PLANT AGE AFFECTS THE LONG-TERM GROWTH RESPONSES TO REDUCED TOTAL PRESSURE AND OXYGEN PARTIAL PRESSURE

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by
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ABSTRACT

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Fundamental to the future of space exploration is the development of advanced life support systems capable of maintaining crews for significant periods without re-supply from Earth. Bioregenerative life support systems harness natural ecosystem processes and employ plant photosynthesis and transpiration to produce food, supply oxygen, and regenerate water while consuming carbon dioxide. Proposed Lunar and Martian exploration has prompted interest into the effects of hypobaria on plant development. Reduced atmospheric pressure conditions will reduce the pressure gradient between the structure and the local environment thereby decreasing the engineering requirements, leakage and mass required to construct the growth facility. To establish the optimal conditions for reduced pressure plant growth structures it is essential to determine the atmospheric pressure limits required for plant development and growth. Due to its physiological importance, oxygen will compose a significant portion of this atmosphere. The effects of reduced atmospheric pressure and decreased oxygen partial pressures on plant germination, growth and development were assessed in the University of Guelph's hypobaric plant growth chambers. Treatments included a range of total pressures from 10 to 98 kPa and oxygen partial pressures from 2 to 20 kPa. Results demonstrated that reduced atmospheric pressure had minimal effect on plant growth, net carbon exchange rate and transpiration if the physiologically important gases including carbon
dioxide, oxygen and water vapour, were maintained above threshold levels. The reduction of oxygen partial pressures below 7 kPa had drastic consequences across all atmospheric pressures with poor germination, seedling establishment and growth. It is evident that the response of plants grown at reduced pressures from young seedlings differs from that of older plants that were established at ambient conditions and then subjected to the atmospheric adjustment. The young plant tissues adapt in response to the extreme conditions and maintain productivity despite the limited atmosphere.
ACKNOWLEDGEMENTS

The journey has been lengthy, but not without abundant rewards. My success was made possible by the support of many. I would like to express my sincere gratitude to my supervisor, Dr. Michael Dixon and the members of my advisory committee, Drs. Terry Gillespie, Youbin Zheng and Michael Stasiak, for their inspiration, thoughtful guidance and encouragement. I am deeply honoured to have had the opportunity to be mentored by such a wonderful group of researchers and extraordinary people.

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Most of all, I wish to thank my parents and family for their nurturing, love, and unwavering dedication throughout my journey. My successes are built upon their confidence in me, the concessions made in support of my pursuits and their understanding along the way. I am humbled by the privilege bestowed upon me.
I would also like to gratefully acknowledge the personal financial support from the Natural Science and Engineering Research Council, the Canadian Space Agency, Ontario Graduate Student Program and the University of Guelph, as well as, infrastructure support from the Canadian Foundation for Innovation and the Ontario Innovation Trust.

I dedicate this work to those who believe in the power of knowledge, stand for the truth and follow their heart. For the next seven generations, I respectfully hope my accomplishments help guide the way for Indigenous youth in the fulfilment of their dreams.

With my greatest respect and thanks, Chi Miigwetch.
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LIST OF SYMBOLS AND ABBREVIATIONS

α - Type-1 error rate
ALS – Advanced life support
ANOVA – Analysis of variance
ATP - Adenosine triphosphate
BLSS – Bioregenerative life support system
°C – Degree Celsius
CAT – Computer-aided tomography
CESRF – Controlled Environment Systems Research Facility
cm – Centimetre
CO₂ – Carbon dioxide
D’ - Diffusion coefficient at P and Ta (cm²s⁻¹)
Dⁿ - Diffusion coefficient in air at 101.3 and 293.16 °K (cm²s⁻¹)
DAP – Days after planting
df – Degrees of freedom
EC – Electrical conductivity (µS)
EVA - Extra vehicular activities
g – Force of gravity (9.8 m s⁻²)
HI – Harvest index (%) 
HNO₃ – Nitric acid
HPS – High pressure sodium
ISS – International Space Station
°K – Degree Kelvin
kcal - Kilocalorie (4184 Joules)
kPa/Pa – Kilopascal/Pascal
KOH – Potassium hydroxide
LA – Leaf area (cm²)
m – Factor which relates to the diffusing substance
m – Metre
M – Molar
mb – Millibars (1 kPa = 10 mb)
mL – Millilitre
mmHg – Millimetres of mercury (1 kPa = 7.50 mmHg)
μS – Microsiemens
MRI - Magnetic resonance imaging
MSE – Mean square error
n – Number of moles of gas
NAD+/NAPH – Nicotinamide adenine dinucleotide
NASA - National Aeronautics and Space Administration
NCER – Net carbon exchange rate
NFT – Nutrient film technique
O₂ – Oxygen
P – Atmospheric pressure (kPa/Pa)
P – Probability value
PC – Personal computer
pCO₂ – Partial pressure of carbon dioxide (Pa)
pO₂ – Partial pressure of oxygen (kPa)
PAR – Photosynthetically active radiation (μmol quanta m⁻² s⁻¹)
ppm – Parts per million
R – Universal gas constant (8.314 Jmol⁻¹ K⁻¹)
R² – Coefficient of determination
RH – Relative humidity (%)
Rubisco – Ribulose-1,5, bisphosphate carboxylase-oxygenase
SLA – Specific leaf area (cm²)
sol – Maritan day
SS – Sum of squares
T – Absolute temperature (°K)
T_a – Temperature of the air (°K)
torr – Measure of pressure (1 kPa = 7.5 torr)
V – Volume of gas (m$^3$)
VPD – Vapour pressure deficit (kPa)
Watt – Unit of energy (1 joule second$^{-1}$)
CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

1.1.1. The Next Frontier

Humans have an innate desire for discovery and exploration. Since the beginning of time, mankind has strived to reach further and advance our understanding of the world around us. As we learn more about our planet and our curiosity deepens, space exploration becomes a logical extension of our ambition. Past space endeavours have contributed to significant scientific achievements, which have translated into a greater understanding of our own planet and higher standards of living on Earth. Space activities have far-reaching impact and help people on Earth in indirect and unexpected ways (Camhi 2002). Advancements in human health, computing, environmental protection, communications, and robotics have resulted in improved weather forecasting, highly specialized agriculture and enhanced image processing for use in computer-aided tomography (CAT) scanners and magnetic resonance imaging (MRI) diagnostic equipment (NASA 1997; Monje, Stutte et al. 2003). Space research has also been credited with developing reflective emergency blankets, an anti-icing agent made from food grade ingredients and a water recovery system capable of producing 28 gallons of clean water per day in remote locations (NASA 2006).
What does the future of space exploration look like? One of the objectives of the National Aeronautics and Space Administration’s (NASA) Mars Exploration Program is to prepare for human exploration of Mars, and suggests that we should plan for additional human exploration within the next two to three decades (Richards, Corey et al. 2006; NASA 2008). Strengthening this aspiration, the Canadian Space Agency has revealed its intention to focus its efforts beyond orbital facilities and transit vehicles to habitation on the Moon and Mars (Bamsey, Graham et al. 2009).

1.1.2. The Red Planet

The recent robotic Mars missions, such as Mars Odyssey (2001), Mars Exploration Rovers and Express Orbiter (2003) and Mars Reconnaissance Orbiter (2005) have intensified the anticipation for the next manned space endeavour. As a stepping stone for future exploration, NASA’s Vision for Space has proposed a revisit to the Moon between 2015-2020 (NASA 2004) and a delegation of international space agency representatives stressed in the Global Exploration Strategy (Unidentified 2007) the value of the Moon as a test-bed for new technologies and skill acquisition. This would allow for the development, assessment and establishment of many technologies with the safeguard of being close enough to abort and return to Earth or the International Space Station (ISS).

Mars has been indicated as the best candidate for future planetary exploration, due to its proximity to Earth and the possibility for in situ resource utilization
Silverstone, Nelson et al. 2003; Wheeler 2003). Although limited, Mars does possess an atmosphere which is composed mainly of carbon dioxide with trace amounts of nitrogen and oxygen (Table 1.1) and is at a pressure of approximately 0.5-1.0 kPa which is less than 1% of that found on Earth at sea level (Owen, Biemann et al. 1977). It is also beneficial that Mars has some surface gravity (0.38 g compared to Earth’s 1 g). With all this in mind, some researchers forecast attempts to terraform the Martian landscape to bring it closer to Earth’s ambient environment (Richards, Corey et al. 2006).

1.1.3. Life Support Considerations

As manned space exploration extends beyond low-Earth Orbit and mission durations increase, so do payload mass and life support demands. Based on current propulsion technologies the proposed Martian mission, including planetary realignment and return travel, is expected to last approximately three years (Wheeler, Stutte et al. 2001). Past and current space endeavours such as the Space Shuttle, MIR and ISS missions have normally been short-duration excursions which have relied on stowage and re-supply of life support consumables from Earth as well as physico-chemical (PC) systems to supply the human requirements for life. However, the launch mass increases linearly with greater mission length or distance from Earth and the mass and energy constraints of this extended travel require that we investigate technologies which mitigate these requirements (Turc, Pintena et al. 1999; Rygalov, Bucklin et al. 2001; Monje, Stutte et al. 2003; Richards, Corey et al. 2006). It has been
Table 1.1. A comparison of the physical parameters between Mars and Earth

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mars</th>
<th>Earth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean distance from Sun</td>
<td>$2.28 \times 10^8$ km</td>
<td>$1.49 \times 10^8$ km</td>
</tr>
<tr>
<td>Rotational rate (hours day$^{-1}$)</td>
<td>24.62 h</td>
<td>24.0 h</td>
</tr>
<tr>
<td>Year</td>
<td>668.59 sols (686.98 days)</td>
<td>365.25 days</td>
</tr>
<tr>
<td>Surface gravity</td>
<td>0.38 g</td>
<td>1.00 g</td>
</tr>
<tr>
<td>Mean surface temperature</td>
<td>-60 °C</td>
<td>+15 °C</td>
</tr>
<tr>
<td>Surface temperature range</td>
<td>-145 - 20 °C</td>
<td>-60 - 50 °C</td>
</tr>
<tr>
<td>Insolation (PAR)</td>
<td>860 $\mu$mol of quanta m$^{-2}$ s$^{-1}$</td>
<td>2000 $\mu$mol of quanta m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>Atmospheric pressure</td>
<td>0.5 - 1.0 kPa</td>
<td>101.3 kPa</td>
</tr>
<tr>
<td>$N_2$</td>
<td>18.9 Pa (2.7%)</td>
<td>$78 \times 10^3$ Pa (78%)</td>
</tr>
<tr>
<td>$O_2$</td>
<td>0.9 Pa (0.13%)</td>
<td>$21 \times 10^3$ Pa (21%)</td>
</tr>
<tr>
<td>$CO_2$</td>
<td>667 Pa (95.3%)</td>
<td>38 Pa (0.038%)</td>
</tr>
</tbody>
</table>

Adapted from (Graham 2004).
suggested that long-term human space habitation will benefit from an autonomous, closed-loop advanced life support system (ALS) in conjunction with traditional PC methods (Wheeler, Stutte et al. 2001; Monje, Stutte et al. 2003; Wheeler 2003; Paul, Schuerger et al. 2004).

Extensions of ALS, bioregenerative life support systems (BLSS) composed of higher plants, have been proposed (Salisbury 1999; Wheeler, Stutte et al. 2001). Bioregenerative life support systems act as artificial ecosystems by harnessing the photosynthetic processes of plants to absorb carbon dioxide and produce food, as edible biomass, generate oxygen and transpire pure water.

Plants have flown in space since the 1960s, when tomato seeds were launched on the Soviet’s Sputnik 4 satellite and later by NASA on STS-3 in 1982 (Porterfield, Neichitailo et al. 2003). Plants are adaptable to a broader range of environmental conditions than humans and it is likely that future space exploration, such as a Martian mission, will take advantage of this adaptability (Corey, Barta et al. 2002; Ferl, Wheeler et al. 2002; Spanarkel and Drew 2002; He, Davies et al. 2003; Richards, Corey et al. 2006; He, Davies et al. 2007). In a BLSS, the crew’s grey water is used to water the plants and the water is purified via transport through the plant. Transpired water is collected as condensate to provide potable water. Microbes or physical and chemical oxidation processes may be used to degrade inedible residues and organic waste. These systems function with minimal surplus inputs or waste as all components must serve their
function and be processed, purified and recycled back into the system. However, creating an equilibrium in biological systems is complicated, therefore it is likely that standard PC methods will be required to buffer the system and act as a backup in unproductive conditions such as periods of low light (Wheeler 2003).

Benefits to this type of system are decreased need for stowage and re-supply, improved nutrition, a perceived psychological connection with Earth, and greater crew safety and survival strategies (Salisbury and Clark 1996; Porterfield, Neichtailo et al. 2003; Silverstone, Nelson et al. 2003; He, Davies et al. 2006).

Research into BLSS began in the 1950s with the United States Air Force and the Soviet Union. It was originally hypothesized that algae might function as a subsystem of a life support system capable of regenerating oxygen and absorbing carbon dioxide (Wheeler, Stutte et al. 2001; Silverstone, Nelson et al. 2003). From this simple conception, continued research has developed highly complex systems composed of plants, algae and microbes (MacElroy, Kliss et al. 1992).

Considerable thought goes into the development of crew diets. BLSS candidate crop selection includes numerous factors such as nutrition, processing time, horticultural conditions, plant stature, quick growth with short cycling, yield and harvest index (HI). It is estimated that at least 15 crop species are required to produce a balanced diet and approximately 40 m² of continuously planted area is required per person to produce 2500 kcal of food per day (Monje, Stutte et al. 2003; Wheeler 2003). Crop lists have been revised several times but generally include dwarf wheat, rice, soybean, tomato, lettuce, pepper, beet, carrot, sweet
potato, lentil, peanut, radish and garlic. One might expect salad crops to be the first grown and consumed due to their quick growth and ease of preparation.

