The $C_2$3A System, an Example of Quantitative Control of Plant Growth Associated with a Data Base

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ABSTRACT

The architecture of the $C_2$3A (Chambres de Culture Automatique en Atmosphères Artificielles) system for the controlled study of plant physiology is described :

1) Modular plant growth chambers and associated instruments (I.R. CO_2 analyser, Mass spectrometer and Chemical analyser).

2) Network of frontal processors controlling this apparatus

3) Central computer for the periodic control and the multiplex work of processors. It also concentrates the data, obtained from processors, and stores them in long term data base.

4) Network of terminal computers able to ask the data base for data processing and modeling .

Examples of present results are given : growth curve analysis, study of CO_2 and O_2 gas exchanges of shoots and roots and daily evolution of algal photosynthesis and of the pools of dissolved CO_2 in sea water.

This system is extremely useful to continue progress in agricultural research. Another application is in Controlled Ecological life Support Systems (CELSS) for space habitats.

AGRONOMY AND ECOLOGY are studies of macrosystems that require basic knowledge from the elementary disciplines of molecular biology, biochemistry, and cellular physiology for the study of botanical systems. None the less, whole plant physiology remains a necessary field of research because the study of the whole system cannot be directly, nor even necessarily deduced, from sublevel or microscopical characteristics, althought these cellular parts are under the control of the genetic program and large-scale environmental factors. This synthesis shows properties which largely remain to be discovered. The integration of studying plant micro-processes with respect to the larger environment is necessary to understand the function of plants and their communities.

The lack of indepth knowledge of plant behaviour becomes both more obvious and more crucial when studying models of whole systems, for example, to predict the long term effect of CO_2 increase on vegetation, (1) or to cultivate plants in chambers under totally artificial conditions in complex ecological cyles such as for the Controlled Ecological Life Support System (CELSS) program (2), suggested for the economical habitation of space stations over periods ranging from months to years.

Conversely, studies at the microscopic level have most frequently been initiated, oriented ans stimulated by observation of macroscopical phenomena. That tends to be forgotten and the interest in whole plant studies has decreased, at least in France, to the profit of new areas like molecular biology.

WHAT IS THE CAUSE of this disinterest, in spite of the needs mentioned above ? It could be that the traditional methods of integrated physiology do not fulfill these needs, or that they have already given their best results using past methodologies, and that answers they can bring now do not justify the experimental effort involved.

Among the many possible reasons for the difficulty of indepth studies in plant physiology , the first is the exagerated diversity of plants studied in plant physiology (many in the juvenile stage), and a seemingly infinite variety of often insufficiently defined experimental conditions. This leads to a multiplication of studies, without allowing a systematic comparison of results and progress (3). Still more important is the separation of scientific specialists.

Studying a complex system where many organs, functions, and a climatic or other trophic factors are interdependent, progress in understanding cannot result without systematically studying the correlations

between and among the various functions and organs.

ANOTHER DIFFICULTY is that the time factor is rarely mastered. The plant system is growing and evolving, it is shaped by the history of its environnent and keeps a memory of it (for example : the stress acclimation). So the constant monitoring and control of all kinetic data is necessary for understanding these processes and relating long-term to short-term responses, long-term responses being the most interesting to agronomists.

This explains why we developed a new experimental system with the purpose of studying plants as integrated systems, our main concern being photorespiration. The equipment should enable us to :

1) Realize an environment approximating natural conditions, especially light conditions.

2) Simultaneously measure the main physiological functions, by non-destructive methods.

3) Quantify the relations between shoot and root metabolism.

4) Analyse time effects by short-term (hour, day) and long-term (month, season) observations, by means of high capacity data files.

5) Relate global behaviour of the system to microscopic characteristics.

THESE AIMS MAY APPEAR AMBITIOUS. Experience has shown that they are attainable using automation and computers. It is enough to apply to plant research the methods common in medical or physical research. Several Laboratories have constructed equipment with similar objectives. The most important to our knowledge is the SPAR system (4). The controlled-environment chambers of Jones et al (1984) is also a good example of the equipment built in American universities for the study of increased atmospheric CO_2 on crop canopies or natural vegetation.

A first version of our system, named C_23A, developed since 1974, has been described (6). It has been improved and the computer system is being rebuilt on a more decentralized mode. We shall use this opportunity to present the new version, its principles and functionning. Experimental possibilities will be illustrated by typical results.

I - PRINCIPLES OF THE C_23A SYSTEM

(for : Chambers for Automatic Cultivation in Artificial Atmospheres).

The general idea is to achieve a direct recording of physiological activities of the plants in culture and store the data in files fit for immediate or delayed use (fig. 1).

