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TESTING PLANT RESPONSES TO RARIFIED ATMOSPHERES FOR INFLATABLE GREENHOUSES

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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. A chamber with high vacuum capability was used to design and begin construction of a system for testing plant responses to reduced pressure atmospheres. Several preliminary tests were conducted to evaluate chamber suitability for plant tests and to determine performance of thermal and vacuum systems at ambient and reduced pressure atmospheres down to 0.1 atm. The first tests consisted of measurements of internal gas volume and leakage rate. The method for volume determination was quite sensitive and will be needed for plant gas exchange measurements and calculations. This information will also be used in conjunction with the leak rate. Measured leak rates on the order of 0.46 mm Hg/min at 76 mm Hg pressure are low enough to conduct sensitive carbon dioxide exchange rate measurements at reduced pressure given an adequate plant sample (0.5 to 1.0 m^2 area). A test rack with lighting provided by 3, high-pressure sodium vapor lamps was built to accommodate both short-term and long-term plant responses. Initial short-term experiments with lettuce showed that a pressure of 77 mm Hg resulted in a 6.1–fold increase in the rate of water loss compared to water loss at ambient pressure. Plants were severely wilted after 30 minutes exposure to 77 mm Hg. Water loss was found to be inversely correlated with atmospheric pressure over the range of pressures from 0.2 to 1.0 atm; the rate of water loss at 0.2 atm was 4.3 times higher than water loss at ambient pressure. Older leaves showed moderate wilting during exposure to 156 mm Hg, but those exposed to 345 mm Hg remained turgid. Results suggest a reduced atmospheric pressure limit of 0.2 to 0.3 atm for lettuce grown in a solid medium. Follow-up experiments with carbon dioxide control and control at high relative humidity (> 90 %) will be needed to further confirm and define safe reduced pressure limits that are feasible for plant tolerance and growth.

SYSTEM DESIGN AND TESTING OF PLANT RESPONSES TO RARIFIED ATMOSPHERES FOR INFLATABLE GREENHOUSES

Kenneth A. Corey

1. INTRODUCTION

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 [3]. 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 inflatable 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. Given the certainty of plant culture outside Earth environments, questions arise 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. These partial pressure values are fairly conservative given the fact that humans are known to adapt to much lower partial pressures of oxygen such as those experienced at high altitude villages (e.g. Jiachan, Tibet).

It may not be necessary to consider habitats designed for integration of both plants and people since pressures selected for human habitation are probably not those that would be optimum for growth of plants. Also, in order to optimize the generation of human resource requirements by bioregenerative means, structures that optimize the total volume to growth area ratio are desirable (< 2). Furthermore, structural requirements to contain a pressure gradient decrease with decreasing pressure. Therefore, a premise of this work is to consider the use of reduced pressure atmospheres in autonomous plant growth structures [2,5] 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.

Several test facilities have been used to assess metabolic and developmental responses of plants to reduced pressure [1,6-10,12,18,20,22]. However, most studies thus far have not provided clear separation of pressure and oxygen effects, nor have they involved complete growouts of large plant samples for assessment of yield. On the basis of enhanced diffusion of gases at reduced pressure, it is expected that water flux will increase. This effect has been documented in several studies [6,8,10,12,18]. 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 suppressing photorespiration. Photorespiratory effects in C_3 pathway plants such as wheat are well documented [4,13,15,16,19,21] and have been reviewed [11,14,17]. Thus, reduced pressure atmospheres provide an additional rationale for testing responses of entire crop stands to reductions in oxygen partial pressure.

2. OBJECTIVES

The objectives of the current research program are to: 1) test the performance of the thermal and pressure control capabilities of the Thermotron, 2) make volume and leak rate determinations to evaluate the suitability of the chamber for use in plant gas exchange measurements, 3) conduct preliminary experiments with small samples of plants to determine the relationship of water flux (transpiration measurements) with pressure and to determine low pressure limits for lettuce, and 4) begin the design and construction of a reduced pressure testbed for the conduct of plant growth experiments in rarefied atmospheres.

3. EXPERIMENTS

3.1 Vacuum Chamber

The thermal vacuum chamber or Thermotron (Thermotron Industries, Holland, Michigan) is 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. The blower and motor are housed internally in the rear of the chamber. The vacuum pump is located external to the chamber. Temperature and pressure measurements were made with thermocouples and a Barotron pressure transducer, respectively.