With sights focused on Lunar and Martian exploration, we must further consider the effects of their ambient environment on the effectiveness of a BLSS. Low pressure, cosmic radiation and the atmospheric composition demand that a BLSS be isolated from the environment. This stipulation imposes many engineering challenges on the system. To overcome these demands, low-pressure plant growth facilities have been proposed (Corey, Barta et al. 2002; Ferl, Schuerger et al. 2002). Reducing the atmospheric pressure of the plant growth facility addresses many of the engineering and system limitations associated with a Lunar or Martian plant growth facility. Decreased atmospheric pressure would decrease the mass of structural components required to construct a growth facility and possibly permit the use of transparent inflatable structures. This mass reduction would thereby decrease the transport costs and ultimately decrease the energy requirements (Simpson and Young 1998; Turc, Pintena et al. 1999; Rygalov, Bucklin et al. 2001). Low pressure environments would also decrease the mass of the start-up consumables, as less nitrogen would be required to augment the physiologically active gasses. Additionally, a low pressure growth structure would reduce the pressure differential between the system and the atmosphere thereby decreasing atmospheric leakage (Goto, Arai et al. 2002; Rygalov, Bucklin et al. 2002).
1.1.4. Physics of Reduced Pressure

On Earth, large variations in atmospheric pressure are uncommon at the surface but are produced by changes in altitude, where a decrease in pressure corresponds to an increase in altitude (Campbell and Norman 1988). The ideal gas law [Equation 1.1]:

\[ PV = nRT \] [Equation 1.1]

where \( P \) is atmospheric pressure (Pa), \( V \) is the volume of the gas (m\(^3\)), \( n \) is the number of moles of the gas, \( R \) is the universal gas constant (\(8.314 \text{ Jmol}^{-1}\text{K}^{-1}\)) and \( T \) is absolute temperature (°K), can be used to explain the relationship between pressure, volume and temperature for a perfect gas (Fritschen and Gay 1979). However, pressure variations significantly affect other environmental variables as well. For example, energy loads are typically dissipated by the re-radiation of thermal radiation or sensible and latent heat flux, but low atmospheric pressures are associated with a decreased molar density which relates to decreased convective heat exchange and therefore increased latent heat flux or evapotranspiration in the case of a leaf.

The rate of molecular diffusion for the transport of mass and heat are also sensitive to changes in temperature and atmospheric pressure [Equation 1.2]. This relationship can be expressed as:

\[ D' = D \left( \frac{101.3}{P} \right)^{\left( \frac{T_a}{293.16} \right)^m} \] [Equation 1.2]
where $D'$ is the diffusion coefficient at $P$ and $T_a$ ($\text{cm}^2\text{s}^{-1}$), $D^\circ$ is the diffusion coefficient in air at 101.3 kPa and 293.16 °K ($\text{cm}^2\text{s}^{-1}$), $P$ is atmospheric pressure (kPa), $T_a$ is air temperature (°K), and $m$ is a factor which relates to the diffusing substance (Campbell and Norman 1988). Low atmospheric pressure is correlated to increased diffusivity for heat, water vapour, oxygen and carbon dioxide and a decrease in the boundary layer. This may be advantageous to plant growth as this decrease in boundary layer resistance coupled with an increased diffusion coefficient should allow for greater gas exchange (Rygalov, Bucklin et al. 2002).

Standard experimental methods used to decrease pressure also act upon the atmosphere. Pressure control is normally established through the use of a vacuum pump which indiscriminately removes all the components of air in order to establish the desired pressure. According to Dalton’s law of partial pressures the total pressure exerted by a mixture of gases, which does not react chemically, is equal to the sum of the partial pressures of the independent gases (Nobel 1991). While this procedure does not change the relative concentrations of the individual gases it does decrease their partial pressures and therefore may affect plant responses. Due to these potential interactions, it is important that low pressure plant growth studies account for these relationships.
1.1.5. Previous Research

Reduced pressure conditions have been employed in previous space missions. The Mercury, Gemini and Apollo missions were designed to function at 34 kPa total pressure with an environment of pure oxygen during periods of human habitation. Skylab was also operated at low atmospheric pressure and was pressurized to a total pressure of 34 kPa with 70% oxygen and 30% nitrogen when manned (Martin and McCormick 1992). The Space Shuttle, MIR and ISS have all been maintained close to ambient Earth pressure, however, the Space Shuttle has been depressurized to 70 kPa for extra vehicular activities (EVA) (Winkler 1992). Despite this, there has been minimal research into the effects of reduced pressure on plant growth (He, Davies et al. 2006; He, Davies et al. 2007) and the available research has focused on the range of 25-100 kPa with the greatest concentration around 50 kPa (Richards, Corey et al. 2006). Much of the previous research has reported inconsistent results and is difficult to interpret due to variations among facilities, methods and treatments (Corey, Barta et al. 1997a; Corey, Barta et al. 2002; Spanarkel and Drew 2002; Richards, Corey et al. 2006).

Early research focused on the influence of the physical principles of low pressure on evapotranspiration and carbon exchange (Gale 1972a; Gale 1972b; Gale 1973b). Gale theorized that the increased diffusivity and decreased stomatal resistance would buffer the effects of decreased carbon dioxide concentrations while increasing evapotranspiration. Using a modified desiccation jar, Gale
confirmed his assumptions by subjecting beans and corn to pressures of 300 and 700 mmHg (40 and 93.3 kPa, respectively). Results demonstrated that the decrease in carbon exchange rates was not as dramatic as the decrease in total pressure and that transpiration rates were significantly increased under reduced pressure.

Further research carried out in controlled environment plant growth chambers reported increased carbon exchange rates as well as increased biomass production (Rule and Staby 1981b; Musgrave, Gerth et al. 1988; Massimino and Andre 1999). Rule and Staby observed increased biomass production in tomatoes at 250 torr (33.3 kPa) total pressure versus ambient (750 torr or 99 kPa). They hypothesized that these results were due to a decrease in photorespiration and increased photosynthesis. Growth of mungbean was not adversely affected by pressures of 21-24 kPa compared to ambient (Musgrave, Gerth et al. 1988). Mungbean growth was enhanced at decreased pressures which may have been a result of decreased partial pressures of oxygen (pO$_2$) and the influence of pressure on ethylene synthesis. Both photosynthesis and transpiration of wheat were enhanced at pressures of 10 and 20 kPa compared to ambient (Massimino and Andre 1999). Increased shoot and root dry weights were similar to those observed with carbon dioxide enrichment and transpiration rates were amplified by over 100%. Conversely, tomatoes exposed to pressures of 40 and 70 kPa showed varying effects (Daunicht and Brinkjans 1996a). Photosynthetic rates at 40 kPa were not significantly different from those at
ambient levels but corresponded to decreased biomass production. The 70 kPa treatment showed enhanced carbon exchange with no increase in biomass production over ambient. The variation in these investigations may be due to the range of species and the treatment differences in partial pressures of oxygen, carbon dioxide and water vapour.

Continued research made an effort to maintain stable partial pressures in order to develop a greater understanding of reduced pressure effects without the complications of hypoxia or drought stress. Corey et al. (1997a) found that a total pressure of 51 kPa enhanced carbon exchange and decreased dark respiration when associated with a decreased partial pressure of oxygen but not with ambient levels of oxygen. Similarly, Iwabuchi et al. (1996) reported no difference in carbon dioxide assimilation and transpiration in spinach exposed to total pressures of 25 versus 100 kPa with oxygen partial pressures of 21 kPa. Carbon exchange rates of lettuce were slightly greater at a pressure of 70 kPa versus ambient with 21 kPa of oxygen. However, no growth parameters demonstrated a significant effect of pressure (Spanarkel and Drew 2002). He et al. (2007) studied lettuce growth at total pressures of 25 and 100 kPa and oxygen partial pressures of 12 and 21 kPa to evaluate the effects of hypoxia and hypobaria. Results suggested that hypobaria did not adversely affect growth however hypoxia did. They also noted that hypobaric plants showed resistance to the hypoxic effects.
Due to the relatively short duration of many low pressure experiments only one growth phase is typically examined. Goto et al. (2002) studied the pressure effects on rice at different growth stages. Germination of rice was found to increase at low pressures with increased partial pressures of oxygen while the vegetative stage demonstrated normal growth at 50 kPa and decreased growth at 34 kPa. There were no differences in the fresh and dry weights during the reproductive stage but there was a decrease in seed set. Additionally, Iwabuchi and Kurata (2003) found that the duration of experiments had a significant influence on the outcome. They reported increased carbon dioxide assimilation and transpiration rates in short-term experiments and no effect in the long-term studies. They concluded that physiological or anatomical changes were modifying the response. Little is known about the mechanisms of adaptive responses associated with low pressure tolerance. *Arabidopsis* plants were subjected to pressures of 10 and 101 kPa along with oxygen partial pressures of 2 or 20 kPa and gene expression was measured (Corey, Barta et al. 2002; Iwabuchi and Kurata 2003; Paul, Schuerger et al. 2004). Over 200 genes were regulated in response to low pressure and while a portion of these genes were similar to those expressed during hypoxic or drought conditions, many were also unique to hypobaria. Further research with *Arabidopsis* studied a greater range of pressures (10-100 kPa) and assessed whether the reported short-term gains would hold up in the long-term (Richards, Corey et al. 2006). Results showed less variation in carbon exchange after 16 hours at reduced pressure indicating a
possible adaptive response. Focusing on the possibility of altered metabolism, radish was grown from seedlings under reduced pressures of 33 and 66 kPa and compared to ambient to assess if pressure had any effect on plant quality. Levine et al. (2008) found that there was little effect of pressure on the sensory perception of radish quality or on the quality of nutrients, antioxidants or glucosinalates.

Previous low pressure results have been confounding due to various technical factors such as environment control, species variations and because most experiments were carried out over short durations with plants that were established at ambient pressure conditions (Corey, Barta et al. 2002; Iwabuchi and Kurata 2003; Paul, Schuerger et al. 2004). These previous investigations have demonstrated that plants have the ability to withstand reduced pressure conditions, however, a long-term comprehensive study has yet to be completed (He, Davies et al. 2007; Levine, Bisbee et al. 2008). Due to these limitations, the optimum pressurization level and specific gas composition are still unknown.

1.1.6. Canadian Contributions to BLSS Research

Canada is recognized for its contributions to space exploration through expertise in optics, automation, robotics and mining, however, due to a strong background of greenhouse horticultural management research it is also a leader in the field of BLSS. The extreme fluctuations in Canadian weather have contributed to the development of a prosperous and expanding greenhouse industry that relies
upon new technologies to increase production efficiency and mitigate environmental impacts. Through technology transfer, research into BLSS facilitates the advancement of the Canadian greenhouse industry while contributing to the future of life support for space and providing opportunities for the training of high qualified personnel and youth outreach in science and technology. The Canadian Space Agency as well as partner educational and industrial facilities such as the Controlled Environment Systems Research Facility (CESRF) at the University of Guelph, are focused on implementing these technologies on the Lunar and Martian surfaces. Combining Canadian strengths, terrestrially and in space, will help to provide a sustainable future for the greenhouse producers while contributing to long-duration, human habitation beyond low-Earth orbit.

1.2 Research Objectives and Thesis Organization

To establish necessary levels of environment control for reduced atmospheric pressure plant growth facilities on the Moon or Mars, it is important to determine plant responses to modified environments. It was imperative that experimental design considered pressure effects independently from those of temperature as well as reduced partial pressures of the atmospheric constituents. In order to do this, partial pressures of carbon dioxide and oxygen and a stable vapour pressure deficit (VPD) must be maintained. Due to oxygen’s biological significance and possibly large contribution to the total pressure of a plant growth facility, oxygen effects were examined to further differentiate the effects of
hypoxia from hypobaria. The objectives of this research were to (1) assess plant responses to reduced pressure conditions and (2) to determine threshold oxygen partial pressures required for sustainable and life-supporting plant production.

The first study examined in Chapter 2, was initiated to test the hypothesis that reduced atmospheric pressure and oxygen partial pressure had no effect on seed germination and seedling growth. Three crop species including: lettuce (Lactuca sativa L. cv. Royal Green M.i. Pvp), tomato (Lycopersicon esculentum Mill. cv. Sub Arctic Maxi) and radish (Raphanus sativus L. cv. Galahad) were germinated and grown for 7 days to assess any changes in germination and seedling viability.

In Chapter 3, the effects of reduced atmospheric pressure and oxygen partial pressure on the productivity of radish are investigated. Radish (Raphanus sativus L. cv Cherry Bomb II) was established from seed and grown for 21 days. Net carbon exchange rate, transpiration and harvest parameters were analyzed.

Following the characterization of pressure and oxygen effects on the growth of radish, Chapter 4 outlines the effects of reduced oxygen partial pressures and total pressures on quantum efficiency and carbon dioxide compensation points. Short-term light response and carbon dioxide drawdown curves were evaluated to determine if the outcomes in Chapter 3 were related to a change in efficiency.

Chapter 5 examines the effects of hypobaria and decreased oxygen partial pressures on the stomatal frequency. As previously noted in the literature, there
may be an adaptation response of plants grown in hypobaric and hypoxic conditions. In order to quantify a change, leaf impressions were taken during harvest and stomatal counts were performed. Stomatal functioning is central to the productivity of the BLSS.

Concluding remarks and suggestions for further research are contained in Chapter 6.
CHAPTER 2
SEED GERMINATION AND SEEDLING GROWTH AT REDUCED ATMOSPHERIC PRESSURES AND OXYGEN PARTIAL PRESSURES

2.1 Related Research

Among the long list of research priorities under hypobaric and variable atmosphere composition conditions is an assessment of seed germination responses. Previous low pressure studies of plant growth and development have documented mixed results however many suggest that plants can be sustained under these conditions (Goto, Iwabuchi et al. 1991; Corey, Barta et al. 1997b; Corey, Barta et al. 2002; Spanarkel and Drew 2002; Richards, Corey et al. 2006). Due to differences in methods used to decrease total pressure, oxygen has been commonly limited in hypobaric environments.

