

INVITED REVIEW

# Responses of CAM species to increasing atmospheric CO<sub>2</sub> concentrations

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## ABSTRACT

Crassulacean acid metabolism (CAM) species show an average increase in biomass productivity of 35% in response to a doubled atmospheric CO<sub>2</sub> concentration. Daily net CO<sub>2</sub> uptake is similarly enhanced, reflecting in part an increase in chlorenchyma thickness and accompanied by an even greater increase in water-use efficiency. The responses of net CO<sub>2</sub> uptake in CAM species to increasing atmospheric CO<sub>2</sub> concentrations are similar to those for C<sub>3</sub> species and much greater than those for C<sub>4</sub> species. Increases in net daily CO<sub>2</sub> uptake by CAM plants under elevated atmospheric CO<sub>2</sub> concentrations reflect increases in both Rubisco-mediated daytime CO<sub>2</sub> uptake and phosphoenolpyruvate carboxylase (PEPCase)-mediated night-time CO<sub>2</sub> uptake, the latter resulting in increased nocturnal malate accumulation. Chlorophyll contents and the activities of Rubisco and PEPCase decrease under elevated atmospheric CO<sub>2</sub>, but the activated percentage for Rubisco increases and the  $K_M(\text{HCO}_3^-)$  for PEPCase decreases, resulting in more efficient photosynthesis. Increases in root:shoot ratios and the formation of additional photosynthetic organs, together with increases in sucrose-Pi synthase and starch synthase activity in these organs under elevated atmospheric CO<sub>2</sub> concentrations, decrease the potential feedback inhibition of photosynthesis. Longer-term studies for several CAM species show no downward acclimatization of photosynthesis in response to elevated atmospheric CO<sub>2</sub> concentrations. With increasing temperature and drought duration, the percentage enhancement of daily net CO<sub>2</sub> uptake caused by elevated atmospheric CO<sub>2</sub> concentrations increases. Thus net CO<sub>2</sub> uptake, productivity, and the potential area for cultivation of CAM species will be enhanced by the increasing atmospheric CO<sub>2</sub> concentrations and the increasing temperatures associated with global climate change.

## INTRODUCTION

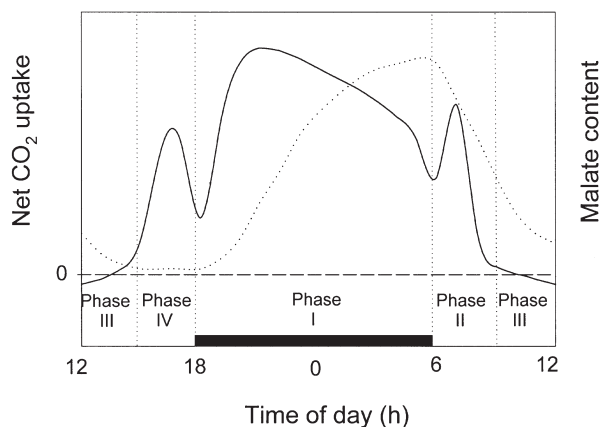
Crassulacean acid metabolism (CAM) is one of three photosynthetic types used by vascular plants. Nocturnal CO<sub>2</sub> fixation by the cytosolic enzyme phosphoenolpyruvate carboxylase (PEPCase) results in the formation of malate,

which is stored in the vacuole of cells in the chlorenchyma. Daytime decarboxylation of the accumulated malic acid releases CO<sub>2</sub>, which is assimilated into carbohydrates using 1,5-ribulosebiphosphate carboxylase/oxygenase (Rubisco) and the C<sub>3</sub> photosynthetic carbon reduction cycle (Winter & Smith 1996a). As is the case for the C<sub>4</sub> type of photosynthesis, primary fixation of CO<sub>2</sub> by PEPCase concentrates CO<sub>2</sub> at the site of Rubisco in CAM plants, thereby suppressing the oxygenase activity of this enzyme (Taiz & Zeiger 1998). Daytime stomatal closure in CAM species reduces CO<sub>2</sub> leakage from the site of the C<sub>3</sub> cycle and effectively decouples the internal CO<sub>2</sub> pool from that of the atmosphere (Monson 1989).

For both C<sub>4</sub> and CAM plants, the CO<sub>2</sub>-concentrating mechanism potentially leads to higher optimal temperatures for photosynthesis (Monson 1989). Moreover, the lower tissue temperatures accompanying the predominantly nocturnal stomatal opening of CAM plants compared with daytime stomatal opening for C<sub>3</sub> and C<sub>4</sub> plants generally result in a three- to five-fold higher water-use efficiency (WUE) for CAM plants than for C<sub>3</sub> or C<sub>4</sub> plants under comparable environmental conditions (Nobel 1996, 1999). The higher WUE reflects the nearly exponential increase in the saturation water vapour content of air as the temperature rises, so stomatal opening during the daytime leads to a much higher transpiration rate than for the same degree of stomatal opening at night when tissue temperatures at the water evaporation sites average 10–12 °C lower.

CAM plants show considerable plasticity, varying in response to environmental conditions and with developmental state (Winter & Smith 1996a; Cushman & Bohnert 1999). Many constitutive CAM species fix CO<sub>2</sub> almost exclusively at night (phase I, Fig. 1; Osmond 1978), even when well-watered, e.g. stem succulents such as *Opuntia ficus-indica* (Nobel 1988). For other species, e.g. *Agave deserti* (Nobel 1988), CO<sub>2</sub> uptake extends into the early morning (phase II, Fig. 1), and also occurs in the late afternoon (phase IV, Fig. 1), when uptake of atmospheric CO<sub>2</sub> involves binding of CO<sub>2</sub> by Rubisco (Winter & Smith 1996a). The high intercellular CO<sub>2</sub> concentrations associated with malate decarboxylation suppress stomatal opening during the middle of the day (phase III, Fig. 1). Most facultative C<sub>3</sub>–CAM intermediates switch between C<sub>3</sub> and CAM in response to changes in environmental

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**Figure 1.** The daily cycle of net CO<sub>2</sub> (solid line) uptake and malic acid accumulation (dotted line) for a typical CAM plant, illustrating the four phases of CAM (Osmond 1978).

factors, especially soil water availability. Reduced water availability favours expression of CAM in the C<sub>3</sub>-CAM intermediates of the tropical tree genus *Clusia* (Lüttge 1996). However, for species of *Peperomia*, the expression of CAM in older leaves is independent of environmental signals, although water stress, light and temperature modify the extent of CAM (Ting *et al.* 1996). For some C<sub>3</sub>-CAM intermediates, e.g. *Mesembryanthemum crystallinum*, environmental stimuli such as drought, soil salinity and low temperature elicit the CAM response only in mature plants (Adams *et al.* 1998). CO<sub>2</sub> uptake patterns in facultative CAM plants may also be regulated by day length (Brulfert *et al.* 1982) and season (Guralnick, Rorabaugh & Hanscom 1984).

For some CAM species, predominantly daytime net CO<sub>2</sub> uptake is accompanied by 'CAM cycling.' This means that, despite nocturnal stomatal closure, malate levels increase during this period as respiratory CO<sub>2</sub> is captured via PEPCase activity, leading to a reduction in daytime stomatal aperture (Martin, Higley, & Wang 1988). For other species, 'CAM idling' occurs during severe drought. Stomatal closure during both day and night then results in no net CO<sub>2</sub> assimilation. A diel cycle in malic acid accumulation during CAM idling, associated with respiration followed by the re-fixation of respiratory CO<sub>2</sub>, apparently keeps the various steps functioning, allowing rapid recovery of carbon assimilation upon rehydration (Bastide *et al.* 1993).

