*New Phytol.* (1996), **133**, 627–635

# The response of *Plantago major* ssp. *pleiosperma* to elevated $CO_2$ is modulated by the formation of secondary shoots

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(*Received 11 October 1995; accepted 1 March 1996*)

SUMMARY

The effect of elevated $CO_2$ on the relative growth rate (RGR) of *Plantago major* ssp. *pleiosperma* was studied during the vegetative stage, in relation to plant development, by growing plants at 350 $\mu l\ l^{-1}$ or at 700 $\mu l\ l^{-1}$ $CO_2$ in non-limiting nutrient solution with nitrate. To minimize interference by the accumulation of non-structural carbohydrates in the interpretation of results, RGR was expressed on a f. wt basis ($RGR_{FW}$), as were all plant weight ratios. Stimulation of the $RGR_{FW}$ of the whole plant by elevated $CO_2$ was transient, and did not last longer than 8 d. At the same time a transient increase in root weight ratio (RWR) was observed.

In order to investigate whether the transient effect of elevated $CO_2$ on $RGR_{FW}$ was size-dependent, the data were plotted versus total f. wt ($\log_e$ transformed). The transient period of stimulation of $RGR_{FW}$ and of RWR by elevated $CO_2$ was still found, but in both $CO_2$ treatments $RGR_{FW}$ decreased after a certain plant size had been reached. This size coincided with the stage at which secondary shoots started to develop, and was reached earlier in plants grown at elevated $CO_2$. The RGR of these secondary shoots ($RGR_{sec}$) was still increased when the period of whole plant stimulation of $RGR_{FW}$ had ended, indicating that the development of these new sinks took priority over a continuation of the stimulation of RWR. It is hypothesized that in this *Plantago* subspecies the response of the $RGR_{FW}$ of the whole plants to elevated $CO_2$ is modulated by the formation of secondary shoots. Apparently, partitioning of the extra soluble carbohydrates at elevated $CO_2$ to this tissue takes precedence over partitioning to the roots, resulting in a cessation of stimulation of plant $RGR_{FW}$ by elevated $CO_2$.

Key words: *Plantago major* ssp. *pleiosperma*, elevated $CO_2$, RGR modulation, sink-source interaction, soluble sugars.

## INTRODUCTION

In a compilation of literature sources on 156 plant species, Poorter (1993) found that the stimulation of growth of whole plants in the vegetative stage by elevated $CO_2$ was, on average, 37%. This enhancement was small compared with what could be expected on the basis of $CO_2$-response curves of photosynthesis. In many species relative growth rate (RGR) was also increased by elevated $CO_2$, but interspecific variation was large (Poorter, 1993). Experiments with *Plantago major* ssp. *pleiosperma* showed that the effect of doubling the ambient $CO_2$ concentration resulted in an increase in whole plant d. wt of 30% after 3 wk of exposure, due to a transient stimulation of the RGR during the first 10 d (Den Hertog, Stulen & Lambers, 1993). The temporary nature of the stimulation of RGR by elevated $CO_2$ was also found for other species, such as *Triticum aestivum* (Neales & Nicholls, 1978), *Brassica pekinenses* (Kriedemann & Wong, 1984), *Cucumis sativus* (Kriedemann & Wong, 1984; Peet, 1986), *Glycine max* (Rogers *et al.*, 1984), *Gliricidia sepium* (Thomas *et al.*, 1991), and *Phaseolus vulgaris* (Jolliffe & Ehret, 1985). Only in rare cases was a continuous stimulation of RGR found (Downton, Björkman & Pike, 1980).

Both source-sink relationship and constraints of size on growth such as pot size or self-shading, can cause the transient stimulation of RGR by elevated $CO_2$ (Poorter, Pot & Lambers, 1988; Farrar & Williams, 1991; Stitt, 1991). Several lines of evidence indicate that growth at elevated $CO_2$ leads to a change in the sink-source balance of the plant: carbohydrates can accumulate in the source leaves if

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Abbreviations: LA, leaf area; $RGR_{FW}$, relative growth rate on a f. wt basis; $RGR_{sec}$, relative growth rate of the secondary shoots; RWR, root weight ratio; SWR, shoot weight ratio; $SWR_{sec}$, shoot weight ratio of the secondary shoots.

the rate of photosynthesis exceeds the capacity of the sinks to utilize the photosynthate, e.g. for growth and storage. According to Stitt (1991) the variability in the response to elevated $CO_2$ in different species, developmental stages or environmental conditions, can be explained in terms of differences in sink strength of the plants.

During development, plants of *Plantago major* start to develop new sinks, namely secondary shoots. The present study was aimed at investigating the influence of the formation of these new sinks on the duration and the degree of stimulation of the RGR by elevated $CO_2$. For that purpose we transferred seedlings of *Plantago major* ssp. *pleiosperma* to elevated $CO_2$ (700 $\mu$l $l^{-1}$) at two developmental stages: (1) before the formation of secondary shoots, and (2) with secondary shoots present at the beginning of the $CO_2$ treatment. A detailed growth analysis was performed including measurement of the $RGR_{FW}$s of the whole plant and of the secondary shoots and of partitioning between root and shoot. At the same time, photosynthetic rate and carbohydrate concentrations in shoot and roots were measured, both during and after the period of stimulation of $RGR_{FW}$, in order to establish whether a diversion of the extra photosynthates from the roots to the developing secondary shoots might be the cause of the transience of the stimulation of $RGR_{FW}$.

