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Water relations and leaf anatomy of the tropical species, *Jatropha gossypifolia* and *Alternanthera crucis*, grown under an elevated CO_2 concentration

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Abstract

In order to address the question of how elevated CO_2 concentration (EC) will affect the water relations and leaf anatomy of tropical species, plants of *Jatropha gossypifolia* L. and *Alternanthera crucis* (Moq.) Bondingh were grown in five EC open top chambers (677 µmol mol^{-1}) and five ambient CO_2 concentration (AC) open top chambers (454 µmol mol^{-1}) with seasonal drought. No effect of EC was found on morning xylem water potential, leaf osmotic potential, and pressure potential of plants of *J. gossypifolia*. In *A. crucis* EC caused a significant increase in morning xylem water potential of watered plants, a decrease in osmotic potential, and an increase of 24-79 % in pressure potential of moderately droughted plants. This ameliorated the effects of drought. Stomatal characteristics of both leaf surfaces of *J. gossypifolia* and *A. crucis* showed time-dependent, but not $[CO_2]$-dependent changes. In *J. gossypifolia* the thickness of whole leaf, palisade parenchyma, and spongy parenchyma, and the proportion of whole leaf thickness contributed by these parenchymata decreased significantly in response to EC. In *A. crucis* EC caused an increase in thickness of whole leaf, bundle sheath, and mesophyll, while the proportion of leaf cross-section comprised by the parenchymata remained unchanged. These effects disappeared with time under treatment, suggesting that acclimation of the leaf anatomy to the chambers and to EC took place in the successive flushes of leaves produced during the experiment.

Additional key words: drought; parenchyma; open-top chamber; osmotic potential; pressure potential; stomatal density; volumetric elasticity modulus; water potential; water supply.

Introduction

Among plant responses to an elevated CO_2 concentration (EC), an increase in growth and productivity and the alleviation of some stresses, such as water deficit, have been reported as typical. Increased productivity may be explained by the increased net photosynthetic rate (P_N) and the improved water status produced by a lower stomatal conductance (g_s), a lower osmotic potential (ψ_s), and a higher water use efficiency (Eamus 1991, Chaves and Pereira 1992, Pospíšilová and Čatský 1999). An increase in P_N with EC has been primarily observed in C_3 plants because at high $[CO_2]$ the ratio P_N/photorespiration rate increases (Woodward *et al.* 1989). Measurements of the responses of g_s of C_3 as well as C_4 plants to $[CO_2]$ indicate nearly a 40 % reduction for a doubling in the ambient CO_2 concentration (AC). Therefore in water-limited environments metabolic activity may be maintained by EC, through improved water conservation (Woodward *et al.* 1989).

Greater values of xylem water potential (ψ) and pressure potential (P) have been observed in droughted plants grown under EC (Tyree and Alexander 1993, Wullschleger *et al.* 2002). In the case of wheat plants grown under EC and subjected to water deficit a higher P was the result of a lower ψ_s and a higher ψ (Sionit *et al.*

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Abbreviations: AB = abaxial leaf surface; AC = ambient $[CO_2]$ chambers; AD = adaxial leaf surface; B = apoplasmic water fraction; EC = elevated $[CO_2]$ chambers; ECD = epidermal cell density; g_s = stomatal conductance; P = pressure potential; P_N = net photosynthetic rate; RWC^0 = relative water content at turgor loss; SD = stomatal density; SI = stomatal index; ε = bulk volumetric elasticity modulus; ψ = xylem water potential; ψ_s = osmotic potential; ψ_s^{100} = osmotic potential at full turgor; $\Delta\psi_s^{100}$ = osmotic adjustment.

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1981). In contrast, there are reports (reviewed by Wullschleger *et al.* 2002) that EC does not necessarily increase ψ; the decrease in g_s may be outweighed by the increase in leaf area, thus cancelling the beneficial effects on soil water use of a reduction in transpiration.

Stomatal density (SD) is an anatomical characteristic important from the physiological viewpoint that it affects both CO_2 uptake and water loss because the diffusion rate of gases through the stomatal pore is directly proportional to SD (Nobel 1974). Hence, changes in SD in response to EC could impact on leaf conductance and water relations of plants. SD is sensitive to the increase in atmospheric [CO_2] since the pre-industrial times. Lower SD and stomatal index (SI, the proportion of epidermal cells comprised by stomata) were found in herbarium samples from 1796, at a [CO_2] = 280 μmol mol^{-1}, than in present day specimens. Therefore, the decrease in SD was due not only to a change in leaf expansion, but also to a decrease in stomatal initiation (Woodward 1987). However, other reports have conflicted with this, suggesting that the effects of EC on SD and SI are highly species- or cultivar-specific (Peñuelas and Matamala 1990, Beerling and Chaloner 1993a,b, Ferris and Taylor 1994, Tognetti *et al.* 2001) or non-existent (Estiarte *et al.* 1994). Since neither SD nor SI decreased in herbarium samples with atmospheric [CO_2] above the current value (Woodward 1987), a lack of change in SD as well as in SI with EC may be the norm rather than the exception.