The majority of the approximately 300 000 species of vascular plants utilize the C<sub>3</sub> pathway; about 2–3% are C<sub>4</sub>, including many agriculturally important species such as maize, sorghum and sugar cane, and 6–7% are CAM (Nobel 1991; Winter & Smith 1996a). CAM has independently evolved many times and occurs in over 30 taxonomically diverse families (Ehleringer & Monson 1993; Smith & Winter 1996). The terrestrial environments and micro-environments favouring CAM are generally characterized by water shortage (Taiz & Zeiger 1998). Seven of the major CAM families – the Agavaceae, Asphodelaceae, Aizoaceae,

Asclepiadaceae, Cactaceae, Crassulaceae (the family for which the type is named) and Euphorbiaceae – occur in arid regions or climates of seasonal water shortage. CAM species in the other two major CAM families, the Bromeliaceae and the Orchidaceae, are predominantly tropical epiphytes whose soil-depauperate substrate has a low water-holding capacity (Winter & Smith 1996a). For epiphytic species of the Bromeliaceae, the proportion of CAM species increases with increasing habitat aridity, indicating a competitive advantage of CAM in water-limited environments (Griffiths & Smith 1983; Smith 1989). The succulent morphology of many CAM species provides a high shoot water-storage capacity (Nobel 1988), and large vacuoles facilitate appreciable storage of the nocturnally accumulated malic acid (Winter & Smith 1996a). The degree of leaf succulence correlates positively with the occurrence of CAM for both the Crassulaceae (Teeri, Tonsor & Turner 1981) and the Orchidaceae (Winter *et al.* 1983), the latter family containing nearly half of the known CAM species (Winter & Smith 1996a).

The pre-industrial atmospheric CO<sub>2</sub> concentration of approximately 280 μmol mol<sup>-1</sup> is predicted to double by the middle of the 21st century due to the effects of deforestation, other land-use changes, and especially the burning of fossil fuels (Neffel *et al.* 1985; Schimel *et al.* 1996). Such observations have resulted in extensive research into the effects of atmospheric CO<sub>2</sub> concentration on the photosynthesis of C<sub>3</sub> plants. For most C<sub>3</sub> species, photosynthesis increases at elevated atmospheric CO<sub>2</sub> concentrations and the accompanying increases in intercellular CO<sub>2</sub> concentration, which suppress the oxygenase activity of Rubisco and thereby reduce photorespiratory loss of carbon. Because the specificity of Rubisco for CO<sub>2</sub> relative to O<sub>2</sub> declines with increasing temperature, the stimulation of net CO<sub>2</sub> uptake by elevated atmospheric CO<sub>2</sub> concentrations is potentially greater at higher temperatures (Morison & Lawlor 1999). Long-term enhancement of photosynthesis is often limited by feedback inhibition of Rubisco activity or by environmental stresses, such as low nitrogen availability or extreme temperatures (Morison & Lawlor 1999; Stitt & Krapp 1999). Increased growth and photosynthesis under elevated atmospheric CO<sub>2</sub> concentrations have been measured for some C<sub>4</sub> species (Poorter 1993; Lecain & Morgan 1998). However, photosynthesis by C<sub>4</sub> species is more readily saturated as atmospheric CO<sub>2</sub> concentrations rise, reflecting the relative insensitivity of PEPCase to the CO<sub>2</sub>:O<sub>2</sub> ratio because of lack of binding of O<sub>2</sub> to the catalytic site (Lawlor & Mitchell 1991).

From a biochemical point of view, obligate CAM species could respond similarly to C<sub>4</sub> species if PEPCase were saturated at close to the current atmospheric CO<sub>2</sub> concentration (Ting 1994; Winter & Smith 1996b). Nonetheless, facultative CAM plants might respond more to elevated atmospheric CO<sub>2</sub> concentrations (Ting 1994). Compared with C<sub>3</sub> and C<sub>4</sub> species, relatively few studies have been conducted on the effects of elevated atmospheric CO<sub>2</sub> concentrations on CAM plants (studies are summarized in Table 1). Most of the CAM species investigated show

**Table 1.** Responses of CAM species to elevated atmospheric CO<sub>2</sub> concentrations

Family/species	Productivity	Morphological changes	CO <sub>2</sub> uptake	Enzymes
<b>Monocotyledons</b>				
<b>Agavaceae</b>				
<i>Agave deserti</i>	Increased (1)	Leaves thicker (1), leaves longer (1), chlorenchyma thicker (1), root cell length increased (2)	Afternoon and night-time uptake increased (1, 3), WUE increased (1)	PEPCase decreased (1), Rubisco decreased but activated <i>in vivo</i> % increased (1)
<i>Agave salmiana</i>	Increased (4)		Afternoon and night-time uptake increased (4, 5)	PEPCase decreased (4), Rubisco decreased but activated <i>in vivo</i> % increased (4), PEPCase $K_M$ decreased (4)
<i>Agave vilmoriniana</i> <i>Yucca schidigera</i> <sup>a</sup>	Increased (6)		Night-time uptake increased (7) Increased (8)	
<b>Orchidaceae</b>				
Mokara Yellow <sup>b</sup>	Increased (9–11)	Root : shoot ratio increased (9, 10)	Nocturnal malate accumulation increased (9)	PEPCase decreased (9), Rubisco decreased (9)
<b>Bromeliaceae</b>				
<i>Aechmea magdalenae</i> <i>Ananas comosus</i>	Increased (12) Increased (13–15)	Root : shoot ratio increased (13), leaf thickness increased (13)	Increased morning and night-time uptake (13–16), WUE increased (15) Nocturnal malate accumulation increased (17)	
<i>Tillandsia ionantha</i>				
<b>Dicotyledons</b>				
<b>Aizoaceae</b>				
<i>Mesembryanthemum crystallinum</i> <sup>c</sup>				
<b>Cactaceae</b>				
<i>Ferocactus acanthodes</i> <i>Hylocereus undatus</i> <i>Opuntia ficus-indica</i>	Increased (3) Increased (4, 19–23)	Chlorenchyma cell volume increased (18) Specific cladode mass increased (19, 20, 24), cladodes thicker (19, 21, 24, 25), chlorenchyma thicker (19, 25), stomatal frequency decreased (24), root : shoot ratio increased (4, 21, 22), root cell length increased (26), chlorenchyma cell length increased (20)	Increased (3) Increased (18) Afternoon, night-time and early-morning uptake increased (20–23, 27), WUE increased (21, 23)	PEPCase decreased (4, 27, 28), Rubisco decreased but activated <i>in vivo</i> % increased (4, 27, 28), PEPCase $K_M$ decreased (4)
<i>Stenocereus queretaroensis</i>			Afternoon and night-time uptake increased (5)	
<b>Clusiaceae</b>				
<i>Clusia uvitana</i> <sup>c</sup>			Night-time uptake decreased (29)	
<b>Portulacaceae</b>				
<i>Portulacaria afra</i> <sup>c</sup>			Night-time uptake decreased (30)	
<b>Crassulaceae</b>				
<i>Crassula arborescens</i>			Nocturnal malate accumulation increased (17)	
<i>Kalanchoë blossfeldiana</i> <sup>c</sup> <i>Kalanchoë daigremontanum</i> <i>Kalanchoë pinnata</i>	Increased (31) Increased (34)		Unchanged (32, 33) Daytime uptake increased (34)	

<sup>a</sup> Seedlings of *Yucca schidigera* as used in this investigation are possibly facultatively C<sub>3</sub> (Huxman *et al.* 1998).

