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Effects of water deficit and its interaction with CO_2 supply on the biochemistry and physiology of photosynthesis in sunflower

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Abstract

Photosynthetic responses of sunflower plants grown for 52 d in ambient and elevated CO_2 (A=350 or E=700 μmol mol^{-1}, respectively) and subjected to no (control), mild or severe water deficits after 45 d were analysed to determine if E modifies responses to water deficiency. Relative water content, leaf water potential (Ψ_w) and osmotic potential decreased with water deficiency, but there were no effects of E. Growth in E decreased stomatal conductance (g_s) and thereby transpiration, but increased net CO_2 assimilation rate (P_n, short-term measurements); therefore, water-use efficiency increased by 230% (control plants) and 380% (severe stress). Growth in E did not affect the response of P_n to intercellular CO_2 concentration, despite a reduction of 25% in Rubisco content, because this was compensated by a 32% increase in Rubisco activity. Analysis of chlorophyll a fluorescence showed that changes in energy metabolism associated with E were small, despite the decreased Rubisco content. Water deficits decreased g_s and P_n: metabolic limitation was greater than stomatal at mild and severe deficit and was not overcome by elevated CO_2. The decrease in P_n with water deficiency was related to lower Rubisco activity rather than to ATP and RuBP contents. Thus, there were no important interactions between CO_2 during growth and water deficit with respect to photosynthetic metabolism. Elevated CO_2 will benefit sunflower growing under water deficit by marginally increasing P_n, and by slowing transpiration, which will decrease the rate and severity of water deficits, with limited effects on metabolism.

Key words: Elevated CO_2, fluorescence, metabolism, photosynthesis, water deficit.

Introduction

Atmospheric CO_2 concentration (C_a), which was about 280 μmol mol^{-1} before the industrial revolution, is now 360 μmol mol^{-1} and is increasing by 1.8 μmol mol^{-1} year^{-1} (Houghton *et al*., 2001). This will affect vegetation, as elevated CO_2 substantially increases photosynthetic CO_2 assimilation rate (P_n), and thereby growth and total biomass, particularly of plants with the C_3 photosynthetic metabolism (Lawlor and Keys, 1993; Bowes, 1996; Drake *et al*., 1997). Also, increased C_a has already resulted in warmer temperatures globally, a trend expected to continue. Changes in temperature are likely to alter precipitation worldwide, decreasing it in many areas (Houghton *et al*., 2001). This, together with the higher evapotranspiration resulting from warmer conditions, is expected to subject vegetation, both natural and agricultural, to greater risk of more severe and prolonged water deficiency (Sengupta and Sharma, 1993; Samarakoon and Gifford, 1995; Ellsworth, 1999). Decreasing water supply will greatly decrease growth. Such changes in CO_2 and water supply affect many key metabolic and physiological processes in plants, the mechanisms of which are still unclear. Particularly, there is limited quantitative understanding of the effects of interactions between CO_2 and

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water deficiency (Chaves and Pereira, 1992; Tschaplinski *et al*., 1995, 1996; Samarakoon and Gifford, 1995).

The effect of increased C_a results from the increased supply of CO_2 to the photosynthetic carbon reduction cycle enzyme ribulose bisphosphate carboxylase-oxygenase (Rubisco), which is not saturated with CO_2 in the current atmosphere (Bowes, 1996; von Caemmerer, 2000). Consequently, more carbohydrates are synthesized, stimulating faster and greater growth of biomass (Lawlor and Mitchell, 1991; Drake *et al*., 1997). Also, carbohydrates accumulate as a result of carbohydrate supply exceeding the demands for growth of the whole plant (Eamus and Jarvis, 1989). Establishment of new sinks, or stimulation of existing ones, is beneficial under elevated CO_2 to consume carbohydrates and avoid imbalance in metabolism (Field and Mooney, 1986; Stitt, 1991). The increased P_n occurs despite smaller g_s caused by elevated CO_2. In spite of the general stimulation in P_n, there is considerable variation in the response of photosynthetic capacity (i.e. the maximum rate of P_n attained under particular conditions of light and temperature at saturating CO_2 supply, etc.) when the plant is grown in elevated CO_2 (DeLucia and Thomas, 2000). The response ranges from greatly decreased capacity via no change to increased capacity (Drake *et al*., 1997; Morison and Lawlor, 1999). Changes in P_n may result (Lawlor and Keys, 1993; Moore *et al*., 1998) from altered regulation of the photosynthetic mechanism without modifications to amounts of components: increased P_n is often called 'up-regulation' and decreased P_n, 'down-regulation'. Also longer-term changes in P_n due to altered photosynthetic capacity may occur: such 'acclimation' may include increased (Habash *et al*., 1995), or decreased capacity (Drake *et al*., 1997). Loss of capacity (using the common terminology it will be referred to as acclimation) is the more common response of plants grown in elevated CO_2. It is associated with altered tissue composition ('machinery') and, particularly, decreased amounts and activities of Rubisco (Lawlor and Keys, 1993; Moore *et al*., 1998). With elevated CO_2, the capacity for carboxylation may exceed the rate of ribulose bisphosphate (RuBP) regeneration, which initially results in decreased activity of Rubisco (down-regulation). Other mechanisms within the leaf may respond to this imbalance and the amount of Rubisco decreases (Stitt, 1991; Sage, 1994; Moore *et al*., 1998). In some cases there may be a shift towards increased electron transport leading to greater production of ATP and NADPH, and thus of RuBP by the PCR cycle. Physiological re-optimization of the photosynthetic machinery away from CO_2 fixation (especially Rubisco) towards increased RuBP synthesis, involving increased components for electron transport and ATP synthesis does not always occur.

The decline in P_n and acclimation in elevated CO_2 is correlated with suboptimal growth conditions, for example, small rooting volume, which affects nutrient supply (Sage, 1994; Drake *et al*., 1997; Morison and Lawlor, 1999). Indeed, such alterations in composition may occur predominantly with deficient nutrient, especially N, supply: in wheat, for example, this depends on the nitrogen supply (Mitchell *et al*., 2000). However, the effects of water deficiency are not well established (Tyree and Alexander, 1993; Samarakoon and Gifford, 1995; Tschaplinski *et al*., 1995, 1996).