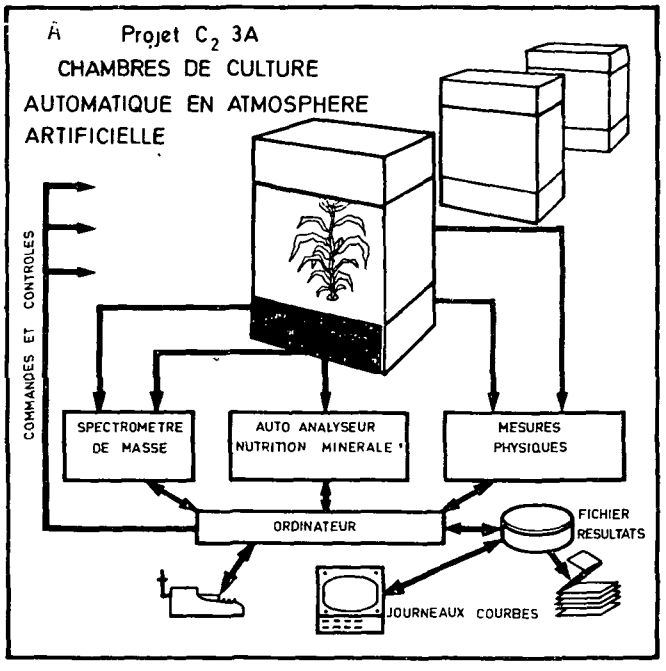


Figure 1 (A) General Principle of C_23A system.

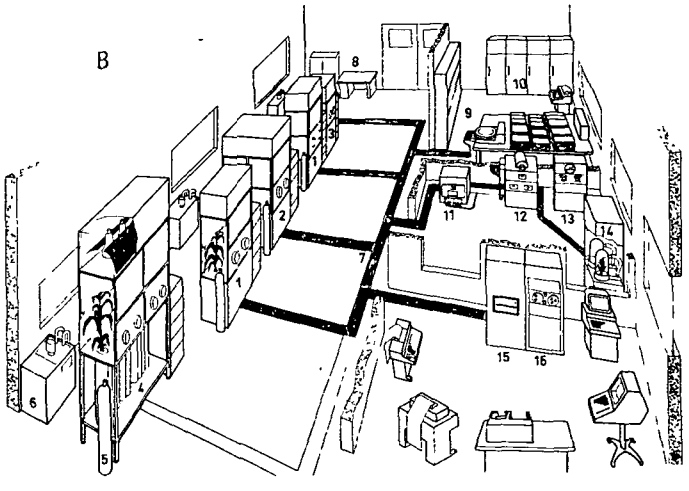


Figure 1 (B) Experimental area : (1, 2, 3) Growth chambers. (4) Twin chambers (5) CO_2 supply. (6) cool water supply. (7) Network conduit : circuits to analyse, monitor and control gas and solutions. (8) Little workshop. Specialized laboratories : (9) Chemical analysis with (10) torage of samples in deep-freeze chambers. (11) Centralized analysis of CO_2. (12) (13) Quadrupolar mass spectrometer. (14) Mini-chambers (4 to 40 l) iters (15) Computer control with (16) board of magnetic disks.

To monitor and direct the growth of plants we associate :

- leakproof culture chambers of various dimensions,
- automatic gas analysis (mass spectrometer and CO_2 infrared analyser) and regulation systems,
- automatic chemical analysis (Technicon),
- a computer system that drives in real time the abovementioned apparatus, provides visual or graphic control and stores all data in short-term and long-term files.

The physiological activities, available by measurements of matter exchanges between the plant and its environnents, are : photosynthesis in O_2 and CO_2, photorespiration measured by $18O_2$ uptake, respiration of shoots and roots, transpiration, nutrition by mineral uptakes.

II - THE CULTURE CHAMBERS

The system comprises three types of chambers, which differ notably, adapted to the study of canopies, isolated plants with a separate root compartment, and acquatic plants.

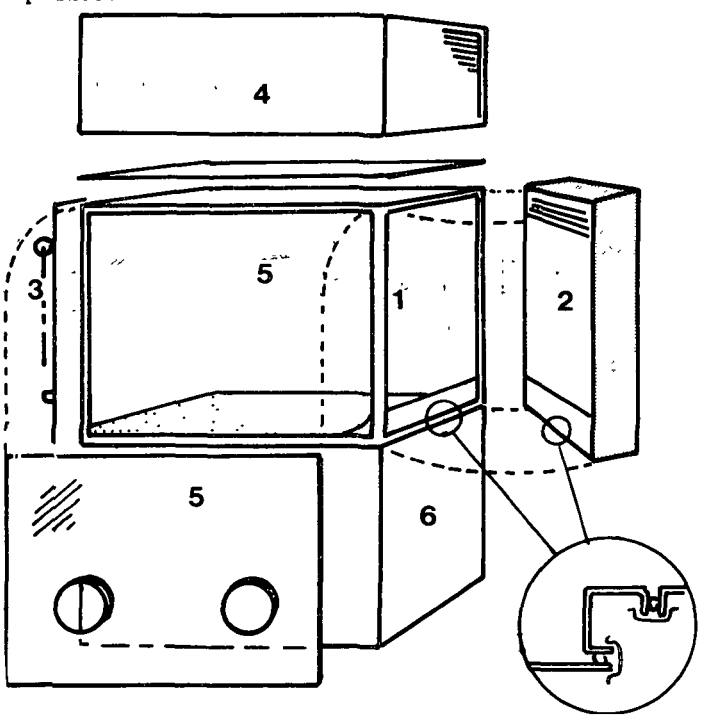


Figure 2A

Culture chambers are made by assembling on a frame unit (1) : an air conditioning unit (air-water exchanger) (2), a gas regulation unit (3), a light unit (4), front and rear panels (5) , and a stand (6). All assemblies are made with clamps and are easily disassembled. A nutrient solution supply unit can be placed on the stand.