<sup>b</sup> *Arachnis hookeriana* × *Ascocenda* Madame Kenny.

<sup>c</sup> C<sub>3</sub>–CAM intermediate.

References: (1) Graham & Nobel (1996), (2) Drennan & Nobel (1996), (3) Nobel & Hartssock (1986), (4) Nobel, Israel & Wang (1996), (5) Nobel (1996), (6) Idso *et al.* (1986), (7) Szarek, Holthe & Ting (1987), (8) Huxman *et al.* (1998), (9) Gouk, Yong & Hew (1997), (10) Gouk, He & Hew (1999), (11) Hew *et al.* (1995), (12) Ziska *et al.* (1991), (13) Zhu, Bartholomew & Goldstein (1997a), (14) Zhu, Bartholomew & Goldstein (1997b), (15) Zhu, Goldstein & Bartholomew (1999), (16) Crewes, Vines & Black (1975), (17) Nowak & Martin (1995), (18) Raveh, Gersani & Nobel (1995), (19) Luo & Nobel (1993), (20) Nobel & Israel (1994), (21) Nobel *et al.* (1994b), (22) Cui, Miller & Nobel (1993), (23) Cui & Nobel (1994), (24) North, Moore & Nobel (1995), (25) Wang & Nobel (1996), (26) Drennan & Nobel (1998), (27) Israel & Nobel (1994), (28) Nobel, Cui & Israel (1994a), (29) Winter *et al.* (1992), (30) Huerta & Ting (1988), (31) Mortensen & Moe (1992), (32) Holtum, O'Leary & Osmond (1983), (33) Osmond & Björkman (1975), (34) Winter *et al.* (1997).

increased productivity, although the effects of elevated CO<sub>2</sub> on the patterns of gas exchange differ. For most species, nocturnal CO<sub>2</sub> uptake is enhanced, while for others, daytime CO<sub>2</sub> uptake is increased with a concomitant decrease in the proportion of nocturnal CO<sub>2</sub> fixation (Table 1). Additionally, a number of changes occur in the morphology, anatomy, and biochemistry of CAM species under elevated atmospheric CO<sub>2</sub> concentrations (summarized in Table 1), which may contribute to their higher net CO<sub>2</sub> uptake rates and the increased biomass productivity as the atmospheric CO<sub>2</sub> concentration increases.

## GROWTH AND BIOMASS

Biomass accumulation is enhanced by elevated atmospheric CO<sub>2</sub> concentrations for all CAM species investigated (Tables 1 and 2). Most of these studies are relatively short-term compared with the life span of CAM species. Nonetheless, dry matter increases averaging approximately 35% are achieved within 3 months at elevated atmospheric CO<sub>2</sub> concentrations of 650–750  $\mu\text{mol mol}^{-1}$ . These increases are similar to the 33% increase averaged for some 430 C<sub>3</sub> crop species when the atmospheric CO<sub>2</sub> concentration is doubled (Kimball 1983). However, they exceed the approximately 10% increase in dry matter production for C<sub>4</sub> plants with a doubling in the atmospheric CO<sub>2</sub> concentration (Kimball 1983; Lawlor & Mitchell 1991; Poorter 1993), despite the similarity in initial fixation of CO<sub>2</sub> by PEPCase for both C<sub>4</sub> plants during the daytime and CAM plants at night. Thus the response to elevated atmospheric CO<sub>2</sub> concentrations cannot be predicted on the basis of the initial carboxylating enzyme alone, nor is the response saturated at atmospheric CO<sub>2</sub> concentrations that are slightly above the current value (Table 2), as would be likely if the

response of CAM plants were mediated solely by CO<sub>2</sub> saturation of PEPCase.

For *Opuntia ficus-indica*, the most-studied CAM plant with respect to the effect of elevated atmospheric CO<sub>2</sub> concentrations, stimulation of biomass accumulation also occurs as the atmospheric CO<sub>2</sub> concentration is increased from 520 to 720  $\mu\text{mol mol}^{-1}$  (Cui *et al.* 1993). Similarly, increasing the atmospheric CO<sub>2</sub> concentration from 675 to 885  $\mu\text{mol mol}^{-1}$  results in a 13% increase in growth rate for *Agave vilmoriniana* (Idso *et al.* 1986). Although a doubling of the atmospheric CO<sub>2</sub> concentration results in a 28% increase in biomass for *A. vilmoriniana* under dry soil conditions (Table 2), a lack of biomass response to elevated atmospheric CO<sub>2</sub> concentrations may occur for *A. vilmoriniana* (Idso *et al.* 1986) and *Ananas comosus* (Ziska *et al.* 1991) when they are heavily watered. For *A. comosus*, such reduced CAM activity and growth under elevated CO<sub>2</sub> concentrations are attributed to water-logging of the soil (Zhu, Goldstein & Bartholomew 1999).

Acclimatization to elevated atmospheric CO<sub>2</sub> concentrations occurs for some C<sub>3</sub> species and has been attributed to a feedback inhibition by increased carbohydrate levels (Heineke *et al.* 1999) that decreases the expression of photosynthetic genes (Moore *et al.* 1999) and affects the activated form of Rubisco (Bowes 1991). Root restriction due to small soil volumes may also result in acclimatization (Arp 1991). For *O. ficus-indica*, dry weight gains under elevated atmospheric CO<sub>2</sub> concentrations are not different from dry weight gains under the current atmospheric CO<sub>2</sub> concentration after 4.5 months of exposure when the root volume is significantly restricted (Nobel *et al.* 1994b). The reduced biomass stimulation that occurs with increasing time of CO<sub>2</sub> enrichment for *Kalanchoë pinnata* (Table 2) may reflect an increase in CAM expression with increasing

**Table 2.** Response of biomass of CAM plants to long-term (> 1 month) exposure to elevated atmospheric CO<sub>2</sub> concentrations as a percentage increase over controls maintained under ambient atmospheric CO<sub>2</sub> concentrations for the same period

Species	Biomass (% increase)	CO <sub>2</sub> ( $\mu\text{mol mol}^{-1}$ ) <sup>a</sup>	Duration (months)	Reference
<i>Agave deserti</i>	30	350–650	12	Nobel & Hartssock (1986)
	31	370–750	17	Graham & Nobel (1996)
<i>Agave salmiana</i>	17	370–730	4	Nobel <i>et al.</i> (1996)
<i>Agave vilmoriniana</i>	28	300–600	6	Idso <i>et al.</i> (1986)
Orchid 'Mokara Yellow'	80	350–10 000	2	Gouk <i>et al.</i> (1997)
	170	350–10 000	3	Gouk <i>et al.</i> (1997)
<i>Aechmea magdalenae</i>	36	354–712	3	Ziska <i>et al.</i> (1991)
<i>Ananas comosus</i>	-10	354–712	3	Ziska <i>et al.</i> (1991)
	23	330–730	4	Zhu <i>et al.</i> (1997a)
<i>Ferrocactus acanthodes</i>	30	350–650	12	Nobel & Hartssock (1986)
<i>Opuntia ficus-indica</i>	32	370–750	3	Cui & Nobel (1994)
	21	370–520	4.5	Cui <i>et al.</i> (1993)
	55	370–720	4.5	Cui <i>et al.</i> (1993)
	40	360–720	12	Nobel & Israel (1994)
<i>Kalanchoë blossfeldiana</i>	37	350–700	1.4	Mortensen & Moe (1992)
<i>Kalanchoë pinnata</i>	51	350/400–700/800	1.9	Winter <i>et al.</i> (1997)
	42	350/400–700/800	2.2	Winter <i>et al.</i> (1997)

<sup>a</sup>Conversion to concentration approximate; the biomass increase occurs from the first concentration to the second concentration.



leaf age, with its associated biochemical changes (Winter *et al.* 1997).