## MATERIALS AND METHODS

### *Plant material*

An inbred line of *Plantago major* L. ssp. *pleiosperma* Pilger (A4), was used (see also Van Dijk & Van Delden, 1981; Dijkstra & Lambers, 1989). The plants were grown from seeds germinated on a sterilized commercial soil mixture, in a glasshouse at 24/17 °C day/night temperature. After germination the seedlings were transferred to a climate room, at 20 °C day/night, 60–65 % r.h. and 12 h light of 500–550 $\mu$mol $m^{-2}$ $s^{-1}$ (400–700 nm, measured with a quantum sensor, SKP 215, Skye, Llandrindod Wells, UK) provided by Philips HPI-T lamps (400 W) and incandescent bulbs (40 W) in a 1:1 ratio. Three weeks after germination the plants were transferred to an aerated Hoagland solution, 1/4 of the strength of that described by Smakman & Hofstra (1982), namely 3·75 mM nitrate, and pH 5·8, at a density of 20 plants in 30 l. The nutrient solution was changed once a week. Neither depletion of the nutrient solution nor change in pH occurred; nitrate concentration decreased by 10 % at most.

### *Experimental design*

One week after transfer to the aerated nutrient solution (4 wk after germination; day 0 in the figures), two groups of randomly selected plants were distributed over two controlled climate chambers kept at 350 and 700 $\mu$l $l^{-1}$ $CO_2$ respectively. The $CO_2$ concentration in the chambers was controlled by infra-red gas analysers (Siemens type ZFPCS and ZFP-DZ). Exposure to elevated $CO_2$ lasted for 20 d. Other growth conditions were as described above.

Two weeks later (6 wk after germination; day 14 in the figures) a third group, randomly selected from the plants growing at 350 $\mu$l $l^{-1}$ $CO_2$, was transferred to elevated $CO_2$. Exposure to elevated $CO_2$ lasted for 6 d. To verify that no effect on population composition had been introduced by removal of this third group of plants, a control experiment was conducted in which the plants were left undisturbed at 350 $\mu$l $l^{-1}$ $CO_2$ until 6 wk after germination, when they were randomly divided into two groups, one of which was transferred to elevated $CO_2$.

In all experiments, thinning to prevent mutual shading was carried out. Experiments started with a plant density of 20 plants per 0·16 $m^3$, and ended with a density of four plants per 0·16 $m^2$. All experiments were repeated at least once; the repetitions gave similar results and, therefore, the results of only one of the experiments are shown. Before repetition of the experiments, the $CO_2$ concentration in the chambers was switched from 350 to 700 $\mu$l $l^{-1}$ $CO_2$ and vice versa in order to eliminate a possible chamber effect. All results were reproducible and independent of the growth chamber used.

### *Growth analysis*

Plants were harvested every 2 d, with $n = 10$, and $n = 20$ at the start and end of the analysis. At each harvest, f. wt of leaves, stems and roots was determined. Dry weight was determined after exposure of the samples to 80 °C for at least 24 h. From day 10 onwards, the fresh and dry weights of leaves and stems of secondary shoots were also determined.

The relative growth rate of the total plant and of the secondary shoots (after day 10) were calculated on a fresh weight basis ($RGR_{FW}$) to minimize interference by accumulated carbohydrates in plants at elevated $CO_2$ (Wong, 1990). The RGR analysis was carried out after Poorter (1989) with data obtained at 2 d intervals. In this method autocorrelation is minimized, since RGR is calculated by skipping one harvest each time, rather than deriving values from adjacent harvests (for further details see Wickens & Cheeseman, 1988; Poorter, 1989). Leaf area (LA) was measured with a digitizer (Hewlett Packard Company, model 9847a, Fort Collins, CO, USA).

The weight ratios (plant part f. wt/total plant f. wt.) of main shoot (SWR), secondary shoot