Water deficits affect plants in very different ways when subjected to elevated CO_2. Long-term, slowly developing water deficits decrease growth, by slowing rates of cell division and expansion due to loss of turgor and increased synthesis of abscisic acid (Lawlor and Cornic, 2002). Also, g_s decreases, thus decreasing transpiration but also limiting P_n. With current C_a, C_i may decrease if photosynthetic capacity is not affected by water deficit, or is decreased less than g_s. With more severe deficits the photosynthetic capacity is decreased (Tezara *et al*., 1999), although there is still lack of agreement about the severity of the deficit at which capacity decreases, and the nature of the changes in photosynthetic mechanism responsible (Lawlor and Cornic, 2002; Lawlor, 2002). The argument may be simplified into stomatal limitation of CO_2 supply or metabolic limitation. If it is accepted that in leaves with mild water deficits, the restriction of water loss and of CO_2 uptake by small g_s predominates, then elevated C_a should ameliorate the development of water deficits and increase or maintain P_n. This would help maintain use of captured light energy for NADPH and ATP synthesis by providing more sink capacity and thus diminish accumulation of excitation energy in the photosynthetic pigments, which is a major cause of photo-oxidative damage (Scarascia-Mugnozza *et al*., 1996). Elevated atmospheric CO_2 should therefore be beneficial for plants in dry environments, offsetting some of the consequences of global environmental change. However, acclimatory changes in the photosynthetic machinery consequent upon growth in elevated CO_2 may reduce the benefits under water deficits. For example, smaller Rubisco carboxylation capacity and greater light capture with greater capacity for synthesis of NADPH and ATP (an apparent benefit in elevated CO_2 for regeneration of ATP) would predispose plants to photo-oxidative damage (Scarascia-Mugnozza *et al*., 1996).

There have been relatively few studies of the interaction between CO_2 and water stress (Vu *et al*., 1987; Tschaplinski *et al*., 1995, 1996) and understanding of the mechanisms is less well developed than that of CO_2 and temperature (Morison and Lawlor, 1999). Does growth in elevated CO_2 cause positive or negative acclimation of the photosynthetic apparatus? Are effects of CO_2 altered by water deficit? Does elevated CO_2 decrease the impact of water deficits on P_n and what processes in photosynthetic metabolism are affected? Is there interaction between elevated CO_2 and water deficits of varying severity? It is hypothesized that elevated, compared with ambient, CO_2

will increase P_n under water deficit and be beneficial for plants in dry environments, thus offsetting some of the consequences of global environmental change. To answer these questions and test the hypothesis, ATP, RuBP and Rubisco (both content and activity) were measured, and photosynthetic energy transduction and dissipation were assessed by measurement of chlorophyll *a* fluorescence. The focus of this study was on sunflower as its responses to water deficits and elevated CO_2, both as single environmental factors,have been analysed in detail (Kramer and Boyer, 1995; Tezara *et al*., 1999).

Materials and methods

Plant material and growth conditions

Sunflower plants (*Helianthus annuus* L. cv. Avante) were grown from seed in plastic pots filled with 3.0 l of sintered argilite clay (Terra-green, Silvaperl Products Ltd, Harrogate, UK), which has high water-holding capacity. Plants were watered daily and fertilized weekly with sufficient nutrient solution (Vitafeed 412: Vitax, Skelmersdale, UK) for maximum growth (based on previous experiments). Plants were grown in naturally lit growth chambers (Lawlor *et al*., 1993) with supplementary light giving an approximately 16 h light period and 8 h dark period. Supplementary light of photosynthetic photon flux (*PPF*: 400–700 nm wavelength) of 350 μmol m^{-2} s^{-1} was applied for 12 h giving an average *PPF* of approximately 500 μmol m^{-2} s^{-1} and a maximum of *c*. 1100 μmol m^{-2} s^{-1}. Temperature was maintained at 25/18 °C (±2 °C day/night) and 70–85% relative humidity in the day and above 90% at night (day/night). Plants were grown in one chamber under ambient CO_2 concentration (*A*, 350±20 μmol mol^{-1}) and one under elevated CO_2 concentration (*E*, 700±20 μmol mol^{-1}). The CO_2 concentrations were continuously measured in each chamber by independent infrared gas analysers (WMA, PP Systems, Hitchin, UK) calibrated at least weekly with standard CO_2 mixtures calibrated against gas mixing pumps (Wösthoff, Bochum, Germany). When the CO_2 concentrations fell below the set-points, pure CO_2 was injected, under control of solenoid valves, into the air flow of the chambers to maintain concentration. Chamber conditions (light, temperature, humidity) were very similar, but to avoid bias due to small differences, which might have had cumulative effects on the plants over the relatively long duration of the experiment, the CO_2 treatments and plants were exchanged between chambers approximately every 4 d.

Water deficit treatments

Water deficit was induced in 45-d-old plants, with the fourth pair of true leaves just fully expanded, by differential watering. Plants were weighed and water lost replaced early each morning. Well-watered (control) plants received sufficient water to maintain soil water content close to pot capacity (an average of *c*. 300 ml d^{-1}), or two-thirds (mild deficit) or one-third (severe deficit) of that. After 12 d of differential watering, plants were not watered the following morning and measurements were made during the day. To accommodate the measurement programme, the experiment was started on different days so that some replicates were measured on different occasions. Data presented are averaged over time**.** Thirty out of 90 grown at each CO_2 concentrations were harvested for growth analyses before the beginning of the water deficit treatments; the remaining 60 plants were subjected to three different water treatments (20 plants for each water treatment). Several experiments were done to measure the physiological parameters described below, and on each experiment not all the parameters were measured concomitantly, except for Ψ_w, *RWC* and Π.

Physiological measurements

Leaf water status: Leaf water potential (Ψ_w) was measured with a custom-made pressure chamber (IACR-Rothamsted, UK) using nitrogen gas on the fourth leaves from five individual plants, chosen at random from the 20 replicate pots per water deficit in each CO_2 treatment chamber. After the measurement of Ψ_w, relative water content was determined using five leaf discs from each leaf; *RWC* was determined as ($RWC=(F_w-D_w)/(T_w-D_w)$, where F_w is fresh mass of the discs, T_w is turgid mass after 6 h floating on distilled water at room temperature, and D_w is dry mass after oven drying for 24 h at 80 °C). Osmotic potential (Π) was determined with a vapour pressure osmometer (Wescor, Logan, USA) on five discs, which were taken from each leaf and frozen and thawed before measurement. Trials demonstrated that *RWC* and osmotic potential were unaffected by using leaves from the pressure chamber or by storage for Π. For these determinations, n=30.

Plant growth: Thirty plants were harvested in each CO_2 treatment when the water deficits were started. Roots were washed from the soil, and plants separated into roots, stems, leaves, and developing flowers. Parts were dried at 80 °C for 48 h.

Gas exchange: All measurements of P_n, g_s and C_i were done on the fourth, fully expanded intact leaf using a six-chamber computerized open gas-exchange system with infrared CO_2 measurements (WA-225-MK3 IRGA; Analytical Development Co., Hoddesdon, UK) as described by Jacob and Lawlor (1991). Ten cm^2 of the lamina (avoiding major veins) of leaves attached to the plant were sealed into each chamber, which was illuminated with *PPF* of 1400 μmol photons m^{-2} s^{-1}; leaf temperature was 25 °C and the air contained 0.21 mol mol^{-1} O_2 and 350 or 700 μmol mol^{-1} CO_2. The vapour pressure deficit between the leaf and chamber air was maintained at 1.2±0.2 kPa. Measurements of gas exchange were taken during steady-state photosynthesis after a 1 h period of adjustment of the leaf to the chamber conditions. The calculations of P_n, g_s and C_i were made according to Farquhar and Sharkey (1982).