THE LARGE CHAMBERS for the study of canopies comprise a modular frame (1 x 0.6 x 1.2 m units), a lighting module, an air conditioning unit, a gas regulation unit, and detachable front and rear panels. Several frame units can be easily assembled for making various volumes or twin chambers (figure 2). The junction between chambers by means of clamps and rubber gaskets ensures the same air-tightness as would O-rings. To optimize the simulation of a crop canopy, the side and rear panels of the chamber are covered with polished aluminium, and the front panel is coated with a semi-reflecting film, so as to reduce the border effect

AIR CONDITIONING UNIT. Dry bulb temperature is regulated by the variation of the rate of air flowing through a large area (18 m^2) heat exchanger. This copper-foil exchanger receives water at the temperature of the desired dew-point.

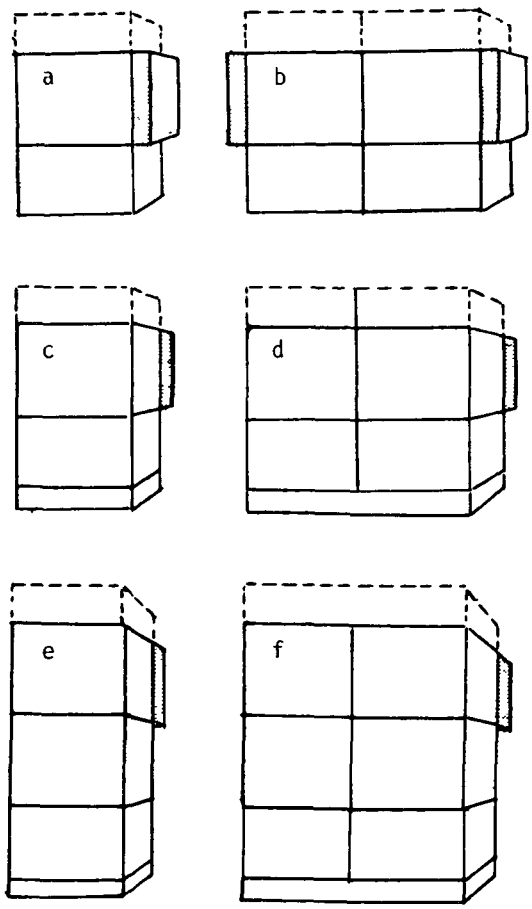


Figure 2B

Example of realization. The growth chambers can be made of 1 module (a,c), 2 modules placed side-by-side (b,d) or single-stacked (e), or made of 4 modules (f). According to need of irradiance two units to control temperature and humidity can be attached to the module (on the rear) (c,d,e,f). The combinations a,b,c,f are in operation in our laboratory.

This has two advantages : hygrometry is very accurate, and the continuous weighing of the water condensed by the exchanger measures the transpiration of the plants.

THE SMALL CHAMBERS (fig.3) from 1 to 40 liters in volume are adapted to the study of one whole plant. They include a metal base containing the heat exchanger.

The plant pot or a flask for hydroponic culture can be fitted below the metal base, and a glass container fits over the top of the plant. A tight seal is ensured by O-rings.

The root and shoot atmospheres are separated by a plastic plate with a hole for the plant(s) in the center. The young plant is inserted though the disk and the hole is sealed around the root-neck with putty a few days after germination. These disks can also be adapted to holes in the base of the large chambers. Two to four small chambers can be placed in a large chamber unit, which gives light and supplementary air-conditioning. Air circulation is ensured by a fan or a Venturi pump.

Humidity is fixed by condensed water, and by measurement of water uptake in the case of hydroponic culture.

III - ANALYSIS AND QUANTITATIVE CONTROL OF ATMOSPHERES

ANALYSIS OF SHOOT AND ROOT ATMOSPHERES. Each chamber is linked by a network of pipes to the multichannel gas introduction systems of the CO_2 analyser and the mass spectrometer. Solenoid valves controled by a microprocessor draw up gas samples which expand either into the CO_2 analyser or into the mass spectrometer. One analysis lasts 15 seconds ; because of necessary vacuum periods, the CO_2 analyser is limited to 60 analyses per hour and the mass spectrometer from 30 to 40.

The analysis of a given chamber atmosphere is programmed to occur at given times, from 1 to 12 times per hour, according to the needs of the experiment. The infrared gas analyser (IRGA) takes 50 ml samples, which prevents its use with small volume chambers. The mass spectrometer uses only 0.5 ml or less gas for each analysis.

