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CLOSED CULTURE PLANT STUDIES: IMPLICATIONS FOR CELSS

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Abstract:

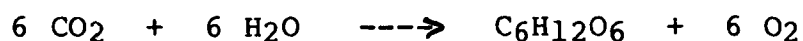
Arabidopsis plants were grown in closed cultures similar to those used in space experiments. A shift in metabolism from photosynthesis to respiration is indicated by the accumulation of CO₂ in the culture atmosphere. Reproductive growth is suppressed. Plant growth and development is apparently related to the atmospheric volume available to each plant. The implications of these findings to CELSS were as follows: 1) need for an open culture having ample gas exchange, 2) CO₂ levels be maintained within prescribed limits, 3) the minimum atmospheric volume required for each plant is thus dependent on the precision of the gas monitors and of the subsystems used used to maintain appropriate levels of various atmospheric components, and 4) volatiles such as ethylene and terpenes emanating from plants be monitored and reduced to benign concentrations.

Introduction:

In a controlled ecological life support system (CELSS), the fundamental system premise is based on the classic photosynthesis reactions of green plants. Plants exposed to light of proper wave lengths and intensities convert CO₂ and water into fixed

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carbon compounds with a simultaneous release of O₂ into the atmosphere. As a result of this photosynthetic reaction, the fixed carbon compounds form the food to be eaten and the uptake of CO₂ and production of O₂ replenishes the air for astronauts. The equation is presented below in an incomplete and simplified form:



This reaction occurs under planet earth conditions where the CO₂ atmospheric concentration is around 300 ppm and O₂ around 20 per cent.

As an example, let us take a green living plant and place it into a small gas-tight glass container. This container when sealed would have the green living plant, a supply of air, water and nutrients for the plant. Being a closed system, no materials are taken out or added. However, energy is supplied in the form of light. Under these conditions, one would expect - following the above photosynthesis equation - the plant to photosynthesize taking up the CO₂ present in the atmosphere until CO₂ is reduced to a low concentration. In theory, an analysis of the gases at this point would be predicted to have a slight increase in O₂ concentration with a concomittent decrease in the CO₂ level. In practice, this is not the case. In closed systems, such as that described, CO₂ was found to increase (Hoshizaki, 1984, Cowles et al., 1984) and O₂ decrease (Cowles et al., 1984). These results

are completely and diametrically opposite from those predicted. Thus, for CELSS being discussed at this meeting, I believe these results have high relevance.

This paper will present background information and various topic matters that relate to this closed system phenomenon. In these discussions, I will attempt to relate and highlight the various bits of information as it might apply to CELSS.

Previous Research and Results:

Tightly sealed test tubes (Hoshizaki, 1982, 1984) and small volume growth chambers (Merkys et al., 1981) have been used to test the effect of space environment, real or simulated, on many species of plants. When attempts were made to carry these plants through a life cycle, there was a general failure to do so. This was especially true in the Soviet space efforts with the plant Arabidopsis thaliana, (L.) Heynh (Merkys et al., 1981). It was only until a "ventilated" open growth chamber was used, that Merkys et al., 1984, were able to successfully grow arabidopsis from seed to seed.

Our ground based experiments with the same species had indicated earlier that a culture system having ample gas exchange with the ambient was required for growing arabidopsis plants from seed to seed in vitro (Hoshizaki, 1982). Whenever the in vitro

cultures were truly sealed and closed, having no gas exchange with ambient, the arabidopsis plants growing inside always failed to grow normally and never set seeds. Thus, we found 1) normal vegetative growth occurred only in cultures having gas exchange with the surrounding ambient atmosphere, 2) abnormal growth occurred in closed gas-tight cultures, 3) reproduction and completion of the life cycle (production of viable seeds) only occurred in cultures having gas exchange with ambient and 4) growth rate, stages of life cycle attained and final size of plants in closed cultures related to the atmospheric volumes surrounding each plant.

The implication of these results is the need for small cultures to have ample gas exchange with the ambient atmosphere surrounding the culture system.