Determination of P_n/C_i *response curves*: Response of CO_2 assimilation (P_n) to intercellular CO_2 concentration (C_i), the P_n/C_i response curve, was measured in the open gas exchange system by increasing C_a from 0 to 1000 μmol mol^{-1}. Carboxylation efficiency was calculated from the initial slope (dP_n/dC_i) of the response curve and P_{nmax} (the CO_2 saturated rate of photosynthesis) from the asymptote of the fitted response function (von Caemmerer and Farquhar, 1981; Farquhar and Sharkey, 1982). Stomatal and metabolic limitations (L_s and L_m, respectively) were analysed as $L_s=100\times(P'_n-P_n)/P'_n$, where P'_n is the photosynthetic rate to be expected when $C_i=C_a$. The relative mesophyll limitation, L_m, was calculated from Jacob and Lawlor (1991) and $L_m=100\times(P_{n(c)}-P_{n(d)})/P_{n(c)}$, where $P_{n(c)}$ is the photosynthetic rate in control leaves at C_i of 500 μmol mol^{-1}, and $P_{n(d)}$ is the rate in droughted leaves at the same C_i. L_m is thus a measure of the capacity of the mesophyll to fix CO_2 at 500 μmol mol^{-1} C_i and is zero in control leaves.

Photosynthetic light response curves: Response of P_n to *PPF* (the light response curve) was measured at two different C_a (350 and 2500 μmol mol^{-1}) and a leaf temperature of 25 °C by illuminating the leaf at increasing *PPF* until P_n was constant. Incident *PPF* on the leaf was modified using neutral density filters (Lee Filters; AC Lighting, Bucks., UK). The apparent quantum yield of CO_2 fixation (Φ_{CO_2}) was calculated as the slope of the linear portion of the response curves between 0 and 150 μmol m^{-2} s^{-1} *PPF*.

Table 1. *Leaf water status of sunflower plants grown under ambient* (A) *and elevated* (E) CO_2 *concentrations, and subjected to no (C), mild (M), and severe (S) water deficit*

Measurements under the conditions of growth. Values are means (n=30); * indicates statistically significant difference at $P \leq 0.05$ (ANOVA) and NS not significant.

	A			*E*			Effects		
	C	M	S	C	M	S	Growth CO_2	H_2O	Growth $CO_2 \times H_2O$
RWC	87.0±0.8	66.8±1.5	53.9±4.3	86.0±0.7	70.5±2.7	51.8±1.1	NS	*	NS
Ψ_w (MPa)	–0.26±0.01	–1.4±0.04	–2.2±0.03	–0.24±0.01	–1.3±0.05	–2.1±0.08	NS	*	NS
Π (MPa)	–1.08±0.05	–1.12±0.2	–1.6±0.3	–1.17±0.05	1.37±0.41	–1.6±0.09	NS	*	NS

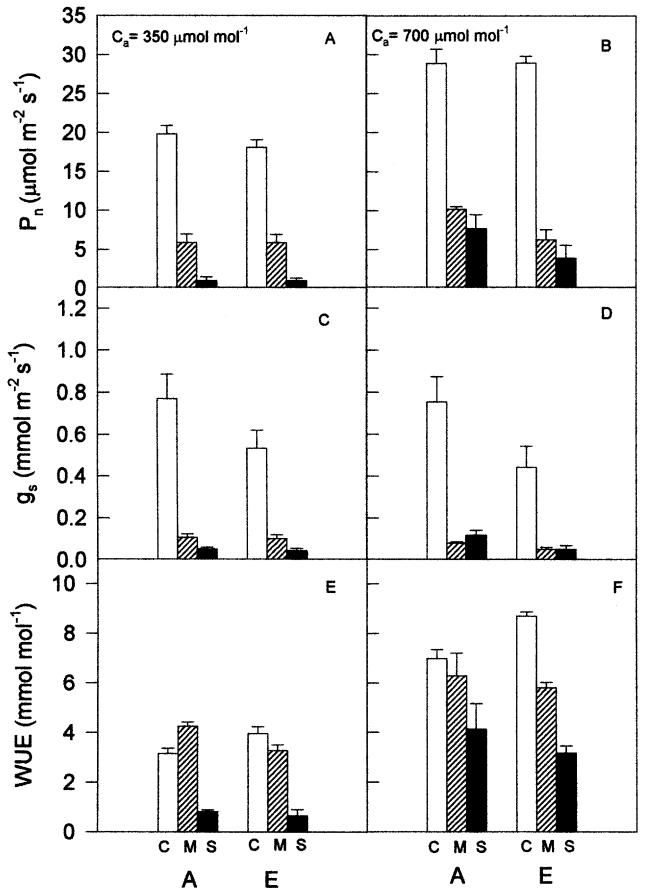

Fig. 1. Net photosynthetic CO_2 assimilation, P_n (A, B), stomatal conductance, g_s (C, D), and instantaneous water use efficiency, *WUE* (E, F) of leaves of sunflower plants grown at elevated (*E*, 700 µmol mol^{-1} CO_2) and ambient (*A*, 350 µmol mol^{-1} CO_2) CO_2 concentrations with three different water deficits: none (control, C, white bars) mild (M, hatched bars), and severe (S, black bars). Measurements were made on leaves attached to the plants at 350 (left panels) or 700 µmol mol^{-1} (right panels) CO_2.

Chlorophyll a *fluorescence measurements*: Fluorescence from PSII chlorophyll *a* of the leaves was measured simultaneously with gas exchange, through the glass window of the chambers at leaf temperature of 25 °C as described by Habash *et al*. (1995), using a modulated fluorometer (MSMF; Hansatech, Kings Lynn, UK). A modulated beam of less than 0.5 µmol m^{-2} s^{-1} *PPF*, set at 580 nm using a narrow-band pass filter (585 DF 44; Omega Optical Inc., Brattleboro, VT, USA) was used and the resulting fluorescence selectively measured at 695 nm using a band pass filter (695 DF 30; Omega Optical). Actinic illumination from metal-halide lamps, was used for both gas-exchange and fluorescence measurements. To reduce Q_A fully, a 2 s saturating flash of 7000 µmol m^{-2} s^{-1} *PPF* of 400–635 nm was applied from a pulse light source (PLS2; Hansatech, Kings Lynn, UK). Far-red light of 15 W m^{-2} from the PLS2 through a far-red filter (RG 715; Schott, Mainz, Germany) allowed the determination of minimum fluorescence (F'_o) immediately after steady-state photosynthesis ceased. The protocol for fluorescence measurement was similar to that described by Genty *et al*. (1989), except that the measurements were performed on attached leaves. The relative quantum yield of PSII (Φ_{PSII}) at steady-state is defined as $(F'_m–F_s)/F'_m$ where F_s and F'_m are steady-state fluorescence and maximum fluorescence in the light, respectively. The coefficients of photochemical (q_P) and non-photochemical (q_{NP}) quenching of chlorophyll *a* fluorescence were calculated from measurements of fluorescence. Whole chain electron transport rate in leaves (*J*) was estimated by the method of Krall and Edwards (1992) from the equation: $J=\Phi_{PSII}\times PPF\times a\times f$ where *a* is the fraction of incident *PPF* absorbed by the leaf, and *f* the absorption of PSII divided by the absorption of (PSI+PSII). It is assumed that the two photosystems are equally involved in linear electron transport, so f=0.5.

