PLANT RESPONSES TO RARIFIED ATMOSPHERES

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Abstract

Reduced atmospheric pressures will likely be used to minimize mass and engineering requirements for plant growth habitats used in extraterrestrial applications. This report provides a brief survey of key literature related to responses of plants to atmospheric variables and a broad rationale for designing minimal atmospheres for future plant growth structures on the Martian surface. The literature and recent work suggest that atmospheric pressure limits for normal plant function are likely to be 10 kPa or perhaps slightly lower. At Kennedy Space Center, a chamber with high vacuum capability was used to design and begin construction of a system for testing plant responses to reduced pressure atmospheres. A test rack with lighting provided by 3, high-pressure sodium vapor lamps was built to conduct measurements of short-term plant responses. Initial experiments with lettuce showed that a pressure of 10 kPa resulted in a 6.1–fold increase in the rate of water loss compared to water loss at ambient pressure (101 kPa). Plants were severely wilted after 30 minutes exposure to 10 kPa, but relative humidity was only 44 %. Water loss was found to be inversely correlated with atmospheric pressure over the range of pressures from 21 to 101 kPa; the rate of water loss at 21 kPa was 4.3 times higher than water loss at ambient pressure. Older leaves showed moderate wilting during exposure to 21 kPa, but those exposed to 46 kPa remained turgid. Relationships between water loss and vapor pressure deficit were nonlinear, suggesting an effect of atmospheric conditions on pathway resistance. Follow-up experiments demonstrated that plant turgidity could be maintained at atmospheric pressures in the range of 10 to 20 kPa, depending on temperature and relative humidity. Further work will be required to separate and clarify the roles of vapor pressure deficit, stomatal conductance, and reduced pressure atmospheres on plant function. Past and present work suggest that deployment of lightweight plant growth structures for Mars using ambient solar flux and atmospheric pressures of one-tenth atmosphere are feasible.

Introduction

Sending organisms to Mars or other extreme environment outposts and sustaining their life-giving functions provides humans with the opportunity to meet one of the major technological challenges of the twenty-first century. There is little doubt that Mars may be recognized as the next frontier. Prior to sending humans to Mars, it will be necessary to develop energetically cost efficient methods for providing them with oxygen, potable water, and food. Early travelers to Mars will likely employ physical-chemical systems to provide the first two requirements with nearly all the necessary food requirements for the journey being launched. Plants may be used to a limited extent primarily to provide supplemental consumables (food, oxygen, and water) to a crew and to help meet human aesthetic and psychological needs.

Long term economic trade-off studies suggest that advanced life support systems for extraterrestrial applications will utilize plants to supply human life support requirements for food, oxygen, and potable water (Barta and Henninger, 1994). There is a need to reevaluate and update such studies to incorporate in situ resource utilization practices for specific scenarios such as reduced atmospheric pressures for plant growth structures on Mars. Even without this rationale, it is certain that plants will at some time in the not too distant future, be a vital and integral component of the human exploration and settlement of space. Life support requirements of plants can be provided more readily from available resources on Mars than those of humans. The Martian atmosphere contains the major essential elements carbon, oxygen, nitrogen, and hydrogen, all of which can be converted into biomass given sufficient solar flux at the Martian surface and an appropriately engineered, controlled environment habitat.

The prospect of plant culture outside Earth environments gives rise to questions regarding the pressure and composition of the atmosphere of growth habitats. If humans and plants share the same atmospheric volumes, then plant culture is constrained by the priority of human requirements. Human requirements will likely not involve oxygen partial pressures below 15 to 20 kPa or total atmospheric pressures below 50 kPa. However, those partial pressure values are fairly conservative given the fact that people are known to adapt to much lower partial pressures of oxygen such as those experienced at high altitude villages in the Himalayas and Andes mountain regions.

It may not be necessary to consider habitats designed for integration of plants and people since pressures selected for human habitation are probably not those that would be optimum for growth of plants. Hypobaric pressures will likely be used to decrease the mass and engineering requirements for establishing and sustaining plant growth habitats on extraterrestrial outposts (Figure 1). The major engineering consideration justifying the use of hypobaric pressures is that the structural requirements to contain a pressure gradient decrease with decreasing pressure. Therefore, a premise of this paper is to consider the use of reduced pressure atmospheres in autonomous plant growth structures [Boston, 1981; Clawson et al., 1999] that would be isolated from human habitation, and provide, in early phase advanced human life support systems, a back-up or perhaps lifeboat to physical-chemical systems. With such a premise, it follows that it will be necessary to define the limits of atmospheric pressure and partial pressures of oxygen and carbon dioxide for growth of plants. Questions related to exploring theoretical and practical limits of atmospheric variables (pressure and composition) for plant growth also have relevance to numerous other fields related to advanced life support program goals (Figure 1).

