DESIGN OF PLANT GAS EXCHANGE EXPERIMENTS IN A VARIABLE PRESSURE GROWTH CHAMBER

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ABSTRACT

Sustainable human presence in extreme environments such as lunar and martian bases will require bioregenerative components to human life support systems where plants are used for generation of oxygen, food, and water. Reduced atmospheric pressures will be used to minimize mass and engineering requirements. Few studies have assessed the metabolic and developmental responses of plants to reduced pressure and varied oxygen atmospheres. The first tests of hypobaric pressures on plant gas exchange and biomass production at the Johnson Space Center will be initiated in January 1996 in the Variable Pressure Growth Chamber (VPGC), a large, closed plant growth chamber rated for 10.2 psi. Experiments were designed and protocols detailed for two complete growouts each of lettuce and wheat to generate a general database for human life support requirements and to answer questions about plant growth processes in reduced pressure and varied oxygen environments.

The central objective of crop growth studies in the VPGC is to determine the influence of reduced pressure and reduced oxygen on the rates of photosynthesis, dark respiration, evapotranspiration and biomass production of lettuce and wheat. Due to the constraint of one experimental unit, internal controls, called pressure transients, will be used to evaluate rates of CO_2 uptake, O_2 evolution, and H_2O generation. Pressure transients will give interpretive power to the results of repeated growouts at both reduced and ambient pressures. Other experiments involve the generation of response functions to partial pressures of O_2 and CO_2 and to light intensity. Protocol for determining and calculating rates of gas exchange have been detailed. In order to build these databases and implement the necessary treatment combinations in short time periods, specific requirements for gas injections and removals have been defined. A set of system capability checks will include determination of leakage rates conducted prior to the actual crop growouts. Schedules of experimental events for lettuce and wheat are outlined and include replications in time of diurnal routines, pressure transients, variable pO_2, pO_2/pCO_2 ratio, and light intensity responses.

INTRODUCTION

Pressure and composition of atmospheres in future life support systems such as lunar and martian bases will likely differ from those of a sea-level, earth-based environment. Establishing an atmosphere for advanced closed life support systems involves consideration of requirements suitable for both autotrophs and heterotrophs. Human life support includes requirements for oxygen supply and carbon dioxide removal, roles served at least in part by plants in advanced life support systems having a bioregenerative component. In recent years, large, closed, controlled environment chambers have been constructed by NASA for gas exchange and plant growth studies (4, 10,22,29,31,32).

Optimum gas environments for plants and people may not be the same even though the atmosphere of the biosphere has evolved to establish gas compositions which reflect the recipricol exchange of oxygen and carbon dioxide. While the atmospheric composition of the biosphere is suitable for the survival of both, it is not optimum for all growth and development processes. For example, it is well established that the carbon dioxide concentration of the biosphere is considerably lower than the level at which photosynthesis of plants is saturated (2,6,12,16,18). This knowledge has been applied to crop production in greenhouses and other closed environment settings where atmospheres are commonly enriched with carbon dioxide to accelerate growth rates.

Hypobaric Effects

Hypobaric pressures will likely be used to decrease the mass and engineering requirements for establishing and sustaining life support systems at extraterrestrial outposts. Recent studies suggest that plant growth may be enhanced at hypobaric pressures particularly when combined with decreased partial pressures of oxygen (1,8,9,11,19,23). At present, it is not clear what may explain effects of hypobaric pressure on plant processes, particularly photosynthesis. However, there are two major possibilities based on well established physical principles and the current state of knowledge in plant metabolism. First, the diffusion coefficient of gases in air increases at atmospheric pressures below those of ambient sea level. The relationship is expressed as

$$D' = D^0(T_1/293)^m(760/P_1),$$

where D^0 is the diffusion coefficient in air at 293 K and 760 mm Hg in cm^2/s, T is the temperature in K, P_1 is the pressure in mm Hg, and m is a factor that depends on characteristics of the diffusing gas and for CO_2 in air is equal to 2. The calculated diffusion coefficients in air for CO_2 and H_2O^v at a range of pressures at 23 C (baseline temperature to be used for crop production tests) are presented in Figure 1. Comparing ambient sea level pressure with the baseline pressure to be used for hypobaric plant growth experiments illustrates that the diffusion coefficients for CO_2 and H_2O increases by a factor of 1.44 at the lower pressure. Increased rates of CO_2 diffusion will result in a more rapid rate of transport to the site of photosynthesis assuming that stomatal conductance and leaf boundary layer resistance also remain unaffected (13). Photosynthetic enhancement attributable to increased carbon dioxide diffusivity would be expected to be greater at carbon dioxide concentrations well below saturation with the effect becoming negligible as

saturating concentrations are approached. Also, the CO_2 concentration required to saturate Ps is highly dependent on genotype (mesophyll resistance), light intensity, and temperature. Water diffusion at reduced pressures will also be enhanced and will lead to

