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Gerbaud, A.
INRA Departement de Biologie Vegetale Route de Saint-Cyr

Andre, M.
CEA Cadarache Departement d'Ecophysiologie Vegetale et de Microbiologie

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Down regulation of photosynthesis after CO_2 enrichment of lettuce; relation to photosynthetic characteristics

A. GERBAUD[1,2] and M. ANDRÉ[2]

[1]INRA Département de Biologie Végétale Route de Saint-Cyr, 78026 Versailles cedex
[2]*CEA Cadarache Département d'Ecophysiologie Végétale et de Microbiologie 13108 Saint Paul Lez Durance cedex, France*

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GERBAUD A. and ANDRÉ M. *Down regulation of photosynthesis after CO_2 enrichment of lettuce; relation to photosynthetic characteristics* *BIOTRONICS* **28**, 33–44. 1999. The effect of CO_2 enrichment was studied in two sets of lettuce which had been cultivated either in summer conditions (summer plants) or winter conditions (winter plants) during the first two weeks of cultivation, then in the same summer conditions in culture chambers during three weeks at either 340 or 1950 ppm CO_2. Summer plants were much heavier, but with smaller and thicker leaves than winter plants, and 6-fold higher specific rates of photosynthesis. Theses differences persisted for more than three weeks after plants were put in the same summer conditions. The stimulation of photosynthesis by elevated CO_2 did not slow off in winter plants, whereas summer plants showed down-regulation of photosynthesis and an inhibition of leaf development at 1950 ppm CO_2, resulting in zero gain weight in elevated CO_2. Photorespiration was inhibited by high CO_2, but this effect was fully reversible with no after-effect of enrichment. Down regulation of photosynthesis in summer plants, consisting in a 25% reduction in photosynthetic capacity and 25% reduction in leaf area, appeared to be due to the enhancement by high CO_2 of limiting factors prevalent only at the high photosynthetic rates attained in summer plants.

Key words: *Lactuca*, elevated CO_2, down-regulation of photosynthesis, gas exchange, growth, photorespiration, $^{18}O_2$

INTRODUCTION

It is well known that some plants fully benefit of the potential increase in photosynthesis brought by elevated CO_2, in terms of growth and dry matter accumulation, whereas others, although they show the same short-term response to increased CO_2, are little stimulated in the long term, due to what is termed down regulation of photosynthesis (*3*). The occurrence of down regulation has

Corresponding author: A. Gerbaud, DEVM, CEA Cadarache, 13108 Saint Paul lez Durance cedex, France
E-mail Gerbaud@DSVCAD. CEA. FR

been shown to depend on several factors, such as age, light intensity and N or P nutrition (*11*). The mechanisms of down regulation have been the subject of numerous studies (see review by Stitt (*12*) ; the most frequently invoqued causes for the observed decrease of the photosynthetic response are limitations by nutrients, translocations, sink capacity and a decrease of Rubisco content or activation (*16*).

Photorespiration might be implicated in down regulation because it constitutes a major loss in the carbon and energy budget (*1, 7*) and is regulated by CO_2 concentration. Due to competition at the Rubisco site, its ratio to photosynthesis should be fixed by the CO_2 concentration, according to the biochemical model of Farquhar (*6*). There are however few actual measurements of photorespiration during down-regulation. Du Cloux (*4, 5*) found no modification of the photorespiration to photosynthesis ratio in CO_2 enriched wheat, whereas Chagvardieff (*2*) found a higher than expected oxygen effect in lettuce, suggesting an adaptive effect of photorespiration. A decrease in catalase activity was observed in acclimated peas (*13*), but not in other photorespiratory enzymes. Other oxidative reactions (dark respiration in the light and Mehler reaction) which occur simultaneously and are also possibly modified by the enrichment have not been measured during CO_2 enrichment.

Lettuce cultivated in greenhouses has been shown to benefit from CO_2 enrichment in winter, but there are no data for summer, as enrichment cannot be economically applied simultaneously with ventilation (*8*). In this study we shall show that down regulation in plants submitted to CO_2 enrichment can occur, or not, according to their previous history: lettuce plants having been cultivated during early growth either in winter or summer conditions will behave quite differently three weeks later. A CO_2 concentration of 1950 ppm (parts per million in volume) for enrichment has been retained so as to maximise effects on the photosynthetic apparatus, without having direct toxicity of CO_2. At this concentration, photosynthesis is CO_2 saturated and photorespiration is largely inhibited, which is not the case at lower concentrations. The components of photosynthesis will be analysed with particular regard to the participation of photorespiration and oxidative processes in the carbon budget and eventual down-regulation.