We initially speculated that the probable cause of the plant responses was most likely related to the initial amount of CO₂ available to the plants in the closed in vitro culture. In a follow-up experiment we measured weekly, over an 8-week period, the level of atmospheric CO₂ in closed in vitro cultures (Hoshizaki, 1984). Arabidopsis seeds were planted one to each container and the growth and CO₂ levels were recorded. As expected, CO₂ levels decreased at the end of the first week with the lowest recorded value of 147 ppm. Surprisingly, the CO₂

level increased from this time on and reached a level of 0.5 percent for the weekly measured cultures and 15 percent for those cultures sampled only at the start and end of the 8-week experiment (Figure 1). The plants continued to grow producing leaves and elongating their stems during the period when the CO₂ levels were around 0.5 percent or 5000 ppm (cf Fig. 1 and Fig. 2) Why did the CO₂ level rise to such high levels? Did photosynthesis stop when CO₂ levels rose above a critical level? Did the plants enter a heterotrophic phase as previously reported by Brown et al, 1979? Ample light and appropriate temperatures were being given to these plants. Do these results mean that CO₂ levels must be maintained within prescribed limits if plants are to grow normally?

At the end of the experiment, lower values of CO₂ were measured but these were considered to be the result of gas leaks induced by the multiple sampling through the rubber septum. Bacteriological tests made for possible culture contamination as a source of the CO₂ proved negative.

A similar increase of CO₂ levels was found in the space shuttle (STS-3) experiments where pine seedlings and germinating oats and beans were flown (Cowles et al., 1982). The pine plants placed in the plant growth container (PGC) as 4-day old seedlings were thus past the initial germination stage where high respiration occurs.