Many plant species can tolerate some level of hypoxia as it can occur in well oxygenated atmospheres such as deep in root tissue or during periods of high rates of cellular metabolism (Geigenberger 2003; Bailey-Serres and Chang 2005). Oxygen partial pressures are known to affect plant growth and development and the plant’s responses are dependant on the cell or tissue types, developmental stage, genotype, severity and duration of hypoxia, light and temperature conditions (Fukao and Bailey-Serres 2004). Low oxygen levels reduce respiration by limiting adenosine triphosphate (ATP) production by oxidative phosphorylation (Geigenberger 2003; Fukao and Bailey-Serres 2004;
Bailey-Serres and Chang 2005). Many plant production studies have shown the importance of atmospheric oxygen in seed development (Quebedeaux and Hardy 1973; Quebedeaux and Hardy 1975; Quebedeaux and Hardy 1976; Musgrave and Strain 1988; Kuang, Crispi et al. 1998) and oxygen is required during seed germination and early seedling establishment (Bewley and Black 1994). Oxygen effects are known to be species specific with most species requiring some oxygen for germination. Results at ambient atmospheric pressure generally show decreased germination associated with reduced oxygen partial pressures (Siegel, Rosen et al. 1963). Generally, monocots require at least 2 kPa of oxygen while dicots demand 5 kPa or more (Al-Ani, Bruzau et al. 1985), however some species benefit from increased oxygen partial pressures. Germination percentages for Giant foxtail were reduced at oxygen levels below 20% (20 kPa pO\textsubscript{2}) and enhanced at greater values until a plateau at 75% (75 kPa pO\textsubscript{2}) (Dekker and Hargrove 2002).

The objective of this study was to test the hypothesis that reduced atmospheric pressure and oxygen partial pressure had no effect on seed germination percentage and seedling growth of lettuce, tomato and radish plants. These results will help establish the optimal oxygen partial pressure required to permit conventional seedling germination at reduced pressures. This level is significant as the oxygen partial pressure required for germination, seed production and seed viability will be a major factor in the determination of the minimum total pressure required for plant production in hypobaric conditions.
2.2 Methodology

2.2.1. Environmental Conditions

The venue for this research was the CESRF, which maintains an expansive array of plant growth facilities and chambers dedicated to the study of biological systems in life support roles for long-term space exploration. Experiments were performed in the large hypobaric plant growth chambers, which have proved to be robust and generate exceptionally reproducible data. To review system design and performance, see the Appendix.

Each of the five chambers has a growing area of 1.5 m\(^2\), and is constructed of non-offgassing materials. The environmental conditions were maintained at a temperature of 25 \(^\circ\)C and a photoperiod of 16 hours of light and 8 hours of dark. The growing area was supplied with a photosynthetically active radiation (PAR) of 800 \(\mu\text{mol m}^{-2} \text{s}^{-1}\) at canopy height provided by six-1000 Watt high-pressure sodium vapour lamps (P.L. Light Systems, Grimsby, Ontario, Canada). This level was achieved using neutral density screens to dim the lighting to the desired light level. The vapour pressure deficit (VPD) was maintained at 1.0 kPa (equivalent to 68 % relative humidity (RH) at ambient pressure and 25 \(^\circ\)C) and the partial pressure of carbon dioxide (pCO\(_2\)) was maintained at 100 Pa (equivalent to a concentration of 1000 \(\mu\text{mol mol}^{-1}\) at ambient pressure) through the addition of pure carbon dioxide from external tanks.

The treatments included four total pressures, and under each total pressure there
were five oxygen levels. Total pressure treatments were 25, 50, and 75 kPa, and an ambient control (approximately 98 kPa in Guelph, Ontario, Canada which is 334 metres above sea level) and oxygen treatments were maintained at 2, 5, 10, 15 and 20 kPa using pure nitrogen to dilute the ambient amounts. Environmental conditions were maintained and logged at one-minute intervals by the control system (Argus Control Systems, White Rock, British Columbia, Canada).

2.2.2. Plant Material

Seeds of lettuce (*Lactuca sativa* L. cv. Royal Green M.i. Pvp), tomato (*Lycopersicon esculentum* Mill. cv. Sub Arctic Maxi) and radish (*Raphanus sativus* L. cv. Galahad) (Stokes Seeds, Thorold, Ontario, Canada) were sown into rockwool plugs (Grodan, Hedehusene, Denmark). The rockwool plugs were divided into mats of 40 plugs and rinsed with deionized water prior to seeding. Each mat was seeded with one species and one seed was placed into the depression of each plug. Seeds were grown hydroponically in 1.40 x 0.15 m stainless steel troughs using a recirculating nutrient film technique (NFT) delivery system. A commercial grade growth solution of 7-11-27 (N:P:K) with micronutrients and added calcium nitrate (Plant Products, Brampton, Ontario) was used. The solution pH was manually maintained at 5.8 with additions of dilute acid (0.5 M HNO₃) and base (0.5 M KOH). Solution electrical conductivity was maintained at 500 μS. Five mats of each species were randomly distributed among the five chamber rows with three mats per row. The doors of the growth chambers were then sealed and the experimental treatments imposed.
2.2.3. Assessment of Seed Germination and Seedling Growth

Seed germination and seedling growth were assessed at seven days after planting (DAP). Germination percent was calculated per rockwool mat separately for each species and germination was defined as the emergence of a radicle. Seedling growth was measured as height in centimetres (cm) from the top of the seedling to the hypocotyl and all germinated seedlings were measured.

2.2.4. Statistical Analysis

Results were analyzed by Analysis of Variance (ANOVA) followed by a multiple comparison test when the ANOVA indicated that there were significant treatment effects. A standard fixed effects ANOVA was used to test the null hypothesis that pressure and oxygen did not have an effect on the germination rate of each species. Germination rate was set as the dependant variable with pressure and oxygen partial pressure set as the independent variables.

A second fixed effects ANOVA, with seedling growth as the dependant variable, and pressure and oxygen partial pressure set as the independent variables, was used to assess their effect on seedling growth for each species.

All values were expressed as a mean ± the standard error of the mean. A statistical significance level ($\alpha$) of 0.05 was applied to all comparisons and the analysis was performed in S-PLUS version 7.0/8.0 for Windows (Insightful Corporation, Seattle, Washington, USA).
2.3 Results

2.3.1. Seed Germination

Percent seed germination demonstrated a species variation in response to both reduced pressure and oxygen partial pressure. Percent germination of lettuce was significantly influenced by oxygen (Table 2.1). Germination of 80 % or greater was maintained at oxygen partial pressures ≥10 kPa and above 50 % at 5 kPa of oxygen (Figure 2.1). At a partial pressure of 2 kPa, there was a sharp decline in germination percent to less than 40%, which would not be acceptable for the sustained functioning of a BLSS. Lettuce germination showed a slight but significant increase due to a total pressure of 50 and 75 kPa, however this promotion was not continued at the lower partial pressures of oxygen (i.e. 2 kPa and 5 kPa pO\textsubscript{2}) of the 50 kPa treatment.

Tomato germination percent showed a significant response to pressure (Table 2.2) and oxygen partial pressure, which became more apparent with decreasing oxygen. Percent germination of tomato at reduced pressures was significantly below that of ambient pressure (Figure 2.2). All reduced pressure treatments dropped to below 80% at an oxygen partial pressure of 10 kPa and less than 50% at 5 kPa of oxygen.
Table 2.1. Results of the fixed effects analysis of variance for the effect of total atmospheric pressure (25, 50, 75 and 98 kPa) and oxygen partial pressure (2, 5, 10, 15 and 20 kPa pO₂) on the germination rate of lettuce (*Lactuca sativa* L. cv. Royal Green M.i. Pvp).

<table>
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<tr>
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Figure 2.1. Germination (%) of lettuce (*Lactuca sativa* L. cv. Royal Green M.I. pvp.) seeds grown at various atmospheric pressures (25, 50, 75 and 98 kPa) and oxygen partial pressures (2, 5, 10, 15 and 20 kPa pO₂). Germination was assessed at seven days after planting and germination was defined as the emergence of the radicle. Error bars represent the standard error of the means (n=4).
Table 2.2. Results of the fixed effects analysis of variance for the effect of total atmospheric pressure (25, 50, 75 and 98 kPa) and oxygen partial pressure (2, 5, 10, 15 and 20 kPa pO\textsubscript{2}) on the germination rate of tomato (*Lycopersicon esculentum* Mill. cv. Sub Artic Maxi).

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<td>30413.9</td>
<td>173.79</td>
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Figure 2.2. Germination (%) of tomato (*Lycopersicon esculentum* Mill. cv. Sub Artic Maxi) seeds grown at various atmospheric pressures (25, 50, 75 and 98 kPa) and oxygen partial pressures (2, 5, 10, 15 and 20 kPa pO₂). Germination was assessed at seven days after planting and germination was defined as the emergence of the radicle. Error bars represent the standard error of the means (n=4).
Radish germination was also significantly affected by reduced pressure (Table 2.3) and oxygen. Radish seed germination percentages were >80 % until the oxygen dropped to 10 kPa (Figure 2.3). At 5 kPa of oxygen, germination percentage was reduced further to <50 %. When the partial pressure of oxygen was ≤5 kPa, the germination percentages were significantly lower under total pressure of 25 and 50 kPa than those under pressures of 75 and 98 kPa.

2.3.2. Seedling Growth

Seedling growth showed greater interaction between pressure and oxygen partial pressure effects than those observed in seed germination. Lettuce seedling growth was reduced by decreased total pressures (Table 2.4) and reduced oxygen partial pressures. Total pressures of 50-75 kPa enhanced lettuce seedling growth when the oxygen partial pressures were 15-20 kPa (Figure 2.4). Lettuce growth at a total pressure of 25 kPa was stunted across all partial pressures of oxygen. Overall, lettuce seedling growth was suppressed by reduced oxygen with minimal growth occurring at 2 kPa of oxygen.

Tomato seedling growth was significantly reduced at decreased total pressures and demonstrated the greatest suppression of growth at reduced oxygen (Table 2.5). At 10 kPa oxygen, tomato growth was significantly reduced and was further suppressed at an oxygen partial pressure of 5 kPa (Figure 2.5). Growth of tomato seedlings at 75-100 kPa total pressure was the greatest at 20 kPa oxygen and growth decreased significantly with reducing oxygen.
Table 2.3. Results of the fixed effects analysis of variance for the effect of total atmospheric pressure (25, 50, 75 and 98 kPa) and oxygen partial pressure (2, 5, 10, 15 and 20 kPa pO₂) on the germination rate of radish (*Raphanus sativus* L. cv. Galahad).

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<tr>
<th>Parameter</th>
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<td>153.16</td>
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Figure 2.3. Germination (%) of radish (Raphanus sativus L. cv. Galahad) seeds grown at various atmospheric pressures (25, 50, 75 and 98 kPa) and oxygen partial pressures (2, 5, 10, 15 and 20 kPa pO$_2$). Germination was assessed at seven days after planting and germination was defined as the emergence of the radicle. Error bars represent the standard error of the means (n=4).
Table 2.4. Results of the fixed effects analysis of variance for the effect of total atmospheric pressure (25, 50, 75 and 98 kPa) and oxygen partial pressure (2, 5, 10, 15 and 20 kPa pO$_2$) on seedling growth of lettuce (*Lactuca sativa* L. cv. Royal Green M.i. Pvp).

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<th>MSE</th>
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<tr>
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<td>2730</td>
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<td>0.23</td>
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Figure 2.4 Height (cm) of lettuce (*Lactuca sativa* L. cv. Royal Green M.I. pvp.) seedlings grown at various atmospheric pressures (25, 50, 75 and 98 kPa) and oxygen partial pressures (2, 5, 10, 15 and 20 kPa pO$_2$). Growth was assessed at seven days after planting and was measured from top of the seedling to the hypocotyl. Error bars represent the standard error of the means (n=4).
Table 2.5. Results of the fixed effects analysis of variance for the effect of total atmospheric pressure (25, 50, 75 and 98 kPa) and oxygen partial pressure (2, 5, 10, 15 and 20 kPa pO$_2$) seedling growth of tomato (*Lycopersicon esculentum* Mill. cv. Sub Artic Maxi).

<table>
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<td>95.46</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pressure:pO$_2$</td>
<td>10</td>
<td>588.34</td>
<td>58.83</td>
<td>26.89</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Residuals</td>
<td>1882</td>
<td>4117.41</td>
<td>2.19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.5. Height (cm) of tomato (*Lycopersicon esculentum* Mill. cv. Sub Artic Maxi) seedlings grown at various atmospheric pressures (25, 50, 75 and 98 kPa) and oxygen partial pressures (2, 5, 10, 15 and 20 kPa pO₂). Growth was assessed at seven days after planting and was measured from top of the seedling to the hypocotyl. Error bars represent the standard error of the means (n=4).
Radish seedling growth also demonstrated a significant response to reduced total pressure (Table 2.6) and oxygen partial pressure. At 10-20 kPa of oxygen and 25-50 kPa total pressure, radish seedling growth was significantly depressed (Figure 2.6). All radish seedling growth was significantly inhibited at a partial pressure of 5 kPa oxygen or lower.

2.1 Discussion

2.1.1. Seed Germination

The effects of oxygen partial pressure on seed germination were species dependent as demonstrated by previous studies (Al-Ani, Bruzau et al. 1985; Kelly, Van Staden et al. 1992; Goto, Arai et al. 2002). These differences may depend on the seed coat, size of the embryo or the composition and size of the endosperm (Mayer and Poljakoff-Mayber 1989). Oxygen is required immediately after germination however cutinized cell layers of the seed coat may impede gaseous diffusion limiting oxygen entry and carbon dioxide exit (Kelly, Van Staden et al. 1992; Geigenberger 2003). A lack of oxygen will constrain the oxidation of inhibitors and impede respiration (Bewley and Black 1994). Scratching or puncturing the seed coat can increase germination and in the case of flax seeds nicking the seed coats resulted in increased germination and root growth (Kuznetsov and Hasenstein 2003).

