Will rising CO₂ protect plants from the midday sun? A study of photoinhibition of *Quercus myrtifolia* in a scrub-oak community in two seasons

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ABSTRACT

Over a large part of the photoperiod, light energy absorbed by upper canopy leaves saturates photosynthesis and exceeds the energetic requirements for light-saturated linear electron flow through photosystem II (J_{PSII}), so that photoinhibition results. From a theoretical consideration of the response of light-saturated photosynthesis to elevated atmospheric CO₂ partial pressure (pCO_2) it may be predicted that, where light-saturated photosynthesis is Rubisco-limited, an increase in pCO_2 will stimulate J_{PSII}. Therefore, the proportion of absorbed quanta dissipated photochemically will increase and the potential for photoinhibition of photosynthesis will decrease. This was tested by measuring modulated chlorophyll *a* fluorescence from *Quercus myrtifolia* Willd. growing in the field in open-top chambers, at either current ambient or elevated (ambient +35 Pa) pCO_2 on Merritt Island, Florida, USA. During spring and summer, light-saturated photosynthesis at current ambient pCO_2 was Rubisco-limited. Consistent with theoretical prediction, J_{PSII} was increased and photoinhibition decreased by elevated pCO_2 in spring. In the summer, when growth had largely ceased, an acclimatory decrease in the maximum Ribulose 1,5 bisphosphate saturated carboxylation capacity ($V_{c\,max}$) removed the stimulation of J_{PSII} seen in the spring, and photoinhibition was increased in elevated pCO_2. It is concluded that, for *Q. myrtifolia* growing in the field, the effects of elevated pCO_2 on J_{PSII} and photoinhibition will reflect seasonal differences in photosynthetic acclimation to elevated pCO_2 in a predictable manner.

Key-words: Atmospheric change; chlorophyll fluorescence; elevated carbon dioxide; photosynthesis; Rubisco; subtropical.

Abbreviations: A_{sat}, net rate of light-saturated CO₂ uptake per unit leaf area (μmol m⁻² s⁻¹); F_o, F_m, minimum and maximum dark-adapted fluorescence yield; F_o', F_m', F_s, minimum, maximum and steady-state, light-adapted fluorescence yield; F_v/F_m, maximum quantum yield of PSII photochemistry; F_v'/F_m', efficiency of excitation energy capture by open PSII reaction centres; J_{max}, maximum capacity for electron transport contributing to RubP regeneration (μmol m⁻² s⁻¹); J_{PSII}, rate of linear electron flow through PSII, estimated from fluorescence and measured at light saturation (μmol m⁻² s⁻¹); pCO_2, partial pressure of CO₂ (Pa); p_i, leaf intercellular partial pressure of CO₂ (Pa); PPFD, photosynthetically active photon flux density (μmol m⁻² s⁻¹); PSII, photosystem II; q_P, the photochemical quenching coefficient; Rubisco, Ribulose 1,5-bisphosphate carboxylase/oxygenase; RubP, Ribulose 1,5 bisphosphate; T_{air}, air temperature (°C); T_{leaf}, leaf temperature (°C); TPU, triose phosphate utilization; v_c, rate of carboxylation of RubP (μmol m⁻² s⁻¹); $V_{c\,max}$, maximum RubP-saturated rate of carboxylation (μmol m⁻² s⁻¹); v_o, rate of oxygenation of RubP (μmol m⁻² s⁻¹); VPD, water vapour pressure deficit (kPa); α, leaf absorptance between 400 and 700 nm; ϕ_{PSII}, quantum yield of PSII photochemistry; ϕ_{CO2}, quantum yield of CO₂ fixation corrected for leaf absorption.

INTRODUCTION

To date, the effects of increases in the partial pressure of carbon dioxide in the atmosphere (pCO_2) on photoinhibition of photosynthesis under field conditions appear contradictory. This is highlighted by two studies of Mediterranean trees. For tree species subject to drought, Jones *et al.* (1995) concluded that elevated pCO_2 protected against photoinhibition, whereas Scarascia-Mugnozza *et al.* (1996) found that photoinhibition was increased in elevated pCO_2.

For young trees in the field, both deciduous and evergreen, elevated pCO_2 has been shown to produce a long-term stimulation of light-saturated photosynthesis (A_{sat}) (Lee & Jarvis 1995; Ceulemans, Jiang & Shao 1995; Curtis 1996; Tissue, Thomas & Strain 1997; Saxe, Ellsworth & Heath. 1998). When linear electron flow through photosystem II (PSII), measured at light saturation (J_{PSII}), increases in elevated pCO_2, the proportion of absorbed photosynthetically active photon flux density (PPFD) used for photochemistry increases. Under these conditions a decrease in photoinhibition may be expected. However, only where