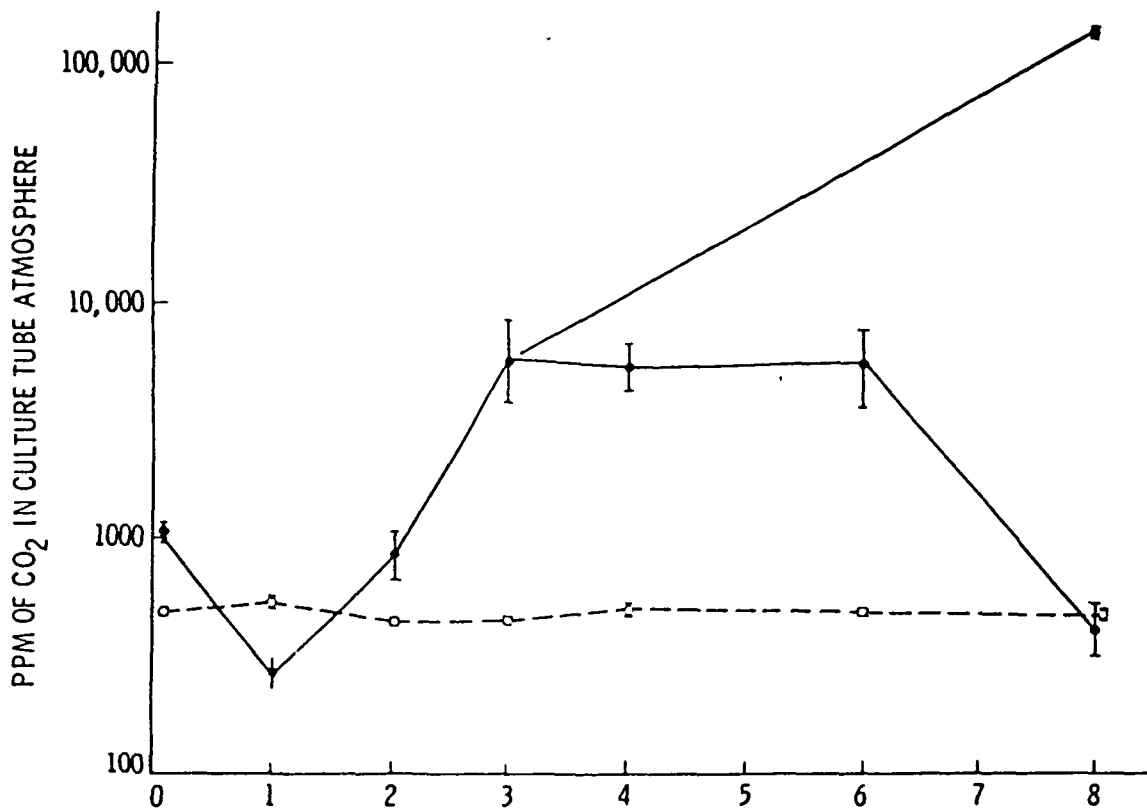


Figure 1. Concentration of carbon dioxide over an 8-week period in the atmosphere of in vitro cultures containing Arabidopsis thaliana (L.) Henyh. plants. Seeds were planted at 0 week. Closed cultures (•) were sealed with vinylidene polymer film held in place by rubber bands. Open cultures (o) were plugged with polyurethane foam permitting air exchange with ambient. Each value represents the mean \pm standard error of CO₂ concentration detected in 40 μ l samples drawn from the head space of cultures. Open culture values for 0 week and 8 weeks are from 4 cultures. Closed culture values for 0 week are also from 4 cultures. All others are from 2 cultures. The solid line drawn from week 3 to week 8 represents the assumed curve for cultures sampled only at the beginning and at the end of the experiment.

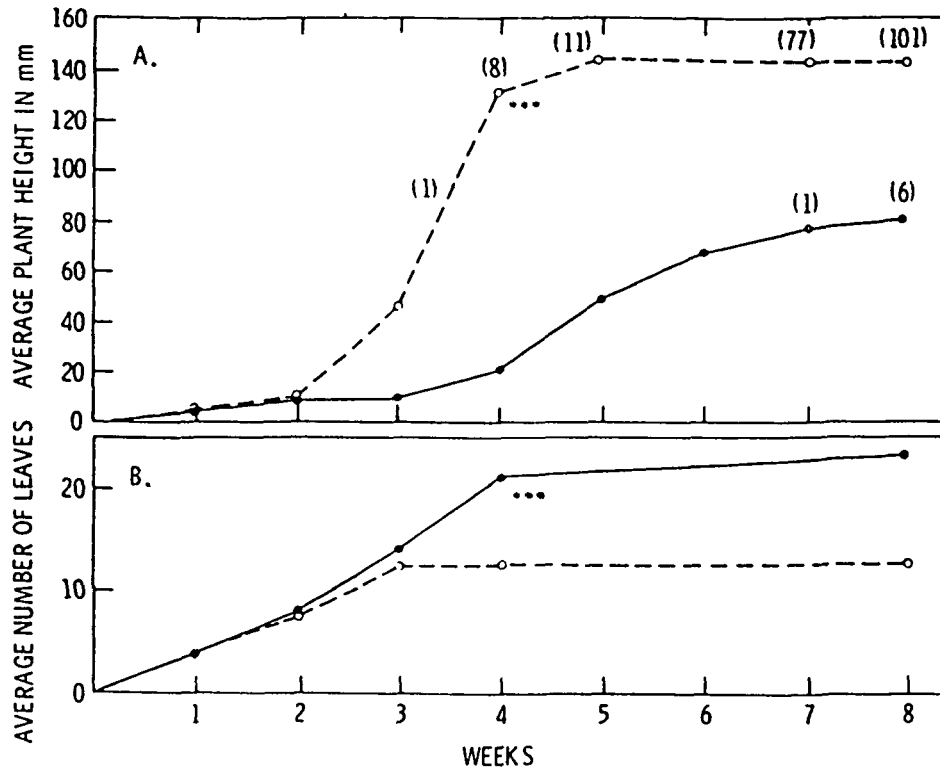


Figure 2. Growth and reproduction response of Arabidopsis thaliana (L.) Henry planted as a seed and grown over an 8-week period in open (o) or closed (•) in vitro cultures. A. Plant height in mm and total number of fertile seed pods () counted on plants of each treatment. Depth of agar medium in the culture tubes was around 50 mm thus leaving 150 mm for stem growth. B. Number of leaves per plant. Leaves with length shorter than 2 mm were excluded from the count. Each value represents the mean derived from 4 cultures. ***Difference between closed and open cultures at $P < 0.001$.