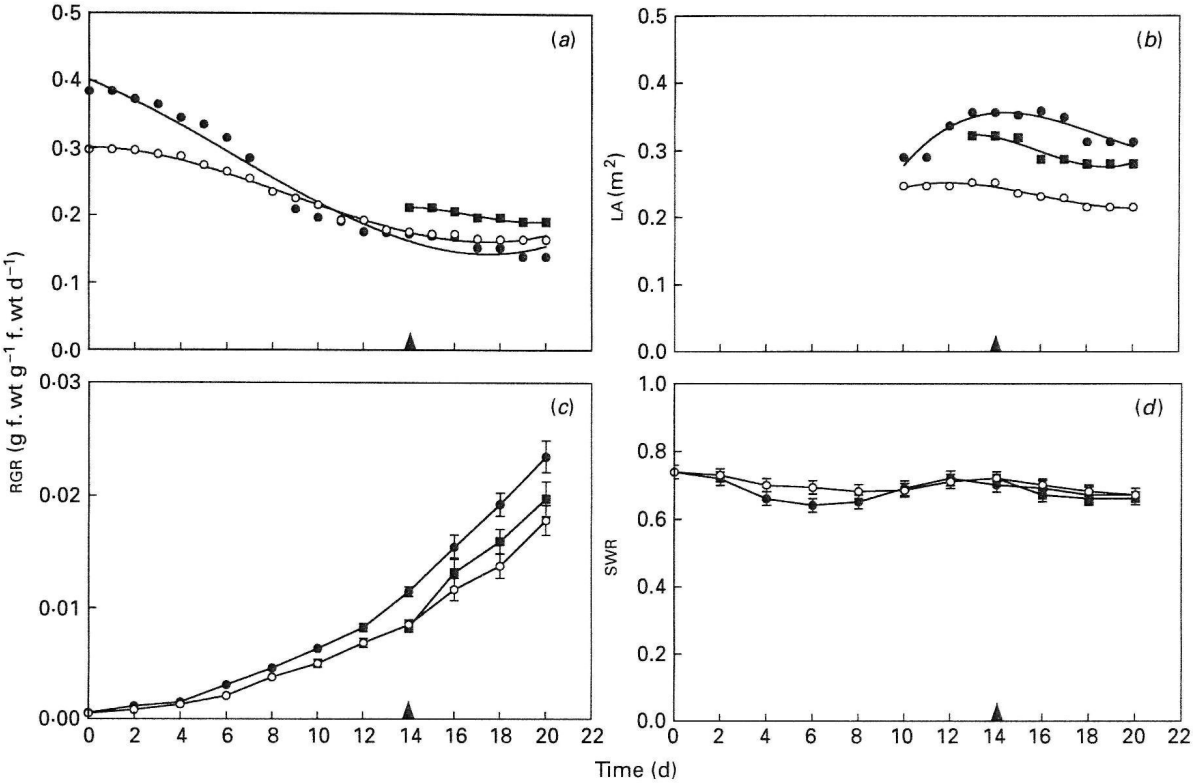


**Figure 1.** (*a*) Relative growth rate ($RGR_{FW}$), on a f. wt basis of the total plant and (*b*) of the secondary shoots. Third order polynomials were fitted through the RGR data. The effects of elevated $CO_2$ on $RGR_{sec}$ were significant at $P < 0{\cdot}001$ for the younger plants and $P < 0{\cdot}05$ for the older plants. (*c*) Leaf area (LA) of the plants sampled for RGR analysis (mean ± SE) (time × $CO_2$ interaction significant at $P < 0{\cdot}0001$ in a two-way analysis of variance, for the younger plants). (*d*) Shoot weight ratio (SWR), on a fresh weight basis (mean ± SE). (●) 700 $\mu$l l$^{-1}$ $CO_2$, (○) 350 $\mu$l l$^{-1}$ $CO_2$, (■) older plants. Black triangles indicate the day when the older plants were transferred to elevated $CO_2$.

($SWR_{sec}$) and root (RWR) were all calculated on a f. wt. basis. The various growth analysis parameters ($RGR_{FW}$, LA, RWR, $SWR_{sec}$ were corrected by plotting each parameter versus a measure of plant size, namely $\log_e$-transformed total plant f. wt ($\log_e$TFW).

### *Carbohydrate analysis*

All plant material for the carbohydrate analyses was harvested at 1000 hours, that is 4 h after the lights had been switched on. Soluble sugar and starch concentrations in shoots and roots were determined on day 4, 7, 9, 16 and 20, using three sets of three plants each. On day 20, shoot material was divided between main and secondary shoots and starch and soluble sugar concentration was determined. For the plants transferred later, soluble sugar and starch in shoot and root were determined on days 16 and 20.

Plant material, dried at 80 °C for at least 24 h, was ground to a fine homogeneous powder with a homogenizer (Retsch, type MM2). Soluble sugars were then extracted with 80% ethanol and the material washed four times with 80% ethanol. The remaining pellet was boiled in 3% HCl for 3 h in order to hydrolyse starch. For both extracts soluble sugar was then determined colorimetrically according to Fales (1951), using anthrone reagent (Merck, Darmstadt, Germany). It was verified that starch and soluble sugar determination data from material dried at 80 °C were comparable with data obtained with fresh material.

### *Leaf photosynthesis measurements*

*In situ* photosynthetic rate was measured with a LICOR portable photosynthesis system (LI-6250, Lincoln, NE, USA; 6000-13 cuvette). The air in the cuvette was circulated by a fan placed inside, and facing the abaxial side of the leaf; the relative humidity during the experiments was 65–70%. Photosynthetic rates were measured on (i) the youngest developing leaf, present at the start of the elevated $CO_2$ treatment (existing leaf), and on (ii) the leaf initiated and developed after transfer to elevated $CO_2$ (new leaf).

For both $CO_2$ treatments net photosynthetic rate was measured on days 4, 8, 15 and 20 (existing leaf), and days 15 and 20 (new leaf), in the respective climate chambers, at the same photon flux density

and temperature as during development. All measurements were carried out between 1100 and 1500 hours, with $n = 5$.