This system has the following advantages :

- it is simple : only one introductory valve per circuit for the spectrometer, one for the CO_2 analyser,
- sampling times can be freely programmed,
- unlike more traditional devices, it is not sensitive to the flow rate in the gas circuit,

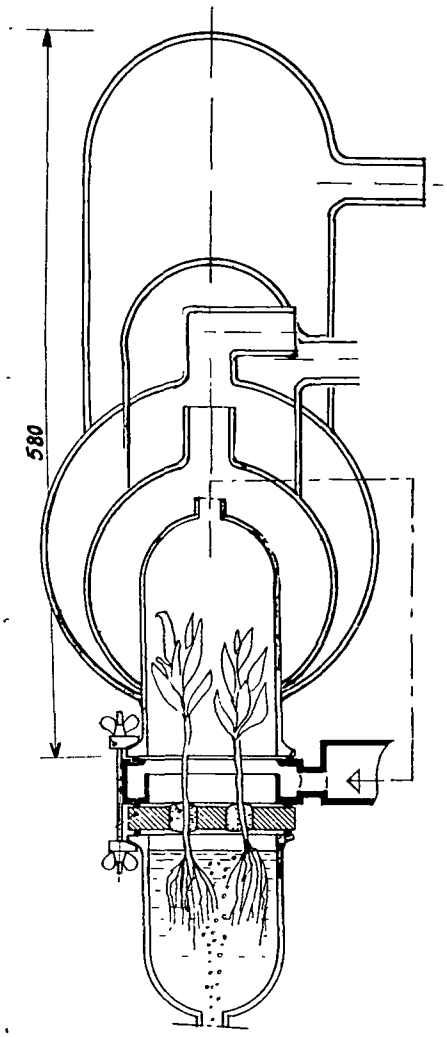


Figure 3 - Small chamber with different types of cloches of glass for shoot compartment. The module of air circulation and cooling is not shown.

- mixing of gas from different circuits never occurs. This is especially important when isotopes ($18O_2$, $14\ CO_2$) are used or when chemical products are tested.

But the main advantage is the quality and the safety of the measurements obtained :

- comparisons between chambers are always allowed, whatever the stability or calibration of the apparatus,
- calibration can be automatized. In the case of the mass spectrometer, this allows a tenfold increase of precision, especially with O_2. Each measurement is numerically compared with the measurement of a reference gas, so that the accuracy approaches that of differential measurements.

QUANTIFICATION OF THE REGULATION OF CO_2. The microprocessors associated with the chambers use the output signals of the centralized (for large chambers) or local (for small chambers) CO_2 analyser, and control injections of CO_2 during photosynthesis, or trapping of CO_2 during respiration, so as to maintain the CO_2 level at the programmed value. CO_2 injections are made by Solenoid valves which give calibrated quantities of CO_2 at each excitation. The number of injections measures the photosynthesis. The regulation adapts the frequency of pulses to the rate of photosynthesis so as to avoid any systematic lag between the real level of CO_2 and the set-point.

Data entered into the computer are the volume of the chamber and that of the injection of CO_2. The range of injection frequency is very large (1 to 100), allowing the measurement of photosynthesis of plantlets or adult plants without modifying any calibration. The regulation of the CO_2 level during respiration operates by opening or closing a CO_2 trap containing soda lime. The amount of CO_2 envolved by the plant, or trapped, is proportional to be duration of the opening of the trap, to the flow rate and to the concentration of CO_2, in the gas. The regulation program takes these experimental parameters into account to calculate the CO_2 exchange rate and to continue the regulation between times of measurement, which can be as far apart as 20 minutes.

THE MASS SPECTROMETER. The computerized control of the mass spectrometer allows repetitive analysis of a sequence of numerous atmospheres, including references gases.
This procedure ensures an exceptional accuracy of the results, although the quadrupolar mass spectrometer per se is not particularly accurate, but is does a lot of measurements rapidly for example of 120 measurements on 6 peaks in 12 seconds, the total duration of the sampling and measurements is 15 seconds. Measurements are taken only on the useful peaks of the spectrum concerning each atmosphere. Results are expressed as relative concentrations. The values given are the difference to a reference gas of reconstituted air.

This corrects the isotopic concentrations from the natural or artifactual background, and corrects the CO_2 concentrations from the background due to the oxidation of the filament. The hourly repetition of the sampling sequence and the adequate programming of the sampling order in the sequence prevent disturbance by memory effects, even at a high frequency of analysis. An example of the performance of the mass spectrometer over 3 days and of its interest in the study of root respiration is shown in Figure 4.