Temperature itself can modify the stomatal characteristics of plants grown under EC. In an experiment on *Lolium perenne*, in which the interaction between EC and temperature was examined, SD was in general lower at a higher temperature, and at a higher temperature *plus* EC, than their controls, but responses were not always consistent and depended on the leaf section sampled (Ferris *et al.* 1996). SD can be decreased by an increase in relative humidity (Tichá 1982). These findings highlight the importance of factors other than EC for stomatal initiation and are a caution against erroneously concluding that EC alone is the cause of the changes observed in stomatal characteristics in experiments with other environmental variables uncontrolled.

In order to answer the question of whether an increase in [CO_2] can be reflected in SD and SI, more research is necessary to address the diversity found in responses of many species to EC. The lack of response of SD and SI to EC has been attributed to the absence of a generalised direct effect of EC on stomatal initiation during either ontogenesis or later stages of epidermal cell expansion in short-term experiments (Ferris and Taylor 1994).

A consistent trend in changes of leaf structure due to EC cannot be found in the literature. For example, whole leaf thickness and the proportion of leaf section occupied by chlorenchymata and epidermes responded differently to EC in different species (Thomas and Harvey 1983, Ferris *et al.* 1996, Tipping and Murray 1999).

The current study describes the response of water relations and leaf anatomy to EC in two tropical xerophytes with contrasting mechanisms of drought resistance (Tezara *et al.* 1998) grown in open-top chambers at AC and EC. The experimental design aimed at simulating as much as possible the conditions to which plants are subjected in their native environment, including natural drought. These plants restrict their annual growth cycle to short and infrequent rainy periods, making them interesting model species to examine the possible impacts of the predicted intensification in drought accompanying increases in atmospheric [CO_2] (Rind *et al.* 1990).

The species studied, the perennial deciduous *Jatropha gossypifolia*, a C_3 shrub, and *Alternanthera crucis*, a C_3-C_4 herb (Fernández *et al.* 1999), grow in the semi-arid zones of north-western Venezuela, where they are important components of the ecosystem. Plants of *J. gossypifolia* shed their leaves at low soil moisture content but show no marked decrease in ψ, while plants of *A. crucis* under drought keep their leaves longer, and show osmotic adjustment and very low values of ψ (Ríos 1993). No osmotic adjustment has been observed in *J. gossypifolia* (A. Herrera, unpublished). In a study done in parallel with the present one (Fernández *et al.* 1999), P_N of both species increased under EC, whereas leaf conductance increased in *J. gossypifolia* (similarly to observations reviewed by Eamus and Jarvis 1989) but decreased in *A. crucis*. Also, P_N was significantly higher in droughted plants of both species under EC than AC.

In order to examine the effects of EC alone and its interaction with drought on the water relations and the leaf anatomy, measurements were taken in watered as well as droughted plants.

Materials and methods

Experimental site and plants: The study was carried out at the experimental site of the Centro de Investigaciones Agrícolas y Pecuarias (CENIAP) in the town of Maracay, Estado Aragua, Venezuela, at 10°20'N – 67°22'W, where the rainy season spans from May until November. During the year of experimentation (1998) rainfall was 1 021 mm. Mean annual temperature was 30±2 °C. Plants were grown in five AC (454±3 μmol mol^{-1}) and five EC (677±10 μmol mol^{-1}) open-top chambers. AC was high due possibly to a high rate of edaphic respiration, since the soil had a significant content of organic matter. The chambers, consisting of cylinders 1.55 m high and 2.00 m wide with walls of 2-mm thick polymethylacrylate sheet, were installed on an area of approximately 800 m^2. The field was divided in 20 square portions of 6×6 m and chambers allotted in such a way that they were as far apart from each other as possible. For details of the construction and distribution of chambers in the field and of plant cultivation see Fernández *et al.* (1999). Three individuals each of *Jatropha gossypifolia* L. and *Alternanthera crucis* (Moq.) Bondingh were planted in the soil in each chamber. Plants were frequently fertilised with commercial N : P : K (15 : 15 : 15) and watered from the beginning of fumigation (week 0) until week 9; plants were allowed to receive water from the rain and additional sprinkling when necessary during the establishment period and were left non-watered during the dry season. Since a significant recruitment took place in the chambers after 23 weeks, some individuals and all leaves of *J. gossypifolia,* and all shoots of *A. crucis* were removed in order to reduce planting density to three individuals per chamber. The leaf dry mass (obtained by drying at 60 °C for one week) of the harvested individuals was then recorded. Whole-plant leaf area was determined multiplying specific leaf area of ten discs per chamber by total leaf dry mass per plant (one plant per chamber, $n = 5$).