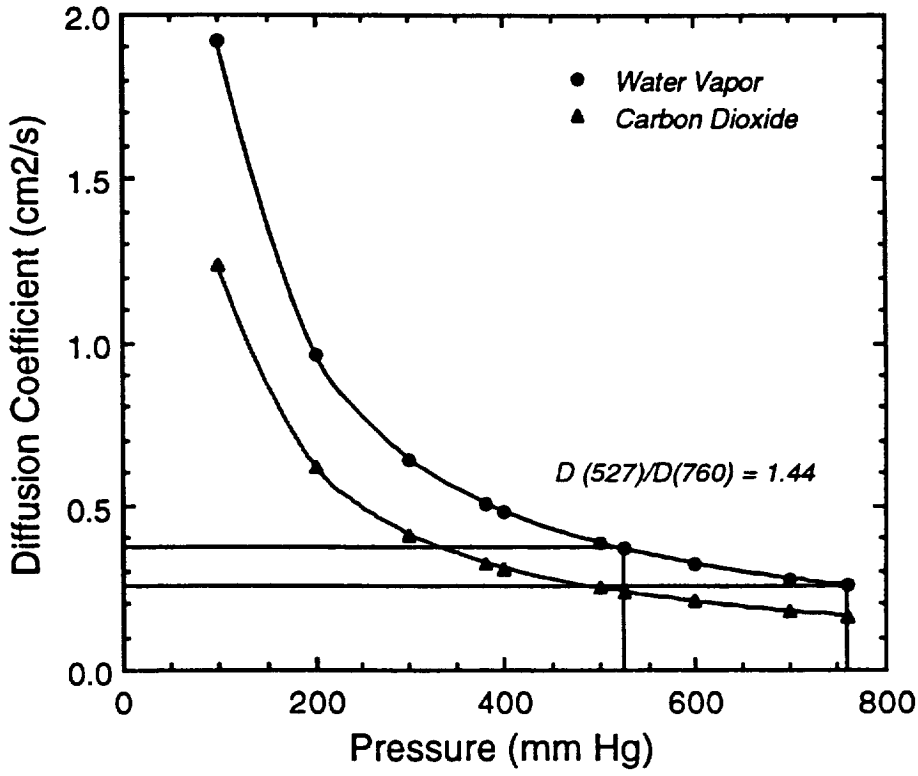

Figure 1.- Diffusion coefficients of CO_2 and H_2O calculated for a range of pressures at 23 C.

greater rates of transpiration (Ts). Increased rates of Ts with decreasing pressure have been reported in several studies (11,14,19). Given that the vapor pressure deficit (VPD) is held constant at different P_t, it should then be possible to determine the influence of P_t on Ts solely through its effect on $D(H_2O^v)$.

A second major possibility for reduced pressure effects relates to the effects of O_2 on plant metabolic processes. If a reduction in atmospheric pressure is accompanied by a reduction in the partial pressure of oxygen, other effects may occur considering the range of oxygenases that regulate plant metabolism. A well established effect of decreased oxygen partial pressure is the decrease in activities of ribulose bisphosphate carboxylase-oxygenase (RUBISCO) and glycolic acid oxidase (12,16). The net effect of the activities of RUBISCO and photorespiratory enzymes in C-3 plant species is an increased carbon loss which can be lessened at lowered partial pressures of oxygen (15,18,20,21,25,26).

Thus, optimizing productivity of plants also may involve a combination of hypobaric pressures and lowered partial pressures of oxygen. In addition, there is an effect of lowered oxygen on background or dark respiration (DR) (7,8,9,17,27,28) which may further increase the rate of biomass production through decreased carbon loss. Since cytochrome oxidase, the terminal electron acceptor in the mitochondrial electron transport chain, has a high affinity for oxygen and the stomatal resistance in the light is low, there may be only minimal effects on background respiration of shoots in the light at pO_2 down to about 1.5 psi. There may be greater affects of pO_2 on root respiration depending on the nature of the culture medium (e.g. solid matrix, solution culture, nutrient film technique), and the density and resistance of the root mass and root surfaces. For certain plant species such as wheat and lettuce, which can be grown under continuous light, the dimunition of overall, whole canopy DR (shoots and roots) may increase the rate of crop growth. However, in some plants there may be intermediates produced at sea level pO_2 values which serve key roles in developmental processes such as seed germination. Perhaps there are different pO_2 optima for the periods before and after attainment of photosynthetic competence during seedling development.