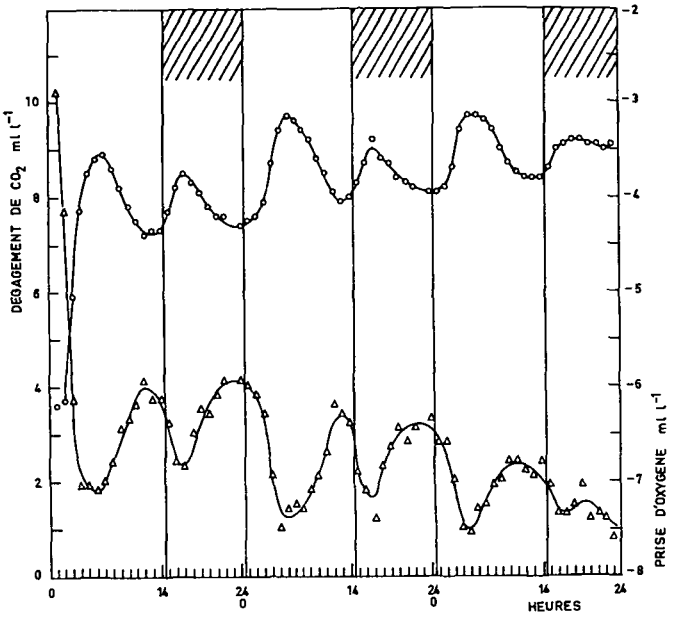


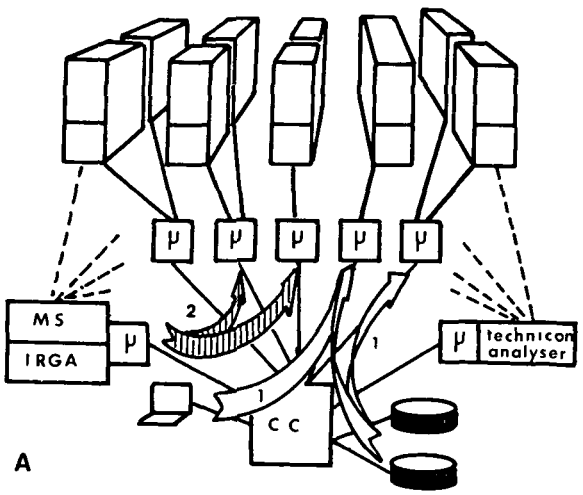
Figure 4 : Rate of root respiration of wheat plant measured by mass spectrometry (M.S) (o) CO_2 evolution, (Δ) O_2 uptake. The root container was aerated by CO_2 free air. The M.S. measured the change of CO_2 and O_2 concentrations due to the root metabolism.

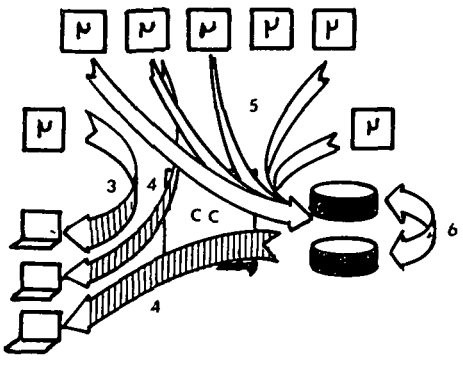
The O_2 uptake was obtained by the difference of O_2 concentration between the entrance and the exit of root system i.e. by the fullscale measurement of about 1.10^{-2} on the background of air, $20.6\ 10^{-2}$.

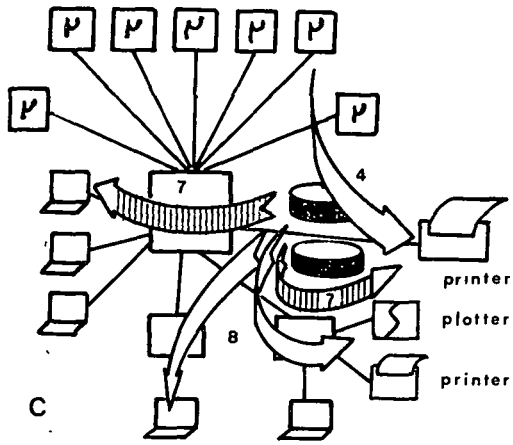
IV - AUTOMATIC CHEMICAL ANALYSIS

The system used (Technicon autoanalyser) is well-known in the field of analysis by colorimetry. It was an original application to associate it with a system of micro-electrovalves and catheteral sampling. The chemical analyzers are also controlled by a specific microprocessor, which commands frequent calibrations with reference solutions, and the accuracy of analysis is always better than 0.5 %. Using experimental parameters (volume of the containers, initial concentrations, etc...) the computer directly calculates uptake rates for each element generally, NH_3, NO3, P, and K but also Ca, S, Mg, Mn and Cl if necessary.

Real time use of the automatic chemical analysis is limited (Massimino et al, 1981), the most frequent use being the delayed analysis of nutrient solution samples that are taken everyday and stored at - 18°C. Then series of samples covering a whole experiment /for example the whole life cycle of a Maize crop (André et al, 1978 b) or the vegetative stage of wheat Ducloux, 1984/ are analysed in one run and daily uptake rates can be calculated as above and filed on disks.







COMPUTEUR SATELLITES

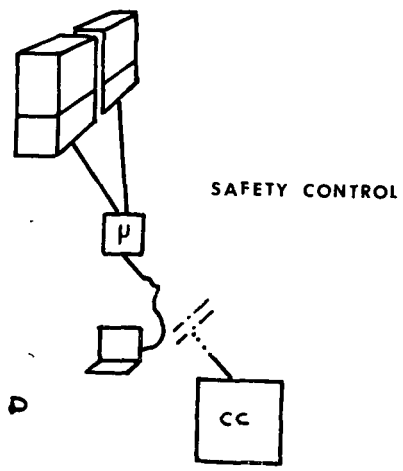


Figure 5 : Functions of the central computer (CC) connected with :
A) The micro-processors (μ) for the control of the growth chambers, the IRGA-mass spectrometer system and the Technicon Autoanalyzer.
B) Two discs as data base.
C) The mini-computers satellites for modeling and tele-processing.
D) Safety connection during maintenance or disturbance of central computer.