The reduced germination percentage of lettuce observed at decreased oxygen was possibly due to the composition of the seed coat and available reserves.
Table 2.6. Results of the fixed effects analysis of variance for the effect of total atmospheric pressure (25, 50, 75 and 98 kPa) and oxygen partial pressure (2, 5, 10, 15 and 20 kPa pO$_2$) on seedling growth of radish (*Raphanus sativus* L. cv. Galahad).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Df</th>
<th>SS</th>
<th>MSE</th>
<th>F Value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure</td>
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<td>201.48</td>
<td>67.16</td>
<td>36.48</td>
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</tr>
<tr>
<td>Oxygen</td>
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<td>1039.52</td>
<td>259.88</td>
<td>141.17</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pressure:pO$_2$</td>
<td>12</td>
<td>953.69</td>
<td>79.47</td>
<td>43.17</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Residuals</td>
<td>2078</td>
<td>3825.30</td>
<td>1.84</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.6. Height (cm) of radish (*Raphanus sativus* L. cv. Galahad) seedlings grown at various atmospheric pressures (25, 50, 75 and 98 kPa) and oxygen partial pressures (2, 5, 10, 15 and 20 kPa pO$_2$). Growth was assessed at seven days after planting and was measured from top of the seedling to the hypocotyl. Error bars represent the standard error of the means (n=4).
Lettuce seeds store their reserves in their cotyledons and contain an endosperm which is only a few cell layers thick (Mayer and Poljakoff-Mayber 1989). Lettuce seeds are known to maintain a quiescent state following imbibition if oxygen is limiting (Fukao and Bailey-Serres 2004; Bailey-Serres and Chang 2005). Similar to this study, Spanarkel and Drew (2002) observed that lettuce germinated at an oxygen partial pressure of 5 kPa however Al-Ani et al. (1985) found that lettuce may require up to 15% oxygen (15 kPa pO$_2$) during the second phase of germination due to the natural barriers limiting oxygen diffusion. To increase lettuce germination one could employ a pinprick through the endosperm near the radicle (Bewley and Black 1994).

Unlike lettuce, tomato seeds are endospermic and Dahal et al. (1996) found that lettuce seed respiration was decreased to 1/3 of normal at 10 kPa of oxygen. Radish seeds are generally endospermic, fatty seeds that store reserves within the cotyledons. Contrary to the results reported here, Al-Ani et al. (1985) reported normal radish germination at 7 kPa of oxygen and no germination at an oxygen partial pressure of 3 kPa. They also noted a varietal difference in both tomato and radish which should be considered in future situations.

2.1.2. Seedling Growth

Seedling growth was greatly affected by reduced pressure and oxygen; however the response characteristics were not the same as those seen in seed germination. This variation in seed germination and seedling growth was previously observed by Siegel et al. (1963) The seedling growth suppression
may be due to diminished storage reserves and a greater respiration demand for oxygen. Seeds differ in cotyledon reserves, expansion rates and initiation of photosynthesis (Mayer and Poljakoff-Mayber 1989). Mobilization is normally a post-germination event however some embryo reserves are available to be used in germination. Under low oxygen conditions, these reserves may be used extensively for successful germination therefore diminishing the available resources for subsequent seedling growth. The reduced oxygen decreases biosynthetic activity to save ATP, limits enzyme synthesis and oxidation of fatty acids (Bewley and Black 1994). Root growth is also inhibited and available roots have limited viability (Saglio, Drew et al. 1988; Andrews, Cobb et al. 1993; Vantapetian and Jackson 1997). Past research has shown that lettuce seedling growth was depressed at an oxygen partial pressure of 15 kPa and that decreased oxygen resulted in reduced growth in tomato due to the lack of endosperm resources available after germination (Mayer and Poljakoff-Mayber 1989).

Schwartzkopf and Mancinelli (1991) found that reduced pressure had no effect on the germination of wheat however it decreased seedling top growth by 75% and Rule and Staby (1981a) noted that tomato growth was reduced at 16 kPa total pressure. In this experiment, reduced pressure effects were secondary to those of oxygen however a few observations should be highlighted. The small increase in germination percentage and seedling growth at 50-75 kPa total pressure may be attributed to an increase in the rate of diffusion of air and water
at these pressure levels. Goto et al. (2002) found that rice germination at reduced total pressures demonstrated greater germination percentages than those at similar partial pressures of oxygen. Alternatively, they noted that *Arabidopsis* germination was not enhanced by reduced total pressure. It is important to mention that the beneficial effects observed at 50-75 kPa of total pressure were not maintained across all levels of oxygen and particularly at 2-5 kPa of oxygen, reduced total pressure had compounded negative effects.

### 2.2 Conclusions

Based on these results, species and varietal selection must be considered when optimizing the gas composition for BLSS that are established from seed. The results suggest that an oxygen partial pressure of 10 kPa or greater would support seedling germination and growth that is adequate for the functioning of a BLSS. These results support the goals of our future research into plant growth under hypobaria and reduced oxygen partial pressures while defining some issues for further consideration. In subsequent studies, optimum environments and pre-germination seed treatments must be established for any priority crops that exhibit negative responses to reduced oxygen levels. If, due to physical and engineering constraints, reduced pressure environments drive oxygen levels below that suitable for seed germination and seedling establishment, it may be necessary to sow and germinate seeds within the human habitat and transfer them to the plant growth facility once established. However, seedling germination should not be the only consideration when establishing the optimal
atmosphere composition and total pressure. Due to the requirement of oxygen throughout the life of the plant and seed development, hypoxic effects must be considered if BLSS are to be sustainable for long periods.
CHAPTER 3
REDUCED ATMOSPHERIC PRESSURE AND OXYGEN PARTIAL PRESSURE IN RADISH: EFFECT ON PRODUCTIVITY, NET CARBON EXCHANGE, AND TRANSPIRATION

3.1 Related Research

As Martian exploration crests the horizon, it is imperative that the development of long-term life support strategies, including bioregenerative systems move forward. The Martian environment has large space radiation loads and low atmospheric pressures. Human habitats and plant growth facilities need to be buffered with significant quantities of atmospheric constituents including oxygen and nitrogen and require isolation from the harsh ambient conditions. Current proposals suggest that a low pressure, less than Earth’s ambient, plant growth facility is optimal and the benefits of reduced pressure have been thoroughly discussed (Corey, Barta et al. 2002; Ferl, Wheeler et al. 2002; Goto, Arai et al. 2002). However, the effects of reduced pressure and oxygen partial pressure on plant growth and development have not been fully characterized.

3.1.1. Effects of Low Pressure

Due to an increased rate of diffusion at low atmospheric pressures, it has been hypothesized that there will be increased gas exchange between the plants and their environment thereby increasing photosynthesis by elevating the availability of carbon dioxide in the mesophyll cells (Gale 1973a; Rygalov, Bucklin et al. 2002). Past experiments have examined a variety of species including wheat
(Massimino and André 1999), tomato (Rule and Staby 1981a; Daunicht and Brinkjans 1996b), lettuce (Corey, Bates et al. 1996; Spanarkel and Drew 2002; He, Davies et al. 2003; He, Davies et al. 2006; He, Davies et al. 2007), spinach (Iwabuchi, Goto et al. 1995), rice (Goto, Arai et al. 2002) and radish (Levine, Bisbee et al. 2008). The results of these studies are difficult to compare and pinpoint the effects of hypobaria or hypoxia due to a limited or sporadic range of conditions (Richards, Corey et al. 2006) and variations in plant exposure times (Iwabuchi and Kurata 2003). However, from these results it is apparent that plants can withstand some degree of atmospheric alteration, and it is likely that adaptation occurs (Paul, Schuerger et al. 2004; Richards, Corey et al. 2006).

In an attempt to define the effects of reduced pressure and oxygen partial pressure on the growth and development of radish the following study was initiated. In this experiment, all other environmental parameters were maintained at stable setpoints and the plants were grown from young seedlings in order to ascertain the effects of reduced total pressure and oxygen partial pressure. Radish was chosen as the test crop due to its rapid growth, high harvest index (HI), nutritional characteristics and it's likelihood as a candidate crop for space-based life support systems (Salisbury and Clark 1996). The treatments covered a range of pressure from 10-98 kPa and oxygen partial pressures from 2-20 kPa with the combinations being limited by the partial pressure of oxygen. Radishes were grown for a standard growth period of 21 days before harvest and analysis.
3.2 Methodology

3.2.1. Environmental Conditions

Experiments were carried out in the large hypobaric chambers at the CESRF. Temperature was held at 22 °C with a photoperiod of 16 hours light and 8 hours of dark. A light intensity of 300 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) PAR at the hydroponic trough level was provided by six-1000 Watt HPS lamps dimmed with neutral density screening. Carbon dioxide and VPD were maintained at partial pressures of 0.12 kPa (equivalent to 1200 \( \mu \text{mol mol}^{-1} \) at ambient) and 0.9 kPa (equivalent to 65 % RH at 22 °C), respectively. Treatments included four total pressures (10, 33, 66 and 98 kPa) and four oxygen partial pressures (2, 7, 14, 20 kPa) with 98/20 kPa combination as the ambient control. Setpoints for carbon dioxide, oxygen and water vapour varied for each pressure treatment because the gas analyzers function at ambient pressure (Table 3.1). To accommodate this, the chamber air samples were pressurized to 100 kPa prior to reaching the gas analyzer (for further description see the Appendix). To minimize errors, gas analyzers were calibrated at the pressure setpoint following each closure. The setpoints were maintained through additions of pure gases from external tanks (BOC Canada Limited, Mississauga, Ontario, Canada).

3.2.2. Plant Material

Radish (\textit{Raphanus sativa} L. cv. Cherry Bomb II) was grown from seed in 1.4 x 0.15 m stainless steel troughs with stainless steel trough covers to minimize
<table>
<thead>
<tr>
<th>Total Pressure (kPa)</th>
<th>Oxygen Partial Pressure (kPa)</th>
<th>Carbon Dioxide (μmol mol⁻¹)</th>
<th>Oxygen (%)</th>
<th>VPD (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>98</td>
<td>20</td>
<td>1200</td>
<td>20.0</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>1200</td>
<td>14.0</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1200</td>
<td>7.0</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1200</td>
<td>2.0</td>
<td>0.9</td>
</tr>
<tr>
<td>66</td>
<td>20</td>
<td>1800</td>
<td>30.3</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>1800</td>
<td>21.2</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1800</td>
<td>10.6</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1800</td>
<td>3.0</td>
<td>0.9</td>
</tr>
<tr>
<td>33</td>
<td>20</td>
<td>3636</td>
<td>60.6</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>3636</td>
<td>42.4</td>
<td>0.9</td>
</tr>
<tr>
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<td>21.2</td>
<td>0.9</td>
</tr>
<tr>
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<td>2</td>
<td>3636</td>
<td>6.1</td>
<td>0.9</td>
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<tr>
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<td>12000</td>
<td>70.0</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12000</td>
<td>20.0</td>
<td>0.9</td>
</tr>
</tbody>
</table>
evaporation and algal growth on the growth medium. Each cover had 4 cm diameter holes placed at 9-10 cm centres within the rows and between troughs. There were five troughs per chamber and 24 plants per trough for a total of 120 plants per chamber. Rockwool slabs (Grodan, Hedehusene, Denmark) were used as the growth medium and a channel was cut from the underside of the slab to facilitate nutrient solution flow. Prior to planting, the rockwool was rinsed twice with deionized water to remove any fabrication residues or particulates. Nutrient solution was provided using a recirculating nutrient film technique (NFT) with a modified, half-strength Hoagland’s solution (Wheeler, Sager et al. 1999). The solution was maintained at an electrical conductivity (EC) of 1200 μS cm⁻¹ using concentrated stock solutions and pH was manually adjusted daily to 5.8 with 0.5 M nitric acid (HNO₃) or 0.5 M potassium hydroxide (KOH). Prior to the experiment, the nutrient reservoirs were ozonated with >10 ppm aqueous ozone and the nutrient stock solutions were autoclaved, to maintain sterility for a concurrent microbial study not reported here.

Three radish seeds were planted per position and allowed to germinate under ambient pressure (98 kPa) for 72 hours. Seedlings were then thinned to one per position and the appropriate pressure treatment imposed.

3.2.3. Assessment of Growth and Productivity

Plant growth and productivity were measured in terms of fresh and dry mass of roots (swollen hypocotyls) and leaves as well as leaf area (LA). Values were obtained using an electronic balance (Sartorius, Gottingen, Germany) and a leaf
area meter (LI-3000, LI-COR, Nebraska, USA). Data were collected on a per plant basis for the entire chamber and harvest index (HI) and specific leaf area (SLA) calculated from the resultant data.

3.2.4. Carbon Assimilation and Dark Respiration

Whole stand net carbon exchange rate (NCER) measurements were calculated from the slope of the carbon dioxide injected into the chamber each day, while dark respiration rates were calculated from the slope of the carbon dioxide evolved over the night period. As it was impossible to access the plants during the experiment, the photosynthesis, respiration and transpiration data could not be reported on a dry weight or leaf area basis and was therefore normalized for growing area. This however does not account for the decreased light interception correlated with reduced canopy cover. The growth period examined was from 3-17 days after planting (DAP) following which subsequent tests of light and carbon compensation points were run and the data reported in the following chapter.

3.2.5. Transpiration

Transpiration rates were calculated from the collected condensate. A calibrated tipping bucket rain gauge was used to collect condensate and then the values were converted to litres of evapotranspiration per day and corrected for evaporation using the evapotranspiration rate from the first day of closure when transpiration would be negligible. Data were also normalized for growing area.
3.2.6. **Statistical Analysis**

Experimental design was a randomized block design with each chamber being considered a replicate and each treatment combination was replicated four times. Replication was achieved by repeating the experiments over time and replicates were cycled through the chambers to minimize chamber effects.

Harvest results were analyzed using a PROC Mixed analysis of variance (ANOVA) to test the null hypothesis that total pressure and oxygen partial pressure did not have an effect on the individual growth parameters (fresh and dry mass, specific leaf area (SLA) and harvest index (HI)). Growth parameters were set as the dependant variable with pressure and oxygen set as the independent variables. Chamber number and its associated interactions were set as random factor effects. Where significance was present, the data was sorted by pressure and the levels of oxygen compared.

Polynomial linear regression analyses were conducted to fit a curve to the carbon exchange and transpiration rates data. The relationships were modeled by the equation: \( y = a + b_1x + b_2x^2 + b_3x^3 \) to test the hypothesis that the data did not fit the regression model.

The regression analysis was performed in S-PLUS version 7.0/8.0 for Windows (Insightful Corporation, Seattle, Washington, USA) and the ANOVA analysis was performed using SAS version 9.1.3 for Windows (SAS Institute Inc., Cary, North Carolina, USA).
3.3 Results

3.3.1. Growth

Visually, there was little difference observed in the quality of the radishes harvested from the control and the reduced pressure treatments (Figure 3.1) however as the partial pressure of oxygen declined there was a visual decrease in radish size with an unacceptable result at 2 kPa of oxygen (Figure 3.2). There were significant decreases in leaf dry mass, radish dry mass, SLA and HI over the range of pressure at an oxygen partial pressure of 2 kPa (Table 3.2).