MATERIALS AND METHODS

Plant Material

Seeds of *Lactuca sativa* (L.) Merr. were sown in Perlite. Culture 1 was sown in April 1994 and kept in the greenhouse (maximum/minimum temperatures 27/19℃, daytime duration around 13.5 hours) during 2 weeks before being transferred to a culture chamber. Culture 2 was sown in November 1995 in the culture chamber set to climatic conditions simulating those seen by culture 1 in the greenhouse (13 hr day/11 hr night with 25/20℃ temperature, 80% HR). Culture 3 was treated like culture 1, except that it was initiated in November 1994, with winter conditions in the greenhouse (maximum/minimum

temperatures around 23/18℃, daytime duration 9 hours). In the following text, cultures 1 and 2 are termed summer plants, and culture 3 is termed winter plants. Plants were watered daily with nutrient solution containing the following ions (mM): K^+ 3.25; Mg^{2+} 1; Ca^{2+} 2; NH_4^+ 0.5; NO_3^- 7.25; SO_4^{2-} 1; PO_4^{3-} 0.5; Fe^{3+} 0.5; and the following microelements (μM): Mn^{2+} 4.5; Zn^{2+} 0.045; Cu^{2+}0.15; Na^+0.05; BO_3^{3-} 23; MoO_4^{2-} 0.05 (half strength Hoagland solution). Conditions in the culture chamber for all cultures from the third week were: 16-hr photoperiod (650 μmol photon m^{-2} s^{-1} flux density provided by Osram HQI 400W lamps), 70% relative humidity, 24℃ day, 19℃ night. When the rosettes were about 8 cm in diameter, plantlets were transferred to special pots, allowing to hermetically isolate the root compartment from the outside air. The roots were separated from the perlite, introduced through a 1 cm hole in the lid of the pot and sealed with putty. Pots were filled with 200 ml of the same nutrient solution as above. An inlet and outlet allowed the aeration of the nutrient solution and the addition of new nutrient solution. Plants were used at an age of 36 to 50 days. Photosynthetic rates ranged from 25 to 100 mL CO_2 hr^{-1} per plant.

CO_2 treatment

The high CO_2 treatment was initiated from the 15th day of cultivation for all cultures. The CO_2 level in the culture chambers was regulated by CO_2 injection by day or CO_2 trapping by night. Then the treatment was done simply by setting the set-point of regulation to 1950 ppm (parts per million in volume). Control plants were cultivated in a chamber regulated at 340 ppm CO_2. The same concentrations were maintained, except for short durations, when the plants were put in the measurement chambers described below.

Gas exchange measurements

For measurements of CO_2 exchange, plants were transferred one at a time to air-tight environmental chambers (Fig. 1). The volume of the chamber was 14 L and it was flushed with 4 L.hr^{-1} compressed air to avoid problems of confinement due to the accumulation of volatile compounds (*15*). Light was provided by 10 Osram HQI 400 W lamps giving a light fluence rate of 800 μmol photons m^{-2} s^{-1}. Temperature controlled by thermocouples placed under shade was 24/19℃ day/night. The relative humidity was set at around 70% by circulation of the air on a cooler.

CO_2 concentration was measured by a Maihak Finor IRGA CO_2 analyzer. The CO_2 concentration was maintained at 340 or 1950 ppm by pulsed injections of CO_2, monitored by a computer. Net photosynthesis P was calculated from the record of CO_2 injections. During the dark period, the CO_2 level was regulated by passing air through a soda lime trap controlled by a regulator. Shoot respiration was calculated from the time of working of the trap. Km and Γ are apparent values characterising the response of *net* photosynthesis to CO_2 concentration. Km, named in analogy to the Michaelis constant, is the CO_2 concentration giving half the maximum net photosynthetic rate; Γ is the CO_2