### *Data analysis*

Third order polynomials were fitted through the RGR data (for details see Poorter, 1989). The significance of the effect of elevated $CO_2$ on $RGR_{FW}$ was tested by a two-way analysis of variance of the $\log_e$-transformed fresh weights and polynomial contrasts, according to Poorter & Lewis (1986). The significance of the effect of elevated $CO_2$ on the other parameters was tested by a two-way analysis of variance or by Student's $t$-test. All statistical analyses were performed with the SPSS/PC computer package or the GraphPad Prism Instat package (GraphPad Software Inc., USA, 1994–95).

## RESULTS

### *Growth analysis*

Stimulation of the $RGR_{FW}$ of the total plant by elevated $CO_2$ was transient in plants transferred 4 wk after germination (younger plants; Fig. 1*a*). The transient nature of the effect was further evident from the significant interaction of time × $CO_2$ as found in a two-way analysis of variance of the $\log_e$-transformed total plant f. wt ($\log_e$ TFW) (Table 1). In plants transferred 6 wk after germination (older plants) the effect of elevated $CO_2$ on the $\log_e$-transformed TWF was significant at $P < 0{\cdot}05$ (two-way analysis of variance). The degree of the stimulation of $RGR_{FW}$ by elevated $CO_2$ was higher for the younger (30 %) than for the older plants (20 %) (Fig. 1*a*). At elevated $CO_2$, both the degree and the duration of the stimulation of $RGR_{sec}$ were increased compared with the whole plant. This increase was evident in the younger as well as in the older plants (Fig. 1*b*). LA of both the younger and older plants was increased by elevated $CO_2$ (Fig. 1*c*). SWR of the younger plants decreased slightly during the period that $RGR_{FW}$ was stimulated, but this decrease was not significant (Fig. 1*d*). In the case of the older plants, SWR was also not significantly changed (Fig. 1*d*).

Plants grown at elevated $CO_2$ started earlier to develop secondary shoots, visible on day 6, when 50 % of the plants used for the RGR analysis had secondary shoots (Fig. 2*a*). In order to correct for ontogenetic drift, $RGR_{FW}$ was plotted versus $\log_e$-transformed total f. wt (TFW). Before a certain plant size was reached, the effect of elevated $CO_2$ on $RGR_{FW}$ was not size dependent; after that there was no difference between the treatments. For both $CO_2$ concentrations it was evident that the decrease in $RGR_{FW}$ occurred when a certain plant size was reached; this size coincided with the beginning of the formation of the secondary shoots (Fig. 2*a*, *b*). RWR was temporarily increased at elevated $CO_2$ (Fig. 2*c*), during the period that $RGR_{FW}$ is stimulated. After this initial increase, RWR was decreased for both $CO_2$ treatments, at the same time that $SWR_{sec}$ started to increase (Fig. 2*e*). In the case of the older plants, RWR was only increased significantly on day 16 (Fig. 2*c*). Correction for ontogenetic drift showed that the increase by elevated $CO_2$, observed for the RWR of the younger plants, was not entirely size-dependent, although the later decrease in RWR was size-dependent, at both $CO_2$ concentrations (Fig. 2*d*). In the younger plants, $SWR_{sec}$ was markedly increased at elevated $CO_2$ (Fig. 2*e*). Correction of the $SWR_{sec}$ data for ontogenetic drift showed that the observed effect was strongly size-dependent for the younger plants (Fig. 2*f*).

**Table 1.** *Analysis of variance of the $\log_e$-transformed total plant fresh weight ($\log_e$ TFW) of the younger plants*

| Source of variation | $\log_e$ TFW |
|---|---|
| Time | a |
| $CO_2$ | a |
| Time × $CO_2$ | a |
| Linear component | b |
| Quadratic component | c |

Independent variables: time (11 harvests) and treatment ($CO_2$ concentration). $P$,$P$-value of the $F$-test.
a, $P < 0{\cdot}0001$; b, $P < 0{\cdot}001$; c, $P < 0{\cdot}01$.

**Table 2.** *Starch and soluble sugar concentration in the secondary shoots (mean ± SE, $n = 3$), on day 20*

| | $CO_2$ concentration ($\mu l\ l^{-1}$) | |
|---|---|---|
| | 350 | 700 |
| Starch | 111 ± 2·9 | 116 ± 6·4 |
| Soluble sugar | 37 ± 3·3 | 56 ± 2·5 a |

a, $P < 0{\cdot}0001$ (Student's $t$-test).

### *Carbohydrate analysis*

Shoot-starch concentration was greatly increased in plants grown at elevated $CO_2$. The difference in concentration decreased with time but the plants grown at elevated $CO_2$ still showed an increase by the end of the experiment (Fig. 3*a*). Soluble sugar concentration of the shoot was also higher at elevated $CO_2$ both during and after the period of stimulation of $RGR_{FW}$ (Fig. 3*b*). In both $CO_2$ treatments soluble sugar concentration decreased with time until day 9, after which it remained constant. The older plants, transferred 6 wk after germination, were also able to increase their shoot starch and soluble sugar con-