These plants were green and under light intensities provided would be capable of photosynthesizing. The oats and bean plants were green and had leaves by the end of the 8-day flight experiment. These plants also appeared to be capable of photosynthesis. However, CO₂ levels from 1.41 to 4.92 percent were measured in flight containers and 2.18 to 4.07 percent in ground controls at the end of 8 days. The CO₂ source was attributed to respiration. The O₂ levels which were initially 24.1 to 24.5 percent in the flight containers decreased to 9.7 to 16.3 percent. Similar differences were found in the ground controls. If one assumes a 1:1 stoichiometry of O₂ uptake to CO₂ production in respiration, a discrepancy appears in the amount of CO₂ found. At this time, one can only speculate about the missing CO₂.

In the arabidopsis closed in vitro cultures, a 15 percent CO₂ level was measured. It was speculated (Hoshizaki, 1984) that an ethylene-CO₂ feedback occurred resulting in a high production of CO₂ by the plants. In a closed system such as CELSS, the same volume of air would be retained for the duration of the mission. Unless steps were taken to remove such compounds, it is more than likely that a similar response might occur. In the STS-3 experiment, ethylene was detected in one of the PGC (Cowles et al., 1984).

Thus, there appears to be a need to carefully monitor the levels of volatiles such as ethylene and terpenes and develop systems to scrub these types of compounds from the atmosphere.

Volume Relationship Relative to Plant Material:

The results of air volume/closed culture experiments performed with arabidopsis indicate that a minimum volume of atmosphere to plant biomass may exist (Hoshizaki, 1984). Below this volume, abnormal growth and metabolic responses may occur. Two closed systems, the STS-3 PGC and the sealed test tubes will now be compared. Mung bean seeds were planted in the PGC (Cowles et al., 1984) and arabidopsis seeds in the sealed test tubes (Hoshizaki, 1984). Seed volumes are estimated from measurements taken of dried seeds. These are only approximate and are presented for the purpose of demonstrating broad generalities. Precise volumes are not implied. Table 1 compares the two closed plant growth systems. The PGC with mung beans is far and above over-loaded in terms of biomass as compared to the sealed tube with arabidopsis. This is expected since the PGC was designed for short duration missions, whereas the arabidopsis culture was selected for long term seed production experiments. The arabidopsis system used here for comparison did permit the arabidopsis plant to complete its life cycle. However, the seed production was low. The estimated value of 550 liters required as air volume to grow mung beans to seed in a closed system is presented to give the reader a grasp of the volume of air

TABLE 1.

COMPARISON OF 2 CLOSED PLANT GROWTH SYSTEMS

Plant	(1)	(2)
	Plant Growth Container	Sealed Test Tube
	Mung bean	Arabidopsis
Atmospheric volume of container - cm ³	1224	455
No. of plants in container	16	1
Atmospheric volume per plant - cm ³	76.5	455
(3) Dry seed volume - cm ³	7.2 X 10 ⁻²	6.0 X 10 ⁻⁵
(4) Atmospheric volume required by one plant for seed production - cm ³	550 X 10 ³	455

(1) Cowles et al., 1984, (2) Hoshizaki, 1984, (3) Volume estimated from measuring the length, width and thickness of a dry seed, (4) Volume of air required for mung seed production in a closed system as estimated from values obtained from arabidopsis data.

required. The use of the very small seeded arabidopsis plant tends to mislead investigators in judging media and gas volume requirements.

Volume Requirements Relative to CO₂ and the CELSS Plant Growth Chamber:

Two closed systems of vastly different sizes will be used to discuss volume effects of CO₂. The planet earth and a 4-person CELSS unit will be compared. The 97 percent food closure unit of CELSS is selected for our example, and such a system will have only 3 percent of the food provided by drawing from stores or obtained by re-supply (Gustan and Vinopal, 1982).