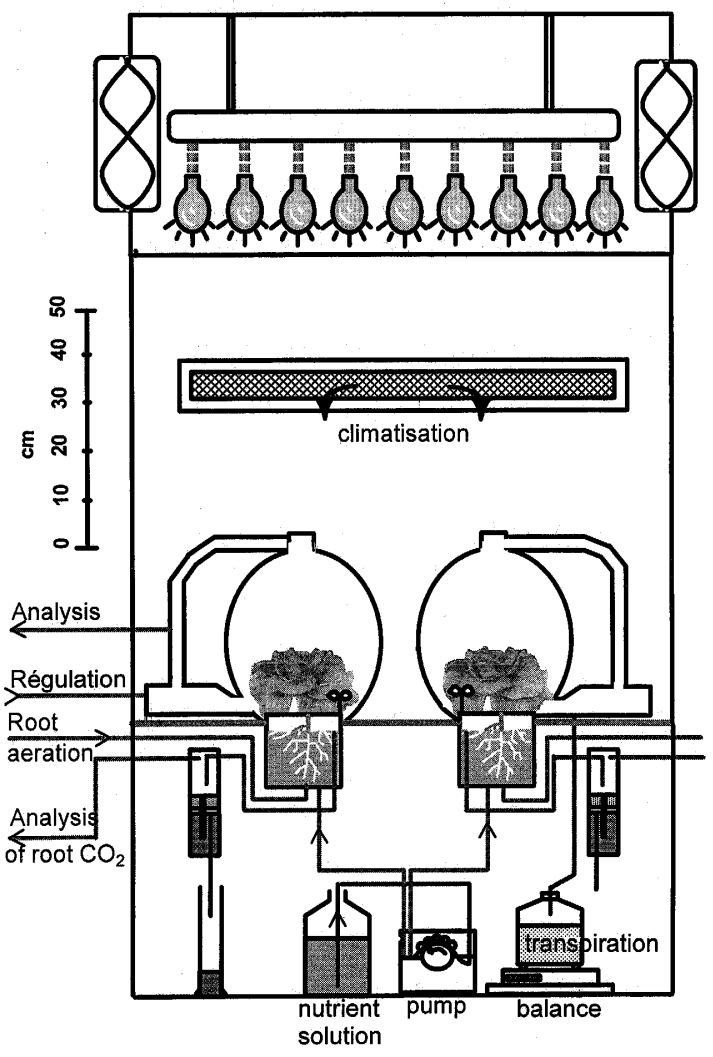

Fig. 1. Diagram of the measuring chambers.
The air–tight, spherical glass vessels containing the plants are inserted in a cabinet providing light and temperature control. The glass vessels are connected to analysis instruments and gas regulation systems. Under the vessels are the root pots, also air–tight, with their nutrient solution and aeration outfits.

concentration giving a zero net photosynthesis. The response of photosynthesis to CO_2 was determined by initially raising the CO_2 concentration inside the chamber to 2000 ppm, and then letting the CO_2 concentration to decrease because of the plant uptake, in the absence of CO_2 regulation.

The water vapour produced by the plant was condensed on a cooler, and then collected in a water jug standing on an electronic balance. Computer recordings of the weight of water were used to calculate the transpiration rate. For the calculation of the substomatal CO_2 concentration, we used the classical method (*9*). In the first step the stomatal resistance to water vapour diffusion is calculated by Fick's law relating the rate of evaporation to the water vapour

pressure gradient. The internal water vapour pressure is calculated from the leaf temperature (approximated by air temperature), assuming that the internal air space is vapour saturated. In the second step, the CO_2 concentration gradient is calculated by multiplying the net CO_2 exchange rate by the CO_2 diffusion resistance, assumed to be 0.625 times the resistance to water vapour. Because this method involves several approximations, mainly concerning leaf temperatures and the uniformity of stomatal closure and internal CO_2 concentration, the result can be considered only as a gross estimation of real internal CO_2 concentration. Therefore we shall use it not as an absolute value, but rather as an index of comparison between different treatments.

Oxygen uptake was measured by introducing $^{18}O_2$ in the chamber to a concentration of around 1% (*7*). The following decrease of $^{18}O_2$ concentration in the chamber is measured by a mass spectrometer and allows the calculation of the oxygen uptake of the shoot. The measurement must be corrected by the dilution of an inert gas (krypton) as an internal standard, which was introduced in the chamber simultaneously with $^{18}O_2$. Photorespiration was calculated as total O_2 uptake minus night (mitochondrial) respiration, hypothesised to occur in light at the same rate as during the night. Photorespiration calculated in this way includes any oxygen uptake due to the Mehler reaction. It can alternatively be calculated from the difference between O_2 uptake at 340 ppm and at 1950 ppm CO_2, keeping in account that only 75% or photorespiration is inhibited at 1950 ppm CO_2 (*10*). The two methods give similar results, showing that the rate of Mehler reaction is small in our experimental conditions; in the figures, photorespiration will refer to the difference of total O_2 uptake minus night respiration. Photosystem activity means the electron transport rate by PSII; it is equal to the rate of gross O_2 production. It is calculated by adding net O_2 and CO_2 uptakes (see (*7*)).

Data treatment.

All parameters were recorded by a computer at 2-min intervals and mean values were calculated and stored every 30 min. A part of data processing was done with the computer program "Voyons" (*14*). The kind of measurements achieved cannot be made on large number of plants. Measurements were independently performed on 4 summer plants and 2 winter plants for each CO_2 concentration. While the small number of replications does not allow the application of statistical methods, confidence in the results is substantiated by the large effects evidenced and by the coherence of results obtained by different methods (e. g. differences in photosynthesis between treatments correspond to differences in dry weights).