N°	Function	Command by	Exchanges from to
1	Loading of	Display consol I	Discs, display μ
2	Exchanges of data	Clocks and soft	μ ⟶ μ
3	Control of process	Display & consols	⟶ Displays & printer
4	Control of data	Display & consols	μ , discs ⟶ Display & printer
5	Storage of data averages	Clock & soft	μ ⟶ Discs
6	Duplication files processing	Soft-Keyboard I	Disc ⟶ Disc
7	Standard calculation	Display consols	Discs ⟶ Display printer
8	Data transfer	Satellites Computers	Discs ⟶ Satellites

V - THE COMPUTER SYSTEM

Its architecture is organized around a central data base in a computer linked to decentralized autonomous terminals :

- Seven microprocessors controling the culture chambers and measuring systems. They attend to the acquisition of measurements of the physiological activity (photosynthesis, respiration, photorespiration, transpiration, root respiration, nutrition) and of environmental parameters.
- Peripherals for data control (visual display terminals, printer).
- Terminals for data processing, calculating, modeling and programming.

THE MICROPROCESSOR. It uses a Motorola 6809 associated with a G64 bus and industrial peripherals ("Europe" card) developed by GESPAC (CH R28 Genève, Switzerland) and Thomson-EFCIS (F 78140 Velizy-VillaCoublay, France).

Its characteristics are a memory of 32 K bytes, 16 analogical inputs, 8 temperature imputs (Pt 100 sensors) 4 analogical outputs, 64 channels of logical imput-outputs.

The control and regulation software is configurable with 30 modules of elementary functions (sequence, comparison threshold, proportional regulation, integration, derivate, input, output, etc...).

One microprocessor can control two chambers. The gas analysis system (IRGA and mass spectrometer) and the Technicon chemical analyzer are controlled by two specific microprocessors, optical fibers connect the central computer and the micro-processors. The general software was studied by the CISI company (F13115 St Paul-Lez-Durance)

THE CENTRAL COMPUTER. We chose an industrial computer the Solar 16-40 produced by Bull-SEMS (Echirolle 38000 France) because a former one had proved very reliable. Its main features are the following :

- memory of 256 K-Words of 16 bits,
- communication processor (IOP) and floating point processor (FPP),
- RTDES real time disk operating systems.

It is associated with two disk units of 10 M-bytes, each with a fixed head disk and a removable disk for data archival. (Figure 5)

THE SATELLITES MICROCOMPUTERS. (Bull-micral 30, compatible IBM PC) They use disk archival files and are programmable in BASIC for non routine data treatment. Programs written on the satellite terminals can be tranferred to be used in the central computer in compiled BASIC. The long distance transfer of the data has been envisioned as part of a European project.

SECURITY. Our experience of using a computer for the direct control of experiments, since 1976, has shown that the computer was the most reliable part in the whole experiment, with a time availability ratio of over 99 %.

This is made possible by the quality of the hardware but also by the safety procedures used, as preventive maintenance, self-testing and diagnosis of the computer. The new descentralized system should be as reliable, thanks to the following precautions :

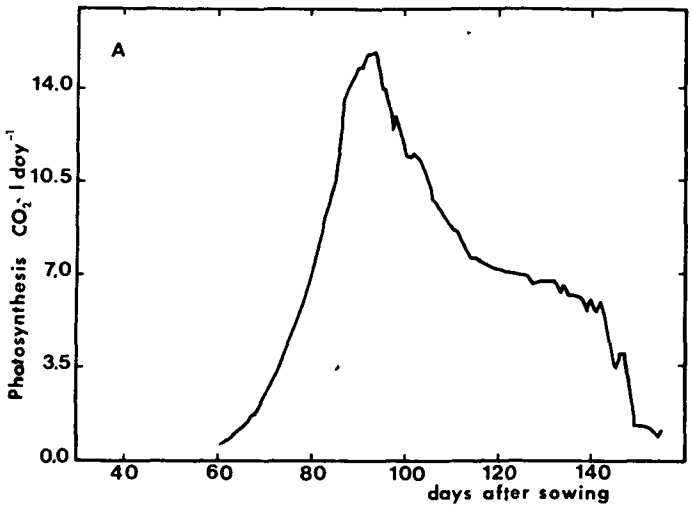
- In case of a default in the central system, the microprocessors associated with the chambers can control the experiments and store the data within three hours.
- They can be directly connected with the control display terminals and a simplified management ot the experiments is possible in case of a lengthy deficiency of the central computer.
- Back up of the software and data is ensured by a systematic copy procedure. The two disks are used symmetrically copy procedure. The two disks are used symmetrically so that one can replace the other.

VI - EXAMPLES OF RESULTS

The leading idea of our experiments was to study the contribution of the main photo-phenomena : photosynthesis and photorespiration, to the energy budget of the plants. We also looked at dark respiration of shoots and roots and even root excretion in some particular studies. These balances were studied at the scale of a plant life cycle, or in a vegetative stage in light of environmental parameters. The parameters (light, CO_2, O_2, water) were chosen to change the value or the proportion of the two photo-phenomena. In a whole plant approach the interactions with other physiological activities were considered to uncover a possible role of the photorespiration.

LARGE CHAMBERS. They have first been used for the simultaneous study of shoot and root physiological activities, either during the life cycle of a Maize plant (8,9) or under the effect of a light reduction simulating a cloudy day (11,12). Data files were used later for the adjustment of growth and maintenance respiration models (fig. 6) (13) (14).