the stimulation of J_{PSII} to photosynthesis exceeds the suppression of J_{PSII} to photorespiration caused by elevated pCO_2, will J_{PSII} increase. The mechanistic model of C_3 photosynthesis proposed by Farquhar, von Caemmerer & Berry (1980) predicts that only when A_{sat}, under ambient pCO_2, is Rubisco-limited will elevated pCO_2 increase photosynthesis, photochemistry and J_{PSII} (Hymus *et al.* 1999). Under these circumstances, the proportion of absorbed energy that needs to be dissipated non-photochemically will decrease, as will the potential for photoinhibition. Three factors could remove this predicted increase in J_{PSII}, and therefore the expected protection against photoinhibition at high light. (i) Acclimation of the photosynthetic apparatus in response to long-term growth in elevated pCO_2 can decrease the stimulation of photosynthesis that is observed on initial transfer to elevated pCO_2; such acclimation has been widely reported in field studies (Oechel *et al.* 1994; Jacob, Greitner & Drake 1995; Nie *et al.* 1995; Osborne *et al.* 1998; Rogers *et al.* 1998). (ii) If photosynthate is accumulated more rapidly in elevated pCO_2, a triose phosphate utilization (TPU) limitation may decrease photosynthesis and J_{PSII} without an effect in the ambient treatment (Pammenter, Loreto & Sharkey 1993). (iii) Elevated pCO_2 may affect the rate of electron use by processes other than photosynthesis and photorespiration. Photoinhibition has been shown to reduce potential carbon uptake (Ögren & Sjöström 1990; Farage & Long 1991; Long, Humphries & Falkowski 1994) and be a major factor affecting seedling establishment (Ball, Hodges & Laughlin. 1991). Therefore, any protection that elevated pCO_2 may provide against photoinhibition is likely to be of ecological significance.

In a plantation forest in North Carolina, elevated pCO_2 increased the proportion of absorbed light used in photosynthesis, and J_{PSII}, decreasing the potential for photoinhibition throughout the growing season. During the winter, elevated pCO_2 depressed J_{PSII} and resulted in increased photoinhibition. By reference to the three factors listed above, there was no evidence of an acclimatory loss of photosynthetic capacity, or of alternative sinks for electrons during the winter. Consequently, the winter reversal could only be explained by TPU-limitation of photosynthesis during this period when growth was inhibited by low temperature (Hymus *et al.* 1999). In subtropical communities, such as the scrub oak palmetto community of Florida, low temperatures rarely limit growth, but high temperatures and drought during the summer may cause a cessation of growth (Breininger & Schmalzer 1990). In this community we hypothesize that elevated pCO_2 will increase J_{PSII} during periods of rapid growth, especially spring, but may result in decreased J_{PSII} during the summer if growth is slow and this in turn will result in increased photoinhibition.

This hypothesis was tested in a scrub-oak palmetto community, on Merritt Island, Florida. Following removal of all above-ground vegetation by fire, randomly selected plots were enclosed by open-top chambers (OTCs) in May 1996, and the new growth continuously exposed to either current ambient or elevated pCO_2. The dissipation of absorbed light energy via photochemistry and photoinhibition was monitored by modulated chlorophyll fluorescence on foliage during the spring and the summer.

MATERIALS AND METHODS

Site

The study site was located on Merritt Island, a barrier island on the east coast of central Florida and part of the Kennedy Space Centre (28°38′ N, 80°42′ W). The substrates were well-drained Pomello (Arenic Haplahumod) and Poala Sands (Spodic quartzipsamment). Both were acidic and low in nutrients, most of which were locked up in the standing biomass and a soil organic layer approximately 30 cm in depth (Schmaltzer & Hinkle 1992). The experimental site was representative of a fire-maintained scrub-oak palmetto community (Breininger & Schmalzer 1990). The shrub layer comprised rhizomatous schlerophyllous evergreen oaks, which re-sprouted from below ground after fire (Breininger & Schmalzer 1990). *Quercus myrtifolia* Willd. was the dominant species (Schmaltzer & Hinkle 1992). The climate was subtropical, warm and humid, with an average annual precipitation of 131 cm but with high year-to-year variability. Mean maximum and minimum temperatures in July, the hottest month, were 33·3 and 21·8 °C, respectively, and 22·3 and 9·5 °C in January, the coldest month (Schmaltzer & Hinkle 1992). The combination of frequent fires, seasonal water stress and nutrient-poor soil conditions maintain the composition and structure of the ecosystem (Breininger & Schmalzer 1990; Guerin 1993).

Experimental design

The study site was burnt in August 1995 with a few remaining areas burnt in January 1996 prior to imposing the CO_2 treatments. The OTCs were octagonal in plan with the largest diameter 3·66 m and the sides 1·4 m long. The entire chamber was 3·3 m high with the frustum at the midpoint, providing a chamber volume of 18·9 m³. Sixteen OTCs, eight maintained at current ambient pCO_2 (ambient) and eight maintained at ambient plus 35 Pa CO_2 (elevated) were paired together according to pre-burn above-ground biomass and species composition (Hungate *et al.* 1999; Stiling *et al.* 1999).

This study reports on measurements made on *Q. myrtifolia*. Measurements were made during the summer (July 1997), when leaves were 3 months old and fully developed. These were compared with leaves formed the following spring (April 1998) measured on completion of expansion. Additional summer measurements were taken in August 1996 and are reported for comparison. Air temperature (T_{air}) inside one OTC was recorded every minute using a shielded copper–constantan thermocouple, located in the centre of the OTC and at a height of 1 m. From this value of T_{air} and absolute water vapour pressure, measured using an infra-red gas analyser (LI-6262, LI-COR, Lincoln,

NB, USA), the relative humidity within the OTC was calculated.