RESULTS

Winter and summer plants

The CO_2 enrichment was applied on winter or summer plants with different effects. To understand these effects as exposed below, we have to note the

differences that were already present before the enrichment took place: summer plants (cultures 1 and 2) were three times heavier than winter plants (culture 3), with a smaller rosette diameter, a 35% smaller leaf area but 4-fold thicker leaves (Fig. 2). They had the same dry matter content, and proportionately more root weight. Net photosynthesis per leaf area was six-fold higher in summer than in winter plants. Leaves of enriched summer plants had a golden colour and were crinkled and affected with necrosis at the margins.

It should be remembered that all plants, summer or winter, were placed in the same "summer" contitions during the whole period of enrichment; the differences noted above were acquired during the first two weeks of cultivation and persisted until the end of the experiments.

Effect of CO_2 enrichment on morphological characteristics (Fig. 2)

Winter plants were quite stimulated by CO_2 enrichment and had more dry matter as the control, for nearly the same leaf area. On the contrary, summer plants cultivated at 1950 ppm CO_2 had nearly the same dry matter than the controls, and they had smaller and thicker leaves. For both summer and winter plants, the proportion of dry matter to fresh weight increased in response to enrichment; the root to shoot ratio was not affected.

Effect of CO_2 enrichment on net photosynthesis (Fig. 3)

The net photosynthetic rates of the whole plants are in accordance with the different weight gains. Summer plants had considerably larger photosynthetic rates than winter plants, but were not stimulated by enrichment. This was essentially due to the smaller leaf area of enriched summer plants (Fig. 2), which compensated for the higher photosynthesis per leaf area at high CO_2.

Effect CO_2 enrichment on respiration (Fig. 4)

Plant respiration has been traditionally separated in maintenance respiration, linked to plant biomass, and in growth respiration, linked to photosynthesis. It appears that in our experiment respiration was essentially maintenance respiration, as it was strongly linked to leaf dry weight, but not to photosynthesis (data not shown). The smallest plants (control winter plants) had a slightly higher ratio or respiration. Only a small fraction of respiration was dependant on the previous day's photosynthesis in plants raised at 340 ppm CO_2, and not at all in plants raised at 1950 ppm for which the night respiration was independent from the previous day's photosynthesis (data not shown).

Effect of CO_2 enrichment on stomatal conductance and transpiration (Fig. 4)

When conditions of light, temperature and relative humidity are equal, transpiration flows are proportional to stomatal conductance. The plot of transpiration per leaf area indicates that stomatal conductances were much higher in summer plants, which had also the higher rates of photosynthesis. The stomatal conductance and transpiration rates were about 20% higher in plants raised in enriched CO_2, but were independent of the measurement CO_2.