Maize is a plant with a very low photo-respiration, only the photosynthesis was concerned, but these studies can be used as base line for experiments manipulating both photosynthesis and photorespiration.



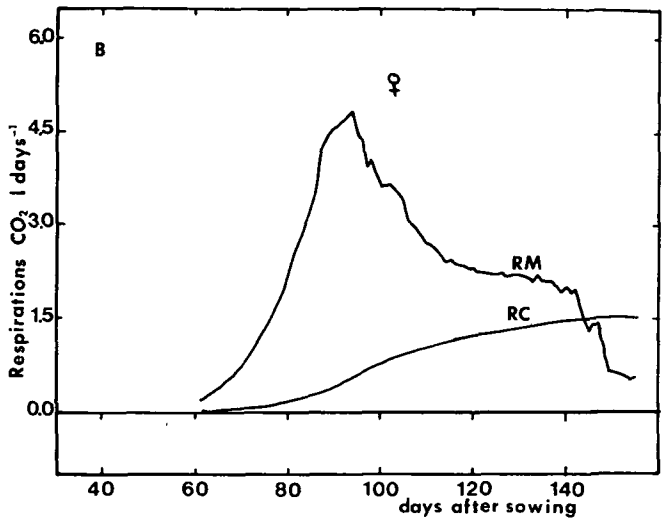


Figure 6 : Paterns of A) Photosynthesis (P) and B) Respiration (R) during the life cycle of maize crop. The measured respiration was shared to the sum of two terms : growth respiration Rc = αP, and respiration of maintenance R_m = bΣ (P - R) following the assumption :
R_t = aP + bΣ (P-R) (adjustment by last square method a = 0.27, b = 0.0032). Rm was very low in the vegetative stage (♀ = silking).

In the case of the wheat plant the acceleration of growth at an increased level of CO_2 is due to both the stimulation of photosynthesis and the inhibition of photorespiration (Fig. 7)

Curves are drawn from data files, without correction or smoothing. The ratio between the two curves is the growth stimulation coefficient. It remains stable throughout the experiment. Contrary to previous studies (15) we do not observe any negative feed-back effect from the enhancement of photosynthesis. The results are part of a more comprehensive study of the effect of CO_2 on photosynthesis, photorespiration, transpiration, nutrition and draught effects (Du Cloux, 1984).

SMALL CHAMBERS

They are adapted to the study of photorespiration with ^{18}O. The mass spectrometry technique allows the continuous measurement of O_2 uptake and evolution during photosynthesis. The first studies in wheat under standard growth conditions, showed that O_2 uptake during photosynthesis was nearly as fast as net photosynthesis (16, 17), which corresponds to a loss of reductive energy of 50 %. This is two times as much as what could be predicted from the biochemical analyses in-vitro of the properties of RuBP carboxylase-oxygenase (18). Research is in progress for understanding the reasons for this difference and possibly finding a physiological role for photorespiration. The current hypothesis of a protective role is supported by the finding that in case of water stress, in the soybean, two-thirds of the reductive power is diverted to photorespiration (19). Also, a study of gas

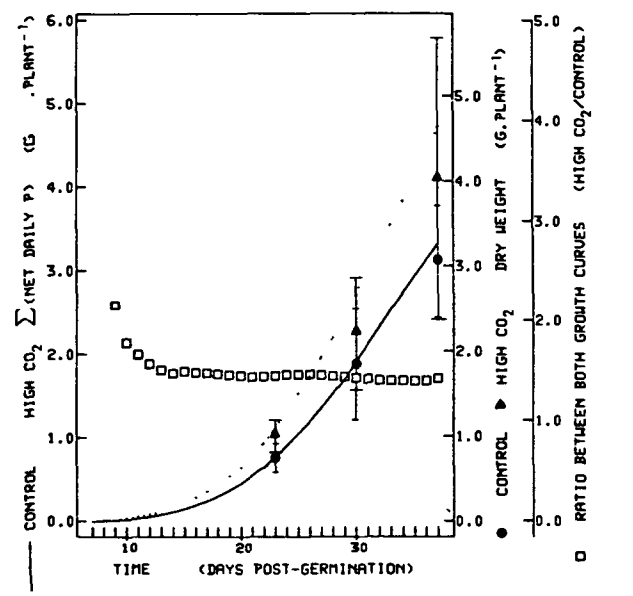


Figure 7 : Growth of a canopy of wheat (Triticum aestivum L. var. Capitole) during the vegetative phase at normal (—) or double (---) concentration of CO_2. Dry weight was calculated by the cumulation of daily CO_2 exchange balance, on the assumption that 1 g dry weight corresponds to 440 mg carbon, stimulation coefficient (□) weights measured on randomly sampled and sacrificed plants (10 plants per sample) (●, ▲). Standard deviation bars show the large dispersion of results obtained by sampling compared with the stability of gas exchange measurement ratios. Plant density was 200 m^{-2}, PAR 600 µmol photons m^{-2} s^{-2}, temperature 24/18°C, photoperiod 14/10 h, R.H. 50/85 %.