The enhancement of growth by elevated atmospheric CO<sub>2</sub> concentrations decreases with plant age for some C<sub>3</sub> species (Bazzaz *et al.* 1989), and elevated CO<sub>2</sub> concentrations may also accelerate ontogenesis (Loehle 1995; Miller *et al.* 1997). For *A. vilmoriniana*, small, younger plants show a greater response to elevated atmospheric CO<sub>2</sub> concentrations than do larger, older plants (Idso *et al.* 1986). Similarly, the maximum relative growth rate for cladodes of *O. ficus-indica*, in addition to being higher, is achieved earlier under a doubled atmospheric CO<sub>2</sub> concentration, but the growth rate subsequently decreases to levels similar to those for cladodes under the current atmospheric CO<sub>2</sub> concentration (Luo & Nobel 1993; North *et al.* 1995). The number of areoles per cladode for *O. ficus-indica* under different atmospheric CO<sub>2</sub> concentrations is similar (North *et al.* 1995), although cladode thickness is greater under elevated atmospheric CO<sub>2</sub> concentrations (Table 1). Nevertheless, the acceleration of development caused by elevated atmospheric CO<sub>2</sub> concentrations increases biomass productivity for *O. ficus-indica* by allowing earlier formation of new cladodes (Nobel & Israel 1994). Similarly, increases in biomass accumulation and leaf thickness for *A. comosus* and *Agave deserti* (Table 1) are also associated with increased leaf production, which is sustained for at least 17 months by *A. deserti* (Table 2; Graham & Nobel 1996).

The increased productivity for highly productive CAM plants under elevated atmospheric CO<sub>2</sub> concentrations may have important ecosystem and especially agricultural consequences (Nobel 1996). In particular, the CAM plants *Agave mapisaga*, *Agave salmiana*, *Opuntia amyoclaea* and *O. ficus-indica* may have an average annual dry mass productivity of 43 tons per hectare per year under the current CO<sub>2</sub> atmospheric concentration (Nobel 1991). This productivity exceeds the average annual above-ground dry-mass productivity of essentially all C<sub>3</sub> plants, and is exceeded by that of only a few C<sub>4</sub> species, notably *Saccharum officinarum* (sugar cane). Based on studies using plants in a field plot that received free-air CO<sub>2</sub> enrichment, the dry mass productivity of *O. ficus-indica* when the plants are closely spaced may be 47 tons per hectare per year at 360  $\mu\text{mol mol}^{-1}$ , increasing to 65 tons per hectare per year when the atmospheric CO<sub>2</sub> concentration is doubled (Nobel & Israel 1994), which has major implications for this widely cultivated species as the atmospheric CO<sub>2</sub> concentration increases.

## MORPHOLOGY AND ANATOMY

Changes in the morphology and anatomy of photosynthetic organs caused by increasing atmospheric CO<sub>2</sub> concentrations influence the photosynthetic characteristics of CAM species, such as gas exchange, reflectance and light absorption (Nobel *et al.* 1994a). Changes in plant morphology reflect changes in carbon allocation patterns, which alter the interaction of the plant with both its abiotic and its

biotic environment (Rogers, Runion & Krupa 1994). For the few CAM species whose morphology and anatomy have been studied under elevated atmospheric CO<sub>2</sub> concentrations (Table 1), a consistent effect of the higher atmospheric CO<sub>2</sub> concentrations is an increase in thickness of the photosynthetic organs. For *O. ficus-indica*, exposure to a doubled atmospheric CO<sub>2</sub> concentration results in increases in cladode thickness of 19% for basal cladodes, 14% for the new daughter cladodes initiated on the basal cladode (first-order daughter cladodes) and 11% for second-order daughter cladodes (initiated on first-order daughter cladodes; Cui, Miller & Nobel 1993; Nobel & Israel 1994; Nobel *et al.* 1994a; North *et al.* 1995). Differences in thickness became apparent within 2 weeks of exposure to the elevated atmospheric CO<sub>2</sub> concentration (Nobel *et al.* 1994b) and were maintained even after these determinate organs had stopped growing (North *et al.* 1995). Similarly, the leaves of both *Agave deserti* and *Ananas comosus* are 11% thicker under approximately twice the current atmospheric CO<sub>2</sub> concentration (Graham & Nobel 1996; Zhu *et al.* 1997a).

More than 65% of the increase in thickness of the leaves of *A. deserti* (Graham & Nobel 1996) and of second-order daughter cladodes of *O. ficus-indica* (Cui *et al.* 1993; Nobel *et al.* 1994a) is accounted for by an approximately 20% increase in the thickness of the chlorenchyma. For *O. ficus-indica*, increases in both the length of chlorenchyma cells and the number of cell layers contributes to the increased tissue thickness (North *et al.* 1995), as can also occur for C<sub>3</sub> species (Thomas & Harvey 1983). An increase in chlorenchyma thickness under elevated atmospheric CO<sub>2</sub> concentrations may be related to a higher CO<sub>2</sub> concentration deeper in the leaf (Powles, Chapman & Osmond 1980). For the CAM species *Crassula argentea*, gradients of  $\delta^{13}\text{C}$  with increasing distance from the upper epidermis are consistent with a diffusion limitation on CO<sub>2</sub> uptake in these succulent tissues (Robinson, Osmond & Giles 1993). For *O. ficus-indica*, the contribution of increased chlorenchyma thickness to overall cladode thickness decreases from 66% for young, second-order daughter cladodes to 16% for two-year-old basal cladodes (Cui *et al.* 1993). Thus increased storage tissue must contribute significantly to the increased thickness for the older cladodes. For *A. comosus*, the ratio of stem mass to total plant mass increases under elevated atmospheric CO<sub>2</sub> concentrations (Zhu *et al.* 1997a). The associated increase in stem dry matter suggests that starch levels are increased under elevated atmospheric CO<sub>2</sub> concentrations, as stem dry matter is positively correlated with starch content for this species (Bartholomew & Paull 1986).

