N86-19931

CAN PLANTS GROW IN QUASI-VACUUM ?

M. André and Ch. Richaud

Département de Biologie
Service de Radioagronomie
CEN Cadarache
F 13108 SAINT PAUL LEZ DURANCE (FRANCE)

The problem of the effect of neutral gas on growth of plants was the motivation of ancient experiments of Schloesing (1897)[1] who observed that nitrogen and argon were not consumed by plants and who concluded that they were probably useless. Since to date we have known that nitrogen can be used by legume plants, but 10 percent of nitrogen is sufficient to supply this process. The question remains to know what happens if, for the same partial pressure of O_2, the nitrogen is suppressed and so, the total pressure falls drastically ? In other words, knowing that only 5 % O_2 (50 mb) is sufficient to maintain plant respiration and that the pressure of CO_2 and water vapour represent around 25 mb in the normal atmosphere, is it possible to conceive of growing plants with only an absolute pressure of 75 mb i.e. a quasi-vacuum ? The predicted answer, following the theory of molecular diffusion of gas is positive but the physiological verification is necessary.

A first positive assay of growth without nitrogen in low pressure was made in the laboratory and mentioned in a space biology meeting (Guérin de Montgareuil et al.)[2], because this theoretical question could also concern space technology. The plant cultivation in space environment is not so far away as we imagined, taking into account the active research of CELSS project (Controlled Ecological life Support System) in which growth of plants and algae is planned for food supply of long distance manned space mission (Moore et al.)[3]. For theoretical and practical reasons it seems useful to analyse experiments in which low pressures were imposed to plantlets by suppression of nitrogen and partly of oxygen.

Materials and methods

Two experiments were conducted in rye-grass and one in barley. They consisted to sow samples of seeds (1 g for rye-grass, 10 grains for barley) in vacuum resistant containers in which was prepared different atmospheres.

Containers. Four glass containers were used in each series of experiments, 0.7 liter of volume for the series I, 3 liters for the series II (Fig. 1). In both cases they had leak proof ground joints and stopcock systems to realize the link with a vacuum bench for the initial preparation of atmospheres and to permit the sampling of gas during the experiment.

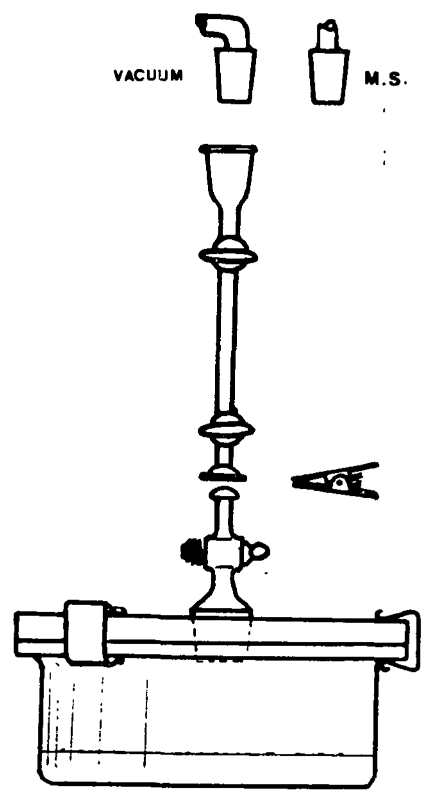


Figure 1. Vacuum resistent glass container for low pressure cultivation of seedling (0.7 liters of volume). On the top, the sampling apparatus which may be fitted on mass spectrometer inlet.

Growing conditions. Each container received 250 g of acid washed quartz sand ; 30 ml of nutrient solution was added and the seeds sown before connection with the vacuum bench. The growth was performed in day light during February (series I) and March (series II) at temperatures between 18 and 22°C. The growth was limited to the juvenile stage because no CO_2 was added and the photosynthesis only used the CO_2 produced by the respiration, mainly by grain, at the heterotrophic stage.

The preparation of atmospheres with the vacuum bench was realized in three steps. 1) Vacuum actively maintained during 2min around 20 mm of Hg to remove initial atmosphere and to purge dissolved gas in nutrient solution. 2) Closure and observation during 5 min to check any increase of presure above water vapour pressure (19 mm Hg at temperature of 20°C). 3) Adding of different amounts of oxygen. The pressure was measured with a mercury manometer. Expressed in m bar for clarity, they were 48 and 205 mb for the oxygen to correspond to the partial pressures of the concentrations of 4.6 and 20 % respectively in normal atmospheric pressure (1030 mb). In two containers, nitrogen was added until normal atmospheric pressure. Two other containers were kept with only water vapor pressure and the two initial pressures of oxygen. Sampling was made to check the preparation and to follow the composition of atmosphere during the growth.