Defining Atmospheres

Given that people and plants have different optima for atmospheric regimes, what atmospheres should be generated which would be suitable for an integrated system, and alternatively, for isolated systems? The issue of designing appropriate atmopheres for people in isolation has been well researched and various regimes have been implemented for space missions (Table 1). An open, but perhaps not critical issue in human physiology is whether there are any detrimental effects of prolonged exposure to lowered atmospheric pressures which are necessarily accompanied by lowered partial pressures of N_2. At this point, there is no strong evidence to indicate that N_2, the major component of the Earth's atmosphere, serves any vital long range function in human physiology.

A set of gas composition and atmospheric pressure values similar to those for human life support is not established for plants, though physiological responses to partial pressures of CO_2 and O_2 have been documented. Furthermore, since there is a greater genetic diversity to consider for plants than for the human species, responses of individual species and genotypes of plants to varied atmospheric conditions are expected to vary more widely than responses of humans. Approaches to the problem of defining atmospheres for plants could be tailored to the situation. One scenario is to define those conditions which are optimum for growth and development regardless of human needs. It is likely that such conditions may not also be suitable to people, necessitating compartment isolation and procedures to enable brief periods of human integration with a plant compartment. If isolation is feasible in future closed environments and if it translates into more efficient production of biomass, then implementation may be advantageous. Working from this assumption, how is the "best" atmospheric regime for plants determined? There are few reports on this subject except for the aforementioned effects of carbon dioxide enrichment and lowered partial pressure of oxygen. What effects, if any, will decreased atmospheric pressures and decreased partial pressures of oxygen have on rates of biomass production?

Another approach to defining atmospheres for plants is to assume a regime which will be integrated with people. This automatically places contraints on the minimum oxygen partial pressure and maximum carbon dioxide partial pressure which can be used safely and comfortably. The partial pressure of carbon dioxide which maximizes plant

TABLE 1.- ATMOSPHERIC REGIMES USED FOR HUMAN LIFE SUPPORT IN THE U.S. SPACE PROGRAM.[a]

Program	Pressure[b]		
	Total (kPa)	Oxygen (kPa)	Carbon Dioxide (Pa)
Apollo	34.5 (5.0)	34.5 (5.0)	----------
Skylab	34.5 (5.0)	22.7 - 26.9 (3.3 -3.9)	660 (5.0)
Orbiter	101.2 (14.7)	20.3 - 23.8 (3.0 - 3.5)	1010 (7.6)
Orbiter (EVA prep)	70.3 (10.2)	18.3 - 19.3 (2.7 - 2.8)	1010 (7.6)
Space Station Freedom	70.3 (10.2)	68.9 - 71.7 (10.0 - 4.0)	1013 (7.6)

[a]adapted from Tables B-1 and B-3 in Wieland, P.O., 1994, pages 184-185 (ref. 33).
[b] psi given in parentheses except carbon dioxide where it is mm Hg.

growth is fortunately well below the defined safety limit for people. The partial pressure of oxygen is perhaps considerably higher than what could be established for optimum plant growth and would have to be a set condition based on human requirements. However, there is considerable latitude in the selection of the total pressure environment, which, for safety issues, would involve the use of a diluent/quenching gas such as nitrogen or helium. During the Skylab missions, the atmosphere consisted of an oxygen partial pressure of 160 mm Hg and 90 mm Hg nitrogen, with carbon dioxide maintained below about 5 mm Hg. The oxygen level was the same as ambient sea level, but the reduced nitrogen gave a total pressure environment of about one-third atmosphere with no documented deleterious effects on human health for a mission that lasted nearly three months. A reduced pressure database for plants needs to be developed which will include conditions that meet the goal of optimizing plant productivity and conditions which would also be suitable for human life support.

Variable Pressure Growth Chamber

The first tests of hypobaric pressures on plant gas exchange and biomass production at the Johnson Space Center will be initiated in January 1996 using the Variable Pressure Growth Chamber (VPGC) with tests of chamber and subsystem function beginning in September 1995. The VPGC, which is rated for a 10.2 psi pressure atmosphere and has a growth area capability of 11.2 m^2 (4) will be used for two complete growouts each of lettuce (*Lactuca sativa* 'Waldmann's Green') and wheat (*Triticum aestivum* 'Yecora Rojo' or a new dwarf genotype, 'Utah-20-1-41'). A primary objective of these experiments is to determine the effects of subambient pressure on gas exchange

and yield of plant species selected as candidate crops for the Controlled Ecological Life Support Systems (CELSS) program. The mass balance of metabolic gases will be important for matching plant requirements and resource generation to the resource needs of humans (3). A set of values for the key environmental variables affecting plant growth will be established and at specific stages of development, experiments will be conducted to answer specific questions about gas exchange responses under the subambient pressure environment. A peripheral, but important issue related to plant growth in closed environments is the evolution of volatiles which may accumulate to physiologically active levels. The plant volatile and hormone ethylene, is of special concern because of its wide range of metabolic effects. Since the synthesis and action of ethylene are dependent on the partial pressure of oxygen, ethylene concentration will be measured routinely and will be of particular interest in the context of variable pO_2 experiments.