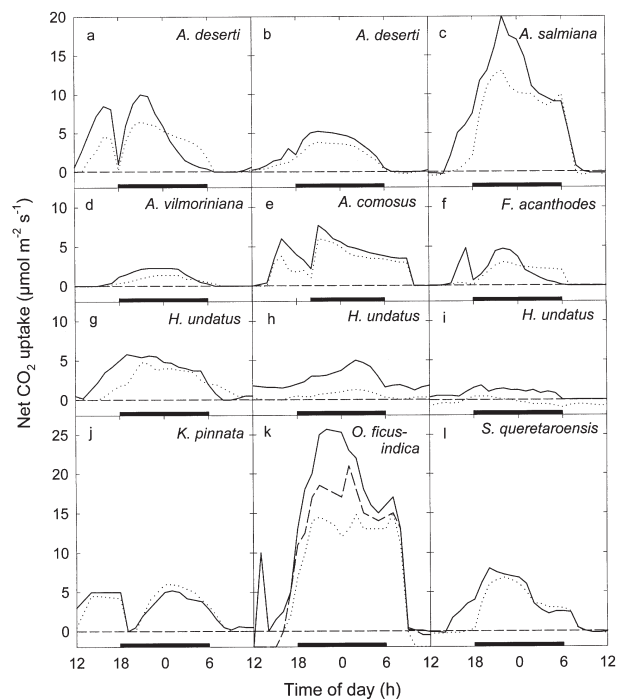
The increase in chlorenchyma thickness with rising atmospheric CO<sub>2</sub> concentrations increases the amount of photosynthetic tissue and the chlorenchyma cell surface area per unit leaf area for *A. deserti* and per unit cladode area for *O. ficus-indica*, thus potentially increasing net CO<sub>2</sub> uptake and WUE (Nobel 1999). The uptake of CO<sub>2</sub> and the water vapour conductance are also influenced by stomatal frequency, which decreases by 20% for *O. ficus-indica* under

elevated CO<sub>2</sub> concentrations, although stomatal pore length is unchanged (North *et al.* 1995). Stomatal frequency is lower under elevated CO<sub>2</sub> for some, but not all C<sub>3</sub> and C<sub>4</sub> species (Thomas & Harvey 1983; Rogers *et al.* 1994). A general decrease in stomatal frequency has accompanied the global rise in atmospheric CO<sub>2</sub> concentration and is suggested to lead to a higher WUE (Beerling & Woodward 1993; Beerling, McElwain & Osborne 1998). In particular, a decrease in stomatal area per unit leaf area decreases transpiration more than photosynthesis (Nobel 1999). Epicuticular wax increases 60% and the cuticle thickness increases 30% for *O. ficus-indica* under a doubled CO<sub>2</sub> concentration (North *et al.* 1995). Cladode reflectance is higher from 400–700 nm but lower from 740–1000 nm for *O. ficus-indica* growing at elevated atmospheric CO<sub>2</sub> concentrations (Nobel *et al.* 1994a). On the other hand, epicuticular wax decreases by 40% for *A. deserti* under elevated atmospheric CO<sub>2</sub> concentrations, which decreases the reflectance of the photosynthetic photon flux from 400–700 nm (Graham & Nobel 1996).

CAM plants typically have extremely low root:shoot ratios of about 0.08–0.14, consistent with their low rates of transpiration and high WUE (Nobel 1988; Nobel & North 1996). Increases in the root:shoot ratio of 33% and 45% have been measured for *A. comosus* and the hybrid orchid 'Mokara Yellow', respectively, under two to three times the current atmospheric CO<sub>2</sub> concentration (Zhu *et al.* 1997a; Gouk *et al.* 1997). Increases of up to 100% in root:shoot ratio occur for *O. ficus-indica* under a doubled atmospheric CO<sub>2</sub> concentration (Cui *et al.* 1993), although the magnitude of the increase may be limited by small soil volumes (Nobel *et al.* 1994b). Not all CAM species show increases in root:shoot ratios under elevated atmospheric CO<sub>2</sub> concentrations; for example, ratios for *Ferocactus acanthodes*, *A. deserti* and *Agave salmiana* are similar under current and doubled CO<sub>2</sub> concentrations (Nobel & Hartsock 1986; Graham & Nobel 1996; Nobel *et al.* 1996). Similar variability in the responses of root:shoot ratios has been reported for C<sub>3</sub> and C<sub>4</sub> species (Rogers *et al.* 1996), and may be the result of interactions of experimental and environmental factors, such as soil volume and temperature, with the response to atmospheric CO<sub>2</sub> concentration (Rogers *et al.* 1994; Morison & Lawlor 1999). Root architecture, especially root diameter and the number of lateral roots, does not change under elevated atmospheric CO<sub>2</sub> concentrations for *A. salmiana* or *O. ficus-indica* (Nobel *et al.* 1996; Drennan & Nobel 1998), although root cell length increases for *A. deserti* and *O. ficus-indica* (Drennan & Nobel 1996, 1998). For these typically shallow-rooting drought-adapted species, root architecture may respond more to water availability, which also will change as the atmospheric CO<sub>2</sub> concentration increases.

## GAS EXCHANGE

The net daily CO<sub>2</sub> uptake for most CAM species is enhanced at elevated atmospheric CO<sub>2</sub> concentrations (Table 1, Fig. 2). The percentage stimulation for plants rep-



**Figure 2.** Daily time-courses of the net CO<sub>2</sub> uptake rate under the current atmospheric CO<sub>2</sub> concentration (dotted line) and, unless otherwise indicated, after acclimatization to a doubled atmospheric CO<sub>2</sub> concentration (solid line) under wet conditions for: (a) *Agave deserti*, 350 and 650 µmol mol<sup>-1</sup> CO<sub>2</sub> (instantaneous increase), day/night air temperatures of 25/15 °C, PPF of 26 mol m<sup>-2</sup> d<sup>-1</sup> (Nobel & Hartsock 1986); (b) *A. deserti*, 370 and 750 µmol mol<sup>-1</sup> CO<sub>2</sub>, day/night air temperatures of 25/20 °C, PPF of 15 mol m<sup>-2</sup> d<sup>-1</sup> (Graham & Nobel 1996); (c) *Agave salmiana*, 360 and 720 µmol mol<sup>-1</sup> CO<sub>2</sub>, day/night air temperatures of 25/15 °C, PPF of 22 mol m<sup>-2</sup> d<sup>-1</sup> (Nobel 1996); (d) *Agave vilmoriniana*, 370 and 750 µmol mol<sup>-1</sup> CO<sub>2</sub> (Szarek *et al.* 1987); (e) *Ananas comosus*, 350 and 700 µmol mol<sup>-1</sup> CO<sub>2</sub>, day/night air temperatures of 30/20 °C, PPF of 17 mol m<sup>-2</sup> d<sup>-1</sup> (Zhu *et al.* 1999); (f) *Ferocactus acanthodes*, 350 and 650 µmol mol<sup>-1</sup> CO<sub>2</sub> (instantaneous increase), day/night air temperatures of 25/15 °C, PPF of 26 mol m<sup>-2</sup> d<sup>-1</sup> (Nobel & Hartsock 1986); (g) *Hylocereus undatus*, 370 and 740 µmol mol<sup>-1</sup> CO<sub>2</sub>, day/night air temperatures of 25/15 °C, PPF of 10 mol m<sup>-2</sup> d<sup>-1</sup> (Raveh *et al.* 1995); (h) *H. undatus*, 370 and 740 µmol mol<sup>-1</sup> CO<sub>2</sub>, day/night air temperatures of 25/15 °C, PPF of 10 mol m<sup>-2</sup> d<sup>-1</sup>, after 24 d of drought (Raveh *et al.* 1995); (i) *H. undatus*, 370 and 740 µmol mol<sup>-1</sup> CO<sub>2</sub>, day/night air temperatures of 45/35 °C, PPF of 10 mol m<sup>-2</sup> d<sup>-1</sup> (Raveh *et al.* 1995); (j) *Kalanchoë pinnata*, 340 and 680 µmol mol<sup>-1</sup> CO<sub>2</sub> (instantaneous increase), day/night air temperatures of 25/20 °C, PPF of 22 mol m<sup>-2</sup> d<sup>-1</sup> (Winter & Engelbrecht 1994); (k) *Opuntia ficus-indica*, 370, 520 (dashed line), and 750 µmol mol<sup>-1</sup> CO<sub>2</sub> (Cui *et al.* 1993); and (l) *Stenocereus queretaroensis*, 360 and 720 µmol mol<sup>-1</sup> CO<sub>2</sub>, day/night air temperatures of 25/15 °C, PPF of 22 mol m<sup>-2</sup> d<sup>-1</sup> (Nobel 1996).

resenting a range of morphologies (i.e. epiphytes, stem succulents and leaf succulents) and maintained at elevated atmospheric CO<sub>2</sub> concentrations for at least several weeks averages 35% (Table 3), which is consistent with the average biomass increase for these species under elevated atmospheric CO<sub>2</sub> concentrations (Table 2). For example,

the biomass enhancement for *Ananas comosus* caused by doubling the atmospheric CO<sub>2</sub> concentration is 23% (Zhu *et al.* 1997b), and the increase in net daily CO<sub>2</sub> uptake is 15% (Fig. 2; Zhu *et al.* 1999). *Agave salmiana* shows a 59% increase in daily net CO<sub>2</sub> uptake under doubled atmospheric CO<sub>2</sub> and a concomitant 55% increase in biomass (Nobel *et al.* 1996).

