of pO_2 in ambient pressure but did germinate at 7 kPa of pO_2. Here, at 10 kPa pO_2 (33 kPa pTotal) the germination was about 82 % compared to 75 % reported for radish at 10 kPa pO_2 with lower total pressures of 25 kPa total pressure (Wehkamp 2009). These results suggest that the lower limit for radish germination in hypobaria or in ambient pressure is at approximately 2 kPa pO_2 although higher levels >10kPa pO_2 are required for germination at levels near normal pressure and oxygen conditions.

Seedling Growth in Hypobaria

Radish plants that were exposed to hypobaria (33kPa) one week after germination (Treatment B) exhibited severe stress or died within a few days in hypobaria (Figure 5-2). However, the plants from the rest of the treatments even those that were germinated in hypobaria appeared healthy and there was no indication of water stress or leaf damage. The poor growth and death of plants after one week of transplant is likely due to the fragile stage of the seedlings at the time of transfer where only the cotyledons were the main area for photosynthesis and transpiration. Indeed, the cotyledons from plants from the one-week-old treatment curled and wilted within the first few days of low pressure exposure (Figure 5-2). In contrast, three-day-old radish seedling that were grown under ambient atmospheric pressure then exposed to hypobaria (25 kPa) grew without any detrimental effects (Wehkamp, 2009). However, those seedlings were grown at higher light conditions than the seedlings from this study and may have already developed primary leaves or may have had a larger root/shoot ratio to keep up with the enhanced transpiration that occurs in hypobaria (Gohil et al., 2010). There may be a critical root to leaf balance that is required for successful acclimation of a plant to hypobaria and this may be met at either the early transfer period of less than one week or after the primary leaves are established at approximately two weeks.

The radish plants that were germinated and grown in hypobaria for the entire duration of the experiment (Treatment A) had similar growth (fresh weight, dry weight and total dry weight) when compared to the control or plants grown for short-term hypobaria (Treatment D, Table 5-3). Previous studies also found no significant differences in the biomass accumulation between radish grown in hypobaria and those grown in ambient atmospheric pressure (Levine et al., 2008; Wehkamp 2009). Other

species such as lettuce (Sparnarkel and Drew, 2002; He et al., 2003, 2007) and spinach (Iwabuchi; Goto et al., 1996) had similar growth between those grown in hypobaria and those in normal pressure.

Interestingly, the leaf area of plants was significantly affected by reduced pressure with a decrease in leaf area the longer the time the plants remained in hypobaria (Figure 5-3C). The leaves from plants grown entirely in hypobaria (Treatment A) were approximately 30 % smaller compared to leaves from plants grown for a short-term in hypobaria (i.e., 2 days, Treatment D) and those grown completely at ambient pressure (Treatment E). The leaves from plants grown in hypobaria for longer-term were also thicker (Table 5-4). Reduced leaf growth can be caused by ethylene accumulation in closed chambers (He et. al., 2003, 2007). Even though ethylene was not measured in the present study, the absence of leaf senescence, leaf epinasty, and leaf pigmentation due to damaged chlorophyll apparatus, all indicators of ethylene build up, suggests that ethylene was not the cause of the reduced leaf area observed in these studies. In addition, other factors that are responsible for mitigating the negative effects of ethylene in hypobaria include the enhanced eviction of ethylene from mesophyll tissues in low pressure environments (Burg and Burg, 1966), the higher CO_2 concentration (0.1 kPa) in low pressure acts as a competitive inhibitor of the ethylene and the larger chamber volume (0.1 m^{-3}) used in these studies compared to other reports. Also, studies on gene expression of Arabidopsis grown in hypobaria compared to plants grown at ambient pressure found no regulation of ACC synthase or ACC oxidase genes which are precursor to ethylene synthesis (Paul et al., 2004; Richards et al., 2006). Therefore, it is likely that the reduced leaf area we observed for plants grown longer terms in hypobaria

was not caused by ethylene and could be a result of enhanced diffusion of CO_2 to the leaf surface or other effects of hypobaria environment such as enhanced transpiration. Plants may acclimate to hypbobaria by reducing leaf area tominimize transpiration.

Gas Exchange, CO_2 Assimilation, and Transpiration in Hypobaria

At the end of the short-term exposure to hypobaria (i.e., 2 days in hypobaria, Treatment D), the CO_2 assimilation rates (C_A) of plants were not significantly different compared to plants grown in ambient pressure (Figure5-3A; Table 5-3). Similar results were found in a previous study with mature radish (26 day old), where after 2 days the C_A rates for plants grown at 33 and 66 kPa total pressure were similar to those of plants grown in ambient pressure for the same CO_2 levels (Gohil et al., 2010). However, for those studies, after seven days in hypobaria, the mature radish plants had increased C_A rates compared to plants grown entirely at ambient pressure. Here, there was no significant difference in the C_A during the first three weeks of growth in hypobaria compared to the C_A of plants grown entirely in ambient pressure aside from an increase for the last week in the experiment($p<0.1$;Figure 5-3A; Figure 5-4A). The C_A of plants grown in hypobaria increased by 25% at the end of the fourth week when compared to the C_A from plants that had only been exposed to two days of hypobaria (Figure 5-3A). Similar results were reported in lettuce grown entirely in hypobaria where C_A rates increased only during the last week of the experiment (day 25 to 30) when compared to plants grown at ambient pressure (Sparnarkel and Drew, 2002). Several studies have also reported increased C_A along with increased biomass for plants that are grown in hypobaria however, these studies were of various durations and gas treatments in hypobaria. (Andre and Massimino 1992, Corey et al. 1996, Sparnakel and Drew 2002, Goto et al. 2002). Here, the C_A reached a saturation level at approximately 21 days after

growth (Figure 5-4A) which is similar to results reported for previous studies with radish grown in hypobaria (Wehkamp 2009). Radish plants reached compensation points at a faster rate as total pressure reduced (100, 70, 50, 25, 10 kPa; Wehkamp 2009). However, after 16 h in hypobaria, Arabidopsis plants exhibited nearly identical CO_2 draw down curves at various low pressure treatments implying that there is an acclimation response (Richards et al., 2006). Taken together, it appears that C_A increases in hypobaria for plants that are transferred to hypobaria from ambient pressures as a short-term response, but this enhancement may not last after a few days or several weeks of growth in hypobaria.

In contrast to C_A, transpiration rates were significantly higher for plants grown in hypobaria ($p< 0.05$) on the last day of the experiment suggesting that transpiration from the leaves was more sensitive to hypobaria treatments than the C_A. Also, during the first two weeks, the transpiration rates of plants that were grown in hypobaria were higher compared to plants grown at ambient atmospheric pressure (Figure 5-4B) but reached a maximal rate by the third week. Wehkamp (2009) also reported that the transpiration rates of radish reached a maximal rate at the end of the third week. In spinach, the transpiration rates were slightly higher during the short-term exposure (1 day) to hypobaria but after 10 days in hypobaria both the C_A and transpiration rates were similar to those of plants grown in ambient pressure (Iwabuchi and Kurata, 2003). This suggests that plants undergo adaptation during the long-term exposure to hypobaria which results in reduced transpiration rates.

Water use efficiency (WUE), based on the rate of CO_2 assimilated over the rate of water lost, increased at a much faster rate for plants grown in hypobaria compared to

plants grown in ambient pressure (Figure 5-4C). However, the overall WUE remained higher for plants grown at ambient pressure throughout the duration of the experiment, except for day 21, compared to plants grown in hypobaria (Figure 5-4C). The WUE of spinach grown for 10 days in hypobaria (25 kPa) was similar to that from plants grown at ambient pressure (Iwabuchi and Kurata, 2003). For many plant species, increases in WUE are observed when water deficit is mild irrespective of the cause of stomata closure (Raven 2002). Changes in WUE of plants could be another acclimation response to water deficit sensing in hypobaria. This may be a concern for plants grown in substrates where water availability is restricted (i.e. soil or agar). In this study, plants were grown in nutrient liquid thus water was not likely limited.