Large scale experiments in growth chambers or in chambers designed as prototype CELSS have been concerned mainly with defining and implementing environmental conditions considered optimum for plant growth. Growouts of specific crops may also involve the conduct of experiments to characterize gas exchange responses to light, carbon dioxide, temperature, and vapor pressure deficit. The VPGC presents the opportunity to conduct similar experiments at subambient pressure. Unique to the scale of this chamber and a pertinent focus to experiments conducted during VPGC growouts will be the use of different partial pressures of oxygen.

OBJECTIVES

A. Database

The following objectives relate to the overall goal of developing a database on crop gas exchange and biomass production for advanced closed life support systems using the VPGC as a tool.

1. Determine gas exchange rates (i.e. Ps, DR, and Et) of lettuce and wheat throughout growth and development.
2. Compare biomass production and rates of gas exchange in hypobaric and ambient pressure environments.
3. Develop photosynthetic response functions for light intensity (PPF) and carbon dioxide concentration.
4. Determine changes in concentration of ethylene in the atmosphere during crop development.

B. Investigative

The following is a list of questions relevant to issues of plant processes and growth in closed life support systems and can be addressed on a large scale plant canopy using the VPGC as an experimental unit.

1. Does hypobaric pressure enhance photosynthesis and biomass production of lettuce and wheat?
2. Are rates of gas exchange (i.e. Ps, DR, and Et) influenced by total atmospheric pressure and by partial pressure of oxygen?
3. What are the physiological explanations for any observed effects on gas exchange? Attempts will be made to make distinctions at the crop canopy level between metabolic and physical possibilities.
4. Are there interactive effects of partial pressures of oxygen and carbon dioxide on gas exchange rates, i.e. is net photosynthesis a function of the pO_2/pCO_2 ratio?

EXPERIMENTAL PROTOCOLS

Baseline Conditions

Baseline environmental conditions were selected based on previous reports of experiments in plant growth chambers (5,6,10,24,31,32) and on the unique capabilities of the VPGC for maintaining reduced atmospheric pressure (Table 2).

TABLE 2.- BASELINE CONDITIONS FOR CROP PRODUCTION TESTS IN VPGC.

Environmental Condition	Lettuce	Wheat
Pressure (psi)[a]	10.2	10.2
Oxygen (psi)[b]	2.1	2.1
Carbon dioxide (μmol/mol)	1200	1200
Temperature (C)	23	23
Dewpoint temperature (C)[c]	17.5	17.5
Photoperiod (light/dark)	18/6	20/4
PPF (μmol/m^2/s)	400	1500

[a]Pressure given is for first growout of each crop; the second growout will use 14.7 psi as the baseline.

[b]Partial pressure of O_2 given is for first growout with the second growout to be set at 3.1 psi.

[c]A dewpoint temperature was selected to give a relative humidity of 70%.

The following sections are the categories of experiments and types of data to be compiled for the two growouts of lettuce and wheat.

Diurnal Routines

Each day, measurements of net photosynthesis (Ps), dark respiration (DR), and evapotranspiration (Et) will be made and continued throughout growth and development. Sensitive measurements of rates of CO_2 uptake will probably not be possible until following the completion of the germination process (several days after planting). Since there will be light/dark cycles used and no attempt at CO_2 removal will be made, the DR measurement will be obtained from the slope of the linear increase in CO_2 concentration that occurs following lamp shutdown. It is assumed that DR is constant over the range of CO_2 concentrations that develop. After the lights are turned on the CO_2 will decrease linearly until the setpoint value of 1200 ppm is achieved. This is referred to as the diurnal photosynthetic drawdown. While the Ps drawdown method of measurement is not steady state, the baseline concentration of 1200 μmol/mol is close to saturation of Ps at the