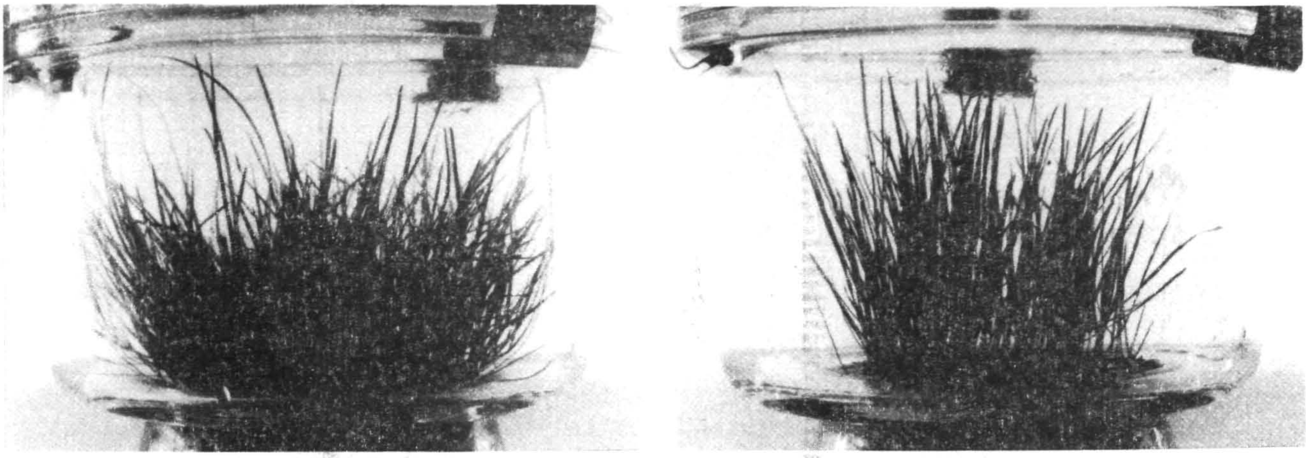
Sampling an analysis of gas. The sampling of 0.2 ml of gas was made by the pre-vacuum of the volume limited by the stopcocks. For analysis, the sampling tube was fitted on the inlet of the mass spectrometer (MAT.CH4). Standard gas and accurate pressure gauge was used to correct the sensitivity of the apparatus for the different types of gas.

Results and discussion

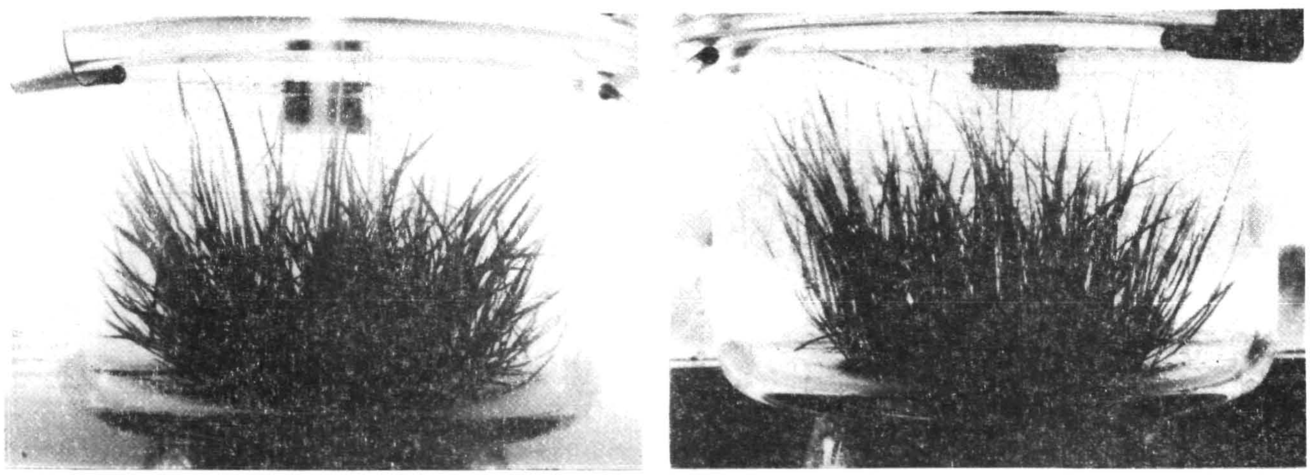
Visual observations. The main result is that the growth of plants is possible under absolute pressure 14 times lower than the atmospheric pressure (Fig. 2d). In first approximation, plants ignore the absence of nitrogen and only react to the partial pressure of O_2. Hence the growth of plantlets was delayed under low pressures of O_2 in both cases with and without nitrogen. The CO_2 availability being limited by the carbon content of the seed, the final results after 20 days were very similar. The differences in the kinetic of growth can be better observed in Fig. 3 and estimated in the examination of gas analyses.