Short-term Acclimation to Hypobaria

Reduced pressure in a closed system increases diffusivity of gases which may result in increased gas exchange rates for plants grown in hypobaria (Rygalov et al., 2004). Although increased C_A is beneficial for plant growth up to limit, the increased transpiration rates may result in water stress if the rate of water uptake is slower than the rate of water lost. For example, for radish that were grown at ambient pressure in soil and then transferred to hypobaria (25kPa total Pressure) plants wilted (Wilkerson 2005). However, these plants were able to recover after several hours in hypobaria. After short-term exposure to hypobaria, Arabidopsis plants showed no wilting or dehydration responses, however, gene expression analyses revealed that almost 200 genes were differentially expressed in response to hypobaria and hypoxia of which about 100 genes were unique to hypobaria (Paul and Ferl, 2006). Of these hypobaria-related genes, about 20 (e.g. LATE EMBRYOGENESIS ABUNDANCE (LEA), COLD RESPONSIVE (COR78), DEHYDRATION RESPONSIVE (DR29), ABSCISIC ACID

(ABA)) were related to dehydration or water stress. These studies suggest that plant perceive water stress when exposed to hypobaria. Therefore, it is not surprising that the response mechanisms that plants use to acclimate to short-term hypobaria stress may be similar to responses that deal with water deficit, high temperatures or very low relative humidity (Iwabuchi and Kurata, 2003). In a review by Chaves et al. (2003) these early mechanisms of adaptation to these stresses are described and summarized here in Figure 5-6. For example, short-term hypobaria such as reduced C_A and stomatal response (Figure 5-6) were observed in the present study whereas other responses including gene-level responses and multi stress sensing have been reported in previous studies for plants grown in hypobaria (Paul and Ferl, 2006).

There are different pathways for stress perception and stress responsive genes. The plant hormone ABscisic Acid (ABA) has been identified as an important chemical signal in regulation of stomata in response to water deficit (Schulze 1986). The ABA signal is localized in roots as well as shoots. Some of the genes are activated are involved in pathways that protect against such stress may be activated for plants transferred from normal pressure to lower pressure. Research on the water deficit sensing and signaling mechanisms suggests that signaling pathways are interconnected and cross talk occurs between the different types of abiotic stresses (Knight and Knight, 2001). For example, hypobaria may result in increased vapor pressure deficit (VPD), a drop in leaf water potential, a reduced leaf temperature (Iwabuchi and Kurata, 2003; Wilkerson, 2005) and an increase in leaf transpiration (Richards et al., 2006; Gohil, 2010). All of these stresses may occur simultaneously in hypobaria. In particular, the drop in leaf temperature in hypobaria may be responsible

for inducing the cold resistance genes that were found for Arabidopsis (e.g. COR78; Paul et al., 2004). A temperature drop of up to 4 °C was reported for leaves of radish when chamber pressure was lowered to 10 kPa (Wilkerson, 2005) and this may induce a cold stress response.

To understand the effects of short-term exposure to hypobaria on plant transpiration, Sparnarkel and Drew (2002) exposed mature lettuce plants to hypobaria (70 kPa) and ambient pressure on alternate days from day 30 to day 38. They found that transpiration rates were higher at ambient pressure and lower at reduced pressure and the pattern was repeating during next 6 days. The reversible trend suggests that short-term effects are not due to morphological changes taking place during the growth but due to stomatal response. Since changes in stomatal density are not possible for short term acclimation to hypobaria, the closure of stomata in response to enhanced transpiration is likely in this study.

Long-term Acclimation to Hypobaria

When mature radish plants were exposed to hypobaria (33 kPa) and similar CO_2 levels in these studies for seven days, the transpiration rates of these plants were almost twice that of plants grown at ambient pressure (Gohil et al., 2010). Based on those studies, the gas exchange rates of radish grown completely in hypobaria treatment were expected to be higher than plants grown in ambient pressures. Although slightly higher transpiration rates were seen for these plants during the first two weeks of growth and on the last day of the experiment, they were only increased by ~20 % (Figure 3-2, Table 5-3). This suggests that plants adjusted their growth so that their transpiration rates were minimized as they grew in hypobaria. Indeed, plants that were grown entirely in hypobaria had reduced leaf area compared to plants grown in ambient

pressure (Figure 5-3C). This reduced leaf area along with increased specific leaf area (SLA), and thus increased leaf thickness, may be a result of a perceived water deficit in hypobaria. Others have also reported increased leaf thickness for plants grown in hypobaria although the leaf area was not reported (Wehkamp, 2009). A reduced leaf surface may result in less water lost through surface evaporation so that plants may decrease leaf area in response to the perceived water stress in hypobaria. However, the decrease in leaf area in hypobaria may also be in response to other environmental conditions such as increased diffusion of CO_2 into the leaf surface in hypobaria.

The stomata are a common gate for CO_2 assimilation and water vapor exit through the leaf air space. Therefore, it would not be surprising that stomatal number and stomatal control would be influenced by reduced pressure since both CO_2 and H_2O diffuse at a faster rate in low pressure. Although leaf area was reduced at 33 kPa, The stomatal density was similar for plants grown in low pressure and the controls in this study. In contrast, Wehkamp (2009) reported highly significant ($p<0.001$) correlation between reduced pressure and stomatal density for radish. The number of stomata increased as total pressure reduced (10, 33, 66, and 98 kPa) and as pO_2 reduced was reduced with up to 44 % increase in stomatal density at 10kPa total pressure and 2 kPa pO_2 compared to plants grown at ambient pressure. The difference in stomata numbers reported previously and in this study might be due to the differences in the methods (leaf imprints vs. SEM analysis), the location and age of the leaf for counts, the different lighting and gas conditions, and the duration of hypobaria treatments. Why plants would increase their stomatal density in response to increased transpiration of hypobaria is not clear but for rice (Meng et al., 1999) and perennial grass species (Xu and Zhou, 2006),

a moderate increase in stomatal density with moderate drought was reported. Xu and Zhua (2006) reported that stomatal density and guard cell size have plasticity in response to large variation in water status. Thus, increase in stomatal density could be a part of adaptation strategy in response to hypobaria stress similar to a water stress. In Arabidposis, differential gene expression profile under hypobaria and hypoxia also included genes associated with stomatal development and regulation (e.g., STOMATAL DENSITY AND DISTRIBUTION ; SDD1) supporting this possibility (Berger and Altmann, 2000; Paul and Ferl, 2006).

Alternatively, changes in stomata development may be attributed to other environmental factors in hypobaria such as enhanced diffusion of CO_2 to the leaf. An increase in CO_2 has shown decrease in stomata density (Knapp et al., 1994) so the reports by Wehkamp (2009) for increase in stomata density in hypobaria do not support this hypothesis. In addition, Case et al., (1998) compared the effects of CO_2 concentration (370 and 680 ppm) on twelve wild radish plants and found that the elevated CO_2 did not significantly affect stomata index or the guard cell length. Therefore, the changes in stomata observed in previous studies (Wekhamp 2009) are not likely due to the enhanced CO_2 in hypobaria.

Shoot or leaf water content has been suggested as a direct indicator of physiological functioning (Sinclair and Ludlow, 1985). In the present study, the percent moisture content of plants grown entirely at hypobaria 86% was similar to moisture content of plants grown at ambient pressure at 88% so that the water status of the plants does not appear to be affected by pressure. Studies to compare this response with different substrates to grow plants in hypobaria i.e., soil or agar, are required.

Radish plants grown in hypobaria for long-term had reduced leaf area but enhanced transpiration compared to plants grown short-term in hypobaria or grown entirely at ambient pressures. The acclimation to hypobaria may include adaption mechanisms that overlap with those for water deficit responses such as reducing the area for transpiration or inducing biochemical pathways that are involved in water stress responses. Further studies on the gene expression and growth of plants in hypobaria are required to identify if acclimation to hypobaria is truly a drought response

Summary

The gas phase in hypobaria can result in reduced germination of radish if oxygen levels are not above 10kPa. Plants acclimate to hypobaria through short-term and long-term mechanisms. For example, short-term responses (few hours to days) to hypobaria include a decrease in stomata aperture (Iwabuchi and Kurata, 2003) and the reduction of gas exchange rates (Sparnarkel and Drew, 2002; Figure 5-3A). Here, the biomass of plants grown long-term in hypobaria was similar to plants grown in ambient pressures. However, the C_A ($p<0.1$) and the transpiration rates ($p<0.05$) were enhanced. Although transpiration was enhanced for plants grown entirely in hypobaria compared to plants grown entirely in ambient pressure, this enhancement was at much reduced levels than that for adult plants that were grown for seven days in hypobaria. For example, long-term acclimated plants to hypobaria had a 20% increase in transpiration (Figure 5-3B) compared to plants that were acclimated for seven days that had 200% increase in transpiration relative to plants grown in ambient pressure (Gohil, 2010).

Long-term exposure to hypobaria may result in the activation of water deficit defense mechanism in plants such as reducing their leaf area. Here, acclimation to

hypobaria resulted in increased WUE as transpiration was reduced after three weeks of growth in hypobaria. Such response has also been reported in several plant species under mild water deficit (Chaves et al., 2003).