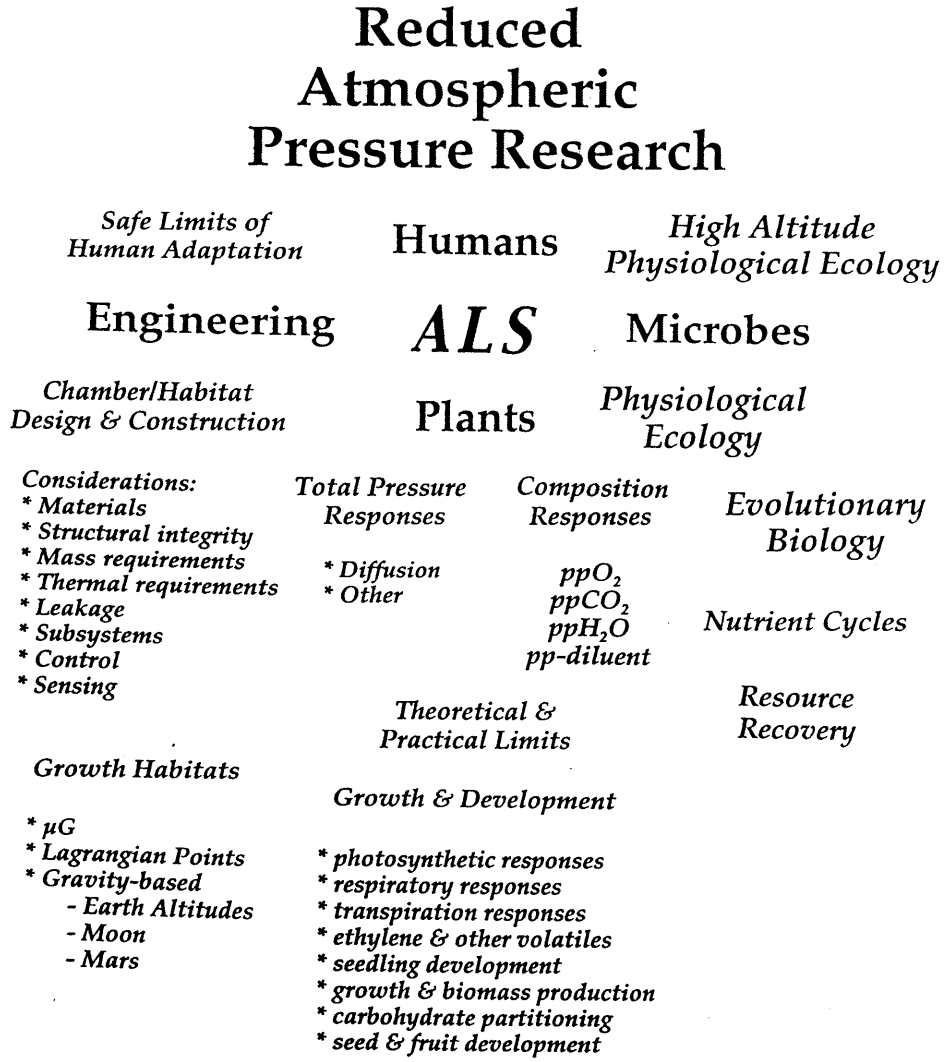

Figure 1. Interdisciplinary nature of advanced life support systems goals and fields of scientific endeavor relevant to reduced atmospheric pressure research with emphasis on future considerations for designing atmospheres for plant growth in extraterrestrial habitats.