Water relations: Morning xylem water potential (ψ) was measured between 06:30 and 08:00 h in the youngest fully expanded leaves (leaf-bearing branches, in the case of *A. crucis*) from one plant per chamber ($n = 5$) with a pressure chamber (*PMS*, Corvallis, OR, USA). A water-vapour pressure osmometer model *5500* (*Wescor*, Logan, Utah, USA) was used to determine ψ_s in the sap of leaves previously used to determine ψ, frozen in plastic syringes and defrosted. P was calculated as the difference between ψ_s and ψ; in the case of *A. crucis* ψ_s was corrected for the apoplasmic water fraction (B) determined in P-V curves. Pressure-volume (P-V) curves of *A. crucis* were done after 9 (drought) and 22 (rains) weeks of treatment. Sun-exposed branches from one individual in each chamber ($n = 5$) were cut under water and re-hydrated for 12 (on week 9) or 3 h (on week 22), based on previous determinations of the time necessary to reach maximum fresh mass. The bulk volumetric elasticity modulus, ε, was calculated using the mean pressure potential from full hydration to turgor loss in the exponential section of the P-V curves. Osmotic adjustment ($\Delta\psi_s^{100}$) in plants of *A. crucis* was calculated as the difference in ψ_s^{100} between watered (week 44) and droughted (week 54) plants, where ψ_s^{100} = osmotic potential at full turgor determined using an osmometer (see above) in the sap expressed from fully re-hydrated leaves. Since values of ψ_s^{100} determined by P-V curves were not statistically different from those determined with the osmometer, P-V curves were not performed after week 22.

Stomatal and epidermal cell counts: Youngest fully expanded leaves (approximately 15-d-old) from watered plants of each species and chamber were collected (one leaf per chamber; $n = 5$). Nail varnish was applied to the lamina on an area of 4 cm^2 midway between the tip and the base, avoiding areas over main veins. Other sections of the lamina were not used for counts, since preliminary observations showed that SD was higher at mid-lamina than near the apex or the basis, and much lower than near the main veins. Varnish replicas were peeled with transparent tape and mounted on glass slides. Cells were counted on photographs taken under a *Nikon Labophot-2* microscope (Tokyo, Japan) at a 150-300× magnification (one microscope field per replica). Care was taken to ascertain that cell distribution on the tape replica was uniform. Sample size was 5 (one microscope-field per chamber). Previous measurements determined that similar means and SE were obtained for $n = 5$ or 10. An eye-piece graticule, pre-calibrated against a stage micrometer, was used to take measurements. Stomatal index was calculated as SI = 100 SD/(SD + ECD), where ECD = epidermal cell density.

Leaf structure: Samples for leaf anatomy were taken, one per chamber ($n = 5$), from youngest fully expanded leaves. Leaf segments, taken from the same area of the lamina as above, were fixed in 75 % iso-propanol and free hand sectioned. Measurements of the thickness of the total leaf and the component tissues were taken.

Data analysis: Significance at $p<0.05$ was assessed through one- and two-way ANOVA using the *Statistica 4.0* package. Values of RWC^0, B, and SI were arc-sin-transformed before performing the ANOVA.

Results

Water relations: Air temperature and relative humidity were not significantly different among the open-top chambers; as an example, in April (week 9 of treatment) values for air temperature and relative humidity were 22.8±0.2/35.5±0.5 °C and 60.5±0.4/100 % (minimum/maximum). Leaf temperature was nearly 5 °C higher than average from weeks 17 until 35 but there were no significant differences between AC and EC.