3.2 Volume Measurement

A first step in the preparation for measurement of rates of gas exchange of plants is a sensitive measurement of the total free gas volume of the atmosphere in the chamber. Since the chamber contains numerous irregular objects such as fans, motors, and blowers, it is not feasible to make internal atmospheric volume determinations by measurements of dimensions and geometries. A practical approach to such a measurement is to introduce a known quantity of gas and measure the change in concentration of the gas associated with the added quantity of gas, followed by application of the ideal gas law. At this time, there are no penetrations with injection ports to permit this procedure. Therefore, an alternative approach was used to circumvent this limitation. A preliminary calculation of the mass of carbon dioxide required to bring the chamber volume to a concentration of 1000 ppm based on an approximate volume of 3.5 cubic meters was made. A piece of dry ice (initial weight = 3.370 grams) was placed on a scale with an accuracy of 10^{-3} grams. A LICOR infrared gas analyzer was used to measure the concentration of carbon dioxide. A small fan was used to ensure rapid mixing of the atmosphere as the carbon dioxide sublimed. Readings of dry ice weight and carbon dioxide concentration were made until all of the initial weight had sublimed. The experiment was carried out under isothermal and isobaric conditions; the average temperature and pressure during the test were 20 C and 765 mm Hg, respectively. The change in weight of dry ice stopped at 0.05 grams, presumably because a small quantity of water condensed out from the atmosphere by the local air cooling of the dry ice or because there was water associated within the structure of the piece of dry ice. Results of the measurements indicated an excellent linear fit of the data yielding a slope of 6.2×10^{-3} grams/ppm (Figure 1A). Volume of the chamber was calculated from eq. [5] using the following steps. Let Δv = volume of CO_2 added and written as:

$$\Delta v = \Delta C \, V_{ch} \qquad [1],$$

where ΔC is the change in CO_2 concentration and V_{ch} is the chamber volume. This equation may also be written as:

$$\Delta v = \Delta w \, V_i / M \qquad [2],$$

where Δw is the change in mass of dry ice, V_i is the molar volume of an ideal gas, and M is the molecular weight of CO_2. From the ideal gas law,

$$V_i = n \, R \, T / P \qquad [3]$$

Rearrangement of eq.[1] as

$$V_{ch} = \Delta v / \Delta C \qquad [4]$$

and substituting eq. [2] and [3] into [4] gives

$$V_{ch} = \Delta w \, n \, R \, T / \Delta C \, M \, P \qquad [5]$$

The volume calculated from eq. [5] was 3,382 liters. This value is reasonable since it is appropriately less than a volume of 3,577 liters calculated by straight dimension measurements assuming a box geometry and not subtracting out the volumes occupied by equipment, motors, blower, and other irregular objects. This straightforward method of volume measurement appears quite sensitive, is rapid, and could be repeated easily when additional equipment is added to the chamber (i.e. test rack, lights, plant growth system, etc.). In order to