The enhancement of daily net CO<sub>2</sub> uptake is maintained in the long term (8–17 months) for those species investigated, i.e. *Agave deserti* (Graham & Nobel 1996), *A. comosus* (Zhu *et al.* 1997b) and *O. ficus-indica* (Nobel & Israel 1994). For *A. deserti*, a small (approximately 10%) decrease in daily net CO<sub>2</sub> uptake may occur over 17 months for both current and doubled atmospheric CO<sub>2</sub> concentrations, and is suggested to be due to the effects of limited soil volume on growth (Nobel *et al.* 1994b; Graham & Nobel 1996). However, the enhancement of CO<sub>2</sub> uptake by elevated atmospheric CO<sub>2</sub> concentrations remains high. Similarly, total net daily CO<sub>2</sub> uptake for *O. ficus-indica* is still 35% higher after 1 year under doubled versus the current atmospheric CO<sub>2</sub> concentration (Nobel & Israel 1994). Thus for the species studied, acclimatization of CO<sub>2</sub> uptake to elevated atmospheric CO<sub>2</sub> concentrations does not seem to occur. Immediate exposure to a doubled atmospheric CO<sub>2</sub> concentration results in only a 2% increase in daily net CO<sub>2</sub> uptake the next day for *A. deserti* (Fig. 2a) and *Ferocactus acanthodes* (Fig. 2f; Nobel & Hartsock 1986). The difference in percentage increase for *A. deserti* for instantaneous versus long-term exposure suggests that the response to elevated atmospheric CO<sub>2</sub> concentrations is maximized once certain physiological and/or morphological changes occur, such as the thickening of the chlorenchyma.

The daily pattern of CO<sub>2</sub> uptake is also altered under elevated atmospheric CO<sub>2</sub> concentrations (Fig. 2). For most species, afternoon (phase IV, Fig. 1) CO<sub>2</sub> uptake increases under elevated atmospheric CO<sub>2</sub> concentrations. Uptake may be initiated earlier in the afternoon, e.g. for *A. salmiana* and *Stenocereus queroetaroensis* (Fig. 2a,l; Nobel 1996);

for those species whose stomatal opening typically occurs in the afternoon at the current atmospheric CO<sub>2</sub> concentration, a marked increase in the maximum rate of CO<sub>2</sub> uptake occurs during this period. For example, for *A. comosus*, the maximum rate of CO<sub>2</sub> uptake in the afternoon is 85% greater under a doubled atmospheric CO<sub>2</sub> concentration, and similar effects occur during phase II (Fig. 2e; Zhu *et al.* 1999).

Increases in the duration and the rate of daytime net CO<sub>2</sub> uptake result in an increase in the percentage contribution of daytime CO<sub>2</sub> uptake to the total daily net CO<sub>2</sub> uptake for CAM species under elevated atmospheric CO<sub>2</sub> concentrations (Table 3). Such responses are consistent with the effect of higher cellular CO<sub>2</sub> concentrations leading to increased saturation of Rubisco, the carboxylating enzyme mediating phases II and IV (Fig. 1) of the CAM cycle (Winter & Smith 1996a), and with the responses of most C<sub>3</sub> species to elevated atmospheric CO<sub>2</sub> concentrations (Kimball 1983; Poorter 1993; Rogers *et al.* 1994). Indeed, the higher carbon-isotope discrimination values for leaves of *A. comosus* grown under a doubled atmospheric CO<sub>2</sub> concentration are indicative of increased Rubisco-mediated CO<sub>2</sub> uptake during the daytime (Zhu *et al.* 1999).

Less expected for CAM species is the increase in PEPCase-mediated uptake of CO<sub>2</sub> at night (phase I, Fig. 2) under elevated atmospheric CO<sub>2</sub> concentrations. PEPCase may be carbon-saturated at current atmospheric CO<sub>2</sub> concentrations (Ting 1994), and elevated atmospheric CO<sub>2</sub> concentrations minimally affect net CO<sub>2</sub> uptake by C<sub>4</sub> plants for which the initial carboxylating enzyme is also PEPCase (Lawlor & Mitchell 1991; Bowes 1993). However, even for those species that achieve relatively high rates of net daytime CO<sub>2</sub> uptake and have large contributions of daytime uptake to overall carbon gain, e.g. *Hylocereus undatus* (Table 3), the observed stimulation of biomass (Table 2) and the enhancement of total carbon gain cannot be sustained by the observed increases in daytime CO<sub>2</sub> uptake alone (Table 3). The most extreme example of this occurs for *O. ficus-indica*, which has very low rates of

**Table 3.** Percentage increase in total daily net CO<sub>2</sub> uptake under elevated atmospheric CO<sub>2</sub> concentrations and percentage contribution of daytime CO<sub>2</sub> uptake to total daily net CO<sub>2</sub> uptake for CAM plants under ambient (340–370 μmol mol<sup>-1</sup>) and elevated (680–750 μmol mol<sup>-1</sup>) atmospheric CO<sub>2</sub> concentrations

Species	Increase in total daily net CO <sub>2</sub> uptake (%)	Daytime net CO <sub>2</sub> uptake (% of total)		Reference
		Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	
<i>Agave deserti</i>	2	22	38	Nobel & Hartsock (1986)
	49	17	24	Graham & Nobel (1996)
<i>Agave salmiana</i>	36	15	23	Nobel (1996), Nobel <i>et al.</i> (1996)
<i>Ananas comosus</i>	15	19	33	Zhu <i>et al.</i> (1999)
<i>Hylocereus undatus</i>	34	24	35	Raveh <i>et al.</i> (1995)
<i>Opuntia ficus-indica</i>				
basal cladodes	41–152	–4	17	Cui <i>et al.</i> (1993)
daughter cladodes	41–61	8	15	Cui <i>et al.</i> (1993)
<i>Stenocereus queroetaroensis</i>	36	7	16	Nobel (1996)



daytime net CO<sub>2</sub> uptake under the current atmospheric CO<sub>2</sub> concentration (Table 3) and may even have net daytime losses (Cui *et al.* 1993). Under a doubled atmospheric CO<sub>2</sub> concentration, the contribution of daytime to total daily uptake rarely exceeds 17%, whereas this species can show a 100% increase in daily net CO<sub>2</sub> uptake (Table 3) and biomass increases of approximately 40% (Table 2). Indeed, the maximal rate of nocturnal CO<sub>2</sub> uptake for daughter cladodes was 43% higher at an atmospheric CO<sub>2</sub> concentration of 520 μmol mol<sup>-1</sup> and 96% higher at 720 μmol mol<sup>-1</sup> CO<sub>2</sub> compared with uptake rates at 360 μmol mol<sup>-1</sup> CO<sub>2</sub> (Fig. 2), indicating that night-time uptake of CO<sub>2</sub> is far from being saturated at the current atmospheric CO<sub>2</sub> concentration for this CAM species. Lack of saturation of PEPCase at the current atmospheric CO<sub>2</sub> concentration possibly results from low diffusion conductances leading to low internal CO<sub>2</sub> concentrations (Nobel 1999). However, for some CAM species, e.g. *A. comosus*, enhanced night-time CO<sub>2</sub> uptake under a doubled atmospheric CO<sub>2</sub> concentration occurs only when day/night temperatures are increased (Zhu *et al.* 1999).