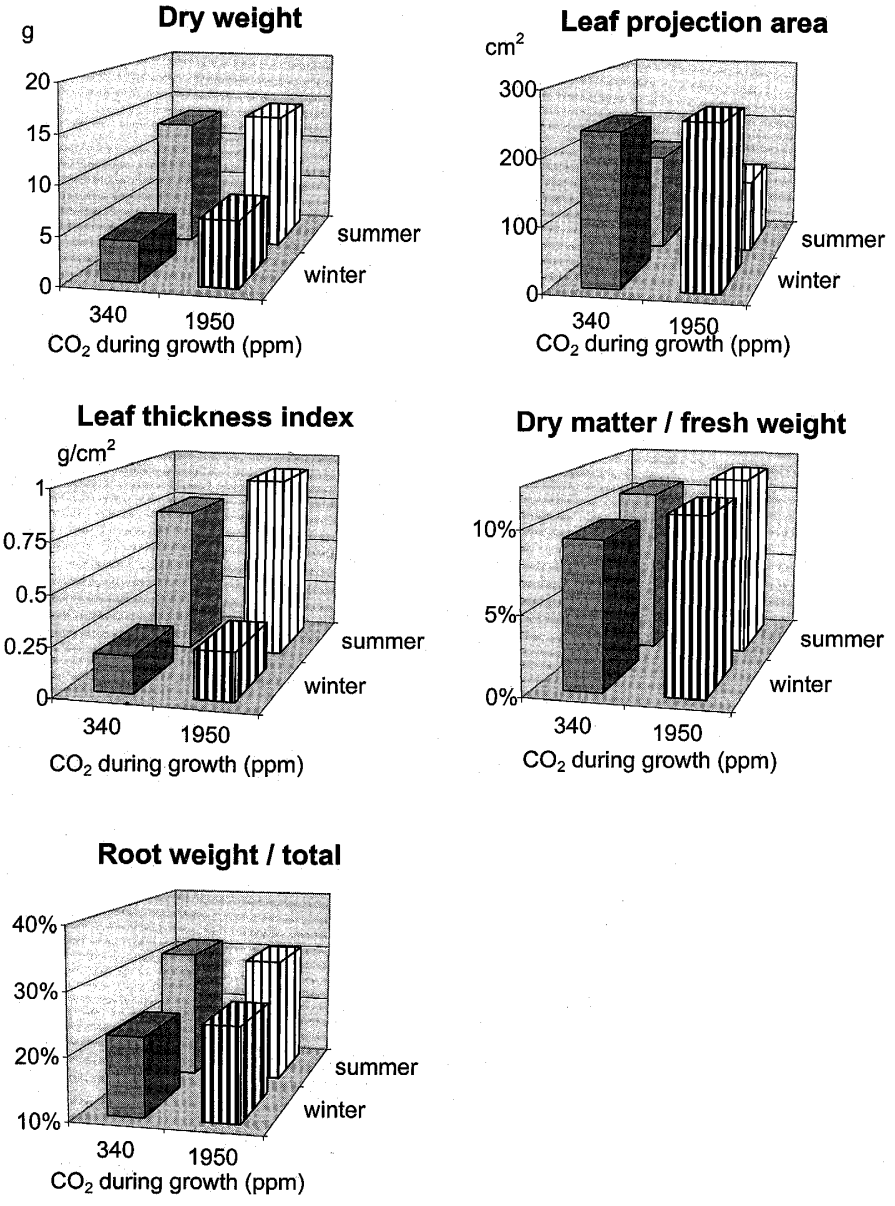

Fig. 2. Effect of CO_2 enrichment during growth on morphological characteristics of lettuce. "Summer" or "winter" refer to the growth conditions of the first 2 weeks, all plants were then grown and measured in the same controlled "summer" conditions.
Leaf projection area is the area of the vertical projection of the plant. The leaf thickness index is the leaf fresh weight divided by the leaf projection area.

Analysis of the photosynthetic performance of enriched plants (Figs. 5 and 6)

To see if there was any effect of the enrichment treatment on the photosynthetic process itself, photosynthesis of the control and enriched groups has to be measured at the same CO_2 concentrations. All comparisons will bear on plants grown at normal or enriched CO_2, but measured at the same CO_2 level

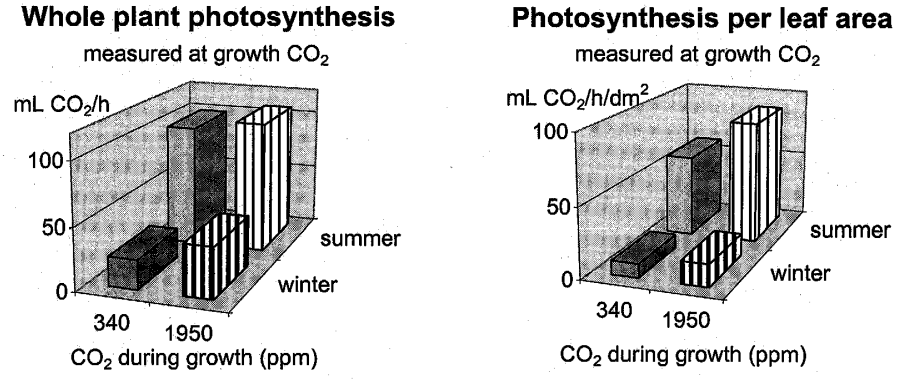

Fig. 3. Effect of CO_2 enrichment on net photosynthesis

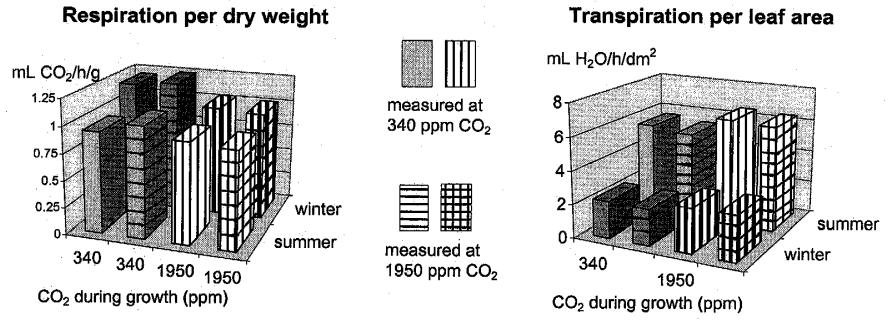

Fig. 4. Effect of CO_2 enrichment on respiration and transpiration measured either at 340 ppm or at 1950 ppm CO_2. Take notice that for clarity, in the respiration per dry weight diagram, summer bars are placed in front of winter bars, contrarily to other figures.

of 340 ppm. Results must however be interpreted in the view that even at the same external CO_2, internal CO_2 may differ. In fact for an external CO_2 of 340 ppm, the calculation based on transpiration rates do not show large variations between internal CO_2 of plants raised in various conditions (Fig. 5). Internal CO_2 stayed in the range 270–290 ppm, except for control summer plants which had a lower internal CO_2 of 235 ppm.