EXPERIMENT AT ONE ATMOSPHERE

20 % O_2 5 % O_2



P. totale	O_2	N_2	H_2O	Partial	O_2	N_2	H_2O	P. totale
985	205	755	25	Pressure	47	916	25	988
230	205	0	25	mbar	48	0	25	73



0,25 Atm. — LOW PRESSURE EXPERIMENTS — 0,07 Atm.

Figure 2. Final result of growing of rye-grass seedling under the different noted pressure conditions. View of the three liter containers 20 days after sowing.

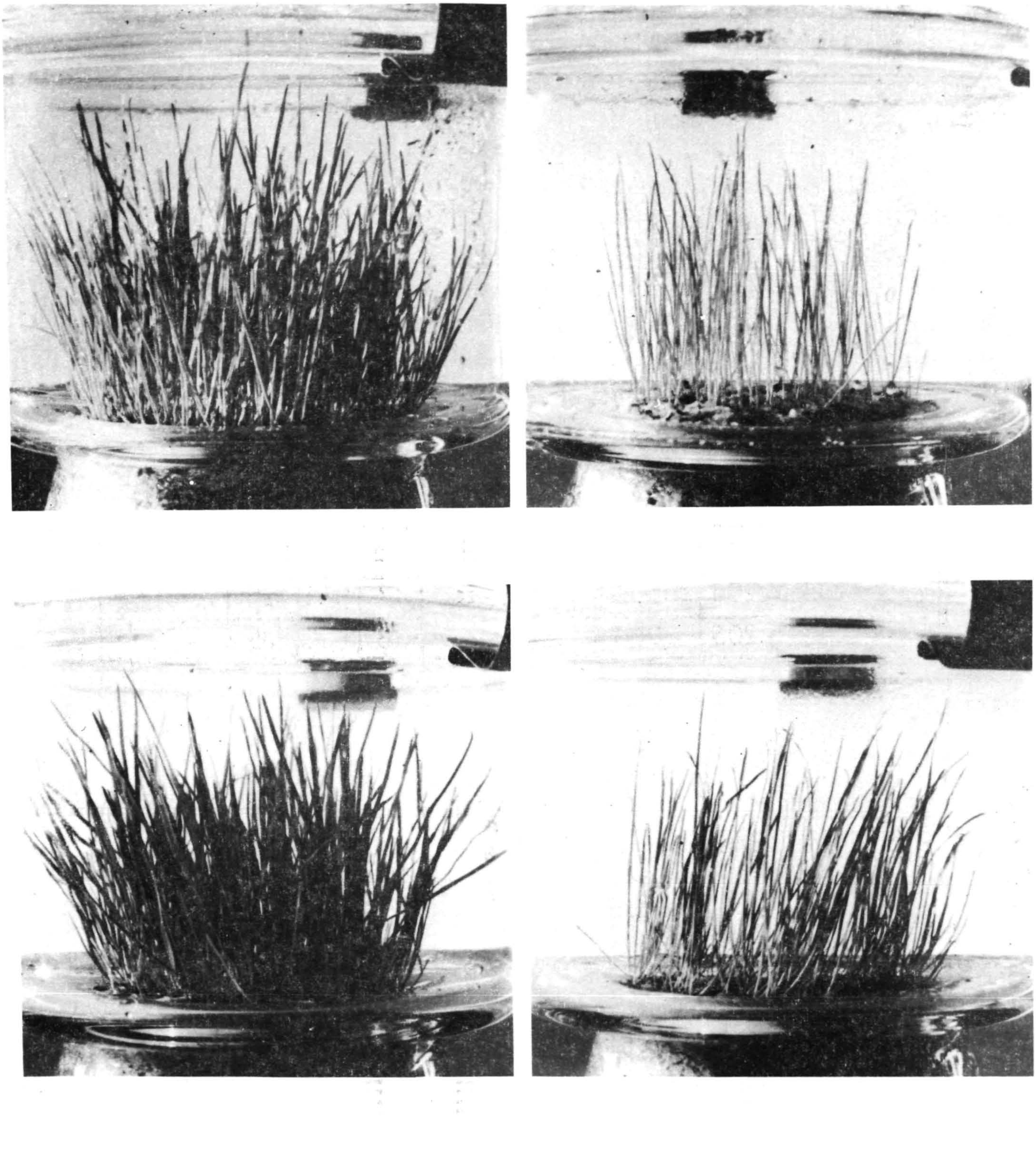


Figure 3. View of the experiment of Fig. 2 - 13 days after sowing. The depressing effect of low O_2 pressure appears(right) and also the depressive effect of nitrogen especially in low oxygen case (right on the top).

In the experiments with barley, only the cases with normal O_2 partial pressure, with and without nitrogen were tested. Seedling was beautiful, no significant differences were observed between both experiments.

Analyses of the atmospheres. Periodic gas analyses have permitted checking the airtightness of the containers. In the case of loss of nitrogen a leak would have been detected by the appearance of nitrogen peak which remained neglectible. Fig. 4 & 5 show the complementary variation of O_2 and CO_2 partial pressures which was amplified in the experiment of Fig. 5 by the effect of the small used volume. Two phases can be observed. In the first phase (0 to 8-11 days) the respiration is the main activity and consequently there is the O_2 decreasing and the CO_2 enrichment. In the second phase, it is the other way round and the photosynthesis re-uses CO_2 faster than it is evolved in a more and more autotrophic way.

The amplitude of the maximal variations was related to the vigour of the plantlets noticed by visual observations in the intermediary phase. The conclusions were as follows :

Slowing down of the growth by low O_2 pressure. Contrarily to the results of Björkman et al.[4] or Quebedeaux & Hardy[5] who observed a growth stimulation on low oxygen conditions, we observed the growth was delayed in the case of low oxygen pressure. The CO_2 and oxygen variation were halved (Fig. 4b, 5) and retarded for one to three days in comparison with normal oxygen pressure. This slowing down can be attributed to a limitation of respiration rate, especially on the grains. Whereas the respiration of the organs such as leaves or roots was generally not modified by a O_2 decrease varying from 20 % to 5 %, the rather strong respiration in particular phases and in not much accessible sites could be limited by O_2 diffusion processes. It was shown by Quebedeaux & Hardy[5] as regarding the phase of the fertilization of the flowers in soybean.

Effect of the presence (of the lack) of nitrogen. If we refer to the visual observations, as well as to the results of gas analyses, the presence of nitrogen significantly slows down the growth of plantlets (Fig. 3).