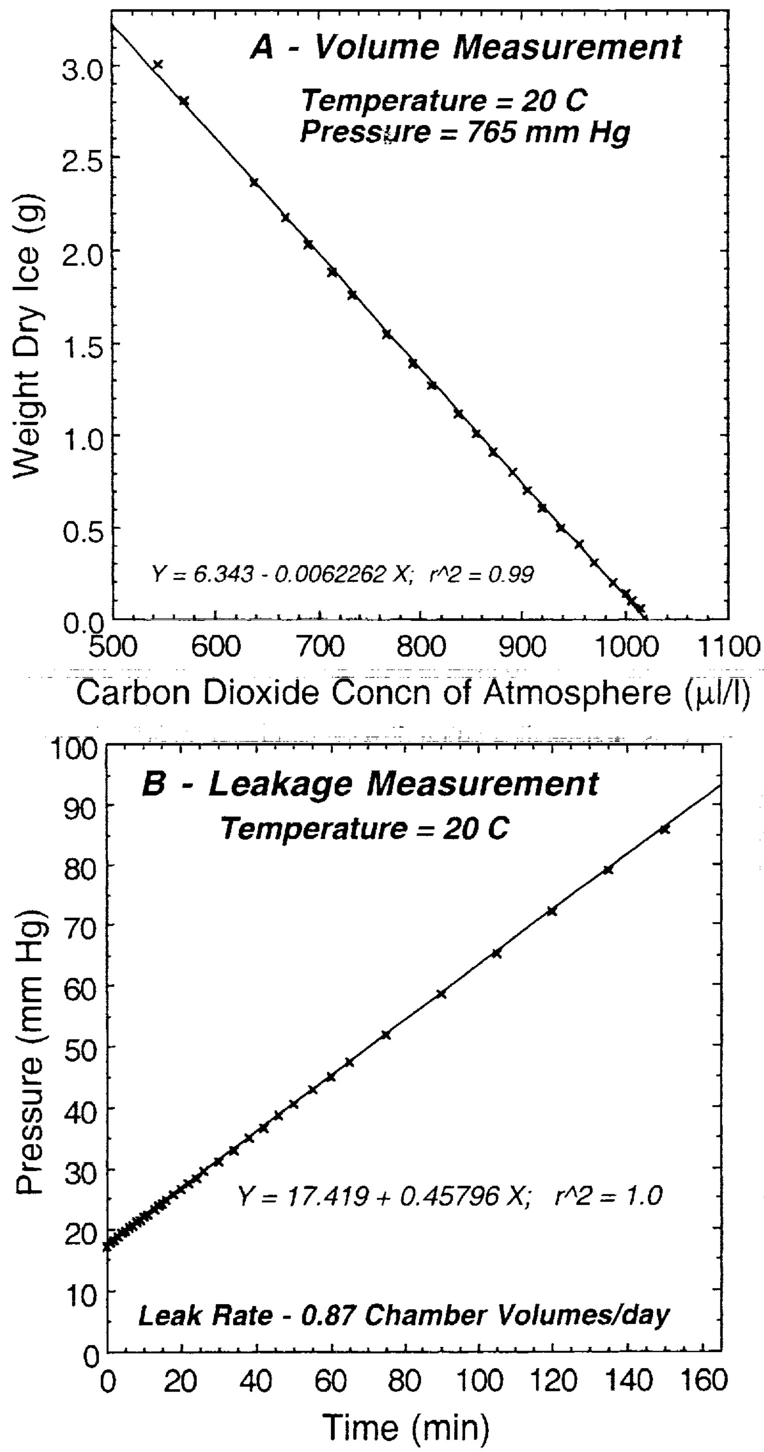

Figure 1. Changes in carbon dioxide concentration and weight of dry ice as it sublimed into the Thermotron atmosphere (A) and changes in atmospheric pressure in the Thermotron due to leakage (B).

apply equations for calculating plant gas exchange measurements, the volume measurement so obtained may be used in combination with leakage measurements.

3.3 *Chamber Leakage Measurement*

Vacuum chambers have penetrations and seals that may lead to leakage of external air into the chamber. 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 has been reported [6,7].

Results of the first of such rate of rise tests 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 (Figure 1B). Using the volume measurement, the air leakage rate of the chamber, L_a, was calculated as 0.87 chamber volumes per day at 20 C. Based upon previous experiences with gas exchange measurements at reduced pressure [8], 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 for the Thermotron is in the range of 0.5 to 1.0 m^2 area.

3.4 *Plant Test Stand*

The development of a test stand for this project involves two phases; the first to conduct short term determinations of physiological responses of crop plants to atmospheric pressure and composition. These experiments will involve measurements of water flux (transpiration) and rates of photosynthesis and dark respiration. The second phase will involve determination of longer-term growth responses in addition to the gas exchange measurements mentioned. A rack was built to accommodate the space and light requirements for both phases. 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 CO_2 of 1200 ppm 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.

3.5 *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 77 mm Hg. 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 (~30 %). Weight loss was over 6-fold higher at 77 mm Hg pressure and plants exhibited severe wilting from which they recovered fully in about 30 minutes after return to ambient pressure (Figure 2). 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 77 mm Hg, the lowest pressure treatment selected was 156 mm Hg (~0.2 atm). There was a progressive increase in rate of water loss with decreasing pressure; the rate at 156 (~0.2 atm) being about 4.3-fold higher than the water loss at ambient pressure (Figure 3A & 3B). Over the range of 156 to 766 mm Hg, the rate of water loss was inversely correlated with pressure (Figure 3B). 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 difference in the leaf to atmosphere vapor pressure deficit for that difference in relative humidity is presumed to be small in comparison to the pressure effect.