Biochemical determinations

Total soluble protein content (TSP), Rubisco content and activity, and RuBP, ATP, and chlorophyll contents were determined on samples immediately after gas exchange measurements at C_a=350 µmol mol^{-1} and 1400 µmol m^{-2} s^{-1} *PPF*. When stable P_n was reached, the section of lamina in the chamber was frozen to –20 °C within 0.1 s by freeze clamping (Lawlor *et al*., 1989) and stored in liquid N_2. The TSP was determined in an aliquot of the crude extract by Coomasie blue binding (Bradford, 1976) with BSA as standard. Rubisco was extracted from a 5 cm^2 area of the freeze-clamped leaf, which had been kept in liquid nitrogen. Frozen samples were ground in liquid nitrogen, and then at 0–4 °C, in 1 ml buffer (100 mol m^{-3} Bicine, pH 8.0; 20 mol m^{-3} $MgCl_2$, 50 mol m^{-3} mercaptoethanol, 10 ml 40 mol m^{-3} phenylmethylsulphonyl fluoride, and 10 mg acid-washed PVP). Initial Rubisco activity was assayed as described by Parry *et al*. (1993). Rubisco content was measured by separation of proteins in the extract on 15% SDS-PAGE gels: Rubisco was identified and quantified by comparison with standard Rubisco protein (Lawlor *et al*., 1989). RuBP was extracted in 5% (v/v) perchloric acid on independent freeze-clamped leaf samples and measured by ^{14}C incorporation into acid-stable products using purified Rubisco and $NaH^{14}CO_3$ (Giménez *et al*., 1992). ATP content was determined enzymatically (Stitt *et al*., 1989).

Table 2. *Maximum* P_n, *carboxylation efficiency* (dP_n/dC_i) *relative stomatal* (L_s) *and mesophyll limitations* (L_m) *to photosynthesis and operating* C_i *of sunflower plants grown in ambient* (A) *and elevated* CO_2 *concentrations* (E) *and subjected to no (C), mild (M), or severe (S) water deficit*

Values are means (n=6); leaves in ambient or elevated CO_2; * indicates statistically significant difference at $P \leq 0.05$ (ANOVA) and NS not significant.

	A			*E*			Effects		
	C	M	S	C	M	S	Growth CO_2	H_2O	Growth $CO_2 \times H_2O$
P_{nmax} (μmol m^{-2} s^{-1})	26.0±1.4	10.5±0.9	3.6±1.8	28.0±1.9	11.6±0.04	11.6±0.05	NS	*	NS
dP_n/dC_i (mol m^{-2} s^{-1})	0.20±0.01	0.03±0.01	0.02±0.01	0.18±0.01	0.03±0.01	0.03±0.01	NS	*	NS
L_s (%)	23.2±0.5	16.3±1.9	56.9±1.1	32.2±2.2	14.5±1.1	47.7±3.2	NS	*	NS
L_m (%)	0±0	61.5±0.1	98.3±0.2	0±0	60.1±3.3	86.3±6.9	NS	*	NS
Operating C_i (μmol mol^{-1})	264.3±6	265.4±8	325.8±12	545.6±11	527.2±22	591.7±27	*	*	NS

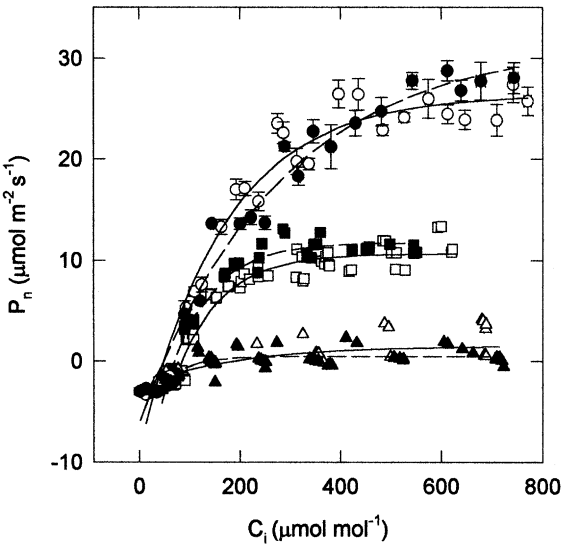

Fig. 2. Response of net photosynthetic CO_2 assimilation (P_n) to intercellular CO_2 concentration (C_i) in leaves of sunflower plants grown at ambient (open symbols) and elevated (closed symbols) CO_2 concentrations and subjected to no (circles), mild (squares) or severe (triangles) water deficits. Values are means (n=6) and standard errors are shown when greater than the size of the symbol.

Statistical analysis

Plants used for measurements were randomly selected from the 20 replicate plants per water stress treatment, grown in each CO_2 chamber. They constitute pseudoreplicates as only a single chamber per CO_2 treatment was used (because of space and costs). Frequent movement of CO_2 treatments and associated plants between rooms, with carefully standardized conditions, is an accepted method of ensuring that the CO_2 treatment can be separated from the effects of other factors (Lawlor *et al*., 1993). The statistical analyses were carried out using Genstat (IACR-Rothamsted), and curve fitting by Sigmaplot. All linear regressions, correlations and variance analyses (single- and two-factor) were tested for significance at $P \leq 0.05$. Results are presented as means ($5 \leq n \leq 10$)±1SE.

Results

Application of three different watering regimes decreased *RWC*, Ψ_w and Π substantially for plants grown under both ambient and elevated CO_2, with no significant effect of elevated CO_2 concentration on them (Table 1). Gas exchange measurements (Fig. 1) showed that decreasing Ψ_w progressively and significantly decreased P_n (Fig. 1A, B) and g_s (Fig. 1C, D) for plants grown and measured in both ambient and elevated CO_2. There were no differences between P_n or g_s related to CO_2 concentration during growth and no interaction between water deficits and CO_2 concentration. P_n of leaves of plants not subjected to water deficit was significantly (P <0.01) larger when measured in $C_{a=}$700 μmol mol^{-1} (Fig. 1B) than in C_a=350 μmol mol^{-1} for both ambient and elevated CO_2-grown plants (*c*. 40% and 55%, respectively; Fig. 1A compared to Fig. 1B). P_n of severely stressed leaves of plants grown in elevated CO_2 was slightly but not significantly smaller than that of ambient grown-plants when measured with C_a=700 μmol mol^{-1} (Fig. 1B), but not when measured in C_a=350 μmol mol^{-1}. P_n of the water-deficient leaves measured in elevated CO_2 was higher than those grown in ambient CO_2 (Fig. 1B compared to Fig. 1A).