Historical

High altitude regions provide us with an excellent Earth-based analog for reduced pressure studies over a limited pressure range. The equation $P = P_0 (1 - 0.0065\ A/288)^{5.255}$ expresses the relationship of total atmospheric pressure with increasing altitude and is illustrated in Figure 2. The curves are expressed over the range of sea level atmospheric pressure up to an altitude of over 30,000 meters or the approximate equivalent of the Martian atmospheric pressure (~ 1 kPa). The summit of Mt. Everest, while rarefied for terrestrial Earth locations, nevertheless has about one-third atmosphere pressure. Based upon theoretical considerations of atmospheric pressure, diffusion, and stomatal resistance relationships, Gale (1972) tested the idea that decreased $ppCO_2$ with increasing altitude led to decreased availability of carbon dioxide for photosynthesis. He concluded that the increased rate of CO_2 diffusion with decreasing pressure should compensate for the effect of lowered $ppCO_2$ with decreasing pressure. Experimental work confirmed this prediction for corn and bean and also demonstrated enhancement of transpiration rates with decreasing pressure (Gale, 1973). The relationship of diffusion rates of carbon dioxide and water vapor with total atmospheric pressure can be expressed by the following equation: $D' = D^0(T'/T^0)^m(760/P')$. Figure 3 illustrates this relationship as a ratio of diffusivity at some pressure, P, with that of ambient sea level pressure (101 kPa). Thus, possible enhancements of rates of carbon dioxide uptake and transpiration with decreasing pressure are predicted based upon enhanced diffusivity of gases (e.g. D at 10 kPa ~ 10 D at 101 kPa).

Several test facilities have been used to assess metabolic and developmental responses of plants to reduced pressure [Andre and Massimino, 1992; Corey et al., 1996, 1997a, 1997b, 1999; Daunicht and Brinkjans, 1992; Ohta et al., 1993; Rule and Staby, 1981, Schwartzkopf and Mancinelli, 1991]. However, most studies thus far have not provided clear separation of pressure and oxygen effects, nor have they involved complete growth tests of large plant samples for assessment of yield. On the basis of enhanced diffusion of gases at reduced pressure, water flux may increase. Although enhancements of water flux with decreased pressures have been documented for several plants, separation of effects of total pressure and water vapor pressure deficit have not been clear.

If reduced pressure is also accompanied by reduced partial pressure of oxygen, enhancement of net photosynthesis and growth may occur through a reduction in carbon loss by suppression of photorespiration. Evolutionary biologists generally recognize that organisms with photosynthetic capacity existed and evolved during a time in geologic history when the partial pressure of oxygen was very low. Thus, the biochemistry of plant metabolism evolved under low partial pressures of oxygen. In 1920, Warburg observed that oxygen release by illuminated *Chlorella* was inhibited by oxygen; the discovery that the photosynthetic mechanism was poisoned by the oxygen it had released. By the 1950's and 1960's, the biochemical pathways for this photoinhibition by oxygen were elucidated. Most lower and higher plants studied thus far, possess this carbon-wasteful process known as photorespiration. Direct and indirect effects of oxygen on photorespiration and growth of C_3 pathway plants are well documented [Bjorkman, 1966; Gerbaud and Andre, 1989; Musgrave et al., 1988, Musgrave and Strain, 1988; Parkinson et al., 1974; Siegel, 1961] and have been reviewed [Ehleringer, 1979; Jackson and Volk, 1970; Quebedeaux and Hardy, 1976]. Photorespiratory carbon losses may be minimized by either low oxygen (i.e. 2 - 5 kPa) or high partial pressures of carbon dioxide (>100 Pa).

Andre and Richaud (1986) and Andre and Massimino (1992) determined physiological responses of wheat to pressures as low as 7 kPa. Their objectives were to determine if plants can grow in a quasi-vacuum and if there was a need for a diluent gas (e.g. nitrogen). Their work demonstrated that wheat was insensitive to depressurization, that an inert 'diluent' gas is unnecessary, and that water loss was accelerated. With lettuce [Corey et al., 1996], carbon dioxide uptake was found to be nearly constant in the range of 50 to 100 kPa atmospheric pressure and that enhancement of photosynthesis with reduced pressure in this range was primarily due to decreased partial pressure of oxygen. A 34-day test and complete growth tests of wheat in Johnson Space Center's Variable Pressure Growth Chamber demonstrated enhancements of photosynthesis and transpiration at 70 kPa atmospheric pressure and reduced ppO_2 of 14 kPa [Corey et al., 1996b, Corey et al., 2000]. Whole stands of wheat exhibit marked responses to ppO_2 down to 5 kPa (Figure 4) despite $ppCO_2$ in excess of 100 Pa (Corey, 2000, unpublished data). Enhanced photosynthesis in those tests also translated into increased total biomass. Thus, the use of reduced pressure atmospheres also provides an additional rationale for modification of gas compositions (O_2, CO_2, H_2O, and diluent) to optimize plant growth and development.