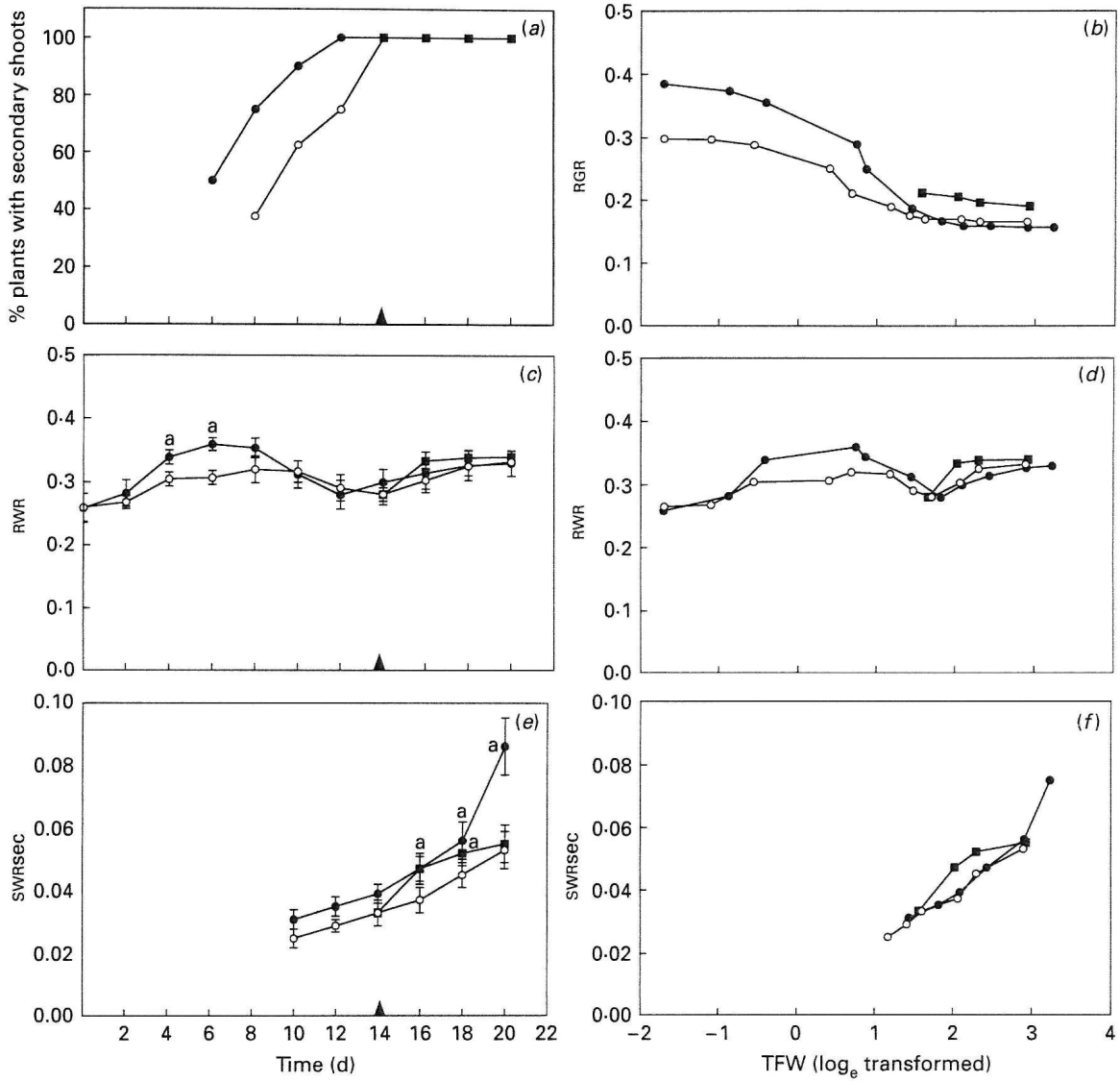


**Figure 2.** (*a*) Percentage of plants, sampled for RGR analysis, with secondary shoots. (*b*) $RGR_{FW}$ data corrected for ontogenetic drift. (*c*) Root weight ratio (RWR) of the plants sampled for RGR analysis (mean ± SE). (*d*) RWR corrected for ontogenetic drift. (*e*) Shoot weight ratio of the plants sampled for RGR analysis ($SWR_{sec}$) (mean ± SE). (*f*) $SWR_{sec}$ corrected for ontogenetic drift. (●) 700 $\mu l\, l^{-1}$ $CO_2$, (○) 350 $\mu l\, l^{-1}$ $CO_2$, (■) older plants. Black triangles indicate the day when the older plants were transferred to elevated $CO_2$. **a**, $P < 0{\cdot}05$ (Student's *t*-test).

centration, although not to the same extent in the case of starch (Fig. 3*a*, *b*). The decrease with time was more marked than for the younger plants. At the last day of the experiment, the starch concentration in the secondary shoots was the same for both $CO_2$ treatments, but the soluble sugar concentration was higher at elevated $CO_2$ (Table 2).

Starch concentration in the roots was not changed by elevated $CO_2$, either in the younger or older plants (Fig. 3*c*). Soluble sugar concentration was only increased at elevated $CO_2$ during the period of growth stimulation (Fig. 3*b*). In both $CO_2$ treatments, soluble sugar concentration decreased with time, until day 9 for plants grown at ambient $CO_2$, and throughout the duration of the experiment for plants grown at elevated $CO_2$. Root-starch concentration of the plants transferred 6 wk after germination was not changed at elevated $CO_2$ (Fig. 3*c*). Soluble sugar concentration, however, was markedly increased and this effect was more pronounced in the older than in the younger plants (Fig. 3*d*).