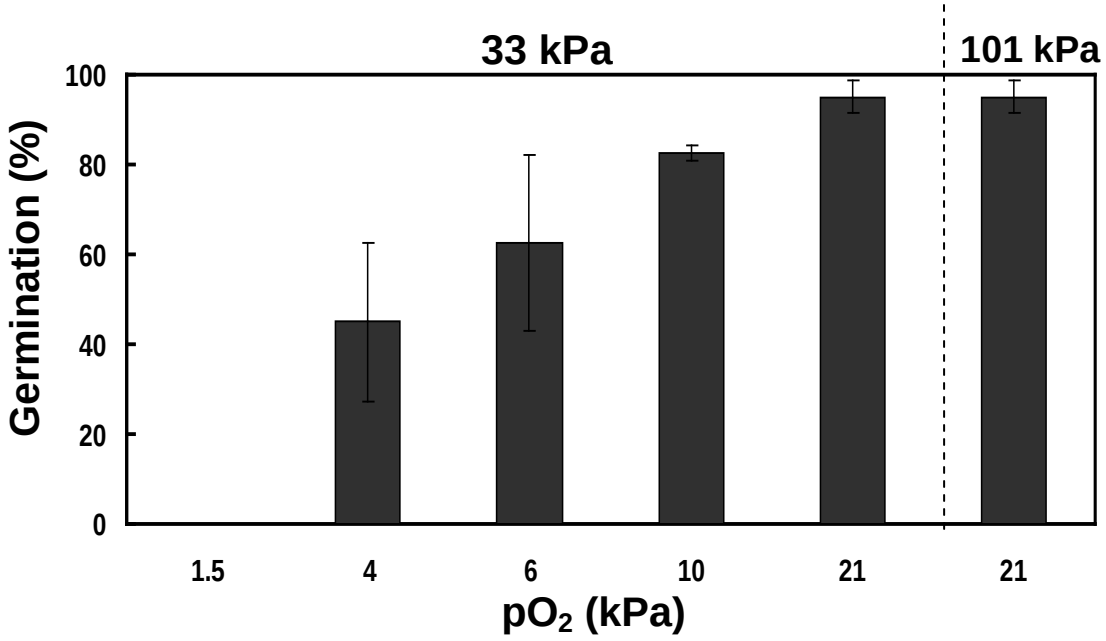


Figure 5-1. Germination of radish seedlings at 33 kPa total pressure and various pO_2. Bars represents the mean ± STDEV (n=2)

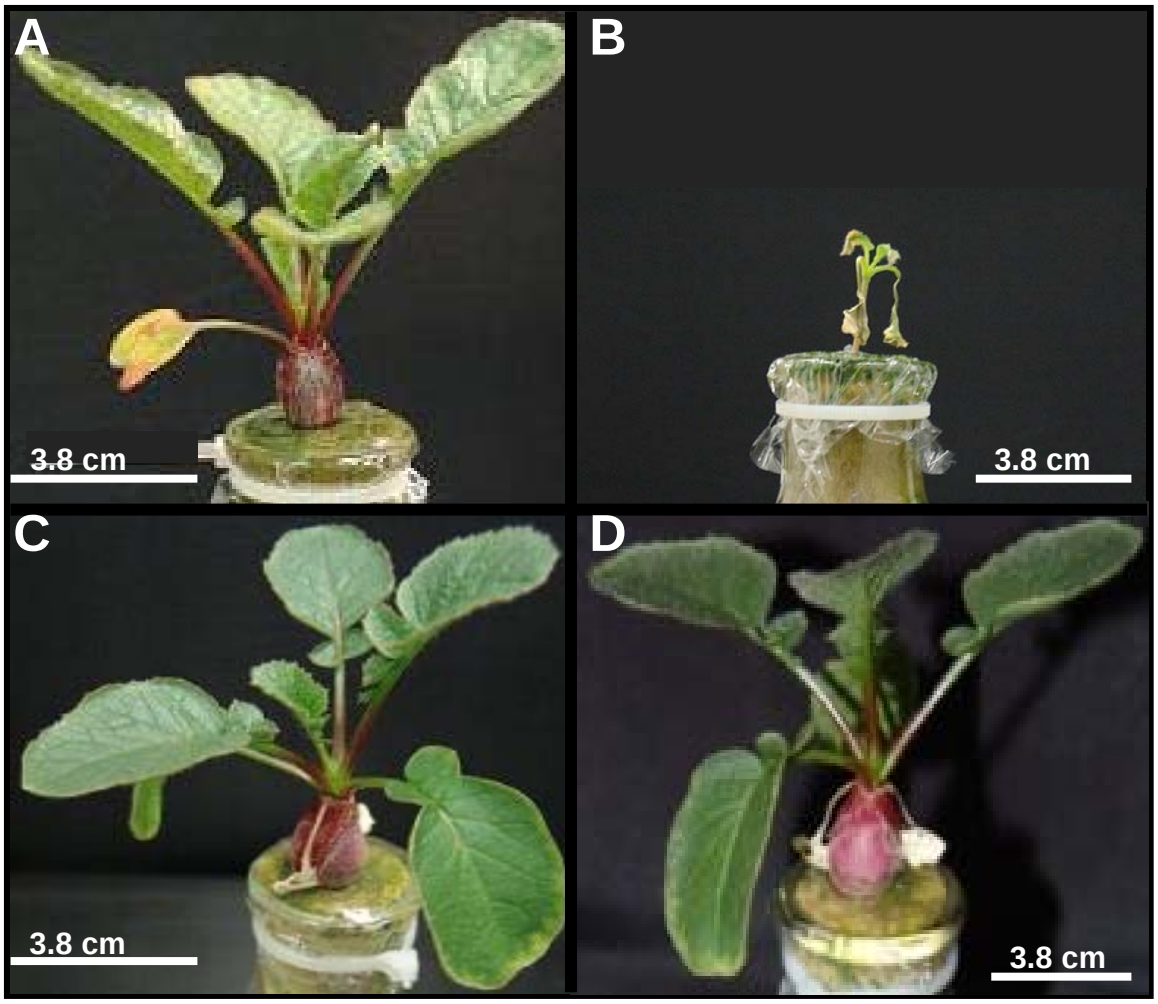


Figure 5-2**.** Radish plants after four weeks of growth Treatment A (28 days at 33 kPa), Treatment B (21 days at 33 kPa), Treatment C (14 days at 33 kPa), and Treatment D (2 days at 33 kPa). All plants in treatment B died within one week of low pressure exposure.