Stomatal conductance was affected by water deficits and CO_2 during growth. For plants not subjected to water deficit, g_s of those grown in elevated CO_2 was 42% lower (significant at P <0.01; Fig. 1C, D) than those grown in ambient CO_2, irrespective of the CO_2 concentration during measurement. With mild and severe water deficit, g_s was much smaller than in the well-watered plants (*c*. 0.05 compared to 0.6 mol m^{-2} s^{-1}), and similar in plants grown and measured in both CO_2 concentrations.

The *WUE* was not significantly altered by the mild water deficit, but was decreased by the severe deficit for plants grown in both ambient and elevated CO_2 when measured at 350 μmol mol^{-1} CO_2. However, *WUE* decreased progressively with water deficit when measured at elevated CO_2 (Fig. 1E, F). Elevated CO_2 concentration during growth did not affect *WUE* when measured in ambient or elevated CO_2. However, *WUE* measured in elevated CO_2 was substantially higher than that in ambient CO_2 (by 230% and 380% in control and severely stressed plants, respectively: Fig. 1E, F).

Table 3. *Calculated rates of electron transport,* J (*μmol e⁻ m⁻² s⁻¹), for sunflower plants grown at ambient (*A*) and elevated (*E*) CO_2 concentrations (350 and 700 μmol mol⁻¹, respectively), and measured at 350 and 2500 μmol mol⁻¹ CO_2 under 800 μmol m⁻² s⁻¹, and subjected to no (C), mild (M), or severe (S) water deficit*

Values are means ±SE (n=6); * indicates statistically significant difference at $P \leq 0.05$ (ANOVA) and NS not significant.

C_a (μmol mol^{-1})	*A*			*E*			Effects		
	C	M	S	C	M	S	Growth CO_2	H_2O	Growth CO_2×H_2O
350	164±15	146±18	98±5	210±20	125±5	98±15	NS	*	NS
2500	284±19	160±10	170±8	258±15	170±12	165±10	NS	*	NS

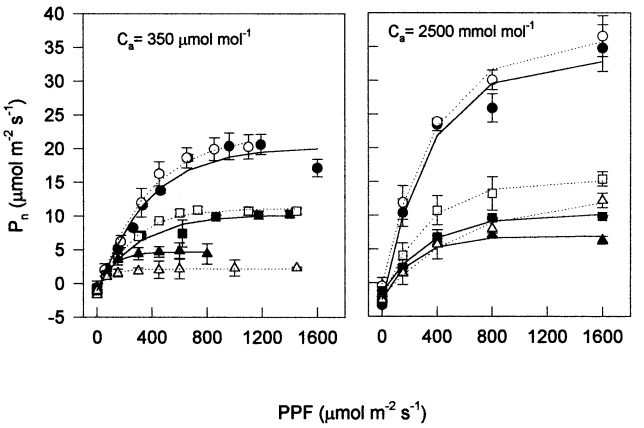

Fig. 3. Responses of photosynthetic rate (P_n) to photosynthetic photon flux (*PPF*) for sunflower plants grown at ambient (open symbols) and elevated (closed symbols) CO_2 concentrations, and measured at ambient CO_2 of 350 and 2500 μmol mol^{-1} CO_2, and subjected to no (circles), mild (squares) or severe (triangles) water deficits. Values are means ±SE (n=6).

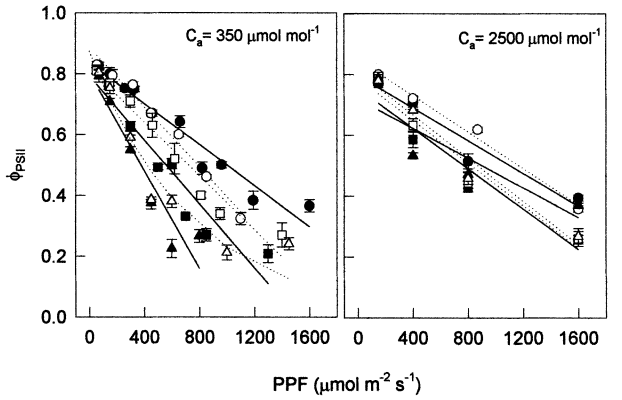

Fig. 4. Quantum yield of electron transport through photosystem II, as a function of photosynthetic photon flux (*PPF*) for sunflower plants grown at ambient (open symbols) and elevated (closed symbols) CO_2 concentrations, measured at 350 and 2500 μmol mol^{-1} of CO_2, and subjected to no (circles), mild (squares) and severe (triangles) water deficits. Values are means ±SE (n=6).

The response of P_n to C_i (Fig. 2), shows that neither P_{nmax} nor carboxylation efficiency, were affected by growth of the leaves in elevated CO_2 at any water deficits. However, both P_{nmax} and carboxylation efficiency declined substantially and significantly with decreasing Ψ_w (Fig. 2; Table 2). Neither stomatal nor mesophyll limitation of P_n (Table 2) was significantly affected by elevated CO_2, although stomatal limitation was somewhat greater in plants grown in elevated CO_2, where g_s was smaller, than in those grown in ambient CO_2. However, L_s with water deficit in elevated CO_2 was smaller than in ambient CO_2 (Table 2). The L_m increased substantially with water deficit, and was slightly (but not significantly) less in leaves grown in elevated than ambient CO_2. The operating C_i was greater with C_a of 700 μmol mol^{-1} than ambient (545 cf. 265 μmol mol^{-1}) (Table 2) but was not affected by the CO_2 during growth when measured in ambient CO_2.

Growth under elevated CO_2 did not significantly alter the parameters of the light response curves (Fig. 3) at any Ψ_w when measurement C_a was 350 μmol mol^{-1}. However, when measurement C_a was 2500 μmol mol^{-1}, P_{nmax} was significantly lower in plants grown under water deficits with elevated CO_2 compared to those grown in ambient CO_2, due to a 43% decrease in g_s (values not shown) of plants grown with mild deficit and 33% with severe deficit. The Φ_{CO_2} (Fig. 3) was not affected by growth under elevated CO_2. However, it was higher when measured at C_a=2500 μmol mol^{-1} at all Ψ_w. At large *PPF*, electron transport rate, *J*, was greater (Table 3), in leaves grown with ample water, in elevated than at ambient CO_2 (210 cf. 164 μmol e⁻ m^{-2} s^{-1}) when measured at C_a=350 μmol mol^{-1}. The difference disappeared when Ψ_w decreased and *J* fell by 28% and 48% in the mildly and severely deficient plants. Elevated CO_2 during measurement increased *J* in all the treatments, particularly in the severely stressed leaves (71% cf. to 45% in the control). However, over all treatments, there was no significant effect of CO_2 during growth, but a significant effect of water deficit.