### *Leaf photosynthesis*

Photosynthesis was increased by elevated $CO_2$ in both the existing and the new leaves (Fig. 4*a*). The plants which were transferred later showed the same pattern as the younger plants, for both existing and new leaves (Fig. 4*a*, *b*).

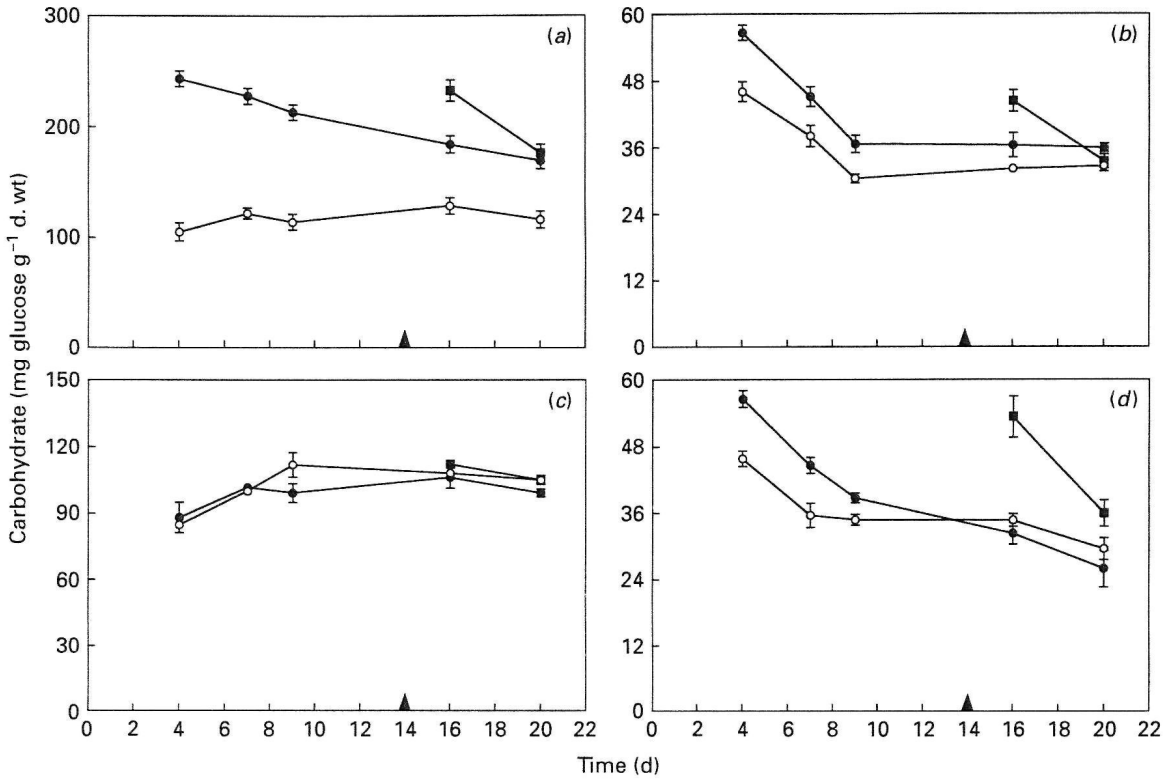


**Figure 3.** (*a*) Starch and soluble (*b*) sugar concentration in the shoots (mean ± SE). The effect of elevated $CO_2$ on soluble sugar concentration of the younger plants was significant at $P < 0{\cdot}0001$ in a two-way analysis of variance. (*c*) Starch and (*d*) soluble sugar concentration in the roots (mean ± SE). Elevated $CO_2$ effects on soluble sugar concentration, tested by a two-way analysis of variance, showed significant time × $CO_2$ at $P < 0{\cdot}001$, for the younger plants and $P < 0{\cdot}05$, for the older plants. (●) 700 $\mu$l l$^{-1}$ $CO_2$, (○) 350 $\mu$l l$^{-1}$ $CO_2$, (■), older plants. Black triangles indicate the day when the older plants were transferred to elevated $CO_2$.

## DISCUSSION

### *Growth parameters*

Plant growth analysis included a separate analysis of the $RGR_{FW}$ of the secondary shoots. For the plants transferred 4 wk after germination, part of the plant, namely the secondary shoots, was still growing at a higher rate than at atmospheric $CO_2$ when $RGR_{FW}$ of the whole plant was no longer stimulated. Because the weight of these secondary shoots is very small compared with the weight of the whole plant, their growth response is masked when calculation of RGR is based on plant total weight. During the period that $RGR_{FW}$ was stimulated by elevated $CO_2$, RWR was also increased. Correction of $RGR_{FW}$ and RWR for ontogenetic drift showed that the transient, stimulatory effect of elevated $CO_2$ on both parameters was not entirely size-dependent. In the older plants the increase in $RGR_{FW}$ and RWR by elevated $CO_2$ was smaller than in the younger plants. At the later stage of development, the ratio between new and old tissue in the shoots is certainly different, possibly affecting the capacity to accelerate metabolic responses.