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. The next experiment simply involved a partial repetition of the previous experiment with a direct comparison of ambient atmospheric pressure and 147 mm Hg. 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).

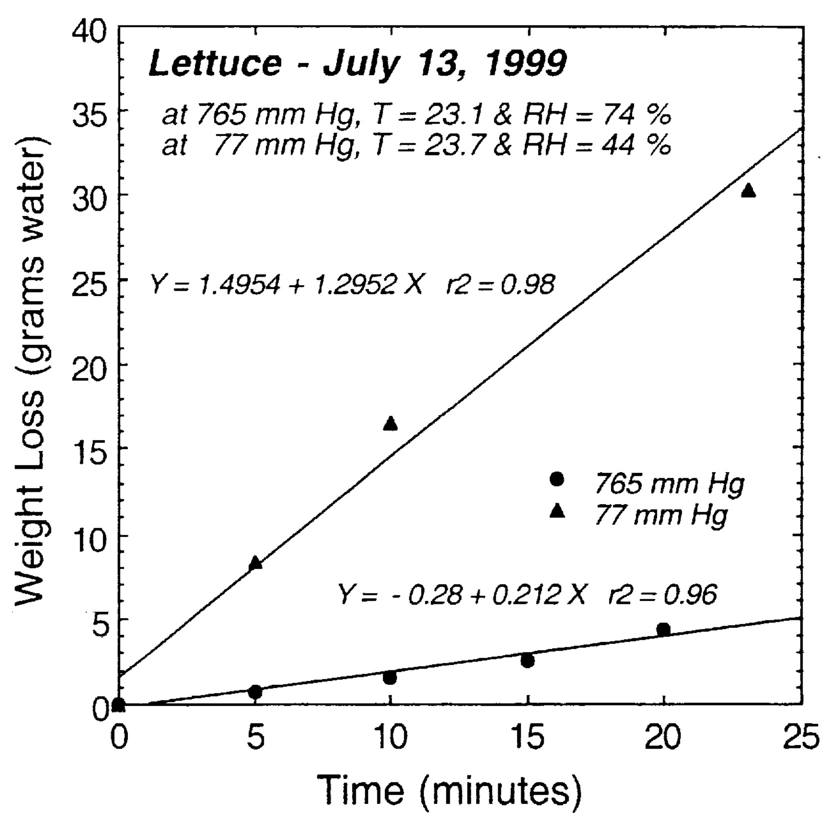

Figure 2. Changes in weight of lettuce plants exposed to ambient and 77 mm Hg atmospheric pressures.