Photosynthesis (Fig. 5)

When measured at 340 ppm CO_2 (or at 1950 ppm CO_2, data not shown), it appears that photosynthetic rates of the enriched plants are slightly enhanced over controls in winter plants, but are reduced by 25% in summer plants. Concurrently the apparent Km and Γ for CO_2 of the whole plants were slightly increased by enrichment in winter plants, but nearly doubled in summer plants.

As net photosynthesis is the resultant of photosystem activity (producing reducing power) decreased by loss of reductants to O_2 (Mehler reaction), then by photorespiration (due to glycollate cycle), we examined how these various components of photosynthesis were affected by enrichment. O_2 fluxes were measured using labelled oxygen.

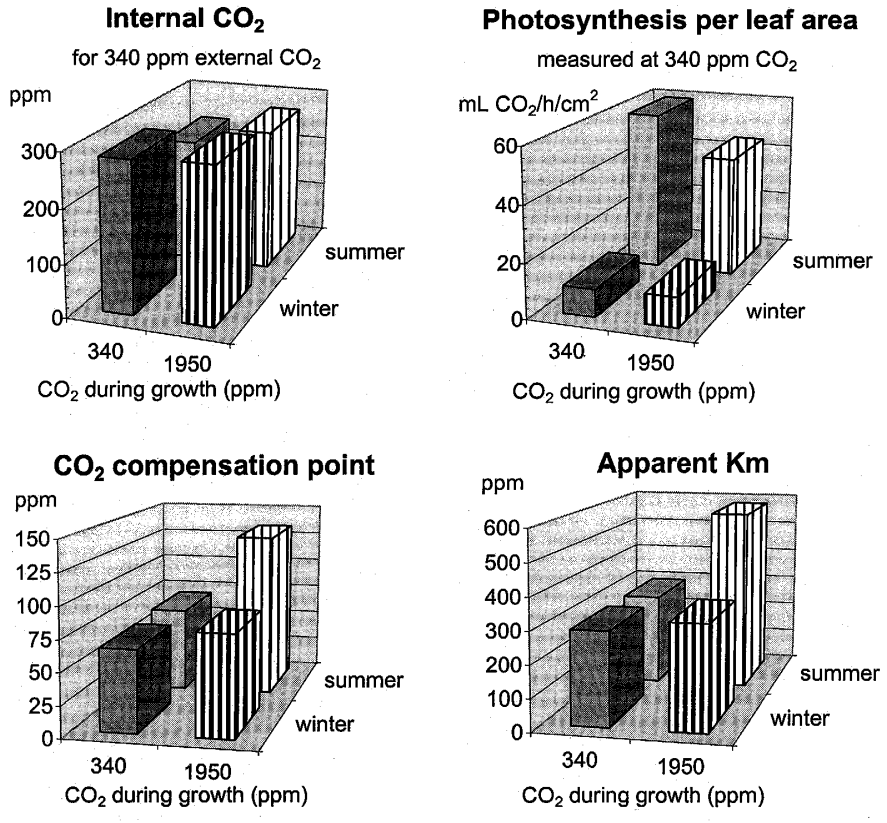

Fig. 5. Effect of CO_2 enrichment on some photosynthetic parameters. Internal CO_2 was calculated from photosynthesis and transpiration data by the method of Jarvis (*9*).

Photosystem activity (Fig. 6)

Photosystem activity, as measured by gross O_2 evolution, was little changed by CO_2 enrichment in winter plants. In summer plants, it was reduced 20% in enriched plants. However the activity was recovered when measured at 1950 ppm CO_2.

Mehler reaction (Fig. 6)

The estimation of Mehler reaction bears a high incertitude because it is estimated by difference. However it appears that Mehler reaction may consume around 5% of the reductants produced by photosystem activity. No clear tendency appears as a result of CO_2 enrichment.

Photorespiration (Fig. 6)

Photorespiration measured at 340 ppm CO_2 remained at the same ratio to photosynthesis of 45% in enriched and control plants, both in winter and summer plants.