3.3.2. Carbon Exchange

There was little change observed in whole canopy net carbon exchange rates over the range of pressures from 33-98 kPa and oxygen partial pressures from 7-20 kPa (Figure 3.3). At 10 kPa total pressure and 7 kPa of oxygen there was a slight but significant decrease in assimilation rate and at 2 kPa of oxygen there was a drastic decline leading to a significant suppression of growth. Similarly, dark respiration also showed no significant change over most of the range of pressures and oxygen levels to 7 kPa, however, was slightly decreased at both oxygen levels at 10 kPa of total pressure.

3.3.3. Transpiration

Transpiration rates showed little effect as a result of the change of pressures from 33-98 kPa and oxygen partial pressures of 7-20 kPa (Figure 3.4). At 98 kPa
Figure 3.1. Visual analysis of radish (*Raphanus sativa* L. cv. Cherry Bomb II) grown at various pressures (from top to bottom: 98, 66, 33 kPa) and ambient oxygen partial pressure (20 kPa pO$_2$). Pictures were taken during harvest which was 21 days after planting.
Figure 3.2. Radish (*R. sativa* L. cv. Cherry Bomb II) grown at various pressures (from top to bottom: 98, 66, 33 and 10 kPa) and oxygen partial pressures (from left to right: 7 and 2 kPa). Pictures were taken 21 days after planting.
Table 3.2. The effect of atmospheric pressure (10-98 kPa) and oxygen partial pressure (2-20 kPa) on the growth radish (*Raphanus sativa* L. cv. Cherry Bomb II) grown over a 21-day period. The partial pressure of carbon dioxide was maintained at 120 Pa.

<table>
<thead>
<tr>
<th>Pressure (kPa)</th>
<th>pO₂ (kPa)</th>
<th>Leaf dry mass (g plant⁻¹)</th>
<th>Root dry mass (g plant⁻¹)</th>
<th>Specific Leaf area (cm² g⁻¹)</th>
<th>Harvest Index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>98</td>
<td>20</td>
<td>0.60 (0.008)a</td>
<td>1.19 (0.019)a</td>
<td>286.9 (1.64)a</td>
<td>65.80 (0.253)a</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.59 (0.007)a</td>
<td>1.16 (0.017)a</td>
<td>284.2 (2.03)a</td>
<td>65.80 (0.227)a</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.57 (0.010)a</td>
<td>1.17 (0.015)a</td>
<td>288.3 (2.93)a</td>
<td>67.24 (0.301)a</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.38 (0.006)b</td>
<td>0.14 (0.004)b</td>
<td>118.2 (1.47)b</td>
<td>26.20 (0.399)b</td>
</tr>
<tr>
<td>66</td>
<td>20</td>
<td>0.62 (0.007)a</td>
<td>1.17 (0.018)a</td>
<td>255.5 (5.05)a</td>
<td>64.64 (0.296)a</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.57 (0.008)a</td>
<td>1.07 (0.016)a</td>
<td>264.2 (1.60)a</td>
<td>64.83 (0.242)a</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.55 (0.007)a</td>
<td>1.06 (0.015)a</td>
<td>274.9 (2.01)a</td>
<td>65.46 (0.284)a</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.47 (0.008)b</td>
<td>0.17 (0.005)b</td>
<td>112.9 (1.76)b</td>
<td>25.17 (0.473)b</td>
</tr>
<tr>
<td>33</td>
<td>20</td>
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<td>1.11 (0.173)a</td>
<td>265.9 (6.29)a</td>
<td>64.79 (0.278)a</td>
</tr>
<tr>
<td></td>
<td>14</td>
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<td>1.00 (0.016)a</td>
<td>254.7 (1.56)a</td>
<td>63.18 (0.232)a</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.55 (0.007)a</td>
<td>0.96 (0.013)a</td>
<td>256.0 (1.69)a</td>
<td>62.97 (0.257)a</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.44 (0.008)b</td>
<td>0.13 (0.006)b</td>
<td>111.5 (1.53)b</td>
<td>22.58 (0.323)b</td>
</tr>
<tr>
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<td>7</td>
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<td>0.76 (0.014)a</td>
<td>243.6 (1.95)a</td>
<td>57.77 (0.369)a</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.36 (0.006)b</td>
<td>0.07 (0.002)b</td>
<td>97.9 (2.10)a</td>
<td>15.43 (0.283)b</td>
</tr>
</tbody>
</table>

¹The means reflect the average of plants harvested from four replications of the experiment. The standard errors of the means are in brackets (n=4).

²Means comparison performed with PROC Mixed lsmeans (p ≤0.05). Means with the same letter within the same column are not different.
Figure 3.3. Whole canopy carbon assimilation and respiration rates (µmol m$^{-2}$ growing area sec$^{-1}$) for radish (*Raphanus sativa* L. cv. Cherry Bomb II) grown with atmospheric pressures of 10, 33, 66, and 98 kPa and oxygen partial pressures of 2, 7, 14, and 10 kPa over a 21-day period. Data shown (3-17 days after planting) represent the period from closure until a series of secondary tests which were performed at 18-20 days after planting. The partial pressure of carbon dioxide was maintained at 120 Pa. The error bars represent the standard error of the means (n=4).
Figure 3.4. Transpiration (litres m\(^{-2}\) growing area day\(^{-1}\)) for radish (*Raphanus sativa* L. cv. Cherry Bomb II) grown at atmospheric pressures of 10, 33, 66, and 98 kPa and oxygen partial pressures of 2, 7, 14, 20 kPa. The pCO\(_2\) was maintained at 120 Pa. Data shown (3-17 days after planting) represent the period from closure until a series of secondary tests which were performed at 18-20 days after planting. Error bars represent the standard error of the means (n=4). Evaporation values (total evapotranspiration at 3 days after planting) were subtracted from total daily evapotranspiration to provide an estimate of transpiration.
total pressure and 20 kPa oxygen, transpiration was decreased at 13 DAP however the change in rate was not significant by the end of the study period. Transpiration at 10 kPa of total pressure and at oxygen partial pressures of 2 kPa was significantly lower than the other treatments and barely measurable at the 10/2 kPa treatment level.

3.4 Discussion

3.4.1. Growth

Across all treatments from 10-98 kPa of total pressure and 7-20 kPa of oxygen partial pressure, radish growth parameters demonstrated minor decreases which were similar to those observed by Levine et al. (2008). However, these differences were minimal as the quantities would likely be evened out during destructive harvesting or food preparation. Results in lettuce (Spanarkel and Drew 2002; He, Davies et al. 2006; He, Davies et al. 2007) and spinach (Iwabuchi, Goto et al. 1995) also suggested that long-term growth at reduced pressure was comparable to ambient levels. Thicker leaves were observed at reduced pressures as indicated by the decrease in SLA. From this observation, one might expect a reduction in growth as the thicker leaves reduce the LA available to capture incident solar radiation. At an oxygen partial pressure of 2 kPa, the suppression of radish growth was most significant and would not be suitable in life support conditions where the crops are the main source of nourishment for the crew and act as the air regeneration system.
3.4.2. Carbon Exchange

Contrary to previous short-term studies in wheat (Massimino and André 1999) and tomato (Rule and Staby 1981a), carbon assimilation rates of radish at oxygen partial pressures greater than 2 kPa demonstrated that reduced atmospheric pressure and reduced oxygen had little effect on photosynthesis and dark respiration. These results correspond with the long-term results of Iwabuchi et al. (1995) in spinach. It is not clear whether the lack of enhanced photosynthesis was due to physical, biochemical or combined adaptations but it is clear that the plant response to hypobaria is indeed complex as demonstrated by the altered regulation of 200 genes (Paul, Schuerger et al. 2004). It is, however, probable that long-term changes are being made as noted by the decreased variation in pressure treatments, observed by Richards et al. (2006), after only 16 hours of acclimation. The enhanced photosynthesis previously observed may have been due to the inhibition of the oxygenase activity of ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) seen when older plants were subjected to reduced oxygen partial pressures as a secondary effect of decreasing the atmospheric pressure (Musgrave and Strain 1988).

3.4.3. Transpiration

Enhanced transpiration at reduced atmospheric pressure has been postulated due to the corresponding decreased aerodynamic resistance and increased diffusion rates (Gale 1973a). However, given that the VPD, for any given temperature, was not affected by pressure one might conclude that transpiration
would remain stable. Similar to the carbon exchange rates in this study, little
effect was noted in transpiration among treatments from 33-98 kPa total pressure
and 7-20 kPa of oxygen. The transpiration trends observed here relate to the
results of Iwabuchi and Kurata (2003) which noted no difference in the
transpiration of spinach, however it contradicts the results of Richards et al.
(2006) where an increase of 50% was noted in Arabidopsis. Terashima et al.
(1995) suggested that increased transpiration would be observed at reduced
pressures even if the VPD remained constant due to the flux driven by the
increased diffusion rates. However, Iwabuchi et al. (1995) explained their lack of
increased transpiration rates as a secondary response in which the increased
diffusivity drives transpiration which lowers the leaf temperature causing the
closure of stomates and therefore a reduction of the enhanced exchange rates,
while (Migge, Kahmann et al. 1999) suggested that the thicker leaves used to
dissipate radiant energy would result in decreased water loss. Our results
suggested that conditions were not optimal for increased transpiration and it is
possible that other factors were responsible such as the aforementioned leaf
adaptation response.

3.5 Conclusions

The current experiment demonstrated the ability of radish plants to withstand the
effects of reduced pressures from seedling to harvest. Contrary to historical
hypotheses and observations, NCER and transpiration were not greatly
enhanced and dark respiration was not suppressed. Clearly, there are a number
of complex factors involved which can not be assessed using past methods of excised plant tissues or through short-term investigation using plants established at ambient conditions, as the short-term gains in photosynthetic capacity are offset by adaptive measures in the long-term. These results represent the most comprehensive analysis of plant growth from seedlings under hypobaric and reduced oxygen partial pressure with respect to the range of atmospheric alteration and control. With these observations the next stage of this investigation was to evaluate the NCER response of radish to light and the carbon dioxide compensation points of plants established at reduced pressure and decreased oxygen. These results should give further insight into past observations and the pressure:oxygen relationships.
CHAPTER 4

LIGHT RESPONSE CURVES AND CARBON DIOXIDE COMPENSATION POINTS OF RADISH GROWN UNDER HYPOBARIO AND REDUCED OXYGEN PARTIAL PRESSURES

4.1 Related Research

The advancement of space travel is creating a need for a life support system with a biological component to supplement the current physical-chemical (PC) systems (Schwartzkopf and Mancinelli 1991; Wheeler, Stutte et al. 2001; Kanervo, Lehto et al. 2005). Controlled environment, plant-based life support systems can provide food, potable water and regenerate the gaseous environment by producing oxygen and scrubbing carbon dioxide. While less defined and less rigidly modeled, BLSS provide a manipulability often lacking in mechanical systems (Tripathy, Brown et al. 1996).

Plant productivity is mediated by numerous environmental parameters, and for a BLSS to supply the needs of the crew and be sustainable with current technological limitations, photosynthesis must be optimized. Reduced oxygen partial pressures are common practice in the models of low pressure environments for space exploration to the Moon or Mars (Migge, Kahmann et al. 1999; Ramonell, Kuang et al. 2001; Spanarkel and Drew 2002) and it is likely that the alteration of the gaseous environment of a plant growth chamber plays a significant role in alteration of the photosynthetic process.

It is well documented that oxygen affects biochemical pathways (Richards, Corey
et al. 2006) and is essential in the oxidative phosphorylation pathway as it acts as the terminal electron acceptor providing adenosine-5'-triphosphate (ATP) for cellular metabolism by regenerating nicotinamide adenine dinucleotide (NAD+) from NADH (Fukao and Bailey-Serres 2004; Bailey-Serres and Chang 2005). When oxygen is limiting, to anoxic levels, it can inhibit ATP formation causing the photosynthetic apparatus to rely on less efficient fermentation processes (Geigenberger 2003). However, an inhibitory effect of high oxygen on photosynthesis has been acknowledged and referred to as the Warburg Effect (Ellyard and San Pietro 1969). Decreased oxygen partial pressures which create increased carbon dioxide to oxygen ratios, can have an enhancing effect on photosynthesis as they limit the activity of ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) as an oxygenase which decreases competition for carbon dioxide in C3 plants (Usuda 2006).

Vartapetian and Jackson (1985) discussed mechanisms to confer tolerance or promote acclimation and adaptation to reduced oxygen in the roots through changes in the energy generating pathways, and in shoots through developmentally passive changes to morphology and physiology. In tobacco, the enhancing effect of decreased oxygen partial pressures on photosynthesis was apparent after one hour however following three days of exposure the rate of photosynthesis declined (Migge, Kahmann et al. 1999). Richards et al. (2006) noted that the short-term gains of reduced oxygen partial pressures may not be significant in the long-term as after only 16 hours of acclimation, the
photosynthetic rate of *Arabidopsis* had slowed and the compensation points increased from their original levels. For the purpose of further exploring the effects of reduced total pressure and oxygen partial pressures on photosynthesis, secondary treatments were added to the end of the growth period of the radish experiments presented in Chapter 3.