The Φ_{PSII} of leaves grown at both CO_2 concentrations, decreased progressively as the *PPF* increased and as Ψ_w became smaller when measurements were done at 350 μmol mol^{-1} CO_2 (Fig. 4A), whilst Φ_{PSII} of plants measured at 2500 μmol mol^{-1} was less affected by Ψ_w (Fig. 4B). In plants grown without water deficit and measured at 350 μmol mol^{-1}, Φ_{PSII} was slightly higher in plants grown in elevated CO_2 than in those grown in ambient CO_2, and this effect disappeared when Ψ_w decreased. However, when measurements were done at 2500 μmol mol^{-1} the effect of

Table 4. *The coefficient of photochemical fluorescence quenching (q_P) and the coefficient of non-photochemical fluorescence quenching (q_{NP}) of sunflower leaves grown at ambient (A) or elevated (E) CO_2 concentration and subjected to no (C), mild (M), or severe (S) water deficit*

Measurements were done at *PPF*=150 or 800 μmol m^{-2} s^{-1} and at 350 μmol mol^{-1} or 2500 μmol mol^{-1} of CO_2. Values are means (n=6); * indicates statistically significant difference at $P \leqslant 0.05$ (ANOVA) and NS not significant.

PPF (μmol m^{-2} s^{-1})	C_a (μmol mol^{-1})		*A*			*E*			Effects		
			C	M	S	C	M	S	Growth CO_2	H_2O	Growth CO_2×H_2O
150	350	q_P	0.939	0.914	0.881	0.933	0.913	0.900	NS	*	NS
		q_{NP}	0.204	0.175	0.207	0.131	0.151	0.182	NS	NS	NS
	2500	q_P	0.941	0.914	0.882	0.937	0.913	0.886	NS	*	NS
		q_{NP}	0.207	0.175	0.146	0.130	0.151	0.173	NS	NS	NS
800	350	q_P	0.688	0.637	0.464	0.717	0.600	0.595	NS	*	NS
		q_{NP}	0.625	0.737	0.737	0.637	0.810	0.705	NS	*	NS
	2500	q_P	0.788	0.62	0.586	0.723	0.639	0.566	NS	*	NS
		q_{NP}	0.525	0.686	0.591	0.618	0.698	0.538	NS	*	NS

Table 5. *Total soluble protein (TSP], Rubisco content, Rubisco initial activity, and RuBP and ATP content in leaves of sunflower grown under ambient (A) and elevated (E) CO_2 concentration, and subjected to no (C), mild (M), and severe (S) water deficit measurements made at ambient CO_2*

Values are means (n=6); * indicates statistically significant difference at $P \leqslant 0.05$ (ANOVA) and NS not significant.

	A			*E*			Effects		
	C	M	S	C	M	S	Growth CO_2	H_2O	Growth CO_2×H_2O
TSP content (g m^{-2})	10.6±0.9	7.1±0.8	6.4±0.8	8.8±0.5	4.5±0.8	6.9±0.7	*	*	NS
Rubisco content (g m^{-2})	3.37± 0.3	1.69±0.3	1.29±0.6	2.56±0.2	0.81±0.1	1.45±0.4	*	NS	NS
Rubisco initial activity (μmol m^{-2} s^{-1})	31.0±2.0	25.4±3.5	3.8±1.3	41.0±2.0	16.7±0.9	3.1±0.2	NS	*	*
RuBP content (μmol m^{-2})	87.7±5.0	21.3±6.6	23.8±5.9	81.0±2.7	48.6±6.3	38.1±4.6	*	*	NS
ATP content (μmol m^{-2})	17.9±1.6	12.3±2.1	9.06±1.6	14.8±2.7	12.9±2.5	7.3±1.1	NS	*	NS

declining Ψ_w on Φ_{PSII} was far smaller and there was no difference between plants grown at different CO_2 concentrations.

The q_P and q_{NP} were significantly affected (Table 4) by water deficiency when plants were grown in both CO_2 concentrations, and measured at 350 and 2500 μmol mol^{-1}, with the exception of q_{NP} measured at low *PPF* (150 μmol m^{-2} s^{-1}) at both CO_2 concentrations. However, growth under elevated CO_2 caused no significant differences in q_P or q_{NP} irrespective of measurement at C_a=350 or 2500 μmol mol^{-1} CO_2 or measurement at a *PPF* of 150 or 800 μmol m^{-2} s^{-1} (Table 4). The q_{NP} measured at *PPF* of 800 μmol m^{-2} s^{-1} was smaller when measured with a C_a of 2500 μmol mol^{-1} than with C_a of 350 μmol mol^{-1}, irrespective of the CO_2 concentration during growth or water deficit. The F_v/F_m ratio was not affected by either the CO_2 or water treatments, averaging 0.84±0.03.

Total soluble protein and Rubisco contents of leaves of well-watered plants were significantly reduced by growth in elevated compared to ambient CO_2 (17% and 25%, respectively); however, the initial activity of Rubisco was 32% higher in well-watered plants grown in elevated than in ambient CO_2 (Table 5). Under both growth CO_2 concentrations, the initial activity of Rubisco decreased with Ψ_w. The RuBP content in well-watered plants decreased slightly with growth in elevated versus ambient CO_2, but increased with water deficit. Severe water deficit decreased RuBP content more in plants grown in ambient CO_2 than in elevated CO_2. The ATP content of well-watered plants was the same, irrespective of the CO_2 concentrations during growth, but decreased by 50% with decreasing Ψ_w in both CO_2 concentrations.

Plant growth was markedly increased by growth in elevated CO_2; after 45 d at the start of the water deficit treatment the total biomass per plant was 27.5±0.92 g in ambient and 35.7±0.7 g in elevated CO_2, a 30% increase. The largest effect was on the roots (53% increase), then stem (40% increase) with little effect on leaves (11%). However, with elevated CO_2, leaf area per plant decreased by 6%, and leaf weight ratio, specific leaf area and leaf area ratio also declined.

Discussion

This study tested the hypothesis that growth of sunflower plants in elevated CO_2 would stimulate the rate of photosynthesis and allow adjustment of cellular water balance, so decreasing the impact of water deficits on

photosynthetic mechanisms (Samarakoon and Gifford, 1995). Elevated CO_2 would also provide a greater sink for electrons and decrease the potential for photoinhibitory damage (Scarascia-Mugnozza *et al*., 1996). As a result, photosynthesis would show a strong interaction between water deficit and CO_2 during growth. Acclimation of photosynthetic mechanisms, frequently caused by growth in elevated CO_2 (Wullschleger, 1993; Bowes, 1996), and the impact on the photosynthetic responses to water deficits were also assessed.