In situ diurnal measurements

A modulated chlorophyll fluorimeter and leaf clip (PAM 2000 & 2030B, H Walz, Effeltrich, Germany) were used to measure diurnal variation in: minimum (F_o'), maximum (F_m') and steady state (F_s) levels of fluorescence *in situ* under prevailing PPFD (Hymus *et al.* 1999). The quantum yield of PSII photochemistry (ϕ_{PSII}), the efficiency of excitation energy capture by open PSII reaction centres (F_v'/F_m') and the photochemical quenching coefficient (q_P) were calculated from each measurement of F_o', F_m' and F_s (Genty, Briantais & Baker 1989; Hymus *et al.* 1999). Leaf absorptance of photosynthetically active radiation (α) was determined using an external integrating sphere and quantum sensor (LI-COR 1800–12, LI-190/LI-1905B, LI-COR), according to the procedure of Rackham & Wilson (1968). The value of J_{PSII} was estimated from ϕ_{PSII} using measured values of α and assuming that 50% of absorbed photon flux was distributed to PSII (Krall & Edwards 1992). Simultaneous measurements of the PPFD incident on the leaves were made with the photometric sensor of the leaf clip, which had been cross-calibrated with a quantum sensor (LI-190, LI-COR). Measurements of F_o and F_m, following dark-adaptation for 15 min, were made with a second fluorimeter (PEA, Hansatech Ltd, Kings Lynn, UK) for the calculation of the maximum quantum yield of PSII photochemistry (F_v/F_m). All the above measurements were made under the prevailing light conditions on at least four, sun-exposed upper-canopy leaves on individual trees. This was carried out in each of three ambient and elevated OTCs in July 1997, and in each of four ambient and elevated OTCs in August 1996 and April 1998. Measurements were made at 2–3 h intervals and completed within 1 h. Absorptance was determined (see above) in July 1997 and April 1998 for two leaves from each of the 16 chambers. To avoid ontogenetic effects, measurements were limited to the fourth or fifth leaf on each shoot.

A/p$_i$ and ϕ_{PSII}/ϕ_{CO2} responses

To separate developmental differences and long-term effects of growth in elevated pCO$_2$ from any change induced by diurnal variation, whole branches were collected for measurements around dawn in July 1997 and April 1998. These branches were cut under water, transferred to a controlled environment and maintained in low light until analysed within 2–3 h of collection. Whole branches were removed from at least six of the ambient and elevated chambers.

The response of A_{sat} to substomatal pCO$_2$ (p_i) was made using a portable gas exchange system (LI-6400; LI-COR). Measurements were at a leaf temperature (T_{leaf}) and VPD reflecting those experienced in the field. In July 1997 T_{leaf} was 29·8 ($\pm$0·2) °C and the VPD 1·9 ($\pm$0·01) kPa. In April 1998 measurements were made at a T_{leaf} of 25·1 ($\pm$0·3) °C

and a VPD of 1·75 ($\pm$0·04) kPa. Measurements were made in 21 kPa O$_2$ and at a PPFD of 1000 μmol m^{-2} s^{-1}, which was sufficient to saturate photosynthesis. Photosynthetic induction was at the growth pCO$_2$, thereafter steady-state photosynthesis was measured with stepwise decreases in pCO$_2$. A second measurement at the growth pCO$_2$ was taken after a measurement at 5 Pa had been made, followed by measurements with stepwise increase in pCO$_2$ up to 150 Pa. The maximum RubP saturated carboxylation capacity ($V_{c\ max}$) and maximum capacity for electron transport contributing to RubP regeneration (J_{max}) were determined from the A versus p_i response curve using the equations and constants of von Caemmerer (2000) and Bernacchi *et al.* (2001).

The response of both A and ϕ_{PSII} to PPFD was determined by simultaneous measurement of CO$_2$ uptake (LI-6400) and modulated chlorophyll *a* fluorescence (PAM-2000). Measurements were made over a range of PPFD from 100 to 1600 μmol m^{-2} s^{-1} and in 1 kPa pO$_2$ (Boggs gases, Titusville, FL, USA). The effect of pCO$_2$ on the ratio of electron transport to CO$_2$ fixation, in the absence of photorespiration, was determined from the relationship of ϕ_{CO2} to ϕ_{PSII} (Edwards & Baker 1993; Fryer *et al.* 1998). The calculated ϕ_{CO2} was corrected for respiratory losses in the light and for α (Fryer *et al.* 1998).

Stem diameter measurements

In order to characterize growth, the stem diameter of 10 *Q. myrtifolia* trees was measured at regular intervals throughout 1997, in each of the eight ambient and eight elevated OTCs. Measurements were made 2 cm above ground level using a digital caliper (NTD13–6, Mitutoyu Corp, Kanagawa, Japan).

Statistical analysis

The effect of pCO$_2$ treatment on light-saturated parameters was tested using *post hoc*, two-tailed Student's *t*-tests (SYSTAT 7·0, Systat, Evanston, IL, USA). A one-tailed Student's *t*-test was used to examine the null hypothesis that there was no increase in F_o from dawn to midday. Regression analysis of variance was used to test the effects of pCO$_2$ on the linear relationship between ϕ_{PSII} and ϕ_{CO2} (Sokal & Rohlf 1981). To avoid pseudoreplication, average values were calculated for each OTC. The average for an OTC was then treated as the individual in deriving mean and variance for the population of the chambers. All derived chlorophyll fluorescence parameters were arc-sine transformed prior to statistical analysis (Sokal & Rohlf 1981).