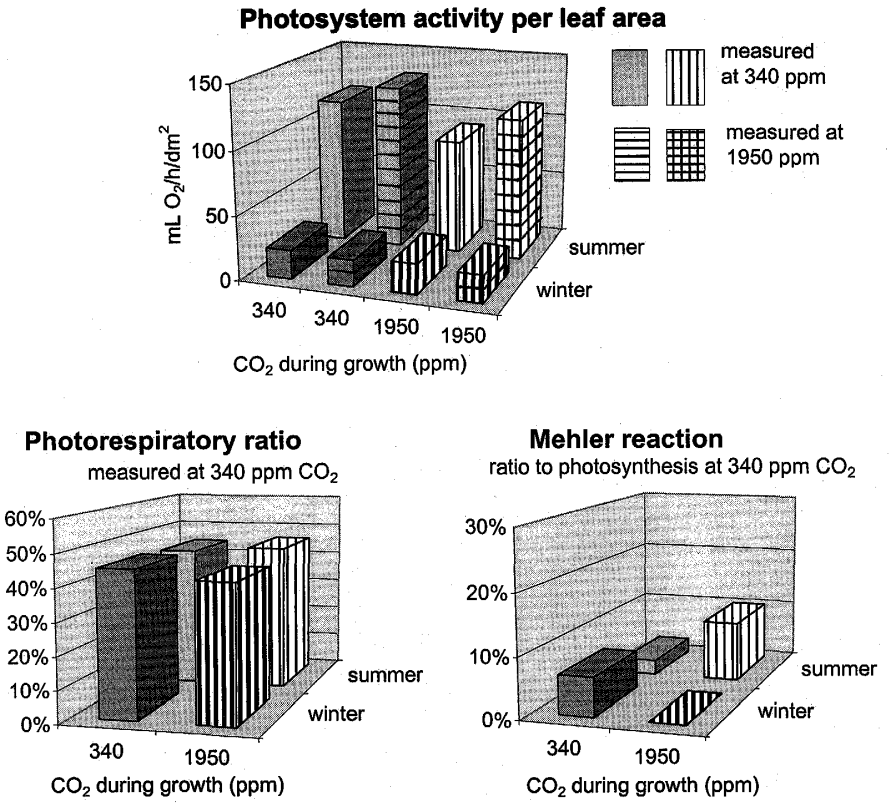

Fig. 6. Effect of CO_2 enrichment on the components of net photosynthesis. Photosystem activity is the gross CO_2 production or electron flow at PSII; photorespiratory ratio is the ratio of true photorespiration to net photosynthesis (see methods).

DISCUSSION

From the preceding results, it appears that winter and summer plants respond completely differently to CO_2 enrichment, as winter plants are largely stimulated by CO_2 and summer plants are not.

Summer and winter plants

In spite of summer or winter climatic conditions being applied only during the first two weeks of growth, after which conditions were identical for all plants, the plants acquired huge differences which persisted until the end of experiments more than three weeks later. This is especially remarkable in the view that most of the plant matter is formed in the latter period. These differences comprise a three-fold gain in weight for summer plants, a 4-fold gain in leaf thickness, but a 35% decrease in leaf area and most importantly, a 6-fold gain in net photosynthesis per leaf area.

Response of winter plants to CO_2 enrichment

In winter plants, the response of the plant to CO_2 enrichment is what would be expected if the short-term response to CO_2 was maintained throughout the enrichment period. The stimulation of photosynthesis by enrichment stays at nearly 50% and is translated in increased dry matter, leaf area and leaf thickness which are enhanced respectively by 60%, 8% and 28%; these results agree with those of Hand in the greenhouse (*8*). Photorespiration is reversibly decreased and returms to the normal value when measured at 340 ppm CO_2. Km and Γ are the same as in control plants. So it can be said that the photosynthetic apparatus of enriched winter plants is fully similar to the controls, the only difference being in plant size.

Response of summer plants to CO_2 enrichment

In summer plants on the other hand, CO_2 enrichment decreases both leaf development (by 25%) (Fig. 2) and the photosynthetic ability of the leaves (Fig. 5): photosynthesis per leaf area measured at 340 ppm CO_2 is decreased by 25% compared to control plants; Km and Γ are doubled, in accordance with a higher instantaneous response to CO_2 (+98% at 1950 ppm CO_2), but in apparent contradiction with the unchanged photorespiratory ratio (Fig. 6). The explanation is that this apparent lower affinity for CO_2 is not due to stomatal closure (no decrease in internal CO_2, Fig. 5), nor to an enhanced photorespiration, but to the increased ratio of dark respiration to photosynthesis. This is due to the increased mass and decreased leaf photosynthesis of enriched, compared to non-enriched summer plants. The application of a doubled ratio of respiration to photosynthesis in a current model of photosynthesis (*6*) will produce about the observed modifications of apparent Γ and Km. So the modifications of Km and Γ do not translate any qualitative change in the photosynthetic apparatus, but only a quantitative decrease of the rate of gross photosynthesis per leaf weight, when measured at 340 ppm CO_2.