specified light intensity, is linear, and is therefore a reliable daily rate measurement (unpublished data). A supplementary method for measuring the photosynthetic rate will be achieved for the complete light period by tracking the absolute quantity of CO_2 injected to maintain the setpoint. This is equivalent to the amount of CO_2 used in Ps plus the quantity leaked from the chamber during the time of measurement. The numbers obtained are referred to as CO_2 mass flow measurements and are considered a steady state method of measurement. Leakage tests performed prior to and following each crop test will be used to apply corrections to calculations made for any long term meaurements (i.e. those exceeding a couple hours). It is anticipated that the leak rate, particularly at the subambient pressures, will be small enough (i.e. < 5 %/day) to assume a negligible loss for short term (<1 hour) rate calculations. Oxygen concentration during the light phase will be maintained at the established setpoint and quantities tracked by continual scrubbing with a molecular sieve. Accounting of the oxygen removed during the photoperiod by the molecular sieve will also serve as a supplementary method of Ps rate measurement and will be compared with the CO_2 mass flow data. It may be possible to use the two measurements to calculate an assimilation quotient for Ps (CO_2 uptake/O_2 evolved).

Daily measurements of water flux will also be made for both light and dark components of the daily cycle. The light measurement actually represents a combined evapotranspiration (Et) value. It will not be possible to separate the two components precisely since the energy budgets differ and a finite, but perhaps small stomatal conductance can be measured during the dark period. An estimate of evaporation will be obtained in the pretest checkouts by running the system at test conditions without plants.

P_t Transients

Since the baseline pressure used is 10.2 psi, it will be necessary to compare measurements of gas exchange rates with those conducted at ambient pressure (see objectives A.2 and B.1). The constraint of one experimental unit and limited replications in time pose an interpretive challenge. Therefore, short-term rate measurements at several stages of development will be made. For the first growout of each crop, the pressure transients will be ambient pressure and for the second growout of each crop where the baseline atmospheric pressure is ambient, the pressure transients will be conducted at 10.2 psi. It will also be necessary to vary the partial pressure of O_2 during the pressure transient experiments. Both the reduced and normal sea level ambient partial pressures of O_2 will be used. These short term experiments will be used as an aid to interpretation of biomass production results and for establishing the extent to which overall rates of plant metabolism can be altered by P_t and pO_2. Pure N_2 and O_2 will be injected to establish increased partial pressures and total atmospheric pressures. The vacuum pumps and a method for O_2 removal (molecular sieve) will be used to reduce levels back to baseline or to change O_2 (for variable O_2 experiments). The pressure transients may be summarized as follows: Growout I (baseline P_t=10.2 psi) - P_t=14.7 psi, pO_2=3.1 psi and P_t=14.7 psi, pO_2=2.1, and Growout II (baseline P_t=14.7 psi) - P_t=10.2 psi, pO_2=2.1 psi and P_t=10.2 psi, pO_2=3.1psi.

Variable pO_2

Crucial to defining the optimum atmospheric environment for plant growth is the

determination of partial pressures of O_2 that will maximize Ps and minimize background respiration (DR). An experiment with variable partial pressure of O_2 will be conducted over the range of 1.1 psi to 3.1 psi using 5 levels for the light period measurements. Due to the constraint of a short dark period, only 2 levels of pO_2 will be used during the dark period. Within each experiment, the appropriate ambient pressure treatments (like those mentioned in the P_t transients section) will be used as internal controls. A sample protocol for the Ps measurements is illustrated in Figure 2; the exact order of the 8 separate measurement periods randomized for each run of the same experiment. The zero-slope portions of the figure represent the quasi-steady state measurements of CO_2 mass flow injections. A fifteen-minute period for total and partial pressure changes is shown and is close to the time limit constraint for changes necessary to complete the set of treatments. Mass flow of CO_2 will be tracked at intervals no greater than 5 minutes in order to establish sufficient time segments for strong statistical estimates of rates. For the dark period experiment, CO_2 will increase and then the starting value of 1200 ppm reestablished using a CO_2 removal system (LiOH) or a photosynthetic drawdown prior to beginning the next treatment. A sample protocol and example of a hypothetical result are shown in Figure 3 to