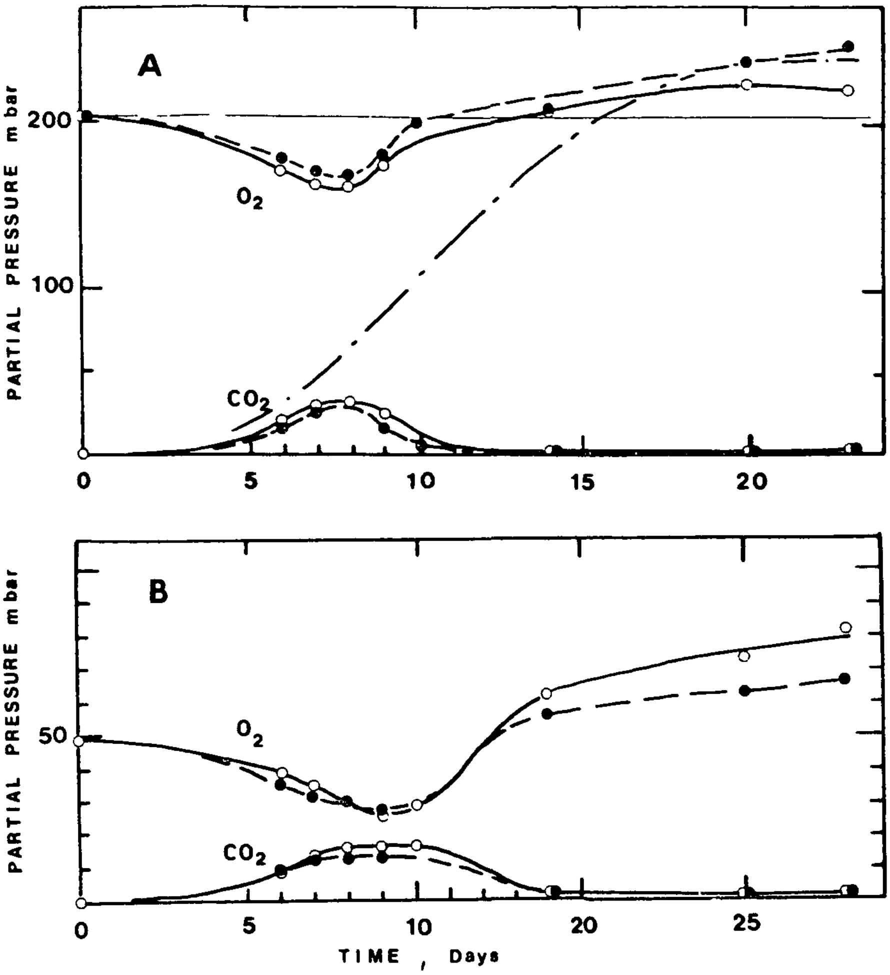


Figure 4. Time evolution of the O_2 and CO_2 pressure in the 3 liter containers. A) With 205 mbar of O_2 initial pressure with (--●--) and without (-O-) nitrogen. B) With 48 mbar of O_2 initial pressure with (-●-) and without (-O-) nitrogen. (— — —) theoretical potential of respiration (see text).

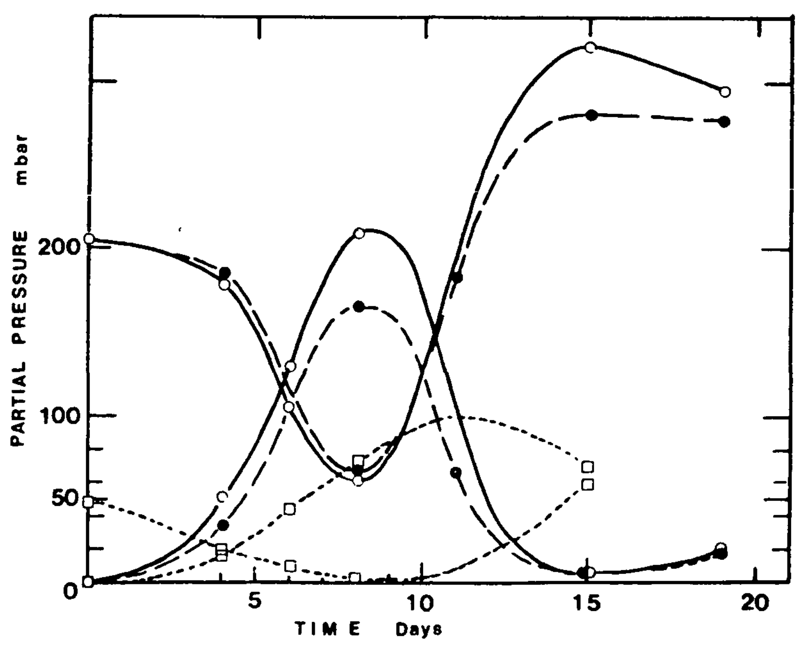


Figure 5. Time evolution of the O_2 and CO_2 pressure in 0.7 liter containers. Same legend as Fig. 4, for 205 mbar of O_2 initial pressure with (-●--) and without (-O-) nitrogen and for 48 mbar of O_2 initial pressure without nitrogen (--□--).

That is noticeable at a high O_2 pressure, in which the CO_2 maximum is always smaller under nitrogen presence. The slowing down effect of the nitrogen presence is more sensitive in the case of low O_2 pressure and stays visible in the final result (Fig. 2). This effect is surprising. It seems to act upon the number of leaves and of tillers and cannot be explained. However, it renforces the previous idea of this experiment of the "uselessness" of neutral gas.