RESULTS

In situ diurnal measurements

On the measurement day in July 1997, T_{air} inside the OTCs reached a maximum of 34·9 °C at 1400 h, from a minimum at night of 23·5 °C, and relative humidity fell from 100% at night, to a minimum of 55·1% at 1320 h (data not shown). On
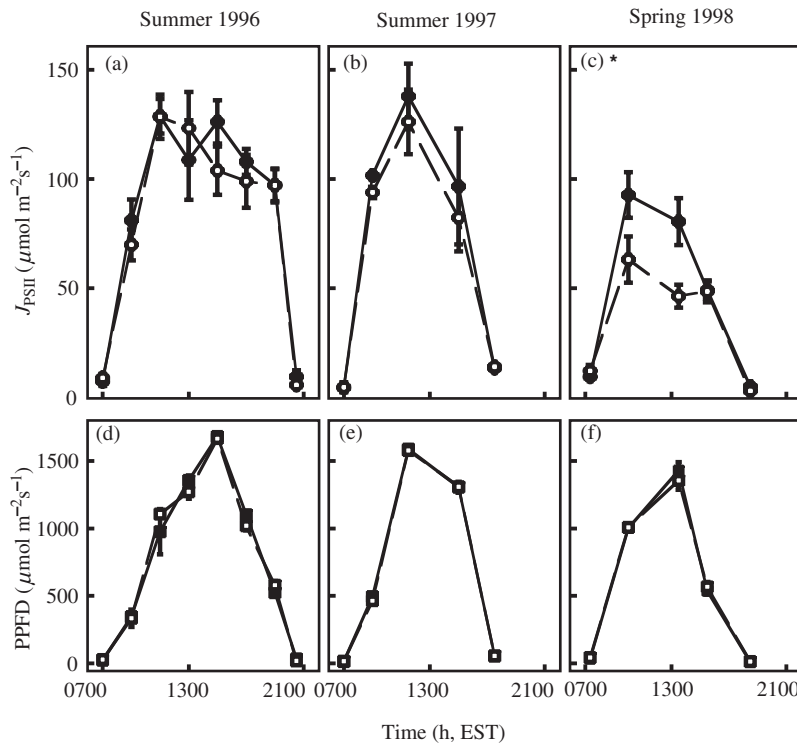
Figure 1. *In situ* diurnal measurements of J_{PSII} (a–c) and PPFD (d–f) for summer 1996, summer 1997, and spring 1998. The symbols illustrated are the means ($\pm$ 1 SE) of measurements made in three replicate OTCs in summer 1997 and four replicate OTCs in summer 1996 and spring 1998, for elevated (solid symbols/lines) and ambient (open symbols/dashed line) OTCs. * denotes a significant effect of pCO_2 ($P < 0.05$) on J_{PSII} measured over the portion of the photoperiod when PPFD was saturating for photosynthesis.

the measurement day in April 1998, T_{air} reached a maximum of 30·7 °C at 1230 h, from a minimum at night of 15·8 °C, and relative humidity fell from 95·6% at night, to a minimum of 40·4% at 1330 h (data not shown). Air temperature and relative humidity were not different between the ambient and elevated OTCs (data not shown). Between sunrise and 1300 h, in both July 1997 and April 1998, T_{air} inside the OTCs rose relative to T_{air} outside the OTCs. On both days T_{air} was up to 4 °C warmer inside the OTCs by 1300 h. Between 1300 h and sunset the difference between T_{air} inside and outside the OTCs decreased and was eventually lost.

Reflecting the typical diurnal courses of solar radiation on these clear sky days J_{PSII} rose and fell with PPFD (Fig. 1). Because PPFD was limiting to photosynthesis at dawn, ϕ_{PSII}, q_P and F_v'/F_m' declined to midday minima as PPFD rose, and then recovered by dusk (Fig. 2). In both summers, J_{PSII} was unaffected by pCO_2 treatment. Conversely, in spring J_{PSII} was significantly increased in elevated pCO_2 ($t_6 = 4·1$; $P = 0·01$) (Fig. 1). In both summers, ϕ_{PSII}, q_P and F_v'/F_m' were also unaffected by pCO_2 treatment (Fig. 2). In spring, the significant increase in ϕ_{PSII} ($t_6 = 3·8$; $P = 0·01$) in elevated pCO_2 was a consequence of a significant increase in both F_v'/F_m' ($t_6 = 2·8$; $P = 0·03$) and q_P ($t_6 = 3·1$; $P = 0·01$) (Fig. 2c, f & i). For both pCO_2 treatments, maximum diurnal J_{PSII} for the young leaves present in spring was approximately half that of the older leaves present in both summers.