In a previous study with the same *Plantago* subspecies no effect on RWR was detected (Den Hertog, Stulen & Lambers (1993). In the present study the increase in RWR was detected because the RGR analysis was done at shorter intervals (2 d). The older root systems did not have the capacity to respond to the same extent as did the younger plants. Probably, at this stage of development, the ratio between new and old tissue in the root system is different from the younger plants, causing a decrease in the effect of elevated $CO_2$ on RWR, or even masking the fact that only the newest parts of the root system may be stimulated while the rest is not. It has been suggested that the extent to which roots may affect the response to elevated $CO_2$ ultimately depends upon the allocation of carbon to coarse or fine roots, i.e. to storage vs. resource capture functions (Körner & Arnone 1992; Curtis *et al.*, 1994; Norby, 1994).

The present data showed that elevated $CO_2$ did not change the pattern of growth, but that it accelerated the rate of development, following a genetically determined inherent pattern of growth, as suggested by Brown (1991), Coleman, McConnaughay & Bazzaz (1993), Poorter (1993) and Lariguaderie, Reynolds & Strain (1994).

One of the reasons for the transient nature of the stimulation of RGR by elevated $CO_2$ might be the occurrence of self-shading during the experiment

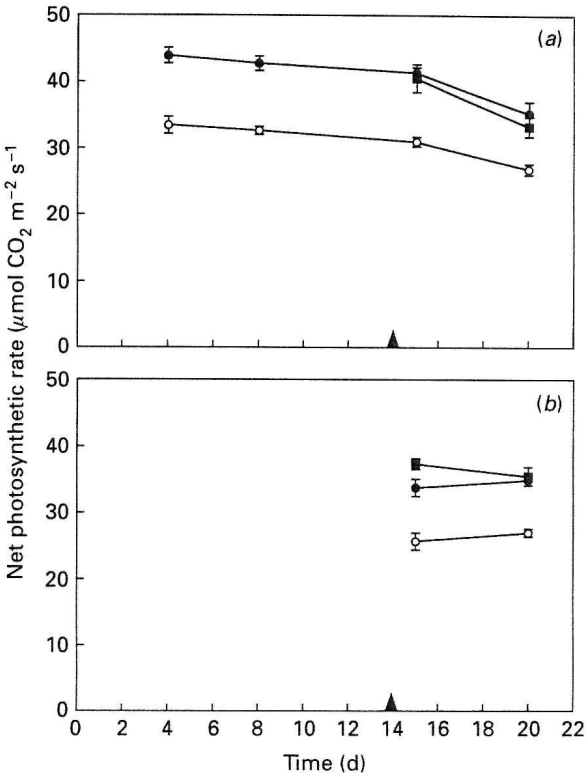


**Figure 4.** (*a*) Net photosynthetic rate of existing leaves and (*b*) of young leaves (mean ± SE). The effect of elevated $CO_2$ on net photosynthetic rate was significant at $P < 0{\cdot}0001$ for existing leaves (younger and older plants), $P < 0{\cdot}0001$ for new leaves of younger plants and $P < 0{\cdot}001$ for new leaves of older plants, in a two-way analysis of variance. (●) 700 $\mu$l $l^{-1}$ $CO_2$, (○) 350 $\mu$l $l^{-1}$ $CO_2$, (■) older plants. Black triangles indicate the day when the older plants were transferred to elevated $CO_2$.

(Poorter *et al.*, 1988). However, in *Plantago major* ssp. *pleiosperma*, the small differences in leaf area between the $CO_2$ treatments would not seem to support the idea that differences in self-shading could be the major cause of the transient nature of the stimulation of the $RGR_{FW}$ of the whole plant. As discussed below, a change in the sink-source relationship in the plant as a result of the formation of secondary shoots might be a more plausible explanation.

### *Sink-source relationships*

In the present study plant total leaf area was increased by elevated $CO_2$. Increases in average leaf size (Ford & Thorne, 1967; Sionit *et al.*, 1981), leaf numbers (Sionit, Strain & Flint, 1987; Sasek & Strain, 1988), total leaf area (Rogers *et al.*, 1984; Cure, Thomas & Israel, 1987; Den Hertog *et al.* 1993), and increased initiation and rate of expansion (Hicklenton & Jolliffe, 1980; Kramer, 1981; Cure, Rufty & Israel, 1989) were found in plants grown at elevated $CO_2$. All these changes in leaf morphology may be regarded as a way of increasing storage capacity for carbohydrates. Stitt (1991) suggested that new temporary storage capacity might not only be formed within the leaves or other existing sinks, but that new sinks could also be initiated. Species can differ greatly in the extent to which this happens (Wardlaw, 1990), and the response of sink metabolism to an increased availability of carbohydrates will depend partly on genetic factors (e.g. determinate versus indeterminate growth, internal genetic constraints on the rate and extent of sink growth) and partly on environmental factors, e.g. nitrogen availability (Stitt, 1991).

The younger plants of *P. major* ssp. *pleiosperma*, responded to elevated $CO_2$ by transiently increasing partitioning to the root, increasing leaf area, and accelerating the development of new sinks for carbohydrates, namely, the secondary shoots. Apparently, the development of these new sinks took priority over a continuation of the stimulation of RWR.