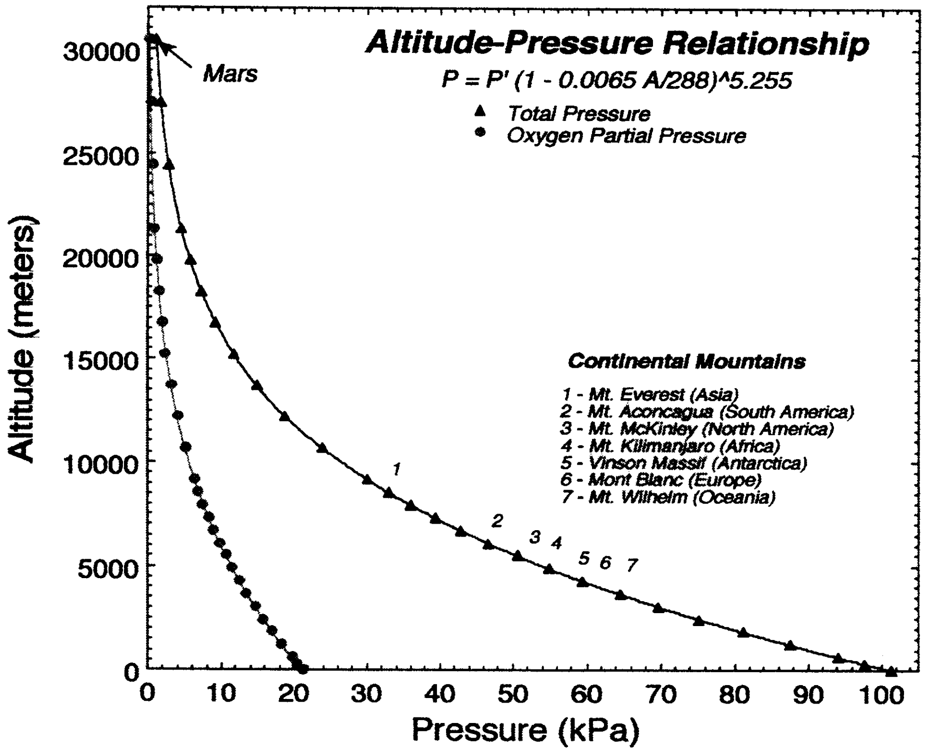

Figure 2. Relationships of total atmospheric pressure and partial pressure of oxygen with altitude. Values for total pressure were calculated after Gale (1972) and values for oxygen partial pressure were estimated assuming a constant mole fraction of 0.209.

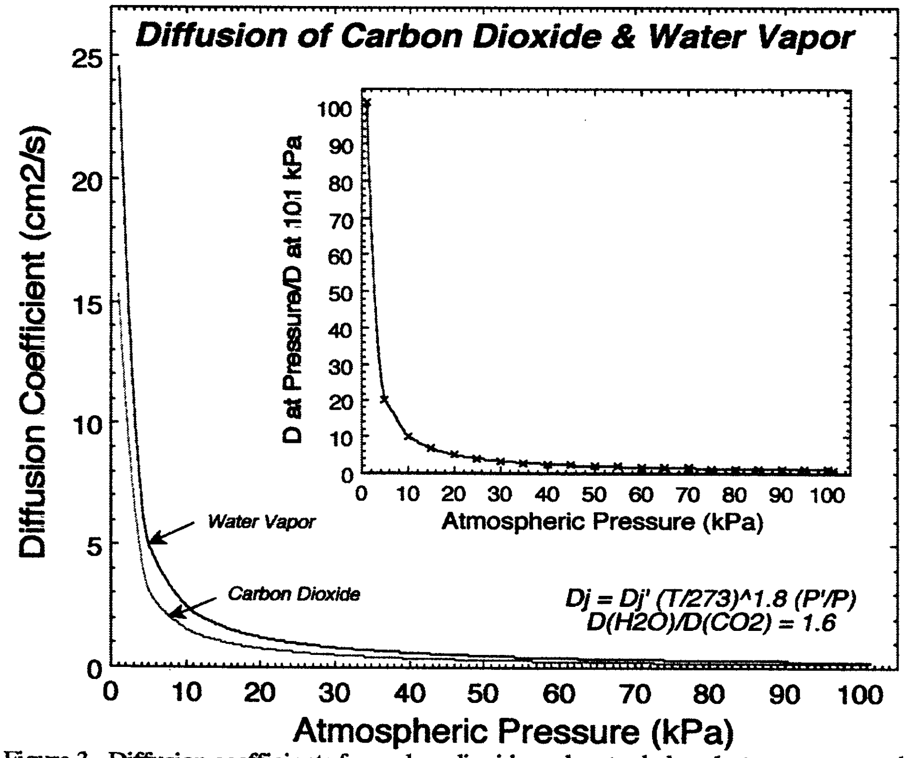

Figure 3. Diffusion coefficients for carbon dioxide and water below 1 atm pressure and the ratio of diffusivities at some pressure, P, to those at mean sea level pressure.