The time-course of ψ, ψ_s, and P of plants of *J. gossypifolia* and *A. crucis* grown under ambient and EC, and of rainfall are shown in Fig. 1. Water status of plants of both species, evidenced by values of ψ, was related to amount of rainfall. No significant effect of EC was found in any of the parameters of water relations in plants of *J. gossypifolia* on any date. The observed increase with drought in ψ_s of AC as well as EC plants of *J. gossypifolia* was possibly an artefact due to an increase in dilution by apoplasmic water in droughted plants,

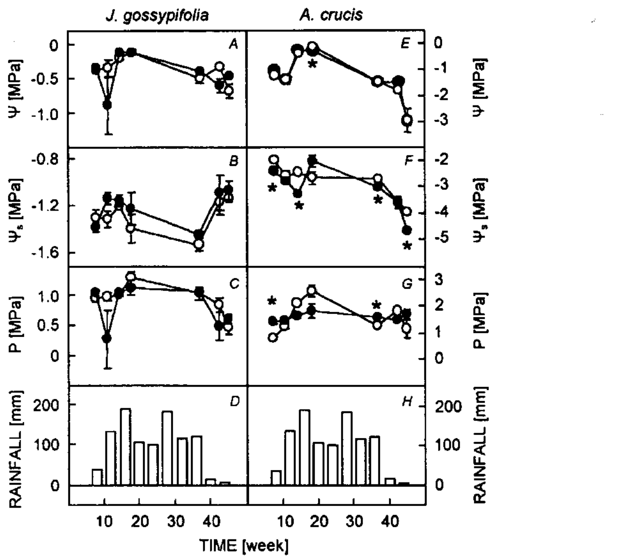

Fig. 1. Time-course of changes in xylem water potential, leaf osmotic potential, and pressure potential of plants of *J. gossypifolia* (*A*, *B*, and *C*, respectively) and *A. crucis* (*E*, *F*, and *G*, respectively) grown under ambient $[CO_2]$ (○) and EC (●). *D*, *H*: seasonal changes in rainfall. Means ± SE ($n = 5$). An *asterisk* indicates significant differences ($p<0.05$) due to EC.

a phenomenon previously noticed in this as well as other xerophytes (A. Herrera, unpublished). In plants of *A. crucis* no effect of EC was found on ψ, except for a decrease after 22 weeks relative to AC plants, whereas EC caused a decrease in ψ_s and, in moderately droughted plants, an increase in P of 79 % (week 9) and 24 % (week 44). Changes with drought in parameters of water relations obtained through P-V curves during the first weeks of the experiment are shown in Table 1; values of ψ are included as a reference for water status. Although significant effects of water status were found on ψ, B, and ε, no effects of EC or its interaction with water status were detected on any parameter, except for ψ, where EC decreased the value in watered EC relative to AC plants.

During the last weeks of the experiment, when watered (44 weeks) and droughted (54 weeks) plants were compared, ψ_s^{100} was not significantly different between watered AC and EC plants (AC, –2.23±0.07 MPa; EC, –2.69±0.18 MPa) but it was lower in droughted EC (–3.37±0.08 MPa) than AC (–2.85±0.13 MPa) plants. Osmotic adjustment was not significantly different between growth CO_2 concentrations (AC, 0.63±0.18 MPa; EC, 0.63±0.13 MPa).

Table 1. Effects of growth $[CO_2]$ and water status on apoplasmic water fraction, relative water content at turgor loss, and bulk elasticity modulus of droughted (9 weeks) and watered (22 weeks) plants of *A. crucis* grown in ambient $[CO_2]$ (AC) and enhanced $[CO_2]$ (EC) chambers. Means ± SE ($n = 5$). Effects of $[CO_2]$, water status (WS), and their interaction, determined by two-way ANOVA, are shown. *$p<0.05$. NS = not significant.

Variable	Time [week]				Effects		
	9		22				
	AC	EC	AC	EC	WS	CO_2	WS×CO_2
ψ [MPa]	–1.23	–1.05	–0.15	–0.29	*	NS	*
ψ [MPa]	–1.52	–1.42	–1.45	–1.67	NS	NS	NS
B	0.27	0.27	0.45	0.40	*	NS	NS
RWC^0	0.91	0.95	0.94	0.93	NS	NS	NS
ε [MPa]	18.2	16.0	24.5	24.3	*	NS	NS