One method of assessing photosynthetic efficiency is through the use of light response curves. Light is commonly a limiting factor in photosynthesis and low light conditions are a frequent occurrence in closed environment growth chambers (Bugbee and Monje 1992). By examining photosynthesis over a series of light intensities, one can extrapolate light saturation values and photosynthetic rates. Generally, as light levels increase so does the rate of photosynthesis until a maximum of carbon fixation at the light saturation point. The response curve is initially linear as light remains the dominant factor. However, when other factors become significant the curve loses the linearity, eventually developing a horizontal phase beginning with the saturation point. Quantum yield, or the increase in carbon gains for each increase in energy absorbed, is represented as the slope of the linear portion of the curve. At high light levels, photosynthesis is limited by the oxidation of Rubisco while at low light the effect is exerted by the rate of the electron transport chain (He, Davies et al. 2006). Light response curves at each combination of pressure and oxygen treatments were examined to compare photosynthetic capacity at varying light intensities.
Another way to assess photosynthetic effects is through photosynthetic drawdown curves which can be examined to identify the carbon dioxide compensation point. This point of equilibrium, between the rate of photosynthetic carbon uptake and carbon dioxide generation from dark and photo-respiration, where there is no net evolution, can be influenced by numerous environmental factors that effect the rate of photosynthesis or photorespiration, including the partial pressures of carbon dioxide, oxygen and water vapour as well as temperature, plant age and genetic variation (Smith, Tolbert et al. 1976; Espie and Colman 1987; Campbell, Sage et al. 2005). In closed environments, drawdown curves are commonly performed by allowing the carbon dioxide to build up from dark respiration and then disengaging the control system during the light period until the plant biomass utilizes enough carbon dioxide to reach the compensation point. Carbon dioxide compensation points of C3 plants, such as radish, are commonly about 40 μmol mol⁻¹. At ambient total pressure, decreasing oxygen partial pressures routinely reduces the carbon dioxide compensation point by decreasing photorespiration and limiting the competition of carbon dioxide for Rubisco.

The examination of the light response curves and carbon dioxide compensation points, while concurrently measuring physiological effects in whole plant growth and development, may provide greater insight into the plants’ ability to withstand considerable atmospheric modification.
The first objective of this research was to evaluate the change in the quantum efficiency of 19 day old radish grown at reduced total pressures and decreased oxygen partial pressures in response to light intensity. The second objective was to determine the carbon dioxide compensation points in order to conclude whether plants grown under hypobaric and reduced oxygen conditions undergo an alteration in efficiency.

4.2 Methodology

4.2.1. Experimental Protocol

The data for the current investigation were collected at the end of the growth period for the radish (Raphanus sativa L. cv. Cherry Bomb II) experiments outlined in Chapter 3. Treatments included four total pressures (10, 33, 66, 98 kPa) and four oxygen partial pressures (2, 7, 14, 20 kPa) in various combinations.

4.2.2. Light Response Curves

Whole canopy net carbon exchange rates in response to seven light intensity levels (0, 119, 154, 213, 287, 375, 600 μmol photons m⁻² sec⁻¹ PAR) were obtained at 19 DAP using a series of neutral density screens to decrease the PAR. Each light level was maintained for a period of two hours. Net carbon exchange rate measurements were calculated from the rate of carbon dioxide injections over a 30-minute period at the end of each light treatment. The data were normalized for chamber growing area due to the inability to access the
plants for destructive harvest until the end of closure and linear regression was used to determine the slope or quantum efficiency.

4.2.3. Carbon Dioxide Compensation Points

Carbon dioxide compensation points for 18-day old radish were established over the standard light period. Carbon dioxide concentration was allowed to build up, over the night period, from dark respiration, and the carbon dioxide setpoints in the control system were disabled until the crop reached the compensation point or the end of the light period of 16 hours.

4.2.4. Statistical Analysis

The experiment was a randomized block design with each chamber being considered a replicate and treatments were cycled through the chambers to account for any chamber variation. All oxygen and pressure treatment combinations were replicated four times.

The slopes of the light response curves were analyzed using a PROC Mixed ANOVA to test the null hypothesis that pressure and oxygen did not have an effect on the slopes. To account for chamber differences, the chamber numbers and the interactions involving the chambers were set as random factor effects. This was followed by a means comparison test when the ANOVA indicated that there were significant treatment effects.

Analysis of the carbon dioxide compensation points also used a PROC Mixed
ANOVA to test the null hypothesis that pressure and oxygen did not have an effect on the carbon dioxide compensation points. Carbon dioxide compensation point was set as the dependant variable with pressure and oxygen set as the independent variables. Once again chamber effects were accounted for and an means comparison was performed when the ANOVA indicated that there were significant treatment effects.

All values were expressed as a mean ± the standard error of the mean and a statistical significance level (α) of 0.05 was applied to all comparisons. The linear regression analysis was performed in S-PLUS version 7.0/8.0 for Windows (Insightful Corporation, Seattle, Washington, USA) and the ANOVA analysis was performed using SAS version 9.1.3 for Windows (SAS Institute Inc., Cary, North Carolina, USA).

4.3 Results and Discussion

4.3.1. Light Response Curves

The regression analysis, with the equation stated as \( \text{NCER} = a + (\text{PAR})b \), revealed that the response of NCER was well correlated to light level (\( R^2 \) values between 0.95-0.97; Table 4.1). The linear nature of all the curves suggests that light was still the dominant factor in the responses observed and that the maximum light level of 600 \( \mu \text{mol} \text{ photons m}^{-2} \text{ sec}^{-1} \) PAR was below the saturation point for radish of this age, density and temperature across all treatment levels (Figure 4.1). A greater intensity of 800-1200 \( \mu \text{mol} \text{ photons m}^{-2} \text{ sec}^{-1} \) PAR may
have been required to reach the light saturation point for radish (Salisbury and Ross 1992) and would have allowed for a subsequent increase in carbon fixation.

There was little difference in the slope, or quantum yield, due to the effect of reduced total pressure (Table 4.2) however there was a minor increasing separation between curves across the total pressures as the light level increased. The quantum yield data showed significant variation among the oxygen partial pressures with the most significant decrease at 2 kPa of oxygen. There was a decrease of 0.0218, 0.0207, 0.0198 μmol CO₂ μmol photons⁻¹ from 20 versus 2 kPa oxygen for the total pressures of 98, 66 and 33 kPa, respectively. And at 10 kPa total pressure, the significant reduction from 7 to 2 kPa was 0.0165 μmol CO₂ μmol photons⁻¹ (t=20.74; p=0.0002). This decreased efficiency at 2 kPa of oxygen supports the significant difference in the fresh and dry mass of radish leaves and roots reported in Chapter 3.

The light response curve results imply that at an oxygen partial pressure equal to or greater than 7 kPa, photosynthetic efficiency at reduced pressures can be expected to be equivalent to that at Earth’s ambient levels.
Table 4.1. The relationship between whole canopy net carbon exchange rate (µmol m⁻² growing area sec⁻¹) and photosynthetically active radiation (µmol photons m⁻² sec⁻¹ PAR) for radish (*Raphanus sativa* L. cv. Cherry Bomb II) grown at atmospheric pressures of 10-98 kPa and oxygen partial pressures of 2-20 kPa. The models are in the form $NCER = a + (PAR)b$.

<table>
<thead>
<tr>
<th>Pressure (kPa)</th>
<th>Oxygen (kPa)</th>
<th>Parameter</th>
<th>Estimate</th>
<th>Std Error</th>
<th>$R^2$</th>
<th>t Value</th>
<th>P</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
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<td>0.96</td>
<td>-0.238</td>
<td>0.8211</td>
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<tr>
<td></td>
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<td>Slope</td>
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<td></td>
<td>11.153</td>
<td>0.0001</td>
</tr>
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<td>0.97</td>
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<td></td>
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<td>-0.658</td>
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<td></td>
<td>Slope</td>
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<td>0.0027</td>
<td></td>
<td>13.146</td>
<td>&lt;0.0001</td>
</tr>
<tr>
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<td>0.0001</td>
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<td>0.9766</td>
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<td></td>
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<td>0.0002</td>
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<td>0.8775</td>
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<td>0.96</td>
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<td>0.6319</td>
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<td>0.0001</td>
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<td>13.868</td>
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Figure 4.1. The linear regressions of whole canopy net carbon exchange rates (µmol m\(^{-2}\) growing area sec\(^{-1}\)) versus photosynthetically active radiation (µmol photons m\(^{-2}\) sec\(^{-1}\); PAR) for radish (*Raphanus sativa* L. cv. Cherry Bomb II) grown at atmospheric pressures of 10, 33, 66, and 98 kPa and oxygen partial pressures of 2, 7, 14, and 20 kPa over a 21-day period. Data were obtained at 19 days after planting using a series of neutral density screens to decrease the photosynthetically active radiation. Each light level was maintained for a period of two hours. The partial pressure of carbon dioxide was maintained at 120 Pa. The models are in the form \(NCER = a + b \times PAR\). The error bars represent the standard error of the means (n=4).
Table 4.2. Results of the fixed effects analysis of variance for the effect of total atmospheric pressure (10, 33, 66 and 98 kPa) and partial pressure of oxygen (2, 7, 14 and 20 kPa pO\(_2\)) on the quantum yield (µmol CO\(_2\) µmol photons\(^{-1}\)) of radish (*Raphanus sativa* L. cv. Cherry Bomb II).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Numerator df</th>
<th>Denominator df</th>
<th>F Value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure</td>
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<td>9</td>
<td>1.62</td>
<td>0.252</td>
</tr>
<tr>
<td>Oxygen</td>
<td>3</td>
<td>9</td>
<td>98.70</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pressure:pO(_2)</td>
<td>7</td>
<td>21</td>
<td>1.67</td>
<td>0.170</td>
</tr>
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</table>
4.3.2. Carbon Dioxide Compensation Points

Both pressure and oxygen had a significant effect on the whole canopy carbon dioxide compensation points (Table 4.3). Hypobaria resulted in a reduction in the carbon dioxide compensation points however the larger effect observed was that caused by reduced oxygen partial pressures (Table 4.4). Compensation points fell with decreased oxygen resulting in the lowest point at 10 kPa total pressure and 2 kPa oxygen which represented only 18 % of that at ambient pressure and oxygen. The reduction of the compensation point is in agreement with Espie and Colman (1987) who reported a significant decline in isolated asparagus mesophyll cells and attached cladophylss or modified leaves. The decreased compensation point allows for increased uptake of carbon dioxide as there is less competition for Rubisco (Richards, Corey et al. 2006; He, Davies et al. 2007).

The relationship of carbon dioxide compensation point and oxygen is in agreement with the work of Byrd and Brown (1989) who reported that the compensation point increased linearly with increased oxygen concentration in C3 plants. They found that over extended periods, increased oxygen levels caused decreased photosynthesis and increased the carbon dioxide compensation point because of increased starch accumulation as a result of interrupted phloem loading. Majeau (1996) also noted elevated starch and soluble sugars were present in leaves when the photosynthetic rate exceeded the translocation to sinks and that Rubisco protein levels were reduced when environmental factors
Table 4.3. Results of the fixed effects analysis of variance for the effect of total atmospheric pressure (10, 33, 66 and 98 kPa) and oxygen partial pressure (2, 7, 14 and 20 kPa \( pO_2 \)) on the carbon dioxide compensation point (µmol) of radish (\textit{Raphanus sativa} L. cv. Cherry Bomb II).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Numerator df</th>
<th>Denominator df</th>
<th>F Value</th>
<th>P</th>
</tr>
</thead>
<tbody>
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<tr>
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<tr>
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<td>18</td>
<td>0.38</td>
<td>0.901</td>
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</table>
Table 4.4. Whole canopy carbon dioxide compensation points (µmol) for radish (*Raphanus sativa* L. cv. Cherry Bomb II) grown under atmospheric pressures of 10, 33, 66, and 98 kPa and oxygen partial pressures of 2, 7, 14, and 20 kPa over a 21-day period. Data were obtained at 18 days after planting from a drawdown of the carbon dioxide buildup from dark respiration over the night period. The partial pressure of carbon dioxide was maintained at 120 Pa.

<table>
<thead>
<tr>
<th>Pressure (kPa)</th>
<th>Oxygen Partial Pressure (kPa)</th>
<th>20</th>
<th>14</th>
<th>7</th>
<th>2</th>
</tr>
</thead>
<tbody>
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<td>98</td>
<td></td>
<td>70.7 (5.78)(^1)(^a)</td>
<td>65.2 (4.37)(a)</td>
<td>42.5 (3.07)(b)</td>
<td>32.2 (10.89)(b)</td>
</tr>
<tr>
<td>66</td>
<td></td>
<td>65.8 (2.79)(a)</td>
<td>53.0 (2.68)(ab)</td>
<td>35.0 (1.32)(ab)</td>
<td>36.3 (16.30)(b)</td>
</tr>
<tr>
<td>33</td>
<td></td>
<td>62.2 (4.67)(a)</td>
<td>50.4 (0.94)(b)</td>
<td>30.4 (2.97)(c)</td>
<td>20.1 (1.85)(d)</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>--</td>
<td>--</td>
<td>20.3 (2.62)(a)</td>
<td>12.9 (2.91)(a)</td>
</tr>
</tbody>
</table>

\(^1\)The means reflect the average of single plants harvested from four replications of the experiment. The standard errors of the means are contained in brackets.

\(^2\)Means comparison performed with PROC Mixed Ismeans (p ≤0.05). Means with the same letter within the same row are not significantly different (n=4).
limited translocation from leaves to sink tissues. The buildup of starch grains is known to decreased photosynthesis by compressing the thylakoids and physically preventing light from reaching the chloroplasts (Salisbury and Ross 1992). Analyzing starch content and phloem unloading would allow for further clarification of the mechanism decreasing the compensation point and contributing to sustained photosynthesis and growth.

4.4 Conclusions

The outcome of the light response measurements and examination of the carbon dioxide compensation points support the results reported in Chapter 3, which demonstrated that plants have a complex adaptive strategy which permits them to be vegetatively productive despite decreased total pressures or reduced oxygen partial pressures. The previous understanding of the effects of hypobaria suggest that due to an increase in the diffusivity of gases there will be a considerable increase in photosynthesis as a result of an increase in the internal concentration of carbon dioxide available for photosynthesis. Additionally, low oxygen partial pressures are known to amplify net carbon exchange by decreasing the oxygenase competition for Rubisco. The results presented here show that hypobaria and hypoxia do exert a physiological response however the limiting factor is likely biochemical rather than physical. It is clear that the adaptation proposed by Paul et al. (2004), due to the extensive change in gene expression, and observed in Arabidopsis by Richards et al. (2006) also occurs in radish however the response is limited in its ability and reaches a threshold at an
oxygen partial pressure between 2-7 kPa. Aside from the biochemical limitations, the acclimation effect may also be a result of a reduction in stomatal conductance possibly facilitated through changes to the stomatal complex (Richards, Corey et al. 2006). To try and further elucidate the mechanisms involved, a supplementary analysis will evaluate stomatal frequency to determine whether there is a modification due to hypobaria or reduced oxygen partial pressures.
CHAPTER 5

EFFECTS OF REDUCED ATMOSPHERIC PRESSURE AND DECREASED OXYGEN ON THE STOMATAL FREQUENCY IN RADISH – A CASE STUDY

5.1 Related Research

As regulators of gaseous exchange, stomata are important in the understanding of hypobaria and oxygen effects on plant growth (Malone, Mayeux et al. 1993). Beside the standard regulation of stomatal pore size, plants can change the number of stomata in newly developing leaves. Stomatal frequency, defined as the number of stomata per unit of leaf area, and changes in stomatal aperture have been found to be affected by a number of environmental stimuli including: light, relative humidity, carbon dioxide, soil water content and nutrients (Poole, Weyers et al. 1996; Morison 1998). However, stomatal functioning is a complex process due to these environmental interactions. There still remains a void in the understanding regarding the physiological controls (Jones 1998) and much of the information on the development of stomata is limited to the effects of carbon dioxide concentration or light intensity.