Exchanges in mosses showed that the maximum oxygen uptake capacity is low in these primitive plants. We suggest that evolution towards a greater capacity of oxygen uptake is a necessary condition to support stress resistance in higher plants.

Small chambers are also well adapted to the study of shoot-root relations, which are a key to the understanding of whole plant physiology (Figure 4 above).

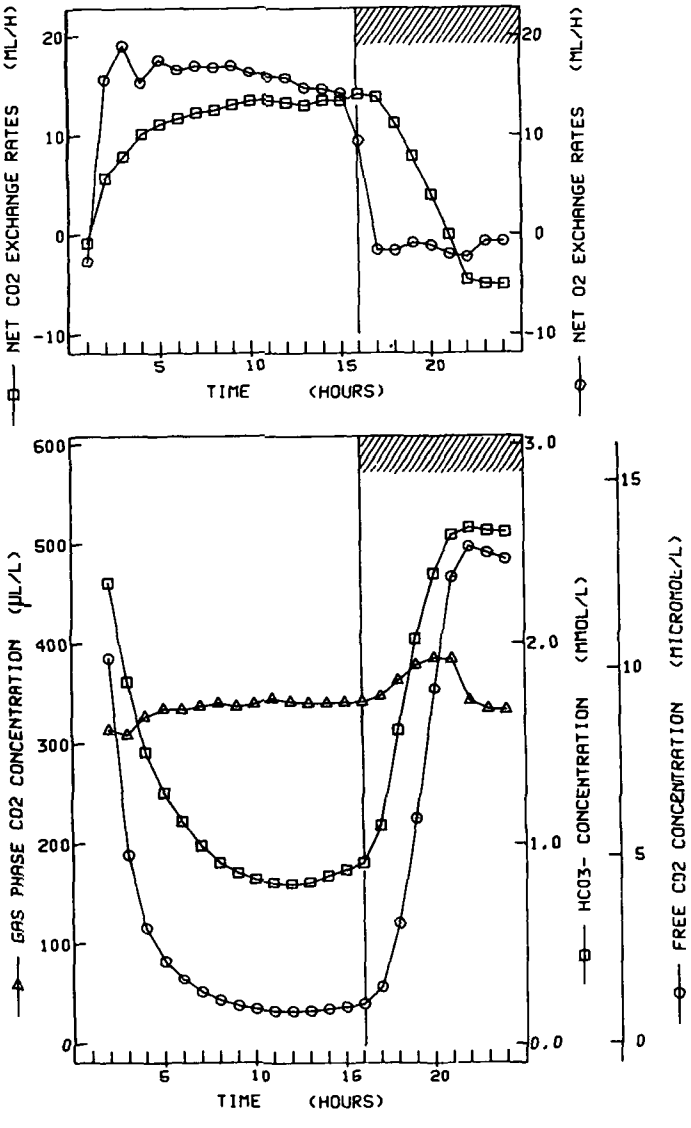


Figure 8 : Photosynthesis in the marine macroalga Chondrus crispus. A) daily cycle of the gas exchanges as measured in the air or in the reactor : (O) O_2 production or uptake, (■) apparent CO_2 uptake (as measured by the regulation)
B) Variations of the various forms of inorganic carbon in the system : (○) free dissolved CO_2, (□) bicarbonate ions, (△) gas phase CO_2 concentration.

REACTORS FOR AQUATIC PLANTS. The study of O_2 and CO_2 uptake in Chondrus crispus has shown that the oxygenation is slow in normal conditions and that it is due only in part to the glycolate pathway. It was shown that, like certain microalgae, bicarbonate can be taken up directly (21,22) and even preferentially to CO_2 (23). Figure 8 shows an example of curves obtained by computer.

The reactor contains 4 liters of sea water in dynamic equilibrium with 1 liter of air, 30 g of algae (fresh weight). PAR : 200 mol photons m^{-2} s^{-1}. The atmosphere was recycled through the water with a flow rate of 120 l h^{-1}.

VII - CONCLUSION

The system described has been shown to advance research in the study of whole plants, compared to previous equipment, thanks to the following advantages :

- It can simulate a real climate or create an artificial one, with the possibility of modifying one parameter or the composition of the atmosphere at any time.
- Cultivation can be prolonged indefinitely in these conditions, while measurements are automatically and continuously taken and stored.
- The large number of parameters measured, and their accuracy, give at every moment a relatively complete picture of the plant activity and of its environnent.
- Mass spectrometry allows the measurement and study of photorespiration, an important component of the gas exchanges (the flow of O_2 is four times that of the dark respiration). The use of other isotopes (^{15}N, ^{13}C, ^{14}C) remains possible.

The price paid for these results is moderate. The initial investment (mainly in the computer system) has been amortized in more than ten years, so that the annual cost of the computer was less than the salary of two gardeners.

The field of possible applications is vast. Long-term as well as short-term effects of any treatment (increase of the level of CO_2, climatic variation, disease, nutrient deficiency, growth regulations, herbicides etc...) can be studied in unequaled conditions of precision. Automatization allows the realization of these experiments with the minimum of staffs : two persons can easily conduct one double experiment in twin chambers.

The capacity of our centralised equipment allows for more possibilities that our limited team, focussed on photorespiration, can use, and gives a possibility for collaboration with other laboratories.

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