As the measurements were made under prevailing PPFD, systematic differences between PPFD incident on each pCO_2 treatment were a possible cause of the different J_{PSII}. However, the mean PPFD measured concurrently with fluorescence was almost identical for the two pCO_2

treatments and was not statistically different, in August 1996 ($t_6 = 0·24$; $P = 0·81$) July 1997 ($t_4 = 0·26$; $P = 0·81$), or April 1998 ($t_6 = 0·6$; $P = 0·57$) (Fig. 1d–f). During the morning and evening, the PVC walls of the OTCs decreased PPFD inside by 10% compared to outside. However, as the sun rose the PPFD increasingly entered the OTCs through the open top, and the difference between PPFD inside and outside the OTC declined (data not shown).

Although predawn F_v/F_m was unaffected by pCO_2 treatment in all three measurement periods, diurnal minimum values of F_v/F_m were affected (Fig. 3a–c). Diurnal minimum F_v/F_m measured after 15 min dark adaptation was significantly lower in the elevated pCO_2 treatment in both summers (1996, $t_6 = 3·0$; $P = 0·02$; 1997, $t_4 = 3·4$; $P = 0·03$). In spring, diurnal minimum F_v/F_m was significantly higher in the elevated pCO_2 treatment ($t_6 = 3·8$; $P = 0·01$) mirroring the effect on F_v'/F_m'. Significant increases in F_o occurred between dawn, and the time of minimal F_v/F_m in the elevated pCO_2 treatments in both summers (1996, $t_6 = 2·1$; $P = 0·04$; 1997, $t_4 = 6·7$; $P = 0·001$). No significant effect on F_o was seen in either pCO_2 treatment in spring.

A/p_i and ϕ_{PSII}/ϕ_{CO2} responses

In the summer of 1997, elevated pCO_2 significantly decreased $V_{c\ max}$ from 113 to 82 μmol m^{-2} s^{-1} ($t_{12} = 2·4$; $P = 0·03$). A parallel decrease in J_{max} from 140 to 133 μmol m^{-2} in elevated pCO_2 was not statistically significant. Despite these decreases, A_{sat} was still significantly increased by 46% ($t_{12} = 5·4$; $P < 0·001$) in leaves grown and measured at elevated pCO_2 compared with controls
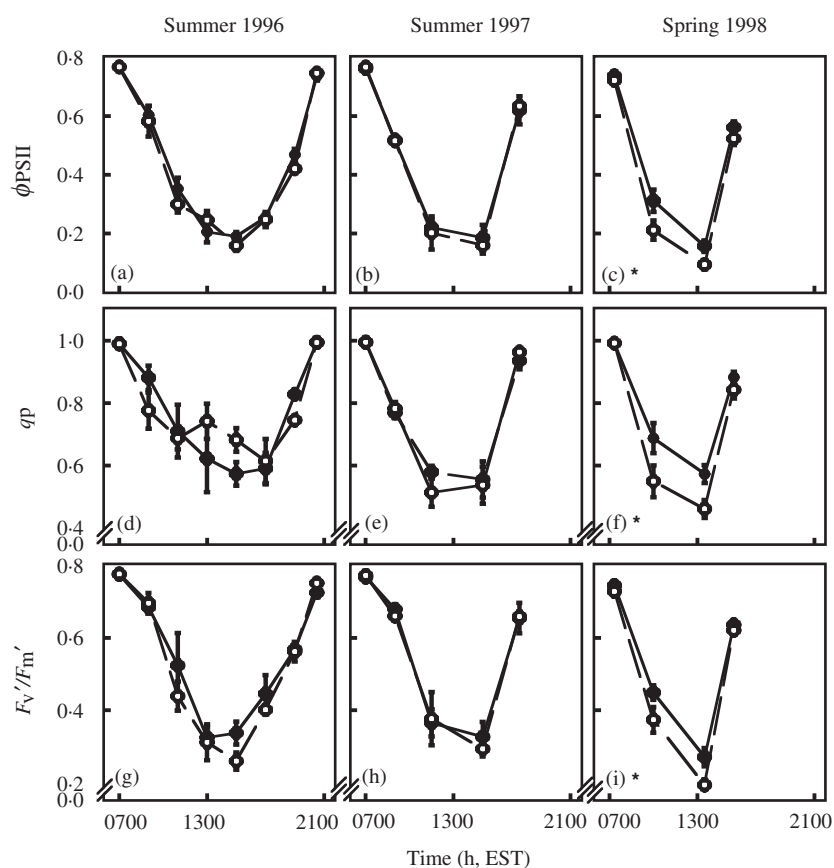
Figure 2. *In situ* diurnal measurements of ϕ_{PSII} (a–c), q_P (d–f) and F_v'/F_m' (g–i) for summer 1996, summer 1997 and spring 1998. The symbols illustrated are means ($\pm$ 1 SE) for three replicate OTCs in summer 1997 and four replicate OTCs in summer 1996 and spring 1998, for elevated (solid symbols/solid lines) and ambient (open symbols/dashed line) OTCs. * denotes a significant effect of pCO_2 ($P < 0.05$) on the parameter measured, over the portion of the photoperiod when PPFD was saturating for photosynthesis.