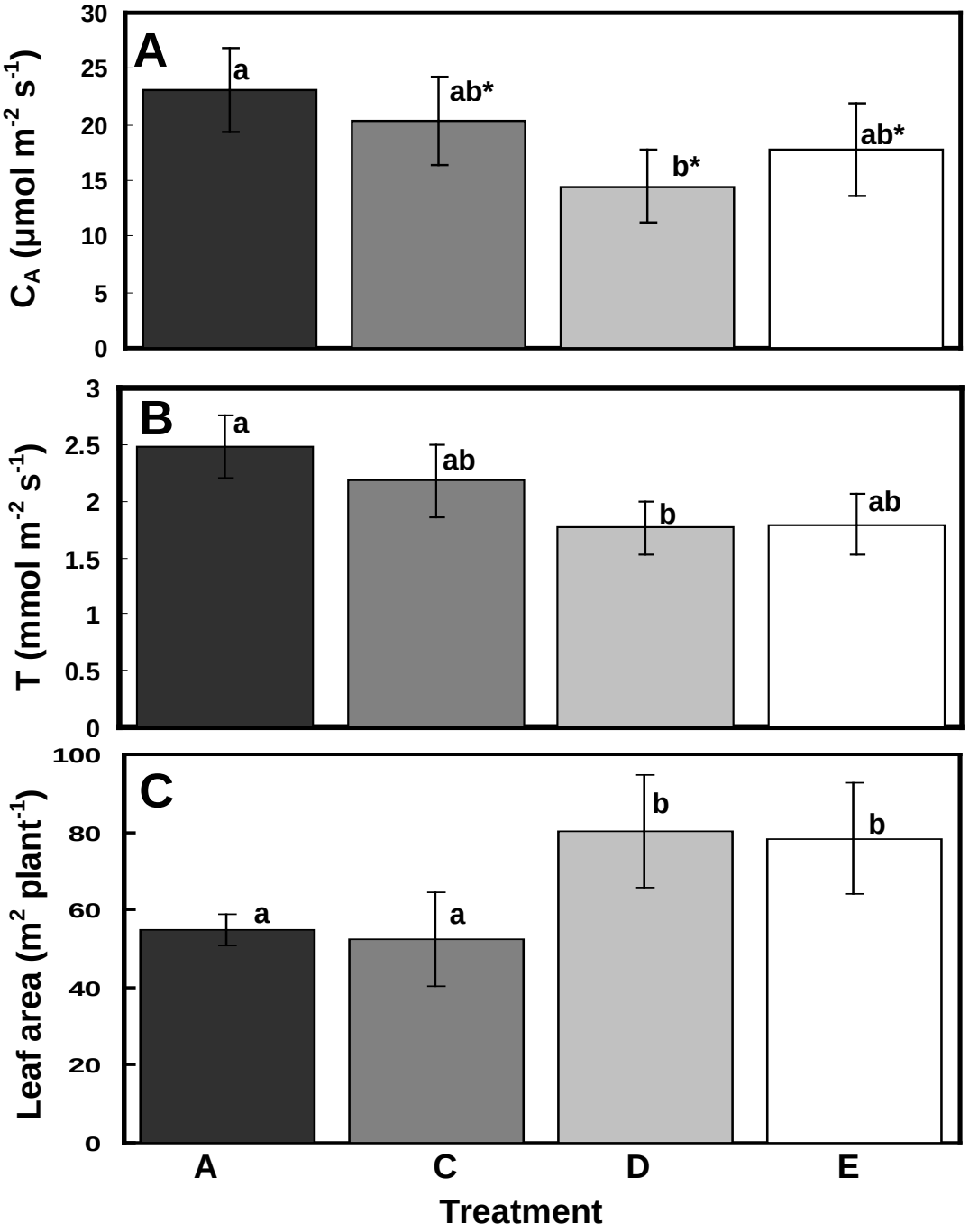


Figure 5-3. Carbon dioxide assimilation (C_A;A), Transpiration rates (B) and leaf area (C) on the last day of the experiment. The bar represent the mean ± STDEV from three experiments (n=3) each having two or three plants. Bars with different letters indicate statistically different means as calculated by multiple t-test for comparing two samples for means ($p<0.05$). The * indicates significant difference at $p<0.1$.

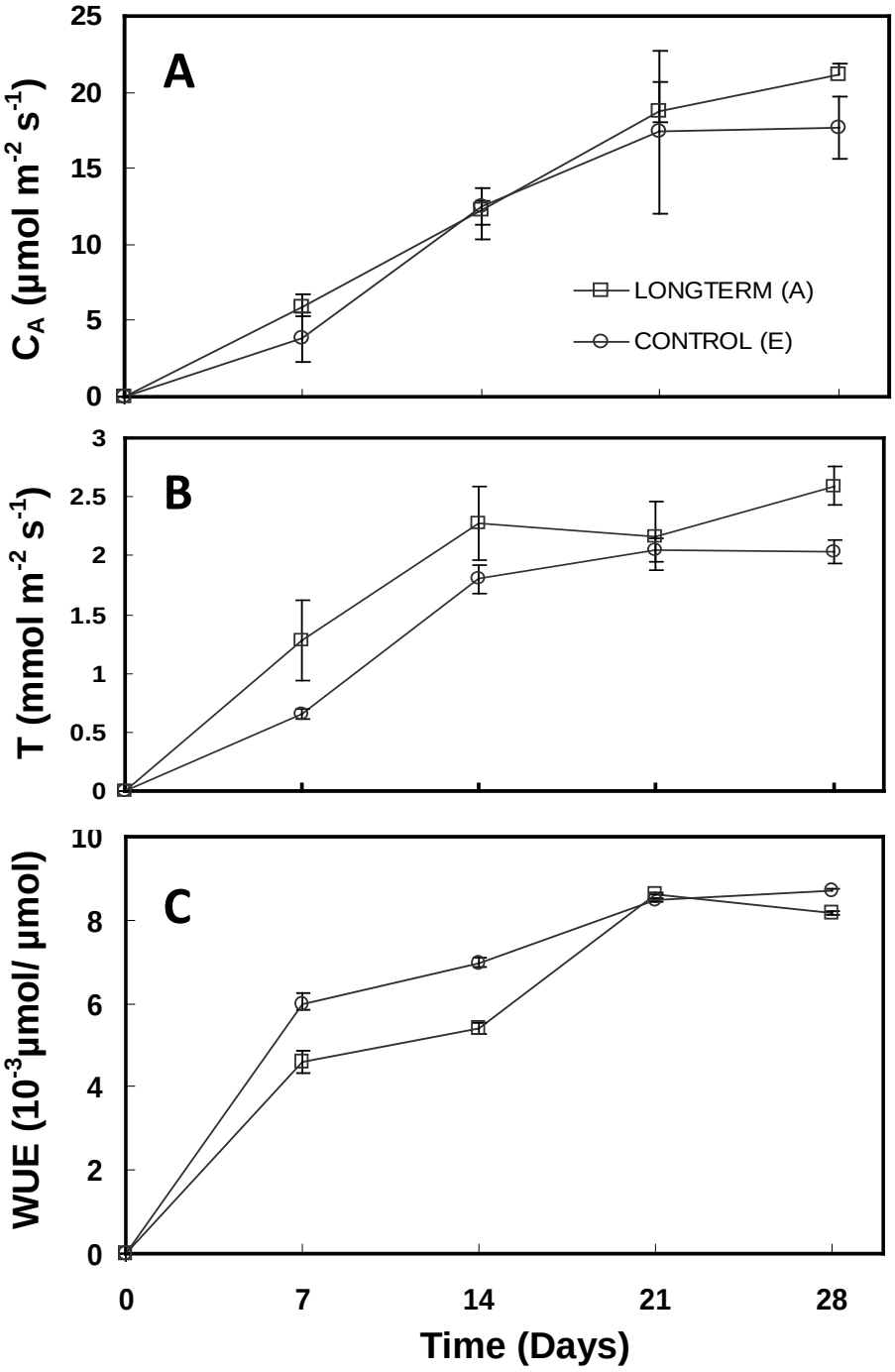


Figure 5-4. C_A (A) and Transpiration rates (B) and WUE (C) on the day 7, 14, 21 and 28 of the experiment. The values are mean ± STDEV from three experiments (n=3) each having at least two plants.

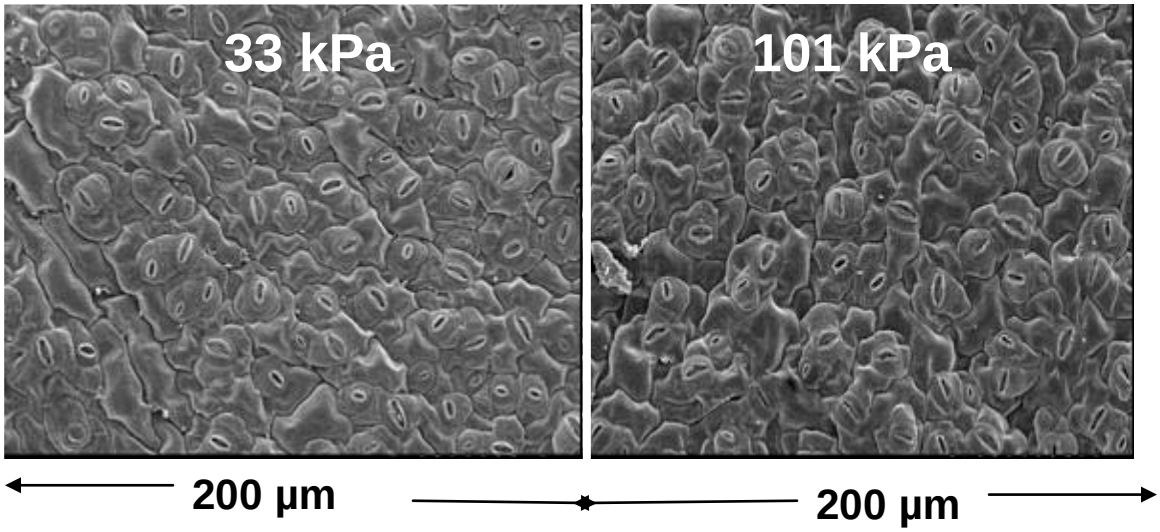


Figure 5-5. Scanning electron microscopy (SEM) image from a youngest leaf of a plant grown entirely in hypobaria (A; 33 kPa) or in normal pressure (B; 101 kPa).

Long term	Short term
Shoot Shoot growth inhibition ***Reduced transpiration area** Gene response Metabolic acclimation Osmotic adjustment	Shoot Stomata closure ***Reduced C_A** **Multi stress sensing** **Gene response** Inhibition of growth
Root Turger maintenance Sustained root growth **Increased root:shoot ratio** Increased absorption area	Root Signal transport Xylem hydraulic changes Assimilate transport Cell drought signaling Osmotic adjustment **Gene response**

Figure 5-6. The long-term and short-term responses of the shoot or root to water deficit, low humidity and high temperature (adapted from Chaves et al., 2003). The * marked responses were observed in present study with the bold indicated in other reports (Paul et al., 2004; Paul and Ferl, 2006).

Table 5-1. Number of days plants exposure to normal pressure and hypobaria.