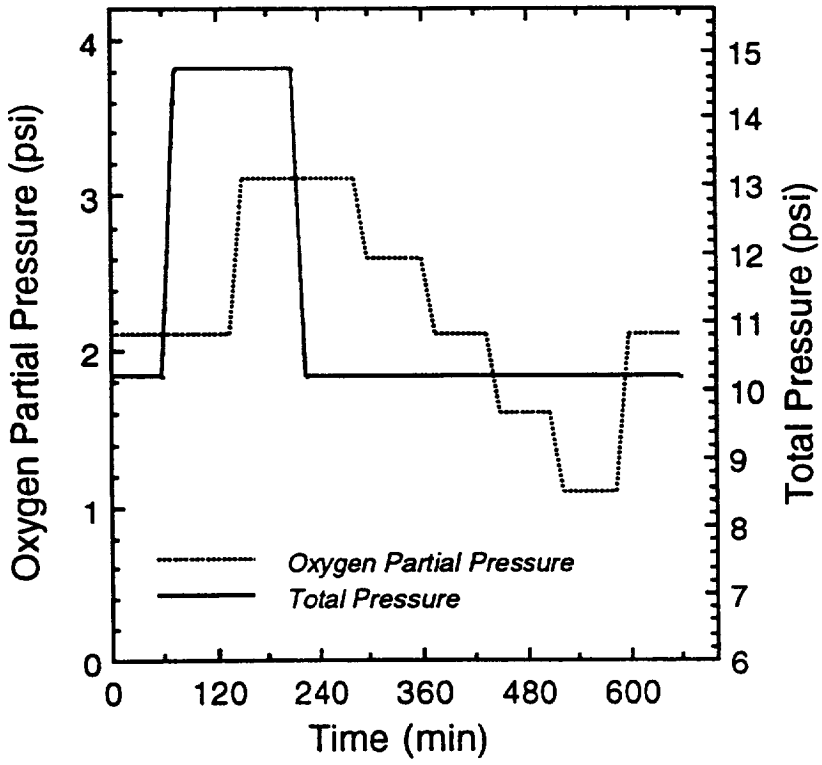

Figure 2.- Example of treatment protocol for the variable oxygen experiments in the VPGC during the light period.

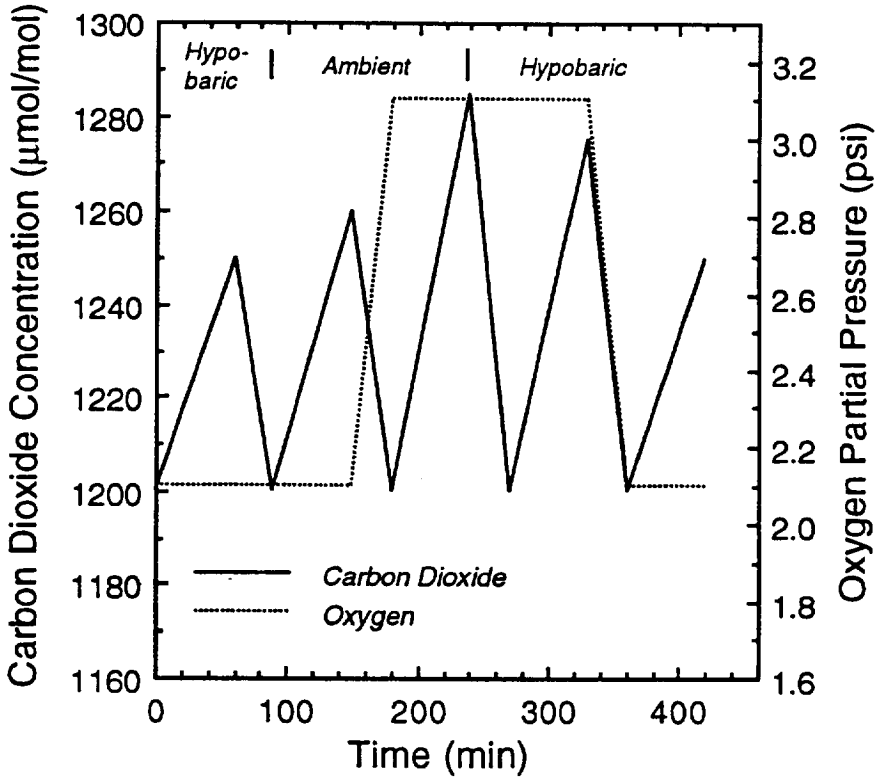

Figure 3.- Sample protocol and example of hypothetical results to illustrate experiments for measuring dark respiration during application of different treatment atmospheres.

illustrate the treatment sequence and possible effects. Also, tracking of water flux for both light and dark experiments would be desirable to determine if the treatments influence evapotranspiration. Given that water vapor pressure deficit is held constant, any treatment effects on Et will likely be attributable to effects on stomatal conductance.