(Fig. 4a). In spring both $V_{c\,max}$ and J_{max} were substantially lower than in summer in both pCO_2 treatments. However, the small reductions in $V_{c\,max}$ from 35 to 32 μmol m^{-2} s^{-1} and J_{max} from 61 to 57 μmol m^{-2} s^{-1} in elevated pCO_2, were not

statistically significant. A_{sat} was significantly increased by 46% ($t_{14} = 3.2$; $P < 0.05$) in elevated pCO_2 when measurements made at the two growth pCO_2 were compared (Fig. 4b). In both summer and spring, light-saturated pho-
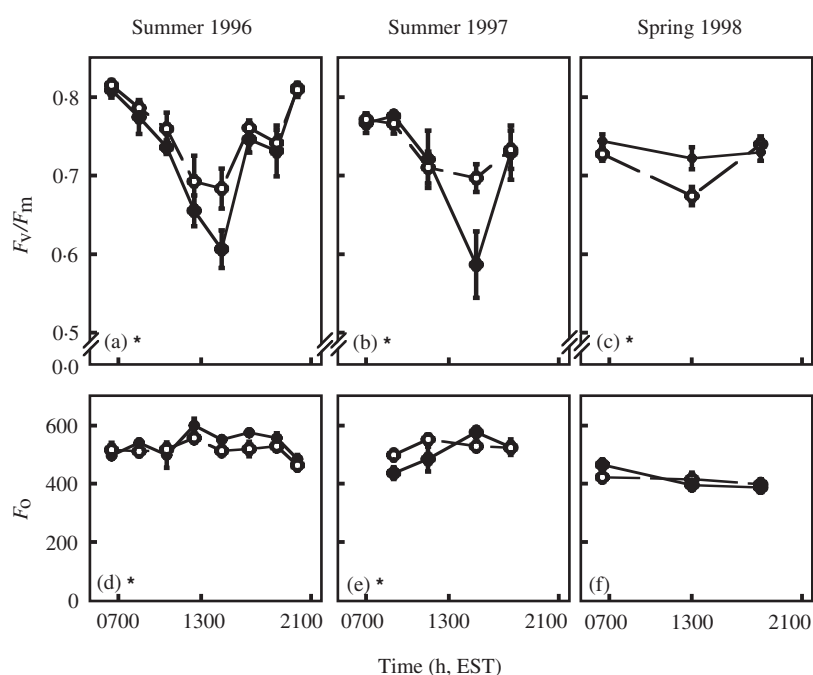

Figure 3. *In situ* diurnal F_v/F_m (a–c) and F_o (d–f) after 15 min dark adaptation, in summer 1996, summer 1997 and spring 1998 . Symbols shown are the means ($\pm$ 1 SE) of measurements made in three replicate OTCs in summer 1997 and four replicate OTCs in summer 1996 and spring 1998 for elevated (solid symbols/solid lines) and ambient (open symbols/dashed line) OTCs. * denotes a significant effect ($P < 0.05$) of pCO_2 on diurnal minimum F_v/F_m (a–c), and also a significant increase between F_o measured predawn and at the point in the photoperiod where F_v/F_m is minimum, for elevated pCO_2 only (d–f).
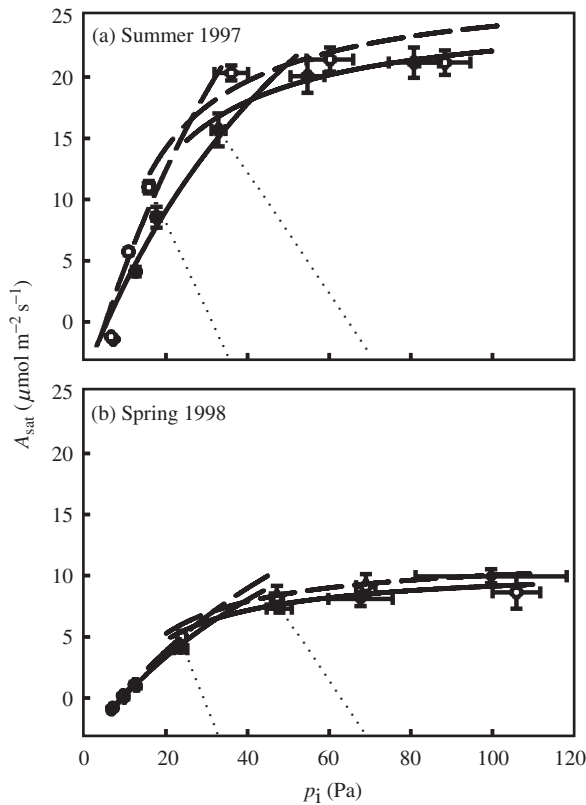
Figure 4. Light-saturated photosynthesis. Plot of A_{sat} against p_i for summer 1997 (a) and spring 1998 (b), for leaves on excised branches grown in elevated (solid symbols, solid lines) or ambient (open symbols, dashed lines) pCO_2 treatments. Also shown are the supply functions for each curve (dotted line). The operating point is at the intersection of the supply function and the A/p_i curve for each treatment. The A/p_i curves are fitted to the data points illustrated by maximum-likelihood regression following the functions of Bernacchi *et al.* (2001). Data points shown are the means (± 1 SE) for measurements made in at least six replicate OTCs. Measurements were made in 21 kPa O_2

tosynthesis under ambient pCO_2 was limited by the amount of Rubisco, as indicated by operating points on the initial slope of the A/p_i responses (Fig. 4).