For several species, e.g. *A. salmiana* (Fig. 2c), *O. ficus-indica* (Fig. 2k) and *S. queretaroensis* (Fig. 2l), increased CO<sub>2</sub> uptake under a doubled atmospheric concentration is most pronounced in the first half of the night. A similar pattern is apparent for *A. deserti* and *F. acanthodes* subjected to an instantaneous increase in atmospheric CO<sub>2</sub> concentration (Fig. 2a,f), which increases the maximum rate of CO<sub>2</sub> uptake by approximately 50%. However, this stimulation for the latter two species is sustained for only about 6 h, after which the rate drops below that for plants exposed to the current CO<sub>2</sub> concentration, resulting in only small net gains in total daily net CO<sub>2</sub> uptake. Such patterns are probably associated with the insufficiency of a CO<sub>2</sub> acceptor or a filled malate pool in plants that have not fully adapted to the elevated atmospheric CO<sub>2</sub> concentration through longer-term exposure. For some species, e.g. *Clusia uvitana* and *Portulacaria afra*, decreases in nocturnal net CO<sub>2</sub> uptake occur in response to short-term exposure to elevated atmospheric CO<sub>2</sub> concentrations (Huerta & Ting 1988; Winter *et al.* 1992). Both species are C<sub>3</sub>-CAM intermediates that exhibit extensive daytime net CO<sub>2</sub> uptake. For *Mesembryanthemum crystallinum* and *P. afra*, the switch to CAM photosynthesis cannot be induced by manipulating the atmospheric CO<sub>2</sub> concentration but readily occurs in response to water stress (Huerta & Ting 1988; Winter 1979). For *C. uvitana*, which also shows increased CAM activity in response to water stress, nocturnal CO<sub>2</sub> uptake may be a function of the net carbon gain during the light period, with daytime decreases resulting in an increase in nocturnal uptake (Winter *et al.* 1992).

The enhancement of daily net CO<sub>2</sub> uptake by elevated atmospheric CO<sub>2</sub> concentrations is influenced by environmental factors, especially photosynthetic photon flux (PPF, 400–700 nm), drought, and day/night air temperatures (Nobel & Israel 1994; Raveh *et al.* 1995; Graham & Nobel 1996; Zhu *et al.* 1999). For *A. deserti*, *H. undatus* and *O. ficus-*

*indica*, daily net CO<sub>2</sub> uptake increases as the PPF increases from 5 to 20 mol m<sup>-2</sup> d<sup>-1</sup> under both current and doubled atmospheric CO<sub>2</sub> concentrations. For these three species, the enhancement of CO<sub>2</sub> uptake by elevated atmospheric CO<sub>2</sub> concentrations is greatest for 10–20 mol m<sup>-2</sup> d<sup>-1</sup>, a PPF range that may allow maximal response of both daytime and night-time CO<sub>2</sub> uptake to the elevated atmospheric CO<sub>2</sub> concentration. When CAM plants are exposed to water stress or high temperatures, net CO<sub>2</sub> uptake is generally depressed more during the daytime than at night (Nobel 1988). For the shade plant *H. undatus*, which shows saturation of net CO<sub>2</sub> uptake at only 10–20 mol m<sup>-2</sup> d<sup>-1</sup> under the current atmospheric CO<sub>2</sub> concentration, increasing photoinhibition of C<sub>3</sub> photosynthesis with increasing PPF and a concomitant decrease in daytime CO<sub>2</sub> uptake is partially offset by the enhancement of CO<sub>2</sub> uptake by the elevated CO<sub>2</sub> concentration (Raveh *et al.* 1995).

For *A. deserti*, *H. undatus* and *O. ficus-indica*, total daily net CO<sub>2</sub> uptake decreases with increasing duration of drought under both current and elevated atmospheric CO<sub>2</sub> concentrations (Nobel & Israel 1994; Raveh *et al.* 1995; Graham & Nobel 1996). However, the rate of decrease is greater under the current atmospheric CO<sub>2</sub> concentration, e.g. for *O. ficus-indica*, 25, 50 and 75% decreases in total daily net CO<sub>2</sub> uptake occur at 17, 23 and 33 d of drought, respectively, under the current atmospheric CO<sub>2</sub> concentration and at 23, 31 and 40 d, respectively, under a doubled CO<sub>2</sub> concentration. Concomitantly, the percentage enhancement of daily net CO<sub>2</sub> uptake under elevated atmospheric CO<sub>2</sub> concentrations increases as the drought progresses, e.g. for *A. deserti*, *H. undatus* (Fig. 2h) and *O. ficus-indica*, the percentage enhancements under a doubled atmospheric CO<sub>2</sub> concentration after 25 d are 300, 340 and 200%, respectively. For *O. ficus-indica*, positive carbon gain is eliminated by 50 d of drought under the current atmospheric CO<sub>2</sub> concentration but still occurs under a doubled atmospheric CO<sub>2</sub> concentration (Nobel & Israel 1994).

The increased ability to withstand drought for CAM species under elevated atmospheric CO<sub>2</sub> concentrations is associated with an increased WUE (Table 4), as has been found for C<sub>3</sub> and C<sub>4</sub> species (Eamus 1991; Ham *et al.* 1995; Jarvis, Mansfield & Davies 1999). The enhancement under a doubled CO<sub>2</sub> concentration occurs despite the greater contribution of daytime CO<sub>2</sub> uptake, which is less water-use efficient, to the total net daily CO<sub>2</sub> uptake. For *A. deserti*, *A. comosus* and *O. ficus-indica*, the increase in WUE is due both to increased daily net CO<sub>2</sub> uptake at elevated atmospheric CO<sub>2</sub> concentrations and also to decreases in stomatal conductance (Cui *et al.* 1993; Graham & Nobel 1996; Zhu *et al.* 1999). Water vapour conductance for *A. deserti* decreases 24% under elevated atmospheric CO<sub>2</sub> concentrations, and most water loss occurs at night. The higher plant water content associated with the increased WUE for these succulent species allows for net CO<sub>2</sub> uptake to occur for longer periods during drought (Raveh *et al.* 1995; Graham & Nobel 1996).