CONCLUSION

The next question is why photosynthesis is not increased proportionally to dry matter in enriched summer plants. The first element of response lies in their different development: a reduced area per leaf weight will inevitably cause a decrease of photosynthesis per unit weight, if light is not saturating. Metabolic perturbations of CO_2 enriched plants have been widely studied. Starch accumulation has been evidenced in many down regulated plants (*13*). We hypothesize that starch accumulates in summer plants (not in winter plants) because of their very high rates of photosynthesis per leaf area (six-fold higher as in winter plants at 340 ppm CO_2), which may cause the emergence of new limiting factors in the transport or metabolism of photosynthates synthetised in the chloroplasts. This theory fits with our observations that although photosynthesis is not directly affected, it is less stimulated by high CO_2 in summer than in winter plants and correlatively surplus photosynthate due to

CO_2 enrichment is not used to build new leaf tissue in summer plants, whereas it is in winter plants.

All these different events are conditioned by the climate of the first two weeks of cultivation. Further cultivation of the plants in the same conditions during several weeks does not lessen the extent of their morphological and physiological differences. This stresses the lasting importance of the intial growth and of the memory of past events in plant physiology.

REFERENCES

1. Canvin D. T., Berry J. A., Badger M. R., Fock H. and Osmond C. B. (1980) Oxygen exchange in leaves in the light. *Plant Physiology* **66**, 302–307.
2. Chagvardieff P., d'Aletto T., Vidal D. and André M. (1994) Physiological responses of lettuce crop to various conditions of light, carbon dioxide and oxygen in artificial systems. pp 233–237. ESA publication SP–366.
3. Cure J. D. and Acock B. (1986) Crop responses to carbon dioxide doubling : a litterature survey. *Agric. For. Meteorol.* **38**, 127–145.
4. du Cloux H., André M., Gerbaud A. and Daguenet A. (1989) Wheat response to CO_2 enrichment : effect on photosynthetic and photorespiratory characteristics. *Photosynthetica* **23**, 145–153.
5. du Cloux H., Richaud C. and Vivoli J. (1984) Effect of increasing atmospheric carbon dioxide on growth, photosynthesis, photorespiration and water use efficiency. *in* C. Sybesma (ed) IV. 3. pp 213–216. Martinus Nijhoff, Den Haag.
6. Farquhar G. D., Von Caemmerer S. and Berry J. A. (1980) A Biochemical Model of Photosynthetic CO_2 Assimilation in Leaves of C3 Species. *Australian Journal of Plant Physiology* **7**, 315–327.
7. Gerbaud A. and André M. (1979) Photosynthesis and photorespiration in whole plants of Wheat. *Plant Physiology* **64**, 735–738.
8. Hand D. W. (1984) Crop responses to winter and summer CO_2 enrichment. *Acta Horticulturae* **162**, 45–63.
9. Jarvis P. G. (1971) The estimation of resistances to carbon dioxide transfer. *in* Z. Sestak, J. Catsky, and P. G. Jarvis (eds) Plant photosynthetic production. Manual of methods. pp 566–631. Dr. W. Junk. N. V. Publ. The Hague.
10. Kent S. S., André M., Cournac L. and Farineau J. (1992) An integrated model for the determination of the Rubisco specificity factor respiration in the light and other photosynthetic parameters of C3 plants in situ. *Plant Physiology and Biochemistry* **30**, 625–637.
11. Sage R. F. (1994) Acclimation of photosynthesis to increasing atmospheric CO_2 the gas exchange perspective. *Photosynthesis Research* **39**, 351–368.
12. Stitt M. (1991) Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. *Plant, Cell and Environment* **14**, 741–762.
13. Thibaud M. C., Cortez N., Riviére H. and Betsche T. (1995) Photorespiration and related enzymes in pea (*Pisum sativum*) grown in high CO_2. *Journal of Plant Physiology* **146**, 596–603.
14. Thiéry J. M. (1985) Voyons programme de simulations conversationnelles en physico-chimie et en agronomie. *In Logiciels pour la Chimie.* pp 156–157. Ed. Soc. Fr. Chimie (Paris).
15. Tibbitts T. W. (1978) Air contaminants. *In A growth chamber manual. Environmental control for plants.* Comstock Publishing, Comstock, Ithaca, New York.
16. Van Oosten J.-J. and Besford R. T. (1995) Some relationships between the gas exchange, biochemistry and molecular biology of photosynthesis during leaf development of tomato plants after transfer to different carbon dioxide concentrations. *Plant, Cell and Environment* **18**, 1253–1266.