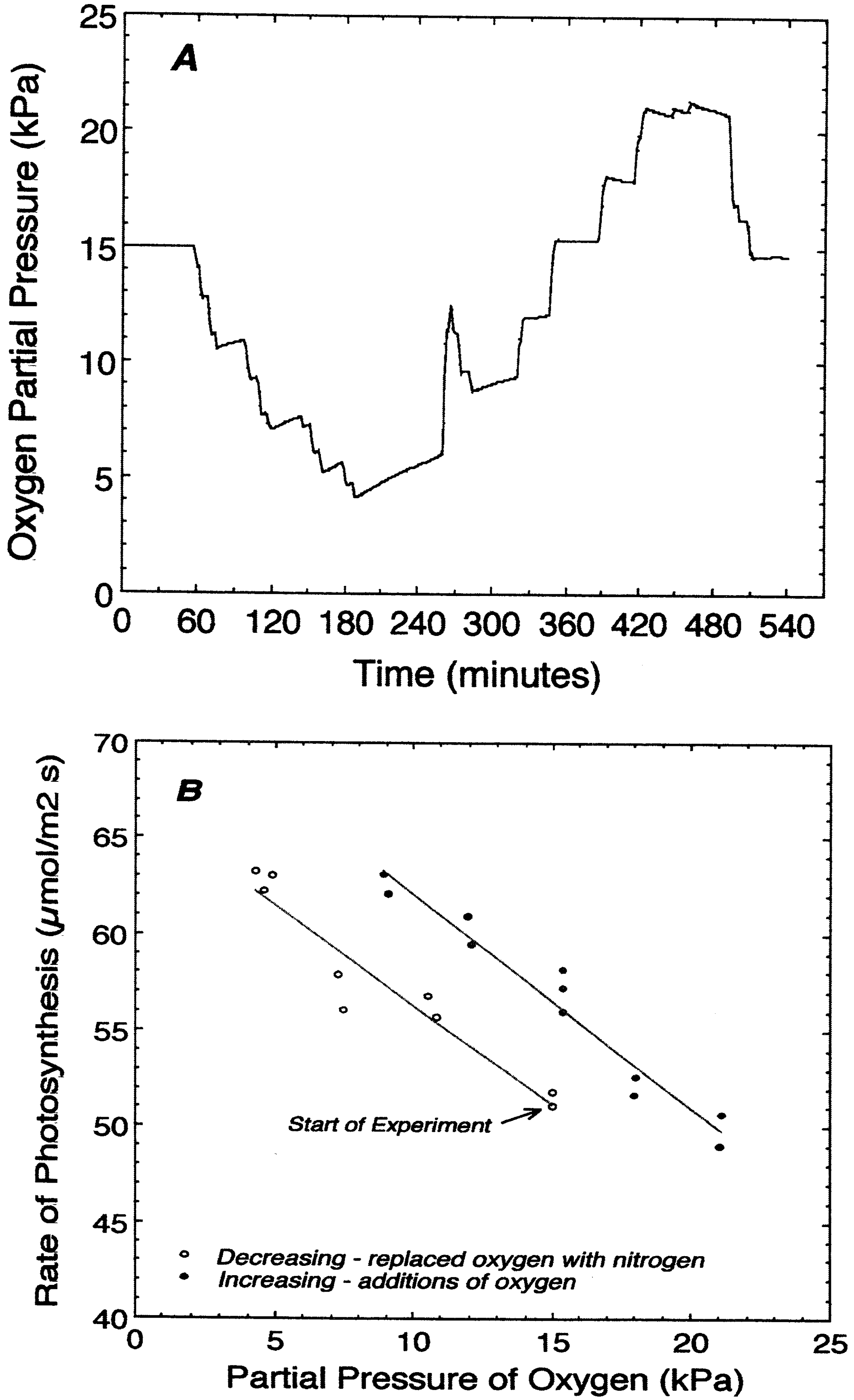

Figure 4. Example of changes made in partial pressure of oxygen of the VPGC atmosphere (A) during the conduct of experiments to determine photosynthetic responses of wheat stands to oxygen (B) [Corey et al, 2000].