The view that the allocation of materials via the phloem is governed by the relative strength of the terminal sinks under control of phytohormones, is well known (Brenner, 1987; Patrick, 1987). This concept attributes a major role to phytohormones in determining allocation patterns, shoot : root ratio and intraspecific shifts in RGR. A contrasting view is the 'functional equilibrium' model (Davidson, 1968; Brouwer, 1983). In this view, root growth is limited by the supply of photosynthates from the shoot and shoot growth by the supply of minerals and water from the root. The model predicts that the functional interdependence of root and shoot results in a balanced outgrowth of the sinks.

A model of whole-plant carbon partitioning in which sucrose plays a central role, with the pools of sucrose in the cytosol of source and sink controlling the capacity for synthetic metabolism, was proposed by Farrar (1992) and Farrar & Williams (1991). According to this model, sink growth should be greater at elevated $CO_2$, given no limitation from nutrients, temperature or inherent capacity. In *Plantago*, new sinks, the secondary shoots, placed along the transport pathway from shoot to root, could have used relatively more of the extra carbohydrates at the expense of the roots, since root carbohydrate concentration decreased, at the time of the formation of secondary shoots and the end of the period of stimulation of RWR. The fact that the secondary shoots have a lower starch : soluble sugar ratio during stimulation of their RGR agrees with the idea that sugars play a central role in synthetic metabolism (Farrar & Williams, 1991).

### *Leaf photosynthesis and carbohydrates*

In some species, changes in leaf properties by elevated $CO_2$ include changes in chloroplasts, in particular the formation of starch grains (Carmi & Shomer, 1979; Yelle *et al.*, 1989*a*). In some cases elevated $CO_2$ caused a decrease in photosynthetic

rate (per unit leaf area) after a period (7–10 d) at elevated $CO_2$ (Aoki & Yabuki, 1977; Mortensen, 1983; Havelka *et al.*, 1984; Spencer & Bowes, 1986), whereas in other cases no decrease in photosynthetic rate was found (Cure *et al.*, 1987). Several hypotheses have been put forward to explain this elevated-$CO_2$ induced decrease in photosynthetic rate, referred to as photosynthetic acclimation.

Spencer & Bowes (1986) suggested that reduced stomatal conductance of leaves at elevated $CO_2$ could partly explain the photosynthetic acclimation. Yelle *et al.* (1989*b*) reported a significant decrease in stomatal conductance of tomato leaves under elevated $CO_2$. However, the constant value of internal $CO_2$ concentration throughout the experiment suggested that reduced stomatal conductance could not explain the photosynthetic acclimation in terms of a counter balancing effect.

Starch accumulation has also been related to the decrease in photosynthetic rate. It has been suggested that starch build-up could act as a feedback inhibitor (Thomas *et al.*, 1975; Mauney, Fry & Guinn, 1978). Other lines of evidence indicated that photosynthetic acclimation cannot be attributed to a build-up of starch (Yelle *et al.*, 1989*a*, Stulen, den Hertog & Jansen, 1993), but rather to a decline of activated Rubisco, with starch accumulation being a symptom, but not the primary cause of the decrease in photosynthetic rate at elevated $CO_2$ (Yelle *et al.*, 1989*b*).

More recently Van Oosten, Wilkins & Besford (1994) proposed a molecular model to account for the photosynthetic acclimation, in which plants exposed to elevated $CO_2$ will progressively accumulate sugars (hexoses) when sink strength is reduced. The mechanism proposed for the acclimation is then mediated by increased hexose concentrations. Accumulation of hexoses might be detected by an as yet unknown protein in plants, involved in carbon metabolism, which will then initiate a cascade of reactions which will repress (Rubisco – ribulose-1,5-bisphosphate carboxylase oxygenase, small subunit, Rubisco activase) or activate (ADP-glucose pyrophosphorylase) specific nuclear genes involved in primary carbon metabolism.

In *Plantago major* ssp. *pleiosperma*, no decrease in leaf photosynthesis was found either in an existing leaf (during or after the transient period of growth stimulation by elevated $CO_2$) or in a new leaf. The observed decrease in photosynthetic rate with time was also present at atmospheric $CO_2$, and was probably caused by ageing of the leaf. Apparently, at elevated $CO_2$, relatively more soluble sugars were used at first for the stimulation of root metabolism, and thereafter for the growth of the secondary shoots. As a consequence of the presence of these sinks, a constant difference in soluble sugar concentration between the $CO_2$ treatments, rather than a progressive accumulation of soluble sugars at elevated $CO_2$ was observed and so changes in genetic expression of carbon metabolism enzymes at elevated $CO_2$ might not occur to the same extent as predicted by the model of Van Oosten *et al.* (1994).

### *Final remark*

It is concluded that the preference of the secondary shoots as a sink over the roots alters the sink-source balance in the roots, which results in a cessation of the stimulation of root metabolism, RWR and whole plant RGR.

ACKNOWLEDGEMENTS

Filomena Fonseca was financed by the University of Algarve (UCTA), J.N.I.C.T. (Programa Ciência) and the Fundação Calouste Gulbenkian, Portugal. J. Den Hertog was financially supported by the Netherlands Organization for Scientific Research.

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