Elevated CO_2 was applied night and day for 45 d and continued for the next 12 d as the water deficits developed. Sunflower increased in biomass, particularly in root and stem, although leaf area decreased a little. Such stimulation of growth has been described for many annual, herbaceous (Lawlor and Mitchell, 1991; Lawlor *et al*., 1993; Drake *et al*., 1997; Morison and Lawlor, 1999) and perennial, woody species (Gunderson and Wullschlegger, 1994). The experimental CO_2 treatment and its duration was sufficient to allow acclimation of photosynthetic processes as shown by the decrease in Rubisco.

The water treatments were not designed to maintain constant water contents or potentials in soil or plants; indeed this is not possible for transpiring plants in soil (Kramer and Boyer, 1995). Because of the strongly non-linear relationships between water content, hydraulic conductivity and water potential in soils, Ψ_w in the plant is not linearly related to soil water content and potential (Kramer and Boyer, 1995)**.** In the plant, *RWC* and Ψ_w may also not be simply related, but in these studies on sunflower they are (Tezara *et al*., 1999) and are used interchangeably for assessing water status. As water deficits decrease g_s and affect metabolism (either directly or via synthesis of abscisic acid), leading to a reduction in growth, the effect of a particular treatment depends on the integral of Ψ_w over the period (Kramer and Boyer, 1995). As the differential watering caused relatively large differences in Ψ_w over 12 d, it was assumed that changes in tissue composition and metabolism would have occurred, particularly as measurements were made when Ψ_w were minimal.

Twelve days of differential watering resulted in *RWC*, Ψ_w and Π which were very different for the three water treatments, but were very similar in ambient and elevated CO_2. Lower values of Ψ_w than Π with water deficits may reflect development of negative wall potential (Kramer and Boyer, 1995; Hopkins, 1995), although not observed in this variety of sunflower in similar experiments (Tezara *et al*., 1999). Similarity in *RWC*, Ψ_w, and Π between the water treatments in the two CO_2 concentrations may have been fortuitous, given the smaller g_s and transpiration rates. Suboptimal watering of plants grown in elevated CO_2 may result in a smaller deficit, i.e. a higher Ψ_w, compared to ambient CO_2 (Tyree and Alexander, 1993), but this is not always so (Ferris and Taylor, 1995; Sgherri *et al*., 1998; Ellsworth, 1999), including the present study. Difference between species in the interaction of CO_2 with water supply has been well demonstrated by Samarakoon and Gifford (1995). Even when Ψ_w is larger in elevated CO_2 (Tyree and Alexander, 1993; Tschaplinski *et al*., 1995; Allen *et al*., 1998; Huxman *et al*., 1998*b*) the increase may not be as large as predicted from the reduction in g_s and transpiration rate measured in leaf chambers. This may be due to decreased evaporative cooling raising leaf temperatures (Lawlor and Mitchell, 1991). The absence of significant differences between components of the leaf water balance resulting from growth in elevated CO_2 may thus be expected.

The relative importance of stomatal and metabolic limitations was assessed from P_n/C_i curves (Fig. 2), which eliminates the effect of decreased g_s resulting from the response of g_s to elevated CO_2 or induced by drought. The validity of calculated C_i, particularly with respect to water deficits (Lawlor and Cornic, 2002) has been questioned but it is considered valid by the authors. Elevated CO_2 during growth did not significantly affect the shape of the P_n/C_i response of well-watered plants, measured in either ambient or elevated CO_2, so that Φ_{CO_2} and light-saturated P_n were not affected, suggesting that there were no changes in photosynthetic capacity (Fig. 3). *Yucca brevifolia* responded similarly (Huxman *et al*., 1998*a*). However, different photosynthetic parameters vary in response to elevated CO_2. Light-saturated P_n was more stimulated than Φ_{CO_2} (i.e. CO_2 increased photosynthetic capacity but not efficiency) in four tree species (DeLucia and Thomas, 2000). The P_n/C_i curves in six C_3 tropical species showed substantial quantitative differences (Bunce and Ziska, 1999). Elevated CO_2 increased capacity in wheat in one experiment (Habash *et al*., 1995), but not in others (Mitchell *et al*., 2000). Photosynthetic rate decreased in *Larrea tridentata* (Huxman *et al*., 1998*b*), *Picea abies* (Urban and Marek, 1999), *Yucca whipplei* (Huxman *et al*., 1998*a*), sorghum (Watling *et al*., 2000), and *Picea sitchensis* (Centritto and Jarvis, 1999).

In this study, stomata limited (L_s) photosynthesis more in elevated than in ambient CO_2, as g_s was smaller. By definition, L_m is zero in watered plants under either CO_2 concentrations. The absence of an effect on P_n in plants grown in elevated CO_2 suggests no acclimation. However, this conflicts with the *c*. 25% decrease in Rubisco content (Table 4), which shows acclimation of tissue composition. Decreased Rubisco content frequently results from growth in elevated compared to ambient CO_2 (Sage, 1994; Drake *et al*., 1997), for example, in *Picea sitchensis* (Rubisco content was 36% lower; Centritto and Jarvis, 1999). Wheat responds variably, but this is probably caused by altered rates of development and leaf senescence, and attendant changes in N-content, than by CO_2 *per se* (Garcia *et al*., 1998; Mitchell *et al*., 2000). The factors determining Rubisco content in leaves grown under elevated, compared

to ambient CO_2 have been extensively discussed (Stitt, 1991; Lawlor and Keys, 1993; Bowes, 1996; Moore *et al*., 1998). Causes of variation in acclimation were discussed in the introduction: the conclusion that variability may reflect nutrition, not CO_2 *per se* (Morison and Lawlor, 1999) is unlikely to apply in this study's experiment, as nutrition was ample. The inhibition of Rubisco synthesis is possible (Moore *et al*., 1998). Decreased Rubisco content in sunflower was compensated by increased activity (32%), indicating much larger carboxylation activity per unit of Rubisco protein, which maintained P_n. This contrasts with the decreased initial activity described by Jacob *et al*. (1995) in *Scirpus olneyi*, which is often seen (Drake *et al*., 1997). The small decrease in RuBP in sunflower leaves grown in elevated CO_2, but measured in ambient CO_2, does not accord with the slightly decreased rate of P_n. Rather it is expected that increased P_n (e.g. under elevated CO_2) would increase demand for RuBP synthesis which is limited by the light reactions (von Caemmerer and Edmondson, 1986; von Caemmerer, 2000).