Thermotron Studies at Kennedy Space Center

During the summer of 1999, low pressure studies were initiated at the Kennedy Space Center as part of a Mars inflatable greenhouse project. A broad goal of the work is to define limits of total pressure and composition for plant growth. Short-term plant responses to reduced pressure atmospheres were measured in a thermal vacuum chamber or Thermotron (Thermotron Industries, Holland, Michigan), a high vacuum chamber rated for 1 torr and thermal control in the range of –72 to 177 C. Effective internal dimensions of the chamber are 1.22 m wide X 1.22 m high X 1.62 m deep. Fans and motors are housed internally in the rear of the chamber and the vacuum pump is located external to the chamber. Temperature and pressure measurements inside the chamber were made with thermocouples and a Barotron pressure transducer, respectively.

Chamber Leakage Measurement

Vacuum chambers have penetrations and seals that usually result in some leakage of external air into the chamber and evacuation of internal gases to maintain constant pressure. Measurements involving the rates of uptake or evolution of a gas such as carbon dioxide during plant photosynthesis and respiration will be affected by significant leakage rates and therefore must be measured. The most rapid and straightforward method for leakage measurement is to disable the vacuum pump and follow the pressure increase over time. Evaluation of the first derivative of this function at the pressure of interest will give a chamber leakage value that can be applied to making corrections to measurements of plant metabolic rates. A detailed treatment of leakage measurements, calculations, and application to gas exchange measurements at reduced pressure has been reported [Corey et al., 1997a; Corey et al., 2000].

The first of such rate of rise tests for the Thermotron was conducted on June 16, 1999. The chamber was pumped down to 17 mm Hg, the pump disabled, and the pressure allowed to increase up to 86 mm Hg. Chamber temperature during the test was between 20 and 21 C. The leakage rate was measured to be 0.46 mm Hg/min. Using a previously determined volume measurement of 3382 liters (Corey, 1999), the air leakage rate of the chamber, L_a was calculated as 0.87 chamber volumes per day at 20 C and pressures < 10 kPa. Based upon previous experiences with gas exchange measurements at reduced pressure and rough calculations, this value is low enough to permit sensitive measurements of CO_2 uptake measurements, given a sufficient plant sample size. The plant sample size required for the acquisition of short-term measurements of good sensitivity and reasonable duration (<30 minutes) for the Thermotron is likely in the range of 0.5 to 1.0 m^2 area.

Plant Test Stand

A rack was built to accommodate the space and light requirements for measuring short term plant responses to reduced pressures. Three, 400-W high pressure sodium (HPS) vapor lamps were mounted on a rack that measured 112 cm wide X 152 cm deep X 116 cm high. Photosynthetic photon flux measurements were made with a LICOR quantum sensor and gave values in the range of 300 to 400 $\mu mol/m^2s$ depending on position and canopy height; more than adequate for testing short-term physiological responses or for growth of lettuce plants. The first phase involved testing small samples of lettuce (*Lactuca sativus* cultivar Waldeman's Green) plants grown in a controlled environment growth chamber. Plants were grown at a temperature of 22 C, 75 % relative humidity, a photosynthetic photon flux of 260 $\mu mol\ m^{-2}\ s^{-1}$, a $ppCO_2$ of 120 Pa, and a light/dark cycle of 18 hr/6 hr. Seeds were sown in a solid medium (1:1 peat-vermiculite mix), transplanted as seedlings into the same medium, and grown in 15-cm diameter plastic pots. Plants were fertilized with half-strength modified Hoagland's solution every other day until 15 days-old, and every day thereafter.