The rest of the food, 97 percent, will be produced in the CELSS unit by recycling materials within the space habitation unit. The comparison is made between planet earth and the defined CELSS system to again highlight the magnitude of change imposed on plants growing in a closed system; and conversely to highlight the biological demands placed on such a CELSS plant growth chamber.

The values entered in Table 2 were derived from various sources and are expressed in a manner as to simplify comparisons. For the planet earth, the CO₂ turnover period is estimated to be between 5 and 10 years. Only the carbon exchange between the

TABLE 2

EFFECT OF PLANT CO₂ UPTAKE ON EARTH AND CELSS ATMOSPHERES

	Plant earth	4 person CELSS
Atmospheric volume - M ³	4.3 X 10 ¹⁸ (1)	1.5 X 10 ² (2)
CO ₂ turnover time	5-10* years (3)	45 min (4)
Supply left after one min	5-10 yr supply no detectable change	44 min supply ~2% decrease

(1) Estimation based on air height being 7600 m when all air is at one atmosphere pressure, (2) Gustan and Vinopal, 1982, (3) Emanuel et al., 1984, (4) Tibbitts and Krizek, 1978. *Only carbon exchange between earth atmosphere and terrestrial plants are considered for simplification and the most conservative time estimates were used. Inclusion of carbonates in the ocean and other carbon sources will greatly increase turnover time.

earth's atmosphere and the terrestrial plants were considered to simplify the discussion and the most conservative time estimates were used. The values used were obtained and then modified from Emanuel et al., 1984. Inclusion of the carbonates in the ocean and other sources will greatly increase the turnover time. On the other hand, the CO₂ turnover time in CELSS is projected to be 45 min using values calculated from the pulldown of CO₂ in a reach-in growth chamber from 320 to 306 ppm in 2 min by butterhead lettuce (Tibbitts and Krizek, 1978). In one min there will be a 6-7 ppm decrease in the CO₂ supply which must then be detected and replenished by the CELSS gas detection and concentration maintenance unit. A \pm 20 ppm variance can be tolerated by plant but this will more than likely reduced harvest yield. For the atmosphere of earth, there will be no detectable global change after one min since the CO₂ turnover time is estimated at 5-10 years. A large buffer system for CO₂ thus exists for the plants of earth. In a CELSS, such a buffer is non-existent. Furthermore, the greater the plant biomass is to the CELSS atmospheric volume, the greater will be the replacement rate. These are engineering problems, but it does indicate the possibility of rapid changes in CO₂ levels in a relatively short time frame. From this, the minimum atmospheric volume required for each plant in a CELSS will depend in part on the precision of the CO₂ gas monitor and the subsystems replenishing the CO₂ gas.

PROTOTYPE PLANT GROWTH CHAMBER OF CELSS QUALITY:

Plant growth chambers in use today are generally very leaky. For a reach-in chamber, a leakage rate equal to 30 percent of the internal volume in 5 min have been measured (Tibbitts and Krizek, 1978). Even chambers specifically designed for gas exchange studies will fall short of the requirements for a CELSS type study where the integrity of the system would have to be maintained for months. Sealing a chamber having multiple ports and utility lines to CELSS quality would require special knowledge and skills. However, with recent advances in space technology and materials, such a CELSS chamber may now be feasible.

The prototype CELSS chamber would have to have the capacity of maintaining the gas ratios in the atmosphere as the plants grow through their life cycle and fill the chamber. Other plant requirements such as temperature, light, water and nutrients will also have to be available while maintaining the integrity of the gas seals. Ethylene, terpenes, CO₂, O₂ and other compounds that are given off by plants will have to be monitored and excesses absorbed or maintained at benign concentrations. In addition to having to meet all of these requirements, the more stringent requirement will be that no materials, solids, liquids or gases, can be added or removed. With such a system, it may be possible to fully understand the

requirements for growing several species of plants singly and simultaneously in a CELSS for a long period of time. Perhaps plant growth systems such as that built by Schwartzkopf and Stofan, 1981, could be used as a starting point for the design of an upgraded second generation system that would fulfill the CELSS requirement.

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