Treatment	Days of treatment	
	(101 kPa)	(33 kPa)
A	0 days	28 days
B*	7 days	21 days
C	14 days	14 days
D	26 days	2 days
E	28 days	0 days

* Plants were severely damaged thus not analyzed for further studies.

Table 5-2. Environmental conditions used for the experiments.

Parameter	Set Point
Total Pressure	101 or 33 ±1 kPa
Light	250 µmol m^{-2} s^{-1}
Air Temperature	22/20 ± 1°C day/night
Relative Humidity	>85%
CO_2	0.1 to 0.02 kPa
O_2	21 kPa

Table 5-3. Average fresh weight (FW), dry weight (DW) of shoots, roots, hypocotyls in hypobaria treatments (A-D) and control treatment (E). The values are mean ± STDEV from three experiments (n=3) each having at least two plants. ANOVA $p<0.05$

Treatment		Parameters								
	Days	Fresh weight (g plant^{-1})				Dry weight (g plant^{-1})				Root/Shoot
	101/ 33	Shoot	Root	Hypo	Total	Shoot	Root	Hypocotyl	Total	ratio
A	0 / 28	3.6 ± 1.3	0.8 ± 0.11	2.4 ± 1.2	6.8 ± 0.7	0.52 ± 0.11	0.53 ± 0.2	0.25 ± 0.06	1.3 ± 0.2	1.02 ± 0.12
*B	14 /14	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C	14 /14	3.9 ± 0.6	0.9 ± 0.23	2.0 ± 0.6	6.8 ± 0.5	0.50 ± 0.10	0.52 ± 0.1	0.23 ± 0.03	1.3 ± 0.1	1.04 ± 0.08
D	26 / 2	3.1 ± 0.3	0.7 ± 0.19	2.1 ± 0.3	5.9 ± 0.5	0.37 ± 0.15	0.34 ± 0.1	0.29 ± 0.02	1.0 ± 0.1	0.92 ± 0.11
E	28 / 0	3.2 + 1.1	1.2 + 0.30	2.2 + 1.1	6.6 ± 08	0.39 ± 0.12	0.33 + 0.1	0.28 ± 0.04	1.0 ± 0.1	0.85 ± 0.06
		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

* Plants were severely damaged thus not analyzed for further statistics.

Table 5-4. Average specific leaf area, stomata density, stomata index and average stomata pore length from leaf of hypobaria (33 kPa) and normal pressure (101 kPa)

	33 kPa	**101 kPa**
Leaf area (cm^2)	58 ± 2.8^a	82 ± 10.2^b
Specific leaf area ($cm^2\ g^{-1}$)	115 ± 12^a	208 ± 10^b
Stomata density ($10^5\ cm^{-2}$)	1.42 ± 0.11^a	1.28 ± 0.16^a
Stomata per SLA ($10^5\ cm^{-2}\ g^{-1}$)	19.2 ± 1.8^a	34.8 ± 2.7^b
Epidermal cell density (cm^{-2})	31 ± 2^a	29 ± 2^a
Stomata index	55 ± 2^a	52 ± 2^a
Average stomata pore length (μM)	9.63 ± 2.2^a	9.98 ± 2.9^a

Values with different letters indicate statistically different means as calculated by t-test for comparing two samples for means ($p<0.05$).

CHAPTER 6

TRANSPIRATION MODEL PERFORMANCE AT REDUCED PRESSURE

Introduction

Low pressure environments have increased gas diffusivity which can result in increased gas exchange rates of plants and thus increased transpiration compared to plants grown in normal pressure. Previously Wilkerson (2005) applied the Penman-Monteith model to predict transpiration rates of radish in hypobaria. Though the model performed well, it was not very effective at very low pressure (10 kPa), was fairly complex, and was developed for evapotranspiration for field grown plants and not transpiration of individual plants. A model developed by Sinclair (1998) described in Chapter 3 is simpler and requires fewer assumptions compared to the Penman-Monteith model (Wilkerson, 2005). Therefore, the simplified model was used here to predict transpiration of radish in low pressure environments. The model incorporated the environmental conditions (gas phase composition, pressure, the temperature, and air velocity) and also the physiology of the leaf (stomata density, stomata dimensions, leaf length etc.). A sensitivity analysis of the model for each parameter was performed to identify the parameters that influenced transpiration to the greatest extent. Many of these parameters could be controlled to minimize water loss and maximize plant growth. The prediction of transpiration based on the model was compared with the observed from plants that were grown in either 33 or 101 kPa (Chapter 5) were compared to the model.

Materials and Methods

A list of the environmental parameters that were used in the model is provided in Table 6-1. The parameters that were adjusted to compare against reference values are listed in Table 6-2. The sensitivity of transpiration predictions to pressure (which also affected gas diffusivity), vapor pressure deficit (VPD) and stomatal width and number at 22 °C , 1 m s^{-1} air velocity, and 100 Pa pCO_2 was determined by varying one parameter at a time while the remaining parameters were held constant (Table 6-2). Transpiration rate was calculated by equation 3-15.

Sensitivity Analysis

Table 6-3 lists the parameters that were evaluated and their reference (101 kPa, 0.6 kPa VPD, 20°C, 100 Pa pCO_2, and 200 μmol m^{-2} s^{-1} light intensity, and 21kPa pO_2 by comparing the % change. The percent change in transpiration was calculated by equation 6-1 for each parameter perturbation (Wilkerson, 2005).

$$\%\ change = \frac{T - T^{0}}{T^{0}} \tag{6-1}$$

Where T = Transpiration rate with one parameter varied, g m^{-2} s^{-1}

T° = Transpiration calculated at reference parameter value, g m^{-2} s^{-1}

The evaluation of the model was done by comparing the calculated values with the measured values for transpiration as presented in Chapter 5 which are averages of three replicated experiments. The VPD was varied by -50 and +50% from the reference value (0.6 kPa). The pressure values were varied from -67% of the reference value (101 kPa). The stomatal width was varied by –0.0001, -0.0002 –0.0003 and stomatal number by -50, +50% from the reference value (n =13500).

Results and Discussion

Transpiration rate was predicted as a function of stomata dimensions, stomata number and the diffusivity of water, as described by Sinclair's (1998) and Chapter 3. Although transpiration rates were calculated at over a range of pressures, they were compared with measured transpiration rate from data from plants grown as described in Chapter 5. These plants were grown for 28 days at 33 and 101 kPa, 20-22 °C, >85% relative humidity and 100 Pa of pCO_2. Measured transpiration rate were on average 2.16 g m^{-2} min^{-1} and 2.76 g m^{-2} min^{-1} for plants grown at 101 kPa and 33 kPa respectively, suggesting that transpiration rate increased by 25 % as total pressure reduced. The model predicted an increase in transpiration rates as total pressure reduced from 101 to 33 kPa (Figure 6-1). The model very closely predicted transpiration at 101kP at 2.2 g m^{-2} min^{-1}. However, the model predicted that the transpiration should be 80 % higher at approximately at 4 g m^{-2} min^{-1} at 33 kPa. The predicted transpiration rates at various stomatal widths (0.0001, 0.0002 and 0.0004 cm) are given in Figure 6-2. The predicted transpiration rate at 33 kPa total pressure and 0.0004 cm stomatal width was 4 g m^{-2} min^{-1} which after adjusting the stomatal width to 0.0003 cm was 3.9 g m^{-2} min^{-1} and at 0.0001 cm width was 3.8 g m^{-2} min^{-1} suggesting that stomatal width adjustment by these values had little effect on the transpiration rates. At normal pressure changing the stomatal width from 0.0004 to 0.0003 and 0.0001 changed transpiration rate only by -0.5 and -0.9%.

To study the effects of vapor pressure deficit (VPD) on transpiration rates, two different values of VPD (0.6 kPa – low VPD and 0.9 kPa – high VPD) were adjusted in equation 2-15. The VPD had significant effect on the transpiration rate. At lower VPD and at normal pressure, transpiration rate was similar to that of predicted using the

model. However, at higher VPD transpiration rate increased by 45 % and 164 % at 101 kPa and 33 kPa respectively compared to measured transpiration rate at 33 and 101 kPa (Figure 6-3). The variability in transpiration could be accounted due to the number of stomata at various pressures. According to Figure 6-4 increases in stomata number from approximately 1350 to 10000 resulted in predicted increased transpiration however further increase in number of stomata did not significantly increased transpiration as the curve approached a maximal transpiration. Thus increased transpiration was restricted beyond stomata number 10000 as a result of limitations in stomatal conductance. When number of stomata was reduced by 50 % in Model, the transpiration rate was reduced by approximately 14% at 101 kPa and 33 kPa (Table 6-3). However, when number of stomata were increased by 50 % (20,500) in model, the transpiration rates was same to reference value at 101 kPa and 82% higher at 33 kPa, suggesting that there is a limit to increase in transpiration due to higher number of stomata, and increasing the stomatal number beyond 13,500 did not further increased transpiration rate.