Disequilibrium of the O_2-CO_2 balance. The present closed experiment is a kind of an environmental microcycle in which is performed the transformation of a system (seed) into another one (plantlet) with conservation of the matter, especially carbon and oxygen. At the end of the experiment, all the components which were at the beginning have to be found. The analysis of the atmosphere shows a significant O_2 enrichment which implies that at the final step the chemical composition of the matter is different, as well as its redox status. The plantlets were in a more reduced state than seeds. This status implicates an energetic gain brought by light for, on the contrary, the final state of a biological process taking place without energetic supplies would be necessarily more oxidized, i.e. with less free oxygen.

Given in terms of concentration, the excess of oxygen seems important, especially in Fig. 5 ; given in volume it is the same : 70 ml for this experiment and between 60 to 90 ml for the experiment of Fig. 4. These values have to be compared not to the oxygen content of the atmosphere but to the turn over of the cycle of transformation mentioned above. For that, the hypothesis is given that the matter of the seeds has been totally transformed and that its structure is - in majority - the carbohydrate one. In that case 1 g of seed correspond to 0.033 mole of CH_2O. Thus we can notice that the excess of oxygen (3.1 m moles) represents about 10 % of the turnover of the total organic matter. Let us notice too that if the CO_2 variation is due to a respiratory activity, it is far from representing the totality of the respiratory potential of the seeds. If all the dry matter of the seeds was consumed by respiration, the hypothetical curve plotted on Fig. 4a could be obtained. It is not excluded that a large part of this matter was thus degraded, but in that case CO_2 was immediatly trapped by photosynthesis and the observed curve results from the equilibrium between the two processes.

Possible consequences for culture in space environment.

Safety this experiment demonstrate that plants are, in first approximation, insensitive to de-pressurization. Only the loss of water, in case of active vacuum, would be crucial.

Space technology. As far as this simple experiment can be generalised, two types of consequences can be suggested. If the cultivation of higher plants must effectively supply a noticeable part of food of future space stations a considerable volume will be devoted to it. Take account of the environment of the space vacuum, the possibility of culture under low pressure proportionally reduces the losses of gas due to unavoidable leaks. But above all, this proceedure reduces the quantity of material required to face vacuum constraints. For the same weight (cost of launching) the gain of volume can be (at least) proportional to the reduction factor of the pressure. If a factor of ten can be expected, this process will be certainly discussed in the future in spite of the great disavantage of space clothes for space gardners.

Advantage in O_2 recycling for ecological system. The elimination of all or part of nitrogen in a cultivation system (as well for higher plants and algae) drasticaly simplifies the management of its atmospheric phases and the control of the oxygen loops between cultures and manned rooms. Cultures are supplied by CO_2 trapped in maned area. O_2 production increases the pressure in culture systems and the its extraction is made by the pump which maintain the depressurization. Dilution process are suppressed. Pure or very enriched oxygen is produced without nitrogen separation. It can be stored for the reserves required by the control of O_2 cycle or to supply the missions out of the space station.

REFERENCES

1. SCHLOESING, P. (1897). C.R. Acad. Sc. Paris. 125 : 719-721.

2. GUERIN DE MONTGAREUIL, P., ANDRE, M. & LESPINAT, P. Mesure instantanée des besoins métaboliques des plantes : applications agronomiques et éventuellement spatiales. In Proceeding of colloquium on Space Biology related to the post-Apollo programme. Eds. ESRO. 1971.

3. MOORE, B., WHARTON R.A. Jr & MAC ELROY R.D. Controlled Ecological Life Support System. First principal investigator meeting. Report NASA CP-2247-1982.

4. BJÖRKMAN, O., HIESEY, W.K., NOBS, M., NICKOLSON, F. & HART, R.W., 1966. *Effect of oxygen concentration on dry matter production in higher* plants. Carn. Inst. Y. Book 1966 : 228-232.

5. QUEBEDEAUX, B. & HARDY, R.W.F. Oxygen concentration : Regulation of crop growth and productivity. In O_2 metabolism and plant productivity. Eds. R.H. Buris and C.C. Black. University Park Press. Baltimore, London-Tokyo, 1976.