pO_2/pCO_2 Ratio

Possible pO_2 effects on the rate of Ps may actually be attributable to the ratio of oxygen to carbon dioxide. This experiment will enable the generation of photosynthetic response functions to CO_2 concentration at two levels of pO_2 (2.1 and 3.1 psi). Control of CO_2 will be disabled and the CO_2 allowed to decrease (photosynthetic drawdown). Drawdowns to the CO_2 compensation point, while desirable for generating a complete function, can take several hours and may result in a substantial decrease in the free carbohydrate pool. The latter effect may influence how the crop canopy is in turn affected

by a subsequent change in the pO_2. The photosynthetic response curve can be characterized down to about 200 ppm CO_2 fairly quickly (i.e. 60-90 minutes) without a major change in the free carbohydrate pool. The method just described is nonsteady state and may not be representative of the internal conditions and time necessary for true photosynthetic steady state to be achieved. Stomatal conductance is strongly dependent on CO_2 concentration and the VPGC has a rather tight configuration (i.e. volume to area ratio = 2.4). Therefore, a steady state experiment will be conducted and mass flow measurements used to calculate the rate of Ps at different CO_2 concentration setpoints. If the two methods do not provide consistent results, then a more time-consuming steady state method (CO_2 setpoints and mass flow measurements) will be used.

Light Intensity Response Function

A routine experiment in constructing gas exchange databases for plants is to determine the photosynthetic response to light intensity (PPF) in the photosynthetically active radiation (PAR) waveband (400 - 700 nm). For lettuce, a reduced baseline light intensity is necessary to minimize the risk of tipburn, a condition exacerbated by controlled environment growth using solution culture or nutrient film techniques. However, given the potential responses to low pressure and decreased partial pressure of oxygen and the potential for accelerating O_2 generation or CO_2 removal, it will be of interest to determine the photosynthetic response to light intensity for differing atmospheric conditions. This can be achieved by conducting an experiment using at least 2 light intensities (baseline and a higher value) in combination with the 2 P_t levels and 2 pO_2 levels as described in the protocol for the variable oxygen experiments. Some exploratory work will be necessary to determine appropriate PPF values to use in combination with the atmospheric treatment variables. The procedure will involve short duration (30 min) drawdowns of CO_2 and calculation of the rate of Ps from the slopes of the line segments (10). For wheat, a photosynthetic response is anticipated throughout the light range capability of up to 1500 $\mu mol/m^2 \cdot s$. This response function will be generated by dimming the lamps to achieve PPF values in the range of 250 to 1500 $\mu mol/m^2 \cdot s$ in steps of approximately 250. The light compensation point will be calculated as the x-intercept from the fitted function. As with the lettuce, an experiment will be conducted to determine if light saturation varies with P_t and pO_2.

A schedule of experimental events for each crop and the estimated times in the growth cycle at which they will occur are summarized in Tables 3 and 4.

TEST REQUIREMENTS

The conduct of experiments outlined and discussed in this report require changes in total pressure and in partial pressures of individual gases in the atmosphere. The required changes must be accomplished in certain time periods in order to impose all necessary atmospheric treatment regimes. The types of changes, methods of implementing the changes, and method capabilities are summarized in Table 5.

Leak Rate Tests

Experiments outlined in this report involve the determination of steady-state and

nonsteady-state rates of consumption and evolution of metabolic gases in a closed environment. These measurements are based upon changes in concentration over time or upon quantities injected to maintain a constant concentration. For time increments more than a couple hours or for measurements integrated over days, calculations will require a knowledge of the rate of leakage of the gas of interest from or into the system. Leakage rates of CO_2 and C_2H_4 from the VPGC will be determined with the chamber in full test mode with the exception of plants. Individual gases will be injected and samples taken from the inside air and the external ambient atmosphere to determine the concentration gradient. The leak rate may be calculated from the formula,

$$L = 1/t_2 - t_1 \ X \ \ln \ [C_1 - C_{amb}/C_2 - C_{amb}],$$

where L is the leak rate in %/day, t is time, C_1 is the concentration of the gas at t_2, C_1 the concentration of the gas at t_1, and C_{amb} the concentration of the gas in the ambient atmosphere (30). In conducting the test, sufficient time should be allowed after injecting CO_2 and C_2H_4 for equilibration with the chamber environment as the gases will dissolve in water, the seals, and perhaps other materials.

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ACRONYMS, ABBREVIATIONS, & SYMBOLS

CELSS	Controlled Ecological Life Support Systems
$D(CO_2)$	Diffusion coefficient of carbon dioxide
$D(H_2O^v)$	Diffusion coefficient of water vapor
DR	Dark Respiration
Et	Evapotranspiration
Hb	Hypobaric
Pr	Photorespiration
Ps	Photosynthesis (net)
P_t	total pressure of atmosphere
pCO_2	partial pressure of carbon dioxide
pN_2	partial pressure of nitrogen
pO_2	partial pressure of oxygen
PPF	photosynthetic photon flux
Ts	Transpiration
VPD	vapor pressure deficit
VPGC	Variable Pressure Growth Chamber

TABLE 3.- SCHEDULE OF TEST ACTIVITIES FOR GAS EXCHANGE EXPERIMENTS WITH LETTUCE IN VPGC.