Conclusion

The transpiration model (Sinclair, 1998) incorporating stomatal conductance, stomatal aperture, diffusivity and VPD performed well at normal pressure. Increased VPD resulted in increased transpiration. At reduced pressure the parameters such as stomatal width and in some cases stomatal number are most likely to change the transpiration rate due change gas diffusivity at reduced pressure.

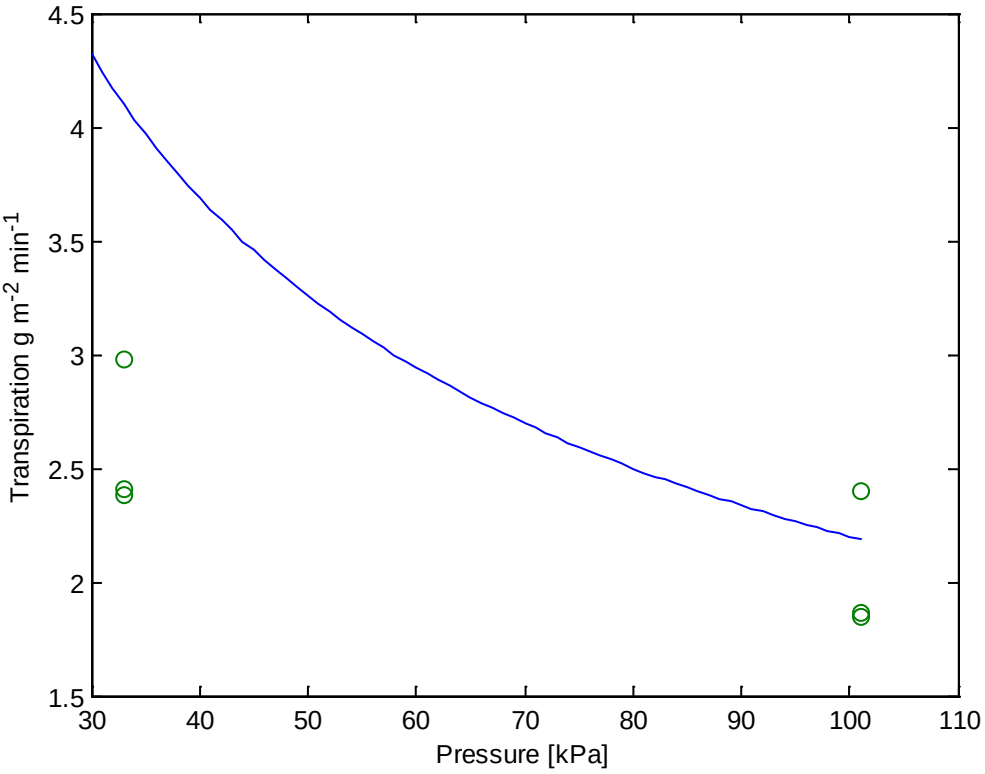


Figure 6-1. Predicted transpiration rates from 33 to 101 kPa with observed data, ‘o’, from plants grown at 33kPa and 101 kPa as described in Chapter 5 of this dissertation.

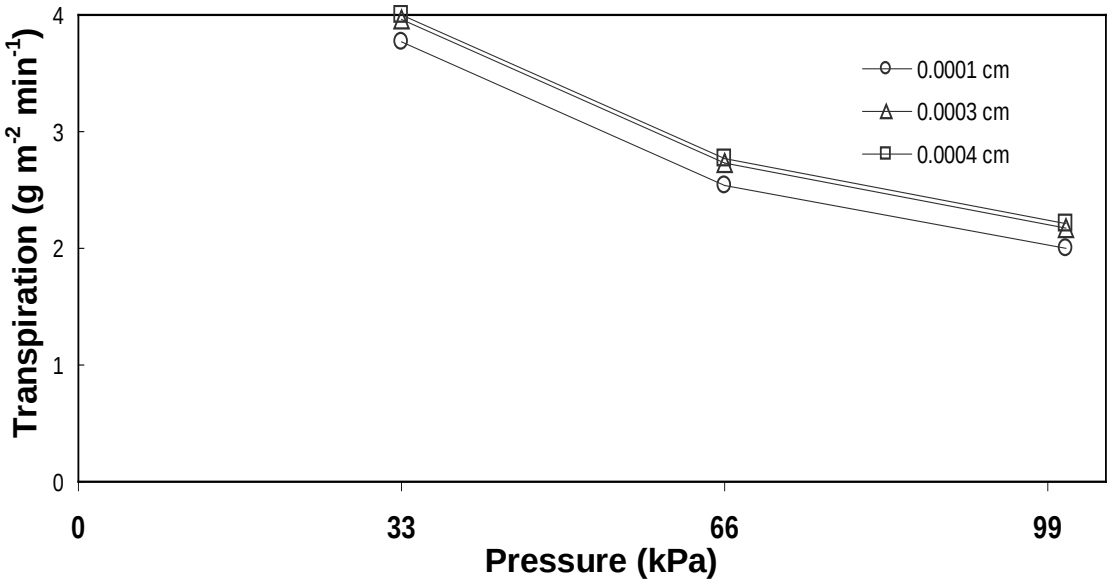


Figure 6-2. Predicted transpiration rate at various total pressures (33, 66, 101 kPa) and various stomatal widths (0.0001, 0.0003 and 0.0004 cm).

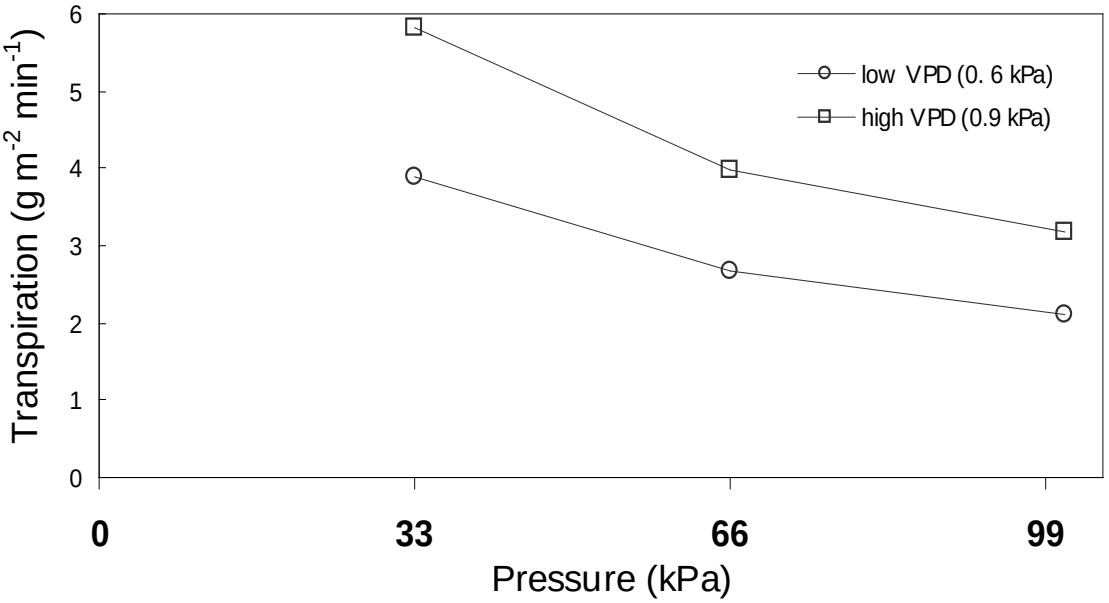


Figure 6-3. Predicted transpiration rate at three different pressures (33, 66 and 101 kPa) and two different vapor pressure deficit (VPD) levels (0.6 kPa and 0.9 kPa).