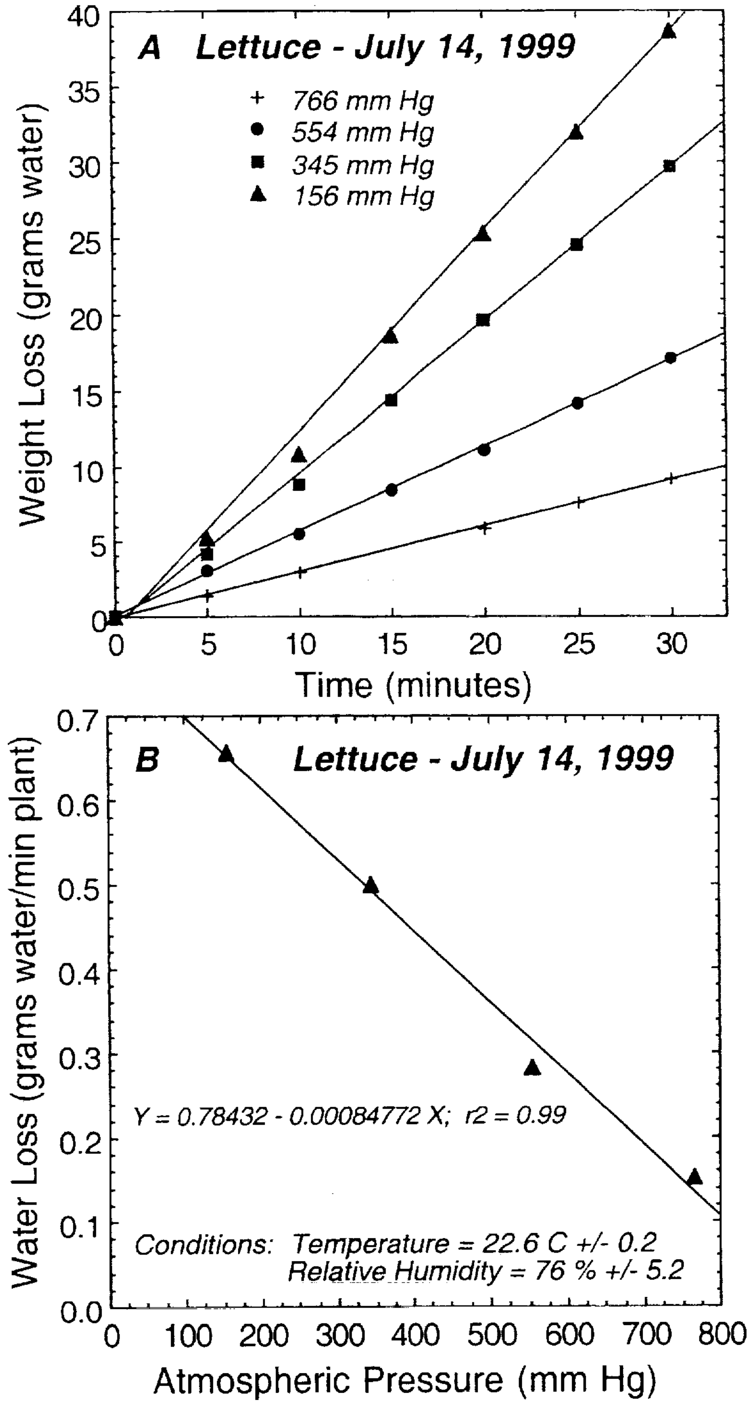

Figure 3. Changes in weight of lettuce plants exposed to progressive reductions in atmospheric pressure (A) and the relationship of water loss to atmopheric pressure (B).

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 $\pm$ 0.1	22.9 $\pm$ 0.3	81 $\pm$ 3	77
147 $\pm$ 1.0	22.8 $\pm$ 0.1	73 $\pm$ 5	522

[a]Values represent means of 7 readings $\pm$ 1 S.D. taken over a period of 30 minutes.
[b]Values represent means of 2 instruments and 7 readings each $\pm$ 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 $\pm$ 0.03 m^2/plant.

4. FUTURE DIRECTIONS

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 (>95 %). The lower limits of atmospheric pressure attainable without adverse effects to plants will depend largely on temperature and relative humidity, the 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. Additional experiments will be designed to determine the safe limits for plant growth at low pressure. It will also be necessary to determine germination of seed and seedling development at low pressures. Perhaps development and growth from seed will lead to developmental, morphological, and physiological adaptations to the reduced atmospheric pressure environment.

Based upon the current study, it appears that lettuce will be able to tolerate pressures as low as 0.25 to 0.35 atm without wilting, provided that high moisture in the root zone and high humidity in the atmosphere are maintained. The preliminary tests of the current study 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. 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. Sensing and monitoring equipment requirements are proposed that assume a broad flexibility in experimental objectives and treatment ranges and will be necessary for accompanying plans to measure rates of transpiration, photosynthesis, and dark respiration. They include sensing capabilities for partial pressures of CO_2 and O_2, total atmospheric pressure, temperature, relative humidity, dissolved oxygen, pH, and solution conductivity (Table 2). 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.

Table 2. Sensor requirements for reduced pressure plant test stand.

Measurement	*Range*
Atmosphere	
Pressure	2 – 50 kPa
Temperature	10 – 30 C
Light Intensity	50 – 800 μmol/m^{2}s
Relative Humidity	50 – 95 %
Carbon Dioxide Partial Pressure	0.01 – 10 kPa
Oxygen Partial Pressure	0 – 10 kPa
Nutrient Solution	
pH	5.0 – 7.0
Conductivity	500 – 2000 μmho/cm
Dissolved Oxygen	0.1 to 9.0 ppm

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