Elevated CO_2 during growth did not affect the energy dissipation of well-watered leaves, judging from the changes in q_P and q_{NP} (Table 4). Nor did it affect whole chain electron transport rate (J) in sunflower, averaged over the water deficits and measurement CO_2 treatments. But J was 34% higher when measured in elevated CO_2 than in ambient. In *Cercis canadensis*, *Liquidambar styraciflua*, *Acer rubrum*, and *Carya glabra, J* was, on average, 10% higher under elevated CO_2 than at ambient CO_2 and P_n was increased by 59% in *A. rubrum* but by 159–190% in the other species (DeLucia and Thomas, 2000). The J, and light-saturated P_n, were also increased in *Pinus taeda* (Hymus *et al*., 1999), and wheat (Habash *et al*., 1995). The Φ_{PSII} of sunflower at low *PPF* was the same for leaves grown and measured in elevated and ambient CO_2, but at large *PPF*,Φ_{PSII} was lower for plants grown and measured in ambient CO_2 than when measured in elevated CO_2. The decrease in Φ_{PSII} due to low Ψ_w measured in ambient CO_2 disappeared in elevated CO_2. Similar responses occurred in *C. canadensis*, *L. styraciflua* and *C. glabra* (DeLucia and Thomas, 2000) and Habash *et al*. (1995). Electron transport and RuBP synthesis were modified; the maximum rate of J was 9% lower in ambient compared to elevated CO_2 and short-term exposure to elevated CO_2 decreased the maximum carboxylation rate by 46% and increased RuBP regeneration by 29% in *Picea abies* (Urban and Marek, 1999). The increase of J of sunflower plants growing at elevated CO_2 corresponded to an increase in Φ_{PSII}, in contrast to *Picea sitchensis* where J decreased at elevated CO_2 (Centritto and Jarvis, 1999).

For sunflower, the slightly higher Φ_{PSII} and possibly lower energy loss related to antenna-based quenching mechanism (q_{NP}), accorded with higher rates of J at large *PPF* when measurements were done at 2500 μmol mol^{-1}, indicating a larger electron sink in carboxylation at elevated than ambient CO_2 concentration. However, the response of q_P was unchanged by elevated CO_2 in this study, in contrast to the response of *C. canadensis*, *L. styraciflua* and *C. glabra* (DeLucia and Thomas, 2000) and wheat (Habash *et al*., 1995). This study's results show that plants exposed to elevated CO_2 have the same proportion of open PSII centres at high *PPF*. At sub-saturating *PPF* (50–200 μmol m^{-2} s^{-1}), P_n of sunflower was strongly limited by electron transport and RuBP regeneration rate, and by photorespiration in ambient CO_2 with the apparent quantum yield smaller than in elevated CO_2. These results suggest that the capacity for electron transport and the requirement for electrons in photosynthesis slightly increase with elevated CO_2. They do not support the hypothesis that elevated CO_2 during growth, under relatively low *PPF*, causes a substantial shift of capacity from carboxylation to electron transport. However, the decrease in TSP and Rubisco protein in well-watered sunflower plants grown at elevated CO_2 (Table 5), shows acclimation involving loss of Rubisco, relative to the capacity for RuBP synthesis, which was retained. Increased activation-state of Rubisco maintained P_n, thus ensuring the sink for electrons and agreeing with the absence of effect of elevated CO_2 on q_P and q_{NP} (Table 4) and the relatively small increase in electron flux. It is concluded that reductions in g_s and Rubisco content, caused by growth in elevated CO_2, are compensated and regulated by changes in electron transport, RuBP synthesis, and Rubisco activity and do not substantially affect photosynthetic energy dissipation.

Considering the effects of water deficiency, P_n decreased at mild and severe deficit but not because of small g_s as C_i increased, so photosynthetic capacity (P_{nmax}) must have decreased (Fig. 2; Table 2). There was no significant response of capacity to substantially elevated CO_2 (note particularly Fig. 3B) at mild and severe water deficits. However, when measured at elevated, compared to ambient CO_2, (Fig. 1A, B), P_n increased as expected from Fig. 2. Because transpiration was decreased in proportion to P_n in the mild deficit, the *WUE* was similar to controls when measured in ambient CO_2 but as P_n fell more with severe deficit, *WUE* diminished substantially.

The changes in P_{nmax} support the earlier conclusion (Tezara *et al*., 1999) that factors associated with decreased *RWC* and Ψ_w progressively inhibited photosynthetic capacity in sunflower. The mechanism was considered to be decreased ATP synthesis, shown by low ATP content and the consequent reduction in RuBP synthesis and content; limitation by inadequate inorganic phosphate supply or triose phosphate transport is not considered to be the cause of the decreased P_{nmax} (Lawlor and Cornic, 2002; Lawlor, 2002). However, the decreases in Rubisco content (62%) and initial activity (88%) were larger than those in RuBP (73%) and ATP (50%) comparing severely water deficient with well-watered plants grown under

ambient CO_2. A larger proportion of Rubisco protein was lost in this study than previously observed (Tezara *et al*., 1999), where the fall in P_n with water deficits was clearly related to RuBP supply and ATP content; Lawlor, 2002). Therefore the limited (or lack of) response of P_n to elevated CO_2, at mild and severe water stress may be related to decreased Rubisco activity in this experiment rather than to impaired ATP synthesis and RuBP limitation.

Water deficiency decreased electron flux, J, through PSII as expected from the decrease in P_n, but much less than the decrease in P_n, due to higher photorespiration at low Ψ_w (Lawlor and Cornic, 2002). Thus Φ_{PSII} was decreased, particularly when measured in ambient CO_2; the reduction was small in elevated CO_2, suggesting that there was an increased sink for electrons. This was not P_n, which decreased despite the large C_a (Fig. 2) and operating C_i, which would have decreased photorespiration. The reason for the lack of an effect of low Ψ_w on Φ_{PSII}, at elevated CO_2 is not known**.** Water deficits decreased q_P, showing that the reduction state of the acceptor Q_A was increased, and increased q_{NP} so a greater proportion of the energy was thermally dissipated at low *RWC*. Such effects have been frequently observed (Lawlor and Cornic, 2002). In droughted *Quercus ilex*, elevated CO_2 decreased photochemical activity and increased photoinhibition (Scarascia-Mugnozza *et al*., 1996). However, in sunflower there was no evidence of photoinhibition as F_v/F_m was unaffected by conditions, as earlier reported by Tezara *et al*. (1999).

It was concluded that in sunflower, elevated CO_2 increased P_n but led to acclimation, with decreased Rubisco protein but increased activity; water deficits decreased P_n with metabolic inhibition involving loss of Rubisco activity; there were negligible interactions between CO_2 concentration during growth and water supply, on photosynthetic processes. Slightly increased P_n in elevated CO_2 under water deficit may have partially compensated for inhibition by mild stress. However, there was no evidence that growth in elevated CO_2 fundamentally altered the photosynthetic metabolism of sunflower under water stress.

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