Stomatal frequency is routinely correlated to stomatal conductance, which is associated with transpiration rates and photosynthetic capacity, making it significant to water regeneration and food production in the bioregenerative life support system (BLSS). However, other factors such as stomatal pore length, depth and width also contribute, and responses are adaptive with different short- or long-term effects. Short-term effects might be due to a change in aperture that
affects stomatal conductance while long-term responses may be morphological including changes in stomatal size, frequency or index (Morison 1998). The changes in stomatal frequency are believed to be signaled from mature leaves to developing leaves and it is suspected that the mechanism may be linked to plant hormones which are significant in leaf development (Miyazawa, Livingston et al. 2006).

The research into hypobaria has demonstrated varied responses to reduced pressure and oxygen. Iwabuchi and Kurata (2003) suggested that this may be due to differences in acclimation responses or the development of morphological changes in stomatal size, density and physiological response. Stomatal distribution, morphology and frequency are species dependant (Miyazawa, Livingston et al. 2006) and the upper and lower surfaces may respond differently to environmental stimuli as observed in some species whose lower surface is more sensitive to light intensity (Malone, Mayeux et al. 1993). Radish is known to be amphistomatic, with stomata on both leaf surfaces, however, there are more on the lower surface. Additionally, radish stomata tend to be in clusters with a larger central stomate and a few smaller ones around its perimeter (Pant and Kidwai 1967).

In chapter 3, an increase in specific leaf area (SLA), calculated as a decrease in leaf area per gram of dry tissue, was reported that supported earlier research, which found that hypobaria and reduced oxygen partial pressures increased leaf
thickness (Quebedeaux and Hardy 1973; Terashima, Masuzawa et al. 1995; Migge, Kahmann et al. 1999; Ramonell, Kuang et al. 2001; He, Davies et al. 2007). Spanarkel and Drew (2002) also noted that increased stomatal conductance may be a factor, however due to the lack of increased transpiration they concluded that a decrease in stomatal aperture may be present. Overall, it is clear that one strategy for anoxia avoidance is often due to changes in morphology (Geigenberger 2003).

With the premise that growth of radishes from seedlings established at reduced total pressure and oxygen partial pressure generates anatomical or morphological changes, the objectives of the current investigation were to assess any effects of the altered environments on the number of stomata on the abaxial (lower) and adaxial (upper) side of the leaves and to attempt to relate these findings to the results observed in chapters 3 and 4.

5.2 Methodology

5.2.1. Experimental Protocol

The data for this supplementary analysis was collected at the end of the experiments presented in Chapters 3. Treatments included four total pressures (10, 33, 66, 98 kPa) and three oxygen partial pressures (2, 7, 20 kPa) with the control treatment represented by the 98/20 kPa combination.
5.2.2. Assessment of Stomatal Frequency

To assess stomatal frequencies, leaf impressions of the adaxial and abaxial leaf surfaces were taken with Oraprint Plus (vinyl polysiloxane) dental impression material (Dental Services Group, Minneapolis, Minnesota, USA). The impression material was mixed at a 1:1 ratio on a strip of paper and immediately placed on a fully expanded leaf for and allowed to cure for 5 minutes. Care was taken in impression placement as there can often be spatial variation in stomatal numbers and aperture across single leaves (Poole, Weyers et al. 1996) and best attempts were made not to place the material across the stem vein even in the smallest leaves.

The dry impressions were then coated with clear nail polish (Sally Hanson - Hard as Nails with Nylon - Nude (Del Labs (Canada) Inc., Barrie, Ontario, Canada) and the thin polish peels were mounted on glass microscope slides. This protocol was similar to that which Weyers and Johansen (1985) used to assess the stomatal aperture of *Commelina communis* L. The slides were observed with a Nikon Eclipse E600 light microscope (Nikon Corporation, Japan) at a magnification of 100X. Pictures were taken using an ORCA II-ER digital camera (Hamamatsu Photonics K.K., Hamamatsu, Japan) mounted to the microscope and Simple PCI 5.1 software (Hamamatsu Corporation, Sewickley, Pennsylvania, USA). Stomata counts were done manually over a randomly selected 0.5 x 0.5 mm area.
Stomatal aperture was not measured as in previous studies (Iwabuchi and Kurata 2003) because it was impossible to access the plants before the pore size changed, within minutes of repressurization, without affecting other parameters being measured.

5.2.3. **Statistical Analysis**

Experimental design was a randomized block design with each chamber being considered a replicate and each treatment was replicated four times. Replication was achieved by repeating the experiments over time and replicates were cycled through the chambers to minimize chamber effects. Imprints were collected from both the abaxial and adaxial surface of three leaves from each pressure combination.

A PROC Mixed ANOVA was used to test the null hypothesis that atmospheric pressure and oxygen partial pressure did not have an effect on stomatal frequency of radish. This was followed by an lsmeans test when the ANOVA indicated that there were significant treatment effects. Stomatal frequency was set as the dependant variable with pressure and oxygen set as the independent variables.

All values were expressed as a mean ± the standard error of the mean and a statistical significance level (α) of 0.05 was applied to all comparisons. The analysis was performed in SAS version 9.3.1 for windows (SAS Institute Inc., Cary, North Carolina, USA).
5.3 Results and Discussion

The abaxial and adaxial stomatal frequencies showed varied responses as reported by Morrison (1998). On the abaxial surface, pressure significantly affected stomatal frequency (Table 5.1) with an increase corresponding to reduced total pressure (Table 5.2). However, on the adaxial surface, oxygen proved significant (Table 5.3) with decreased oxygen partial pressures increasing stomatal numbers (Table 5.4). The abaxial increase became more apparent with decreasing oxygen partial pressures with the greatest stomatal frequency at the 10/2 kPa treatment. This increase in frequency might be a response to the reduced oxygen, resulting in increased stomatal conductance, in addition to the increased diffusivity of gases at reduced pressures. The adaxial response was only significant across oxygen partial pressures at ambient total pressure (98 kPa), suggesting that adaptations to reduced total pressure might lessen the adaxial response to reduced oxygen. The increase in stomatal frequency across all treatments on both surfaces proved similar with a 36 % increase on the abaxial surface and a 44 % on the adaxial surface between the ambient treatment versus the 10/2 kPa treatment.

The difficulty with carrying the analysis of these results further is the complexity of the stomatal reaction to secondary stimuli. This can create an ambiguous response and results in the inability to directly relate stomatal frequency to stomatal conductance due to a need to understand stomatal aperture, pore length, width and depth (Morison 1998). Experimental protocol involved with the.
Table 5.1. Results of the analysis of variance for the effect of total atmospheric pressure (10, 33, 66 and 98 kPa) and oxygen (2, 7, and 20 kPa pO$_2$) on stomatal frequency (stomata mm$^{-2}$) on the abaxial surface of radish (*Raphanus sativa* L. cv. Cherry Bomb II) leaves.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Numerator df</th>
<th>Denominator df</th>
<th>F Value</th>
<th>P</th>
</tr>
</thead>
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<td>6</td>
<td>36.89</td>
<td>0.0003</td>
</tr>
<tr>
<td>Oxygen</td>
<td>2</td>
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<td>Pressure:Oxygen</td>
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<td>74</td>
<td>0.67</td>
<td>0.5726</td>
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Table 5.2. The effect of atmospheric pressure (10, 33, 66 and 98 kPa) and oxygen partial pressure (2, 7, 20 kPa pO₂) on the stomatal frequency (stomata mm⁻²) on the abaxial surface of radish (*Raphanus sativa* L. cv. Cherry Bomb II) leaves grown over a 21-day period. The partial pressure of carbon dioxide was maintained at 120 Pa.

<table>
<thead>
<tr>
<th>Pressure (kPa)</th>
<th>Oxygen (kPa)</th>
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<th>2</th>
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<tr>
<td>98</td>
<td>377.0 (6.52)¹</td>
<td>381.8 (16.40)a²</td>
<td>392.9 (12.25)a</td>
</tr>
<tr>
<td>66</td>
<td>---</td>
<td>382.7 (18.98)a</td>
<td>419.7 (13.46)ab</td>
</tr>
<tr>
<td>33</td>
<td>---</td>
<td>464.4 (11.30)b</td>
<td>466.4 (10.65)bc</td>
</tr>
<tr>
<td>10</td>
<td>---</td>
<td>496.8 (11.57)b</td>
<td>515.1 (89.88)c</td>
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</table>

¹The means reflect the average of single plants harvested from four replications of the experiment. The standard errors of the means are contained in brackets.

²Means comparison performed with PROC Mixed lsmeans (p ≤0.05). Means with the same letter within the same column are not significantly different.
Table 5.3. Results of the analysis of variance for the effect of total atmospheric pressure (10, 33, 66 and 98 kPa) and oxygen (2, 7 and 20 kPa pO$_2$) on stomatal frequency (stomata mm$^{-2}$) on the adaxial surface of radish (*Raphanus sativa* L. cv. Cherry Bomb II) leaves.

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<th>Denominator df</th>
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<tr>
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<tr>
<td>Pressure:Oxygen</td>
<td>3</td>
<td>74</td>
<td>1.34</td>
<td>0.2683</td>
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</table>
Table 5.4. The effect of atmospheric pressure (10, 33, 66 and 98 kPa) and oxygen partial pressure (2, 7, 20 kPa pO$_2$) on the stomatal frequency (stomata mm$^{-2}$) on the adaxial surface of radish (Raphanus sativa L. cv. Cherry Bomb II) leaves grown over a 21-day period. The partial pressure of carbon dioxide was maintained at 120 Pa.

<table>
<thead>
<tr>
<th>Pressure (kPa)</th>
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<tbody>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td>98</td>
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<tr>
<td>66</td>
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<td>33</td>
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</tr>
<tr>
<td>10</td>
<td>---</td>
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</tbody>
</table>

$^1$The means reflect the average of single plants harvested from four replications of the experiment. The standard errors of the means are contained in brackets.

$^2$Means comparison performed with PROC Mixed Ismeans (p ≤0.05). Means with the same letter within the same row are not significantly different.
hypobaric chambers precluded the collection of more detailed measurements.

However, the change in frequency signaled by older leaves presents one explanation to relate the differences between the short- and long-term findings reported as a result of hypobaric and reduced oxygen partial pressure effects in past literature. Schoch *et al.* (1980) revealed that stomatal differentiation in cowpea (*Vigna sinensis* L.) was shown to be signaled by changes in light intensity between days one and six prior to leaf unfolding and that there was a maximum response at day three. Since many of the previous studies involved the use of plants that were grown at ambient conditions and then subjected to reduced pressure and oxygen for only short periods, it is clear that the results of past research demonstrated the stress response of the mature leaves which differs greatly from this research which involved young adapted leaves.

From research that involved young plants or those grown from seed, similar results to those presented in Chapters 3 and 4 were demonstrated. From their research at high altitudes, Terashima *et al.* (1995) suggested that the distribution of stomata and the increase in leaf thickness may override the increased diffusivity found at reduced pressures. Additionally, it is clear that boundary layer effects on stomatal control must not be underestimated (Jones 1998). In lettuce, there was a lack of increased transpiration associated with reduced total pressures causing Spanarkel and Drew (2002) to propose that a decrease in stomatal aperture and thin boundary layer might have lessened water loss from
the leaves without reducing photosynthesis. While Iwabuchi and Kurata (2003) found that the stomatal pore length and width were reduced on both leaf surfaces of spinach and suggested that the increased diffusivity of gases might relate to a decreased humidity and elevated carbon dioxide response which are both known to cause stomatal closure. Correspondingly, Richards et al. (2006) proposed that the acclimation in *Arabidopsis* occurred due to a reduction in stomatal conductance and changes in stomatal aperture. Results of this work support preceding research indicating that the lack of apparent response to reduced pressure and oxygen partial pressures above 7 kPa is due to a plant adaptation response partially exhibited by a change in stomatal development.

5.4 Conclusions

Stomata provide the key interface between plants and their atmospheric environment therefore an understanding stomatal development and function under the proposed hypobaric and hypoxic conditions of a BLSS are clearly among the most important research questions. Although the results presented here do not elucidate the change in stomatal morphology or its affect on stomatal conductance, it is clear that there is an adaptive plant response that mitigates the reduced pressure effects and minimizes the effects of decreased oxygen to a threshold level. This adaptation is demonstrated in lack of adjustment of both NCER and transpiration across the range of total pressures. Further research should be undertaken to assess aperture size and changes to stomatal pore length, width and depth to allow for additional comprehension of the effects.
CHAPTER 6

GENERAL DISCUSSION AND FUTURE CONSIDERATIONS

6.1 Discussion

Current space missions that are focused on the ISS rely solely on PC systems and resupply, however, as interest builds in long-duration exploration to the Moon, Mars or beyond, the notion of human life support broadens and BLSS become a feasible option. These BLSS, composed of plants and other biotic species, provide a method of atmospheric regeneration and food production with a great degree of flexibility and sustainability that is not possible with PC methods alone. Biological life support systems also allow for a greater range of atmospheric fluctuation as well as a buffer from control issues and minor accidents. Since higher plants will likely play a central role in the BLSS, it is imperative that there is an understanding of plant development and physiology under the functioning environmental parameters of the BLSS.