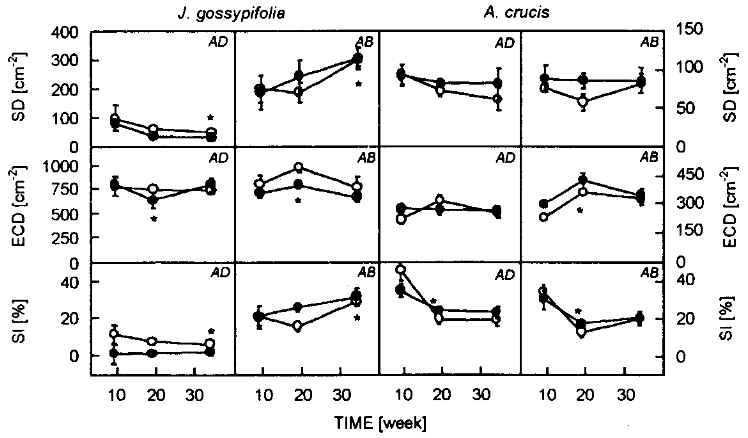

Fig. 2. Time-course of changes in stomatal density, epidermal cell density, and stomatal index of plants of *J. gossypifolia* and *A. crucis* grown under ambient (○) and elevated (●) $[CO_2]$. *AD*, adaxial surface; *AB*, abaxial surface. Means ± SE ($n = 5$). An *asterisk* indicates significant differences due to time relative to week nine ($p<0.05$).

Leaf anatomy: EC did not affect any of the stomatal characteristics examined in plants of *J. gossypifolia* or *A. crucis* (Fig. 2). An effect of time under treatment with no interaction with [CO$_2$] was found after a two-way analysis of variance on the adaxial and abaxial values of the three variables in *J. gossypifolia*, and on the adaxial SI and the abaxial ECD and SI of *A. crucis*. EC affected the thickness of some of the leaf tissues, the thickness of both epidermes remaining unaffected (Fig. 3). After 19 weeks, there was a significant decrease due to EC in the thickness of leaf, and palisade and spongy parenchyma of *J. gossypifolia* and an increase in *A. crucis* in whole leaf, bundle sheath, and mesophyll. All these changes, except for leaf thickness of *A. crucis*, disappeared after 34 weeks. A significant decrease in the proportion of whole leaf thickness comprised by the palisade and spongy parenchymata was found in *J. gossypifolia* after 19, but not after 34 weeks of growth. In *A. crucis* the proportional thickness of the bundle sheath and the mesophyll was unresponsive to either EC or time under treatment ($p<0.05$).

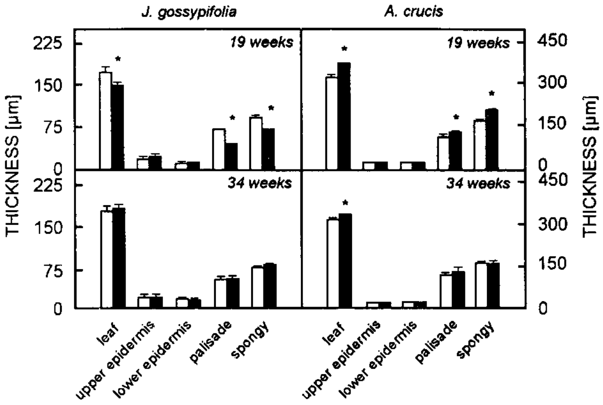

Fig. 3. Time-course of changes during the rainy season in whole leaf and tissue thickness of plants of *J. gossypifolia* and *A. crucis* grown under ambient (*white bars*) and elevated (*black bars*) [CO$_2$]. Means ± SE ($n = 5$). An *asterisk* indicates significant differences due to EC ($p<0.05$).

Specific leaf area was not affected by EC after 22 weeks of treatment; values were 15.5±0.5 m^2 kg^{-1} (AC) and 17.8±1.4 m^2 kg^{-1} (EC) in *J. gossypifolia* and 12.3±0.5 m^2 kg^{-1} (AC) and 13.1±0.5 m^2 kg^{-1} (EC) in *A. crucis*. No significant differences due to EC in total leaf area per plant were found after 23 weeks: 0.4±0.1 m^2 (AC) and 1.9±1.1 m^2 (EC) in *J. gossypifolia* and 4.3±0.3 m^2 (AC) and 4.2±0.7 m^2 (EC) in *A. crucis*.

Discussion

Water relations: In the present study no effect of EC on ψ of plants of either *J. gossypifolia* or *A. crucis* was observed, in agreement with findings reported in soybean (Rogers *et al.* 1984), *Pinus taeda* (Ellsworth 1999), and pea (Páez *et al.* 1983). Previous studies on the interaction between EC and drought have been done measuring watered and droughted plants simultaneously (*e.g.* Clifford *et al.* 1995) in order to avoid time effects, specially on growth and development. In the present investigation, selective watering inside each chamber was not possible because plants were grown directly in the soil to avoid the possible down-regulation of photosynthesis due to limited rooting volume (Drake *et al.* 1997). Nevertheless, subjecting the plants to the natural rain regime makes our approach more ecological, in that it pretends to mimic natural conditions, *i.e.* enhanced growth during the rainy season and a possibly improved drought tolerance due to EC. In plants at moderately low water status, pressure potential was higher under EC, similarly to the effect of EC observed in droughted groundnut plants (Clifford *et al.* 1995). Thus, even though EC did not increase the value of ψ it did alleviate the effects of water deficit through an increase in turgor.