Lettuce Transpiration Experiments

The first chamber test with lettuce involved placing 2 plants on a scale (0.1 g sensitivity) and monitoring weight loss at ambient pressure, followed by pumping the chamber down to a pressure of 10 kPa. Plants were watered to bring the soil up to an approximate field capacity moisture content prior to the start of the experiment, and then placed in plastic bags that were tucked loosely under the foliage to minimize the evaporative water loss component. Temperature control for the comparison was excellent, but relative humidity was lower at reduced pressure (~44 %). Weight loss was over 6-fold higher at 10 kPa pressure and plants exhibited severe wilting from which they recovered fully in about 30 minutes after return to ambient pressure. The next test involved an incremental step down in atmospheric pressure from ambient with plants held at each pressure for about 30 minutes each. Since plants exhibited severe wilting at 10 kPa, the lowest pressure treatment selected was 21 kPa. Rate of water loss increased with decreasing pressure; the rate at 156 mm Hg (~ 21 kPa) being about 4.3-fold higher than the water loss at ambient pressure (Figure 5A & 5B). Over the range of 156 to 766 mm Hg, the rate of water loss was inversely correlated with pressure (Figure 5B). Relative humidity was controlled fairly well, though it was lower (68 %) for the 156 mm Hg treatment than the average of 76 % across all treatments. The relationships of water loss and vapor pressure deficit for those experiments were nonlinear (data not shown) suggesting an effect of atmospheric conditions on stomatal resistance.

The next experiment simply involved a partial repetition of the previous experiment with a direct comparison of ambient atmospheric pressure and 147 mm Hg (21 kPa). At the end of the experiment, all leaves of each plant were detached and area determinations made with a LICOR portable area meter (model LI-300A). Results of the previous experiment were confirmed, with water loss expressed on a leaf area basis being 6.8 times higher at 147 mm Hg than that of ambient pressure (Table 1). Only slight wilting of the older leaves was observed on the low pressure treatment. Several months later, better control of relative humidity and $ppCO_2$ enabled measurements at pressures down to 10 kPa. This particular batch of lettuce plants held at 10 kPa and 75 % relative humidity for several hours exhibited no signs of wilting.

Table 1. Water loss from lettuce plants held for 30 minutes in the Thermotron at ambient and reduced atmospheric pressures.

Pressure[a] (mm Hg)	Temperature[b] (C)	Relative Humidity[b] (%)	Water Loss[c] (mg/min·m^2)
777 ± 0.1	22.9 ± 0.3	81 ± 3	77
147 ± 1.0	22.8 ± 0.1	73 ± 5	522

[a] Values represent means of 7 readings ± 1 S.D. taken over a period of 30 minutes.
[b] Values represent means of 2 instruments and 7 readings each ± 1 S.D. taken over a period of 30 minutes.
[c] Water loss was expressed on the basis of an average leaf area of 0.31 ± 0.03 m^2/plant.

Future Directions in Reduced Pressure Research

This report presents a brief overview of the rationale, history, and current directions of research on plant responses to reduced atmospheric pressure for extraterrestrial crop production. If the atmospheric requirements of plants are considered, the two broad categories of necessary research are to define the limits of total pressure and of partial pressures of oxygen, carbon dioxide, water vapor, and diluent gas (Figure 1). The simulation of reduced atmospheric pressure environments involves the use of vacuum systems or the use of terrestrial, Earth-based analogs such as mountains or high altitude flights. The terrestrial analog limit of pressure is about one-third atmosphere. Testing the limits of plant function and growth and attempting to make the delta pressure for a Martian growth structure a minimum, will involve testing at pressures in the range of 2 to 20 kPa. Perhaps short-term responses could be measured using high, constant altitude flight experiments conducted at altitudes of 13,000 to 30,000 meters.

Based upon current work at KSC, it appears that lettuce will be able to tolerate pressures as low as 10 kPa without wilting, provided that high moisture in the root zone and high humidity in the atmosphere are maintained. The preliminary tests of the Thermotron experiments did not involve control of carbon dioxide partial pressure, a variable known to effect stomatal physiology. Therefore, future tests will require modifications that will enable carbon dioxide measurement, injection, and control to hold partial pressure constant for comparisons of different atmospheric pressures.