Although the A/p_i responses indicated that A_{sat} was Rubisco-limited at both ambient and elevated pCO_2 in

summer 1997 (Fig. 4), J_{PSII} was similar for both treatments in the field. Table 1 explains this apparent contradiction. A 37% increase in J_{PSII} would have been expected for these leaves when pCO_2 was increased from 35 to 70 Pa, if $V_{c\ max}$ was unaffected. However, the acclimatory decrease in Rubisco in elevated pCO_2 offset this expected increase. Table 1 also showed that J_{PSII} calculated from gas exchange agreed closely with J_{PSII} calculated from fluorescence.

The ratio of electron transport to CO_2 fixation, measured in the absence of photorespiration, was unaffected by pCO_2 treatment in both summer ($F_{1,52} = 1\cdot5$; $P > 0\cdot05$) and spring ($F_{1,58} = 3\cdot1$; $P > 0\cdot05$; not illustrated) (Fig. 5). For each pCO_2 treatment, in both time periods, the correlation between ϕ_{CO2} and ϕ_{PSII} was statistically highly significant ($r^2 = 0\cdot71–0\cdot94$; $P < 0\cdot001$). In summer α was $0\cdot79 \pm 0\cdot007$ and $0\cdot75 \pm 0\cdot01$ in spring with no significant effect of pCO_2 at either time of year.
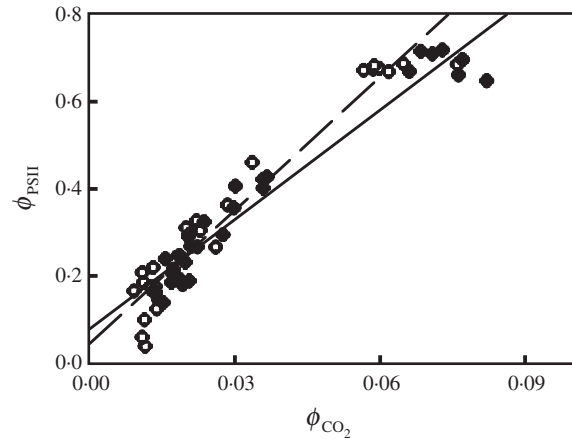

Figure 5. Ratio of electron transport to CO_2 fixation. Plots of ϕ_{PSII}/ϕ_{CO2} for leaves measured in summer 1997. Symbols are for measurements from multiple leaves made over a range of PPFD (60–1600 μmol m^{-2} s^{-1}). Measurements were made in 1 kPa O_2 and at the growth pCO_2, for either elevated (solid symbols/solid line) or ambient (open symbols, dashed line) pCO_2 treatments. Linear regressions were fitted using the least squares method. The equations of the fitted regressions were $\phi_{PSII} = 10\cdot2\ \phi_{CO2} + 0\cdot04$ and $\phi_{PSII} = 8\cdot4\ \phi_{CO2} + 0\cdot07$ for the ambient and elevated pCO_2 treatments, respectively.

Table 1. Comparing measured and modelled J_{PSII} in summer 1997. Rubisco-limited velocities of carboxylation (v_c) and oxygenation (v_o) are calculated from $V_{c\ max}$ and p_i measured for each OTC, using the equations of von Caemmerer (2000) and Bernacchi *et al.* (2001). By assuming that four electrons are required for each carboxylation and oxygenation J_{PSII} is calculated ($J_{PSII\ CAL}$) and compared to J_{PSII} measured on leaves in the field. All values are the means (± 1 S.E) for six replicate ambient OTCs (A) and seven replicate elevated OTCs (E). The J_{PSII} that would have been found in leaves in elevated pCO$_2$, had they not shown an acclimatory decrease in $V_{c\ max}$ (E_{NA}), is also calculated. Field measurements and model predictions were made at 27 °C

	$V_{c\ max}$	p_i	v_c	v_o	$J_{PSII\ CAL}$	J_{PSII}
A	$113\cdot1 \pm 7\cdot4$	$15\cdot95 \pm 0\cdot97$	$21\cdot4 \pm 1\cdot2$	$15\cdot6 \pm 1\cdot1$	$148\cdot1 \pm 7\cdot9$	$128\cdot5 \pm 34\cdot8$
E	$82\cdot0 \pm 6\cdot9$	$32\cdot98 \pm 2\cdot55$	$26\cdot9 \pm 1\cdot9$	$9\cdot4 \pm 0\cdot9$	$144\cdot9 \pm 10\cdot4$	$139\cdot8 \pm 30\cdot1$
E_{NA}	$113\cdot1$	$32\cdot98$	$37\cdot5$	$13\cdot0$	$202\cdot1$	–

$V_{c\ max}$, v_c, v_o, $J_{PSII\ CAL}$ and J_{PSII} are all expressed in μmol m^{-2} s^{-1}; p_i is expressed in Pa.
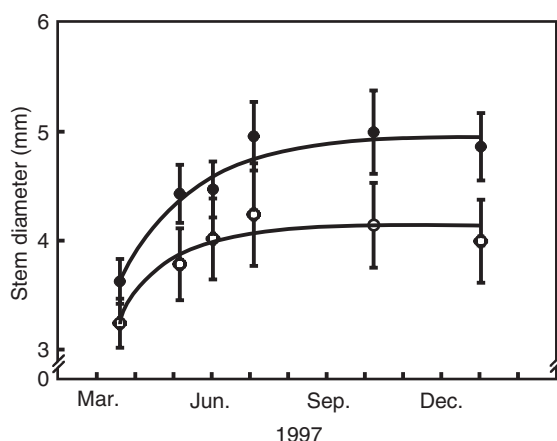
Figure 6. Stem diameter measurements. Plots of the increase in stem diameter of *Q. myrtifolia* trees in 1997. Symbols shown are the mean (± 1 SE) for measurements made in eight elevated (solid symbols, solid lines) or ambient (open symbols, dashed lines) pCO_2 treatments.