Increases in whole-plant leaf area beyond that which can be compensated for by CO$_2$-induced reductions in leaf conductance may lead to greater rates of soil drying and associated plant water stress (Wullschleger *et al.* 2002). Total leaf area per plant was not affected in either species by EC; therefore, we rule out that change in transpiratory water loss due to increases in leaf area affected ψ. For *J. gossypifolia,* an increase with EC in leaf conductance was previously reported (Fernández *et al.* 1999); this should affect leaf water loss and therefore ψ. Nevertheless, EC may increase hydraulic conductivity or water absorption by the roots, thus preventing an increased transpiratory water loss and consequently a decrease in ψ (Wullschleger *et al.* 2002). The response of ψ and transpiration to EC seems to vary widely: no evidence of water saving was found in plants of *P. taeda* grown under EC, and predawn and midday ψ were unaffected by EC (Ellsworth 1999). In contrast, droughted groundnut plants grown under EC had a greater ψ and extracted substantially less water from the soil than droughted plants under AC (Clifford *et al.* 1995).

The magnitude of $\Delta\psi_s^{100}$ in plants of *A. crucis* was not affected by EC, similarly to previous reports but in contrast with others (reviewed by Wullschleger *et al.* 2002). Plants of *A. crucis*, having a higher P_N under EC, may have allocated the surplus assimilates produced to growth (roots and stems) rather than to the production of compatible solutes for a higher osmotic adjustment. However, droughted EC plants of *A. crucis* had a lower ψ_s^{100} on week 54, when ψ was low and similar for both [CO$_2$]; this implies a higher solute accumulation possibly as a response to a decrease in sink strength under water-limited conditions (Wullschleger *et al.* 2002). EC apparently has minimal effect on ψ_s^{100} and osmotic adjustment, and should not be considered beneficial with respect to this variable (Wullschleger *et al.* 2002).

Alternatively, the dry season may not have been long enough for the development of a higher osmotic adjustment in plants of *A. crucis* that could highlight differences between AC and EC plants. An osmotic adjustment of 1.07 MPa was measured in plants of *A. crucis* in the field, at a $\psi = -8.0$ MPa (Ríos 1993), a value not reached during the present experiment. The decrease in RWC^0 and ψ_s^{100} in droughted EC compared to droughted AC plants, coupled to more elastic walls, suggests that EC increased drought tolerance through the maintenance of turgor during the dry season, as suggested by Robichaux *et al.* (1986).

Leaf anatomy: None of the stomatal characteristics examined responded to EC in *J. gossypifolia* or *A. crucis*, similarly to previous reports (Radoglou and Jarvis 1992, Estiarte *et al.* 1994). These observations, together with our results, suggest that responses of stomatal characteristics to EC cannot be generalised. The possibility exists that the lack of changes in stomatal characteristics were due to developmental effects through incomplete leaf expansion but this may be ruled out because stomatal index would correct the effects of leaf expansion on stomatal and epidermal cell density.

Stomatal characteristics along the experiment were apparently unrelated to the changes with EC in leaf conductance previously reported in *J. gossypifolia* and *A. crucis* (Fernández *et al.* 1999). Therefore, effects of EC on leaf conductance were direct, rather than mediated by indirect effects on SD.

The absence of response of stomatal characteristics to EC may have been due to phylogenetic reasons: in plants of *Nothofagus cunninghamii* changes (increases or decreases) in stomatal characteristics in response to depleted [CO_2] (170 *vs.* 350 µmol mol^{-1}) were strongly dependent on genotype. This was interpreted as long-term evolutionary effects of EC that may differ from the short-term acclimation effects commonly seen in growth experiments (Hovenden and Schimanski 2000).

In our experiment, the absence of significant differences in leaf temperature between the AC and EC chambers may partly warrant that differences in response between chambers were due solely to EC. An increase in leaf temperature of nearly 5 °C in both AC and EC from week 17 until week 35 may help explain the time-dependent changes observed in leaf anatomy. Irradiance may influence the stomata response to EC (Tichá 1982) but this may not have been the case in this study, since values of abaxial SD were similar to those of plants growing during the rainy season under full sun exposure in their natural habitat (Herrera and Cuberos 1990). The possibility is not ruled out that time-dependent changes in microclimatic conditions, including those created by closing of the canopy, exerted a far stronger influence than EC on the anatomical characteristics examined.