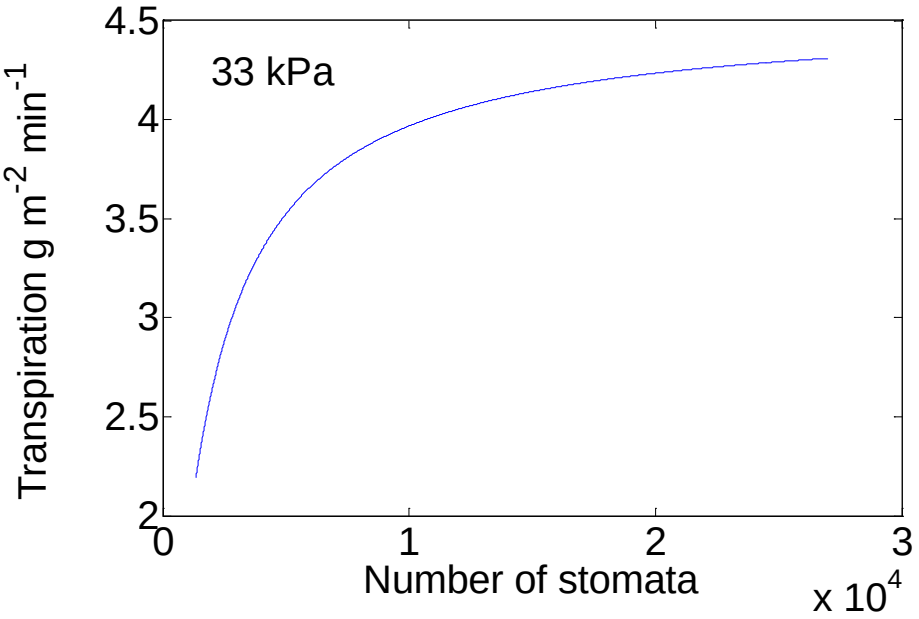


Figure 6-4. Predicted transpiration rates as stomata number varies from 1350 to 27000 at 33kPa.

Table 6-1. Parameters and reference values used in the model.

Parameter	Variable (units)	Reference value (at 101 kPa)
Stomata Length	a (cm)	0.0010
Stomata Width	b (cm)	0.0002
Stomata Depth	d (cm)	0.0001
Diffusivity of Water	D (cm^2 s^{-1})	0.8331
Number of. stomata	n (no. cm^{-2})	13500
Stomatal Conductance	hs (cm s^{-1})	17.7
Boundary Layer Conductance	hb (cm s^{-1})	1.6
Constant		0.6450
Thermal Diffusivity of Water	K (cm^2 s^{-1})	10
Length of leaf	L (cm)	0.4250
Kinematic Viscosity	Kv (cm^2 s^{-1})	100
Air Speed	u (cm s^{-1})	2353
Reynolds Number	Re	0.0002
Viscosity of Air	μ (g cm^{-1} s^{-1})	0.0004
Density of Air	ρ (g cm^{-3})	0.5101
Schmidt Number	Sc	0.507

Table 6-2. Parameter description and reference values used for sensitivity analysis

Parameter	Description	Reference value
P	Atmospheric pressure	101 kPa
D	Diffusivity of gases	According to Figure 3-1
VPD	vapor pressure deficit	0.6 kPa (Low)
b	Stomatal width	0.0004 cm
n	Stomatal number	13500

Table 6-3. Sensitivity analysis of the transpiration model at various conditions. Given are the transpiration rates and percent change from the reference conditions (101 kPa, 0.6 kPa VPD, and stomatal width of 4×10^{-4} cm and stomatal number of 13500) when one parameter is varied. The reference transpiration is 2.2 g m^{-2} min^{-1}.

	Tran (g m^{-2} min^{-1})	% change
Pressure		
101 kPa	2.2	0 (reference value)
33 kPa	4	82
$VPD_{air-leaf}$		
0.6 kPa		
101kPa	2.1	-4
33kPa	3.9	77
0.6 kPa		
101kPa	2.2	0 (reference value)
33kPa	4	82
0.9 kPa		
101 kPa	3.2	45
33 kPa	5.8	163
Stomatal width		
0.0001 cm		
101 kPa	2	-9
33 kPa	3.8	72
0.0003 cm		
101 kPa	2.1	-5
33 kPa	3.9	78
0.0004 cm		
101 kPa	2.2	0 (reference value)
33 kPa	4	82
Stomatal number		
6750	1.9	-14
	3.7	68
13500		
101 kPa	2.2	0 (reference value)
33 kPa	4	82
20250		
101 kPa	2.2	0
33 kPa	4	82

CHAPTER 7

SUMMARY AND FUTURE WORK

Plants will be an integral part of an Advanced Life Support (ALS) system for space exploration for many reasons but particularly as a source of fresh food. The plant growth facility in an ALS will most likely be maintained at reduced pressure due to the lower costs associated with low pressure facilities compared to those that would be run at normal pressures. The overall growth of plants will depend on the environment within the chambers. Important environmental factors that will be monitored and controlled include temperature, relative humidity, light, total pressure, and overall gas composition (CO_2, O_2, ethylene among other gas volatiles). Here, the effects of reduced pressure on radish gas exchange, growth and adaptation to hypobaria were studied. These studies were performed using unique environmental chambers that could monitor all of the important environmental conditions that would be needed for growing plants on long-term missions to the Moon or Mars in hypobaria. However, these chambers had limitations since the relative humidity, gas phase composition, light and temperature were not controlled by the chambers themselves but by either a larger environmental chamber in which they were housed (temperature and light) or through manual control mechanisms such as the use of saturating salt solutions to maintain humidity levels within the chamber or through manual injection of gas into the chamber on daily intervals to maintain various gas phase concentrations set points. Also, the nutrient solution was not monitored except for the first and final days of the experiments. Improvements to the system would include developing an automated system for controlling humidity and gas phase composition as well as a hydroponics system that could monitor and control nutrient levels, pH and be adjusted throughout the growth of

plants. Despite these limitations of the chambers, radish plants grew well in low pressure and their growth and gas exchange rates could be monitored.

Radish plants grown short term (1 week) in hypobaria had increased biomass (DW), CO_2 assimilation, dark respiration (DR), and transpiration compared to plants grown in ambient pressures. Transpiration was reduced and growth enhanced by enrichment of the gas phase with CO_2 for all pressure treatments (33, 66, and 101kPa). Plant transpiration rates remained constant over the seven days in hypobaria suggesting that plants grown short term in hypobaria did not acclimate to hypobaria by reducing stomata aperture during this period. Very high pCO_2 (180 Pa) when combined with hypobaria (33 or 66 kPa) induced leaf damage. The leaf damage was not found at lower pCO_2 (100 Pa) treatments suggesting that the threshold for pCO_2 uptake had been reached at 180 Pa pCO_2. However, the cause of this leaf damage is unknown. Further studies to identify the cause of this damage in low pressure are required. These studies should include the analysis that compare chlorophyll content, rubisco activity, and starch accumulation in the leaves of damaged plants.

In addition, the stage at which plants are transplanted from high to low pressure can affect the growth and survival of the plant in hypobaria since plants at early stages may not have the proper root to shoot balance to deal with the increased transpiration that often occurs in hypobaria. Overall, it appears that radish plants do acclimate to hypobaria long term by reducing leaf area and increasing leaf thickness. This may be an adaption to water stress caused by enhanced transpiration, an adaption to the increased diffusion to CO_2 in low pressure or other adaptations to the interactions of the gas phase and other conditions such as lower leaf temperature in hypobaria. Further

studies on these interacting factors are required to understand the mechanisms of plant adaption to hypobaria.

Transpiration and photosynthesis are both sensitive to the total pressure of the atmosphere. Due to the increases in diffusivity of gases in hypobaria, and hence gas flux, plant growth and water loss can be influenced by the pressure of the growth chamber. If water flux, for example, is a critical aspect of a water purification system or for water conservation, the transpiration rate of the plants can be increased or decreased by simply adjusting the plant chamber pressure or pCO_2. Models predict that both CO_2 assimilation and transpiration will increase in hypobaria due to the increased diffusivity of gases. However, these models still require improvements since many assumptions are required. For example, the stomatal dimensions and densities are difficult to measure for plants grown in hypobaria and further studies are required that compare stomata and leaf development from plants grown long term in hypobaria with those grown at normal atmospheric conditions.

Although utilizing plants as part of a BLSS seems promising, it is far from mature. More studies are required to identify the effects of the challenging environments on plant growth and development that will be encountered on space missions including increased radiation and reduced water supplies. Gene expression analyses suggest that plants are undergoing stress in hypobaria. This stress response could divert some of the metabolic energy from plant growth to other pathways that may result in reduced food supply. Further integration of molecular information in response to hypobaria may provide information to breed or genetically engineer plants that can mitigate these stresses in hypobaria. The knowledge gained from research on plant adaption to low

pressures for long-term space missions can be applied for terrestrial agriculture particularly in areas of the world where water and other resources are limited.