Stem diameter measurements

In 1997, the stem diameter of *Q. myrtifolia* trees, growing in both ambient and elevated pCO_2 increased after the spring and reached an annual maximum by the end of June. After June the stem diameter remained constant for the rest of the year (Fig. 6).

DISCUSSION

It was hypothesized that elevated pCO_2 would affect J_{PSII} and photoinhibition differently according to time of year. This hypothesis has been validated. *In situ,* J_{PSII} was unaffected by pCO_2 treatment in the summer but significantly increased in the spring (Fig. 1). In the summer photoinhibition was significantly increased by elevated pCO_2, whereas in the spring it was significantly decreased (Fig. 3).

In spring there was no acclimation of either $V_{c\,max}$, J_{max}, or the ratio of electron transport to CO$_2$ fixation in response to growth in elevated pCO_2 (Figs 4 & 5). Given that light-saturated photosynthesis under current ambient pCO_2 was Rubisco-limited, a large stimulation of J_{PSII} was predicted and observed (Fig. 1). In the spring F_v'/F_m' measured at light saturation, and F_v/F_m measured after 15 min dark adaptation were significantly increased by elevated pCO_2 (Figs 2 & 3). These findings supported our hypothesis that elevated pCO_2 would partially protect PSII from photoinhibition when growth provides adequate sinks for additional photosynthate.

In summer, when growth had ceased (Fig. 6), elevated pCO_2 did not stimulate J_{PSII} and photoinhibition was increased (Fig. 3), as was hypothesized. Given that photosynthesis at ambient pCO_2 was Rubisco-limited (Fig. 4), a stimulation of J_{PSII} similar to that observed in spring could have been expected. However, although long-term growth

in elevated pCO_2 did not result in any change in the ratio of electron transport to CO$_2$ fixation in summer, it did result in an acclimatory decrease in $V_{c,max}$. In the absence of acclimation a 37% increase in J_{PSII} was predicted due to a net increase in $v_c + v_o$ (Table 1). From theory this would be almost completely offset by the observed 27% decrease in $V_{c\,max}$ (Fig. 4). In *Pinus taeda* a TPU limitation in winter was suggested to decrease J_{PSII} in elevated pCO_2 (Hymus *et al.* 1999), here the cause is different, acclimation of Rubisco activity. The link between photosynthetic acclimation and the restriction of sinks for photosynthate is well established (Arp 1991). Currently, changes in gene expression triggered by accumulation or fluxes of additional photosynthate are thought to be the cause of acclimatory decreases in $V_{c\,max}$ in elevated pCO_2 (Drake, Gonzalez-Meler & Long 1997; Moore *et al.* 1999). It is wholly in keeping with these ideas that a decrease in $V_{c\,max}$ was seen in summer when growth was restricted by heat and drought in this ecosystem.

In both summers, ϕ_{PSII}, q_P and F_v'/F_m' were unaffected by pCO_2 treatment (Fig. 2). However, diurnal minimum F_v/F_m measured after 15 min dark adaptation was lower in elevated pCO_2 (Fig. 3). Where light-induced quenching processes are considerably greater than those that remain in the dark-adapted leaves, the effect of elevated pCO_2 on F_v/F_m may be absent in F_v'/F_m' (Hymus *et al.* 1999). In both summers there was a significant increase in F_o between predawn and the point at which F_v/F_m was minimal, in elevated pCO_2. This may reflect photoinactivation or photodamage to PSII reaction centres in elevated pCO_2 (Fig. 3). The slow recovery, of the order of minutes to a few hours, will significantly decrease the total carbon gain of natural canopies (Long *et al.* 1994). This loss will therefore be greater in the elevated pCO_2 treatment. Two earlier studies of the effects of elevated pCO_2, on photoinhibition in Mediterranean trees (Jones *et al.* 1995; Scarascia-Mugnozza *et al.* 1996) produced conflicting results. This study extends on these earlier observations to explain how seasonally dependent acclimation to elevated pCO_2 underlies increased resistance in spring and increased susceptibility in summer.

In conclusion, for *Q. myrtifolia* growing in the field, the effects of elevated pCO_2 on J_{PSII} and photoinhibition changed during the year. In the spring, during periods of growth, light saturated J_{PSII} was increased by elevated pCO_2, with the result that photoinhibition was decreased. In summer, when growth was restricted, this stimulation of J_{PSII} was lost, largely due to an acclimatory loss of carboxylation capacity, and photoinhibition increased.

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