Since in the present study AC was greater than 350 µmol mol^{-1}, AC plants were under a supra-atmospheric [CO_2]; this narrowed the experimental range of [CO_2], possibly obscuring any possible anatomical response to EC. Studies done on herbarium samples suggested that SD may not change significantly with further [CO_2] elevation above the current value of 350 µmol mol^{-1}, since the main changes occurred from the pre-industrial era till present (Woodward 1987).

Effects of EC on leaf cross-sectional anatomy were time-dependent and species-specific. These species keep functional leaves for over two months; this suggests that, since the experiment was extended for over one year, acclimation of the leaf anatomy to the chambers and to EC took place in the successive flushes of leaves produced during the experiment. An increase in the thickness of parenchyma, such as found by Radoglou and Jarvis (1990), would result in an increase in the proportion of leaf area comprised by mesophyll surface area (Nobel 1974), possibly enhancing P_N. Changes in relative mesophyll area are not likely to have influenced P_N in these species, since changes in proportional mesophyll thickness disappeared with time, whereas the direct stimulation of P_N by EC did not (Fernández *et al.* 1999).

EC exerted no effect on specific leaf area, in contrast with previous reports (Radoglou and Jarvis 1990, Hovenden and Schimanski 2000). Increases in poplar due to EC were found in the thickness of parenchymata as well as in cell size, thus explaining both a lower specific leaf area and a higher leaf thickness (Radoglou and Jarvis 1990). We found changes in tissue thickness which were not directly associated to changes in specific leaf area; differences (not evaluated) in the proportion of sclerenchyma, in cell size, or in starch content could help explain this discrepancy.

References

Beerling, D.J., Chaloner, W.: The impact of atmospheric CO_2 and temperature change on the stomatal density: Observations from *Quercus robur* Lammas leaves. – Ann. Bot. **71**: 231-235, 1993a.

Beerling, D.J., Chaloner, W.: Stomatal density responses of Egyptian *Olea europaea* L. leaves to CO_2 change since 1327 BC. – Ann. Bot. **71**: 431-435, 1993b.

Chaves, M.M., Pereira, J.S.: Water stress, CO_2 and climate change. – J. exp. Bot. **43**: 1131-1139, 1992.

Clifford, S.C., Black, C.R., Roberts, J.A., Stronach, I.M., Singleton-Jones, P.R., Mohamed, A.D., Azam-Ali, S.N.: The effect of elevated atmospheric CO_2 and drought on stomatal frequency in groundnut (*Arachis hypogaea* (L.)). – J. exp. Bot. **46**: 847-852, 1995.

Drake, B.G., González-Meler, M.A., Long, S.P.: More efficient plants: a consequence of rising atmospheric CO_2? – Annu. Rev. Plant Physiol. Plant mol. Biol. **48**: 609-639, 1997.

Eamus, D.: The interaction of rising CO_2 and temperature with water use efficiency. – Plant Cell Environ. **14**: 843-852, 1991.

Eamus, D., Jarvis, P.G.: The direct effects of increase in the global atmospheric CO_2 concentration on natural and commercial temperate trees and forests. – Adv. ecol. Res. **19**: 1-47, 1989.

Ellsworth, D.S.: CO_2 enrichment in a maturing pine forest: are CO_2 exchange and water status in the canopy affected? – Plant Cell Environ. **22**: 461-472, 1999.

Estiarte, M., Peñuelas, J., Kimball, B.A., Idso, S.B., La Morte, R.L., Pinter, P.J., Jr., Wall, G.W., García, R.L.: Elevated CO_2 effects on stomatal density of wheat and sour orange trees. – J. exp Bot. **45**: 1665-1668, 1994.

Fernández, M.D., Pieters, A., Azkue, M., Rengifo, E., Tezara, W., Woodward, F.I., Herrera, A.: Photosynthesis in plants of four tropical species growing under elevated CO_2. – Photosynthetica **37**: 587-599, 1999.

Ferris, R., Nijs, I., Behaeghe, T., Impens, I.: Elevated CO_2 and temperature have different effects on leaf anatomy of perennial ryegrass in spring and summer. – Ann. Bot. **78**: 489-497, 1996.

Ferris, R., Taylor, G.: Stomatal characteristics of four native herbs following exposure to elevated CO_2. – Ann. Bot. **73**: 447-453, 1994.

Herrera, A., Cuberos, M.: Stomatal size, density and conductance in leaves of some xerophytes from a thorn scrub in Venezuela differing in carbon fixation pathway. – Ecotrópicos **3**: 67-76. 1990.