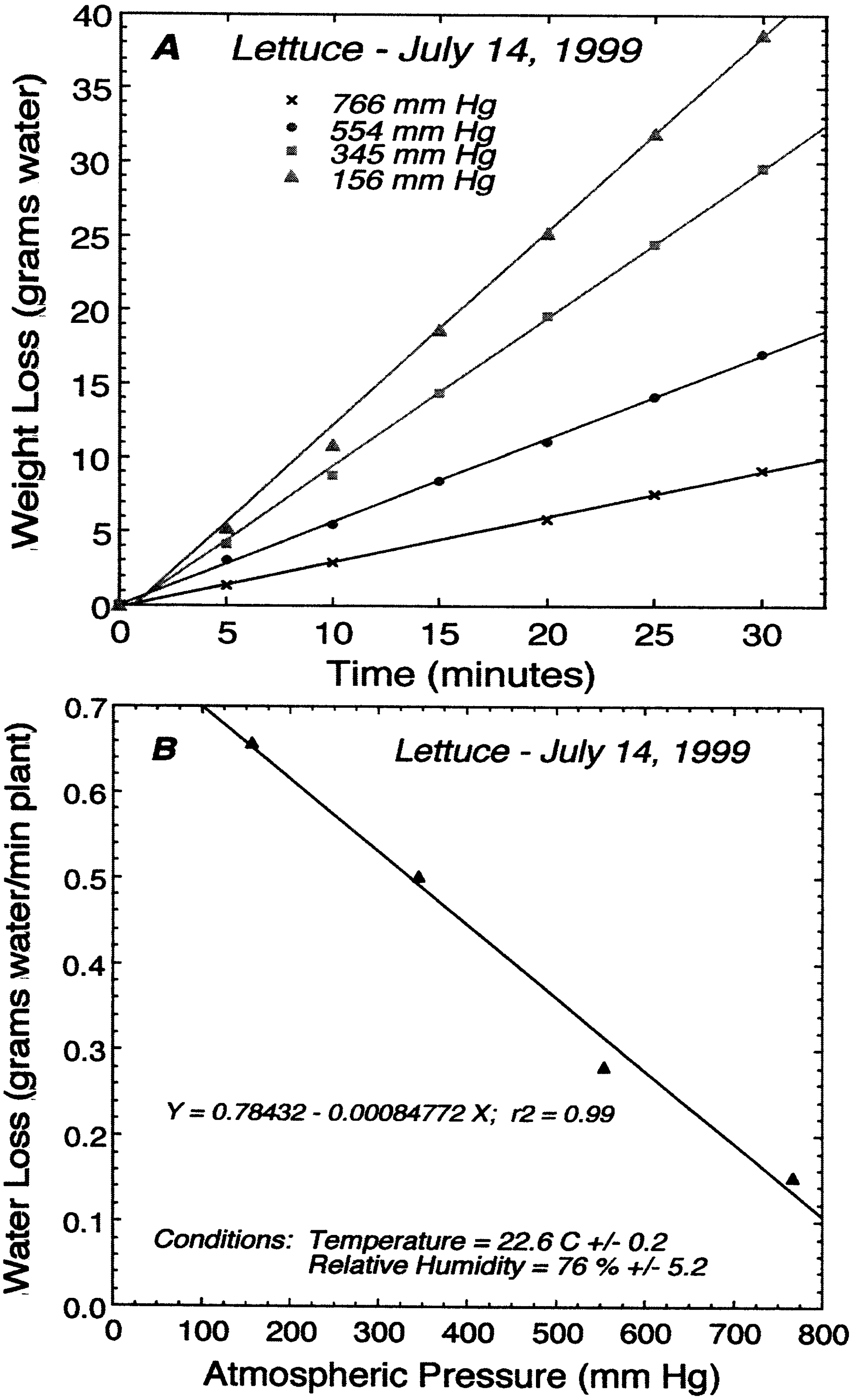

Figure 5. Changes in weight of lettuce plants exposed to progressive reductions in atmospheric pressure (A) and the relationship of water loss to atmospheric pressure (B).

In future studies, it will be important to have a higher degree of control of relative humidity and to be able to control at a higher value (>90 %). The lower limits of atmospheric pressure attainable without adverse effects to plants will depend largely on temperature and relative humidity, the primary factors controlling the leaf-to-atmosphere vapor pressure deficit. Higher relative humidity and lower temperature will both have the effect of decreasing the gradient for water transfer from the leaf to the atmosphere. Considerable research will be needed to determine the safe limits for plant growth at low pressure for growth from seed to harvest maturity of different species. Perhaps development and growth from seed will lead to developmental, morphological, and physiological adaptations to the reduced atmospheric pressure environment.

Beyond such studies, there will be additional needs to control other atmospheric gases such as oxygen and nitrogen, construct an appropriate hydroponic nutrient delivery system, and monitor key atmospheric and nutrient solution variables. Following testing with at least two crop species, it will then be possible to use results of such tests to define some of the requirements for inflatable structures and specifically for near term prototype testing of such structures on ISS or on the Moon. In the long run, it will be economically and psychologically necessary to have plants, microbial life, and other life forms connected with the human settlement beyond the boundaries of planet Earth.

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