The harsh Lunar environment with its lack of appreciable atmosphere and the Martian environment with its minimal atmosphere, necessitate the need for a pressurized, controlled environment plant growth facility. And although research into plant growth at reduced pressures has been undertaken since the early 1970s, there is still an incomplete understanding of the role of hypobaria on the development and growth of plants. The deficiencies are related to the lack of common facilities, methods, treatments and plant species as well as previous difficulties segregating the effects of reduced atmospheric pressure from those of
other environmental variables. It is now widely recognized that the use of a vacuum pump, to impose pressure treatments in hypobaric plant growth chambers, reduces the quantity of the atmosphere’s constituent gases including oxygen and water vapour whose physiological importance is critical to the functioning of the BLSS.

This research represents the most extensive series of hypobaric plant growth experiments involving the broadest range of reduced oxygen partial pressures and contributes to the future of advanced life support research globally by providing an overview of long-term vegetative plant responses in large-scale, controlled environment studies. Results demonstrated that plant responses to their atmospheric environment varies greatly with species, varietal and genotypic differences as well as the distinct life stages of the plant. In order for the BLSS to be sustainable, it must be productive from seed germination until harvest of foodstuffs and provide viable seeds for the next generation. It is for this reason, that an evaluation of germination was undertaken for three candidate crop species. Seedling germination and growth was negatively affected by both reduced total pressures and decreased oxygen partial pressures. To diminish these effects in the functioning BLSS, it is important to consider seed morphology when choosing crop varieties and developing seed treatments and germination protocols to ensure successful crop establishment.

Once seedlings are established, growth of edible biomass is of the utmost importance as atmospheric regeneration is in excess and secondary when
compared to food production limits. Net carbon exchange rate proved not to be greatly affected by hypobaria, however hypoxia was a limiting factor. Radish growth proved to be comparable across the range of oxygen until a partial pressure of 7 kPa. The reduced oxygen partial pressure did not prevent growth however, the 2 kPa oxygen treatment showed significantly impeded growth and development. The imposition of hypoxic stress lengthened the period required for establishment and thereby decreased yield and the overall harvest capacity of the BLSS. This differs from the well known results of short-term experiments in which photorespiration was mitigated at oxygen partial pressures of less than 2 kPa causing increased NCER. However, from the current study, it appears that radish plants adapt in the long-term and lose this efficiency.

The mechanism for adaptation is most likely a complex merger of biochemical and morphological changes. Previous short-term evaluations don't account for the fact that mature leaves are fully developed and are therefore unable to acclimatize to the considerable atmospheric changes, however seedlings or young plants established at reduced pressures and oxygen partial pressures are able to adapt during the development of new tissues. While it is difficult to conclusively account for all of the stomatal adaptation using the methods employed in this research, it is clear from the changes in stomatal index that the environmental conditions affect the development of leaves and likely the stomatal functioning. With these morphological changes, there were no significant modifications to NCER or transpiration over the range of pressure treatments.
however oxygen proved to have a threshold response, which is likely between 2 and 7 kPa.

The negligible of effect of low total pressure on the rate of transpiration was unexpected because of the physical properties of the environment, which demand an increase in water flux and past research, which supports this premise. It is apparent that the plants acclimate to the altered environment and overcome the driving forces of latent heat exchange through changes in leaf thickness, stomatal development and functioning and possible biochemical modifications.

These results suggest that a reduced pressure plant growth facility could be established over a range of pressure from 10-20 kPa and be as vegetatively efficient as an ambient facility on Earth as long as the oxygen partial pressure was maintained above the threshold for the crop which is likely between 2-14 kPa. This low limit is possible with the current gaseous composition of 120 Pa of carbon dioxide and 1 kPa of water vapour but may not be feasible from an engineering standpoint with the high concentration of oxygen required to maintain sufficient plant growth.

6.2 Future Considerations

Proposed Lunar and Martian greenhouses need to address numerous environmental requirements including temperature, light quality and quantity, humidity, root zone moisture and atmospheric pressure while withstanding a
harsh external environment. Many of these factors are routinely managed in greenhouses on Earth, with the exception of pressure. The total atmospheric pressure and the gaseous composition of space environments are a considerable challenge as similar conditions are not found on Earth, requiring the development of sophisticated systems to carry out this research and answer the question of how to best design a space greenhouse.

The size, shape and materials used in construction of a space greenhouse would be greatly defined by the total atmospheric pressure required for plant production. Since reduced total pressure does not greatly influence productivity in the long-term if seedlings are used to establish the crops, a useful extension to this investigation would be to further analyze changes in leaf morphology, stomatal conductance and underlying biochemical adaptations to better understand the alteration occurring in response to decreased pressure and oxygen.

Further research should focus on the oxygen partial pressures between 2-7 kPa, to determine the threshold level and to better predict the optimum gaseous composition for plant-based life support systems. Once threshold limits are identified, it may be possible to select particular species and genotypes or to adjust the atmospheric environment and plant culture conditions to establish the optimum range for BLSS functioning.

For accurate evaluations of the optimal low pressure growth environments,
additional studies are required to assess the developmental stages from seed to the next generation of the crop. One must carefully consider the effects on seed production, fruit ripening and subsequent seed viability, ethylene production during fruit ripening, harvest index, respiration and gene expression for a range of candidate crop species.

With international goals to return to the Moon in the near-term, an experimental focus of the mission should be the establishment of a Lunar test base to develop and assess strategies for Martian habitation. This analogue site would allow for the testing of space greenhouse and startup procedures as well as acting as a facility for assessing environmental parameters, material cycling, the degree of closure required and attainable, optimal multi-cropping combinations, harvestable products, processing requirements and ultimately the size of the crew that can be sustained. Based on the results of Lunar simulations, Martian BLSS best practices could be evaluated and any potential risks and problems addressed.
REFERENCES


Guelph, Ontario, University of Guelph. MSc.


APPENDIX

HYPOBARIC CHAMBERS FOR BIOLOGICAL LIFE SUPPORT RESEARCH:
SYSTEM DESIGN AND PERFORMANCE

The Controlled Environment Systems Facility (CESRF) at the University of Guelph is focused on research and development in plant production in closed environments including investigation into the use of plants and microbes in bioregenerative life support systems (BLSS). The facility houses a group of five canopy-scale hypobaric plant growth chambers that are designed to monitor numerous environmental and plant growth parameters and are capable of maintaining a wide variety of atmospheric compositions (Figure A1). For a detailed description of the canopy-scale hypobaric plant growth chambers design and system specifications see (Chamberlain 2004). This report consists of an overview of system design and an examination of the baseline data used to evaluate systems performance under typical experimental conditions.

Plant Material

Radishes (*Raphanus sativa* L. cv. Cherry Bomb II) were grown from seed in rockwool slabs in the CESRF hypobaric chambers. Seeds were spaced in the rockwool at 9-10 cm centres within rows and between trays. Three seeds were planted at each position and allowed to germinate for 72 hours at ambient pressure. Seedlings were then thinned to one seedling per position, resulting in 24 plants in each of the five trays for a total of 120 plants per chamber. Nutrient
Figure A1. One of the five hypobaric plant growth chambers in the Controlled Environment Systems Research Facility at the University of Guelph. Visible are the main door and closing mechanisms, lighting canopy, nutrient system and mass flow controllers used for gas composition control.
solution was provided using a recirculating nutrient film technique (NFT) delivery system with a modified half-strength Hoagland’s solution. A stainless steel tray cover with 4 cm holes at the appropriate spacing was placed over the rockwool to minimize algae growth and reduce evaporation rates. Pressure treatments were an ambient control (approximately 98 kPa), 66, and 33 kPa. Temperature was set at a constant 22 °C with a photoperiod of 16-hours light and 8-hours dark, with a light intensity averaging approximately 300 μmol m⁻² s⁻¹ PAR at the hydroponics tray level. Carbon dioxide was set at 120 Pa (equivalent to 1200 μmol mol⁻¹ at ambient pressure), oxygen was set at 20 kPa, and vapour pressure deficit (VPD) was set at 0.9 kPa (equivalent to 65 % relative humidity at 22 °C).

**Temperature and VPD**

Control of temperature averaged +/- 0.5 °C over the course of an 18 day full closure experiment with 120 radish plants at ambient, 66 kPa, and 33 kPa total pressures (Figure A2). As expected, there were greater deviations in the 33 kPa treatment when the lamps turned on or off due to a rapid change in energy flux and decreased sensible heat exchange to transfer the load to the hot and cold exchangers. VPD remained within +/- 0.5 mb of setpoint for the duration of the experiments (Figure A2), although there were slight fluctuations during the change between day and night due to energy alterations from lamp heat load. As VPD is coupled to temperature, the changes were greater in the 33 kPa pressure treatment. Relative humidity was controlled within +/- 2.5 % of setpoint for the duration of the experiment.
Figure A2. Temperature (°C; top) and vapour pressure deficit (kPa; bottom) control over 18 days of closure with radish (*Raphanus sativa* L. cv. Cherry Bomb II) at 33, 66 and 98 (ambient) kPa total pressure.
Evapotranspiration was measured by passing condensate from the chamber through a tipping bucket. The number of tips were recorded by the data acquisition and control system and converted to litres by calibrating the tipping bucket in mL per tip. At 18 DAP, radish grown at 33 kPa had the highest rate of transpiration (Figure A3).

**Atmospheric Pressure and Gas Composition**

Pressure control utilized one of the three available vacuum pumps (model NC0070.ABMG.000F, Busch Vacuum, Boisbriand, Québec, Canada) while the others provided backup in case of failure. Used together, the three pumps are capable of reducing total pressure to less than 1 kPa. The available control range was +/- 0.1 kPa, however a larger range was used to minimize potential volume losses during heating and cooling cycles which alter system pressure, and could signal a requirement for air removal.

The gas sampling system, for carbon dioxide and oxygen, was based on pressurization of a hypobaric chamber air stream (Figure A4). Chamber air was continuously removed by a vacuum pump (Model UN820.3 FTP, KNF Neuberger Inc., Trenton, New Jersey, USA) and repressurized in a sampling loop. Ambient pressure treatments were not pressurized as use of the vacuum sampling pump at ambient conditions would lower its life expectancy. Prior to introduction of the gas stream to the gas analyzer (Model 200: California Analytical Instruments, Inc., Orange, California, USA), the air stream was chilled to approximately 5 °C.
Figure A3. Total evapotranspiration (litres) over a 24-hour period in 18 day old radish (*Raphanus sativa* L. cv. Cherry Bomb II) grown at 33, 66 and 98 (ambient) kPa total pressure. The growing area was 1.5 m².
Figure A4. Schematic representation of the Controlled Environment Systems Research Facility’s hypobaric gas sampling system. Air is removed from the plant growth chamber with a high vacuum pump, recompressed, dehumidified, and pressure regulated for analysis using a carbon dioxide/oxygen gas analyzer.
to remove water vapor. Condensate and the sampled air stream were returned to the chamber to ensure full system gas loop closure.

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

Pressure levels shown over 18 days of closure of a radish crop demonstrated the high level of control and pressure containment (Figure A5). The highest oscillations were in the ambient treatment, which were the most susceptible to the influence of the atmospheric pressure fluctuations of the ambient environment. System leakage, estimated by measuring the loss in pressure over a 24-hour period, was less than 1 % per day for all pressures (Figure A6).

System control of carbon dioxide levels at 18 days after planting (DAP) of radish was +/- 20 μmol mol\(^{-1}\) and showed a linear injection profile at the three pressure
Figure A5. Pressure (kPa) control over 18 days of full closure with radish (*Raphanus sativa* L. cv. Cherry Bomb II) at 33, 66 and 98 (ambient) kPa total pressure.
Figure A6. Pressure (kPa) changes demonstrating leakage over a 24-hour period at 5, 10, 33 and 66 kPa total pressure. Hydroponics and lighting systems were in operation however no plants were in the chambers.
levels tested (Figure A7). Oxygen levels were not controlled in the same manner as carbon dioxide.

In order to mitigate vacuum system losses due to percent level adjustments in oxygen, required levels were obtained at the beginning of each experiment and then allowed to fluctuate with dark respiration and as growth conditions changed. Adjustments were only made when large deviations in the desired setpoint were observed due to periodic system adjustments or failures.

**Nutrient Delivery**

The nutrient delivery system utilized a NFT design (Figure A8). Water was stored in a 200-liter, temperature-controlled, external stainless steel tank. Dilute nutrient solution was pumped to the chamber troughs, circulated through a sensor loop, and returned to the reservoir by gravity. All external storage tanks for stock nutrients, acid, and base were maintained at chamber pressure through a series of pressure compensation lines. Concentrated acid, base, and nutrient solutions were added using gravity feed from stainless steel reservoirs. Electrical conductivity (EC) sensors were used to measure nutrient concentration. EC control with a setpoint of 1200 μS was +/- 10 μS and pH levels were manually adjusted daily to +/- 0.1 pH units.

**Conclusions**

The CESRF represents a core of analytical infrastructure specifically related to international advanced life support research objectives in the areas of:
Figure A7. Carbon dioxide control (µmol mol⁻¹) and accumulation (mmol) over a 24-hour period in 18 day old radish (*Raphanus sativa* L. cv. Cherry Bomb II) grown at 33 kPa, 66 and 98 (ambient) kPa total pressure.
Figure A8. Schematic representation of the CESRF hypobaric, nutrient film technique, hydroponic delivery system. All wetted components are maintained at chamber pressure through a series of pressure compensation lines. An externally mounted pump moves water from the outer reservoir to distribution lines within the chamber. Water is returned by gravity to the external reservoir.
atmosphere composition and gas phase management, hydroponic nutrient recycling, plant physiological and gene expression adaptation to reduced pressure and other environmental variables, productivity studies, candidate crop selection studies and analytical techniques for measurement of plant and microbial interactions. The current performance assessments on radish have demonstrated temperature control of +/- 0.5 °C, VPD control of +/- 0.5 mb, carbon dioxide injection control of +/- 20 μmol mol\(^{-1}\), and leakage rates of less than 1 % per day. The evaluation of system performance data suggests that the CESRF hypobaric chambers are an effective tool for assessing the effects of modified gas composition and reduced pressure on plant growth and development.