Hovenden, M.J., Schimanski, L.J.: Genotypic differences in growth and stomatal morphology of Southern beech, *Nothofagus cunninghamii*, exposed to depleted CO_2 concentrations. – Aust. J. Plant Physiol. **27**: 281-287, 2000.

Nobel, P.S.: Introduction to Biophysical Plant Physiology. – W.H. Freeman and Co., San Francisco 1974.

Páez, A., Hellmers, H., Strain, B.R.: CO_2 enrichment, drought stress and growth of Alaska pea plants (*Pisum sativum*). – Physiol. Plant. **58**: 161-165, 1983.

Peñuelas, J., Matamala, R.: Changes in N and S leaf content, stomatal density and specific leaf area of 14 plant species during the last three centuries of CO_2 increase. – J. exp. Bot. **41**: 1119-1124, 1990.

Pospíšilová, J., Čatský, J.: Development of water stress under increased CO_2 concentration. – Biol. Plant. **42**: 1-27, 1999.

Radoglou, K.M., Jarvis, P.G.: Effects of CO_2 enrichment on four poplar clones. I. Growth and leaf anatomy. – Ann. Bot. **65**: 617-626, 1990.

Radoglou, K.M., Jarvis, P.G.: The effects of CO_2 enrichment and nutrient supply on growth morphology and anatomy of *Phaseolus vulgaris* L. seedlings. – Ann. Bot. **70**: 245-256, 1992.

Rind, D., Goldberg, R., Hansen, J., Rosenzweig, C., Ruedy, R.: Potential evapo-transpiration and the likelihood of future drought. – J. geophys. Res. **95**: 9983-10004, 1990.

Ríos, L.: [Occurrence of Osmotic Adjustment in *Alternanthera crucis*.] – Licenciado Thesis. Univ. Central de Venezuela, Caracas 1993. [In Spanish.]

Robichaux, R.H., Holsinger, K.E., Morse, S.R.: Turgor maintenance in *Dabautia* species: the role of variation in tissue osmotic and elastic properties. – In: Givnish, T.J. (ed.): On the Economy of Plant Form and Function. Pp. 353-579. Cambridge University Press, Cambridge 1986.

Rogers, H.H., Sionit, N., Cure, J.D., Smith, J.M., Bingham, G.E.: Influence of elevated carbon dioxide on water relations of soybean. – Plant Physiol. **74**: 233-238, 1984.

Sionit, N., Strain, B.R., Hellmers, H., Kramer, P.J.: Effects of atmospheric CO_2 concentration and water stress on water relations of wheat. – Bot. Gaz. **142**: 191-196, 1981.

Tezara, W., Fernández, M.D., Donoso, C., Herrera, A.: Seasonal changes in photosynthesis and stomatal conductance of five plant species from a semiarid ecosystem. – Photosynthetica **35**: 399-410, 1998.

Thomas, J.F., Harvey, C.N.: Leaf anatomy of four species grown under continuous CO_2 enrichment. – Bot. Gaz. **144**: 303-309, 1983.

Tichá, I.: Photosynthetic characteristics during ontogenesis of leaves. 7. Stomatal density and sizes. – Photosynthetica **16**: 375-471, 1982.

Tipping, C., Murray, D.R.: Effects of elevated atmospheric CO_2 concentration on leaf anatomy and morphology in *Panicum* species representing different photosynthetic modes. – Int. J. Plant Sci. **160**: 1063-1073, 1999.

Tognetti, R., Sebastiani, L., Vitagliano, C., Raschi, A., Minnocci, A.: Responses of two olive tree (*Olea europaea* L.) cultivars to elevated CO_2 concentration in the field. – Photosynthetica **39**: 403-410, 2001.

Tyree, M.T., Alexander, J.D.: Plant water relations and the effects of elevated CO_2: a review and suggestions for future research. – Vegetatio **104/105**: 47-62, 1993.

Woodward, F.I.: Stomatal numbers are sensitive to increases in CO_2 from pre-industrial levels. – Nature **327**: 617-618, 1987.

Woodward, F.I., Thompson, G.B., McKee, I.F.: The effects of elevated concentrations of carbon dioxide on individual plants, populations, communities and ecosystems. – Ann. Bot. **67** (Suppl.): 23-38, 1989.

Wullschleger, S.D., Tschaplinski, T.J., Norby, R.J.: Plant water relations at elevated [CO_2] – implications for water-limited environments. – Plant Cell Environ. **25**: 319-331, 2002.