APPENDIX
CR10 PROGRAM

```
;{CR10};
```

Table 1 Program

```
   01: 1        Execution Interval (seconds)
  ; Reference Temp Reading
1:  Internal Temperature (P17)
   1: 1        Loc [ temp_int  ]
  2:  Z=X*F (P37)
   1: 1        X Loc [ temp_int  ]
   2: 1.0      F
   3: 35       Z Loc [ temp_int2 ]
  3:  Z=X+F (P34)
   1: 35       X Loc [ temp_int2 ]
   2: 0.0      F
   3: 36       Z Loc [ temp_int3 ]
```

O_2 Reading

```
  4:  Volt (SE) (P1)
   1: 1        Reps
   2: 15       2500 mV Fast Range
   3: 1        SE Channel
   4: 21       Loc [ O2sensor  ]
   5: 1.0      Mult
   6: 0.0      Offset
```

O_2 Calibration

```
  5:  Z=X*F (P37)
   1: 21       X Loc [ O2sensor  ]
   2: 419.43   F
   3: 47       Z Loc [ O2sensor2 ]
  6:  Z=X*F (P37)
   1: 47       X Loc [ O2sensor2 ]
   2: 1.07     F
   3: 54       Z Loc [ mult      ]
  7:  Z=X+F (P34)
   1: 54       X Loc [ mult      ]
   2: 487.07   F
   3: 48       Z Loc [ O2sensor3 ]
  8:  Z=X*F (P37)
   1: 43       X Loc [ Prs3      ]
   2: 1.2975   F
   3: 49       Z Loc [ factor1   ]
  9:  Z=X*F (P37)
   1: 43       X Loc [ Prs3      ]
```

2: 2.4183 F
3: 50 Z Loc [factor2]
10: Z=X+F (P34)
1: 50 X Loc [factor2]
2: -7.1664 F
3: 51 Z Loc [factor3]
11: Z=X+Y (P33)
1: 48 X Loc [O2sensor3]
2: 49 Y Loc [factor1]
3: 52 Z Loc [factor4]
12: Z=X+F (P34)
1: 52 X Loc [factor4]
2: -164.56 F
3: 55 Z Loc [factor5]
13: Z=X/Y (P38)
1: 55 X Loc [factor5]
2: 51 Y Loc [factor3]
3: 53 Z Loc [O2output]

RH Reading 1

14: Volt (SE) (P1)
1: 1 Reps
2: 15 2500 mV Fast Range
3: 3 SE Channel
4: 22 Loc [RH]
5: 1.0 Mult
6: 0.0 Offset

RH Calibration

15: Z=X*F (P37)
1: 22 X Loc [RH]
2: 1 F
3: 65 Z Loc [RH3]
16: Z=X+F (P34)
1: 65 X Loc [RH3]
2: -.793 F
3: 63 Z Loc [RH2]
17: Z=X*F (P37)
1: 63 X Loc [RH2]
2: .03 F
3: 64 Z Loc [RHoutput]

Pressure Reading

18: Volt (SE) (P1)
1: 1 Reps
2: 15 2500 mV Fast Range
3: 4 SE Channel

```
 4: 24       Loc [ Prs       ]
 5: 1.0      Mult
 6: 0.0      Offset
```
Pressure Calibration

```
19:  Z=X+F (P34)
 1: 24       X Loc [ Prs       ]
 2: -122.59  F
 3: 42       Z Loc [ Prs2      ]

20:  Z=X*F (P37)
 1: 42       X Loc [ Prs2      ]
 2: .0456    F
 3: 43       Z Loc [ Prs3      ]
```
Pump Relay Control

```
21:  If (X<=>F) (P89)
 1: 43       X Loc [ Prs3      ]
 2: 3        >=
 3: -5       F
 4: 46       Set Port 6 High
22:  If (X<=>F) (P89)
 1: 43       X Loc [ Prs3      ]
 2: 4        <
 3: -7       F
 4: 56       Set Port 6 Low
;solenoid control (normally closed)
23:  If (X<=>F) (P89)
 1: 43       X Loc [ Prs3      ]
 2: 3        >=
 3: -5       F
 4: 48       Set Port 8 High
24:  If (X<=>F) (P89)
 1: 43       X Loc [ Prs3      ]
 2: 4        <
 3: -7       F
 4: 58       Set Port 8 Low
25:  Do (P86)
 1: 10       Set Output Flag High
; IRt/c
26:  Thermocouple Temp (DIFF) (P14)
 1: 1        Reps
 2: 14       250 mV Fast Range
 3: 6        DIFF Channel
 4: 3        Type K (Chromel-Alumel)
 5: 1        Ref Temp (Deg. C) Loc [ temp_int  ]
 6: 66       Loc [ IRtc        ]
```

7: 1.0 Mult
8: 0.0 Offset

CO_2 Reading

27: Volt (Diff) (P2)
1: 1 Reps
2: 15 2500 mV Fast Range
3: 04 DIFF Channel
4: 25 Loc [CO2]
5: 1.0 Mult
6: 0.0 Offset

Light Reading

28: Volt (SE) (P1)
1: 1 Reps
2: 15 2500 mV Fast Range
3: 05 SE Channel
4: 26 Loc [light]
5: 1.0 Mult
6: 0 Offset

Light Calibration

29: Z=X*F (P37)
1: 26 X Loc [light]
2: 0.0353 F
3: 29 Z Loc [light2]
30: Z=EXP(X) (P41)
1: 29 X Loc [light2]
2: 30 Z Loc [light3]
31: Z=X*F (P37)
1: 30 X Loc [light3]
2: 0.0027 F
3: 31 Z Loc [light4]

Thermocouple Reading

32: Thermocouple Temp (DIFF) (P14)
1: 1 Reps
2: 14 250 mV Fast Range
3: 05 DIFF Channel
4: 1 Type T (Copper-Constantan)
5: 1 Ref Temp (Deg. C) Loc [temp_int]
6: 37 Loc [thermocou]
7: 1.0 Mult
8: 0.0 Offset
33: Z=X*Y (P36)
1: 68 X Loc [O2ppa]
2: 43 Y Loc [Prs3]

 3: 69   Z Loc [O2pp   ]
34: Z=X*F (P37)
 1: 53   X Loc [O2output]
 2: 0.01   F
 3: 68   Z Loc [O2ppa   ]

***Program**

02: 600   Execution Interval (seconds)
1: Do (P86)
 1: 10   Set Output Flag High
2: Real Time (P77)
 1: 110   Day,Hour/Minute (midnight = 0000)
3: Sample (P70)
 1: 1   Reps
 2: 43   Loc [Prs3   ]
4: Sample (P70)
 1: 1   Reps
 2: 37   Loc [thermocou]
5: Sample (P70)
 1: 1   Reps
 2: 53   Loc [O2output]
6: Sample (P70)
 1: 1   Reps
 2: 31   Loc [light4   ]
7: Sample (P70)
 1: 1   Reps
 2: 25   Loc [CO2   ]
8: Sample (P70)
 1: 1   Reps
 2: 64   Loc [RHoutput]
9: Sample (P70)
 1: 1   Reps
 2: 69   Loc [O2pp   ]

***Subroutines**

End Program
-Input Locations-
1 temp_int 1 3 1
2 _____ 1 0 0
3 _____ 0 0 0
4 _____ 0 0 0
5 _____ 0 0 0
6 _____ 0 0 0
7 _____ 0 0 0
8 _____ 0 0 0
9 _____ 0 0 0
10 _____ 0 0 0
11 _____ 0 0 0

12 _____ 0 0 0
13 _____ 0 0 0
14 _____ 0 0 0
15 _____ 1 0 0
16 _____ 0 0 0
17 _____ 0 0 0
18 _____ 0 0 0
19 _____ 0 0 0
20 temp 1 0 0
21 O2sensor 1 1 1
22 RH 1 1 1
23 CO2sensor 1 0 0
24 Prs 1 1 1
25 CO2 1 1 1
26 light 1 1 1
27 exponent 1 0 0
28 lightcorr 1 0 0
29 light2 1 1 1
30 light3 1 1 1
31 light4 1 1 1
32 temp2 1 0 0
33 temp3 1 0 0
34 temp4 1 0 0
35 temp_int2 1 1 1
36 temp_int3 1 0 1
37 thermocou 1 1 1
38 expone 1 0 0
39 therm2 1 0 0
40 __light__ 1 0 0
41 lig 1 0 0
42 Prs2 1 1 1
43 Prs3 1 8 1
44 light5 1 0 0
45 light6 1 0 0
46 light7 1 0 0
47 O2sensor2 1 1 1
48 O2sensor3 1 1 1
49 factor1 1 1 1
50 factor2 1 1 1
51 factor3 1 1 1
52 factor4 1 1 1
53 O2output 1 2 1
54 mult 1 1 1
55 factor5 1 1 1
56 CO2_____ 1 0 0
57 RH_____ 1 0 0

58 CO2_____ 1 0 0
59 o2ave 1 0 0
60 o2ave2 1 0 0
61 mult2 1 0 0
62 o2ave3 1 0 0
63 RH2 1 1 1
64 RHoutput 1 1 1
65 RH3 1 1 1
66 IRtc 1 0 1
67 _____Prs3 1 0 0
68 O2ppa 1 1 1
69 O2pp 1 1 1
-Program Security-
0000
0000
-Mode 4-
-Final Storage Area 2-

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BIOGRAPHICAL SKETCH

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