Test Day	Activities/Experiments
1-8	Germination and seedling establishment/dark respiration measurements[a]
9	Gas exchange measurements[b]
10-14	Stand establishment/Daily routines[c]
15	Daily routines/Pressure transient experiment (dark)
16	Pressure transient experiment (light)
17	Variable pO_2 experiment
18	Ps response to CO_2 at 2 pO_2 (drawdowns) or pO_2:pCO_2 ratio experiment
19	PPF experiment at 2 pO_2
20-21	Daily routine
22	Daily routines/Pressure transient experiment (dark)
23	Pressure transient experiment (light)
24	Variable pO_2 experiment
25	Ps response to CO_2 at 2 pO_2 (drawdowns) or pO_2:pCO_2 ratio experiment
26	PPF experiment at 2 pO_2
27	Daily routine
28	Daily routine/Return to ambient P_t - measure rates/Harvest[d]

[a]Respiration rate measurements during germination will be possible by about day 2 or 3.
[b]Estimate of day for obtaining reliable Ps and Et measurements.
[c]Daily routine includes measurements of Ps, DR, and Et at baseline conditions.
[d]Day of harvest is estimate.

TABLE 4.- SCHEDULE OF TEST ACTIVITIES FOR GAS EXCHANGE EXPERIMENTS WITH WHEAT IN VPGC.

Test Day	Activity/Experiment
1- 4	Germination and seedling establishment/Dark respiration measurements[a]
5	Gas exchange measurements[b]
6-14	Stand establishment/Daily routines[c]
15	Daily routines/Pressure transient experiment (dark)
16	Pressure transient experiment (light)
17	Variable pO_2 experiment
18	Ps response to CO_2 at 2 pO_2 (drawdowns) or pO_2:pCO_2 ratio experiment
19	PPF experiment at 2 pO_2
20-21	Daily routines
22	Daily routines/Pressure transient experiment (dark)
23	Pressure transient experiment (light)
24	Variable pO_2 experiment
25	Ps response to CO_2 at 2 pO_2 (drawdowns) or pO_2:pCO_2 ratio experiment
26	PPF experiment at 2 pO_2
27-35	Daily routines[d]
36-40	Repeat of experiments conducted days 15-19 and days 22-26
41-42	Daily routines
43-47	Repeat of experiments conducted days 36-40
48-64	Daily routines[e]/Return to ambient P_t - measure rates/Harvest[f]

[a]Respiration rate measurements during germination will be possible by about day 2 or 3.
[b]Estimate of day for obtaining reliable Ps and Et measurements.

[c]Daily routine includes measurements of Ps, DR, and Et at baseline conditions.
[d]Flowering and seed set will take place during this interval and atmospheric manipulations will be minimized.
[e]Long duration of daily routines at baseline conditions occurs during the latter stages of growth and development which includes seed fill and senescence.
[f]Day of harvest is estimate.

TABLE 5.- TEST REQUIREMENTS TO IMPLEMENT ATMOSPHERIC CHANGES IN THE VPGC DURING PLANT GROWTH STUDIES.

Atmospheric Change	Direction of Change	Change		Method	Method Capability
		Minimum	Maximum		
P_t	increase	4.5 psi	4.5 psi	injection	[a]1000 l/min
	decrease	4.5 psi	4.5 psi	vacuum	[b]2000 l/s
pO_2	increase	0.5 psi	2.0 psi	injection	[a]1000 l/min
	decrease	0.5 psi	0.5 psi	Rs vacuum & injection	variable [b]2000 l/s [a]1000 l/min
		maintenance		molecular sieve	[c]2 l/min
pCO_2	increase	0 - 10 ppm	1000 ppm	injection	[d] 3 - 30000 cm^3/min
	decrease	0 - 100 ppm	1000 ppm	Ps LiOH	variable [e]74 mmol/min
pN_2	increase	0.5 psi	4.5 psi	injection	[a]1000 l/min
	decrease	0.5 psi	4.5 psi	vacuum	[b]2000 l/s
pH_2O^v	increase	maintenance		mist injection	
	decrease	maintenance		heat exchanger cold H_2O coils	

[a]Tescom Corp. dome-loaded pressure regulators, wide range of capacities available.
[b]approximate requirement of tests is only 20 l/s based on an approximate chamber volume of 27,000 liters at STP.
[c]effective only for continuous scrubbing to maintain pO_2.
[d]Brooks Instrument Mass Flow Controller Model 5850i.
[e]Maximum capability needed, system specific method may need to be developed.