For both current and elevated atmospheric CO<sub>2</sub> concentrations, maximum daily net CO<sub>2</sub> uptake for many CAM



Species	WUE (mmol CO <sub>2</sub> mol <sup>-1</sup> H <sub>2</sub> O)		Reference
	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	
<i>Agave deserti</i>	20	42	Graham & Nobel (1996)
<i>Ananas comosus</i>	9.5	13	Zhu <i>et al.</i> (1999)
<i>Opuntia ficus-indica</i>			
basal cladodes	4	7	Cui <i>et al.</i> (1993)
daughter cladodes	10	16	Cui <i>et al.</i> (1993)

**Table 4.** Water-use efficiencies (WUE) for CAM plants under ambient (350–370 μmol mol<sup>-1</sup>) and elevated (700–750 μmol mol<sup>-1</sup>) atmospheric CO<sub>2</sub> concentrations

species occurs at day/night air temperatures of approximately 25/15 °C, reflecting the optimal night-time temperature for carboxylation by PEPCase (Winter 1985; Nobel 1988). Increasing or decreasing the day/night air temperatures from the optimum reduces the daily uptake of CO<sub>2</sub>, but the reduction is generally greater at current than at elevated atmospheric CO<sub>2</sub> concentrations. For *H. undatus* (Fig. 2i), daytime net CO<sub>2</sub> uptake becomes negative at a day/night air temperature of 45/35 °C (Raveh *et al.* 1995), the latter reflecting the sensitivity of C<sub>3</sub> photosynthesis to high temperatures. Increases in leaf temperature increase the O<sub>2</sub>:CO<sub>2</sub> ratio at the site of CO<sub>2</sub> fixation via Rubisco, thus increasing photorespiration (Taiz & Zeiger 1998). Elevated atmospheric CO<sub>2</sub> concentrations can offset this increase and may thus contribute to the large percentage enhancement in total daily net CO<sub>2</sub> uptake that occurs under elevated temperatures for several CAM species, such as *A. deserti*, *O. ficus-indica*, *H. undatus* and *A. comosus* (Nobel & Israel 1994; Raveh *et al.* 1995; Graham & Nobel 1996; Zhu *et al.* 1999). The enhancement of total daily net CO<sub>2</sub> uptake at high temperatures for CAM species is likely to be important under a changing global climate that may result in average annual temperature increases of 2–4 °C associated with a doubled concentration of atmospheric CO<sub>2</sub> and increases in other greenhouse gases (Kattenberg *et al.* 1996).

For CAM species at the current atmospheric CO<sub>2</sub> concentration, respiration becomes more important at high temperatures (Kluge & Ting 1978). Relatively little is known about the effect of elevated atmospheric CO<sub>2</sub> concentrations on their respiration. In any case, elevated atmospheric CO<sub>2</sub> concentrations may reduce mitochondrial/dark respiration, as occurs for C<sub>3</sub> species (Amthor 1991; Drake *et al.* 1999).

## CELLULAR ASPECTS OF PHOTOSYNTHESIS

### Chlorophyll

The chlorophyll content for *A. deserti*, *A. vilmoriniana* and *O. ficus-indica* is approximately 20% less under doubled compared with the current atmospheric CO<sub>2</sub> concentration (Table 5; Szarek *et al.* 1987; Nobel *et al.* 1994a; Graham & Nobel 1996). Similar decreases occur in C<sub>3</sub> species and suggest increased photosynthetic efficiency (Wullschlegel,

Norby & Hendrix 1992; Moore *et al.* 1998). For *O. ficus-indica*, the chlorophyll *a*:*b* ratio is 9% lower under a doubled CO<sub>2</sub> concentration (Table 5; Nobel *et al.* 1994a), indicating an increase in light-harvesting chlorophyll *b* and photosystem II activity, as found for shade versus sun leaves (Nobel 1999). In contrast the chlorophyll *a*:*b* ratio increases approximately 12% with a doubling of the atmospheric CO<sub>2</sub> concentration for *A. deserti* and *A. vilmoriniana* (Table 5; Szarek *et al.* 1987; Graham & Nobel 1996). The different responses of the chlorophyll *a*:*b* ratio may reflect changes in light penetration to the mesophyll resulting from different responses of the photosynthetic surfaces to elevated atmospheric CO<sub>2</sub> concentrations. In particular, increased cuticular thickness and decreased transmission of PPF occur for *O. ficus-indica* as the atmospheric CO<sub>2</sub> concentration is raised (Nobel *et al.* 1994a), but decreased cuticular thickness and increased PPF transmission occur for *A. deserti* (Graham & Nobel 1996).

### Enzymes

Carboxylation activities for the enzymes PEPCase and Rubisco decrease in response to a doubled atmospheric CO<sub>2</sub> concentration for the few CAM species that have been studied (Table 5; Nobel *et al.* 1994a; Graham & Nobel 1996; Nobel *et al.* 1996). Decreases in PEPCase and Rubisco activities also occur for the orchid 'Mokara Yellow' under 1 and 5% atmospheric CO<sub>2</sub> concentrations in photosynthetic shoots and roots (Gouk *et al.* 1997). Many C<sub>3</sub> species show decreases in Rubisco content or activities in response to elevated atmospheric CO<sub>2</sub> concentrations (Drake, González-Meler & Long 1997), potentially resulting in acclimatization to the higher CO<sub>2</sub> concentrations (Bowes 1993; Moore *et al.* 1999). However, transforming the C<sub>3</sub> *Nicotiana tabacum* such that Rubisco production is decreased approximately 15% reduces net carbon uptake at the current but not at a doubled atmospheric CO<sub>2</sub> concentration, indicating a reduced requirement for Rubisco at elevated atmospheric CO<sub>2</sub> concentrations (Masle, Hudson & Badger 1993). Furthermore, as CO<sub>2</sub> is an activator as well as a substrate for Rubisco (Lorimer, Badger & Andrews 1976), increases in internal CO<sub>2</sub> concentrations in response to elevated atmospheric CO<sub>2</sub> concentrations can result in increases in the enzyme activation state, thus compensating for decreases in total activity. Indeed, for *A. deserti*, *Agave salmiana* and *O. ficus-indica*, decreases in Rubisco

**Table 5.** Chlorophyll and carboxylation enzyme properties under current (370  $\mu\text{mol mol}^{-1}$ ) and doubled (720–750  $\mu\text{mol mol}^{-1}$ ) atmospheric  $\text{CO}_2$  concentrations

Species	Current $\text{CO}_2$	Doubled $\text{CO}_2$	Reference
Chlorophyll content			
<i>Agave deserti</i> ( $\text{g m}^{-2}$ )	0.80	0.64	Graham & Nobel (1996)
<i>Agave vilmoriniana</i> ( $\mu\text{g g}^{-1}$ FW)	132	116	Szarek <i>et al.</i> (1987)
<i>Opuntia ficus-indica</i> ( $\text{g m}^{-2}$ )	0.65	0.52	Nobel <i>et al.</i> (1994a)
<i>O. ficus-indica</i> ( $\mu\text{g g}^{-1}$ FW)	320	280	Nobel <i>et al.</i> (1994a)
Chlorophyll <i>a:b</i>			
<i>A. deserti</i>	2.3	2.6	Graham & Nobel (1996)
<i>A. vilmoriniana</i>	2.5	2.8	Szarek <i>et al.</i> (1987)
<i>O. ficus-indica</i>	3.2	2.9	Nobel <i>et al.</i> (1994a)
PEPCase ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )			
<i>A. deserti</i>	55	37	Graham & Nobel (1996)
<i>O. ficus-indica</i>	32	20	Nobel <i>et al.</i> (1994a)
Rubisco ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )			
<i>A. deserti</i>	40	35	Graham & Nobel (1996)
<i>Agave salmiana</i>	28	23	Nobel <i>et al.</i> (1996)
<i>O. ficus-indica</i>	41	29	Nobel <i>et al.</i> (1994a)
<i>O. ficus-indica</i>	34	29	Nobel <i>et al.</i> (1996)
Activated:total Rubisco (% of total)			
<i>A. deserti</i>	59	74	Graham & Nobel (1996)
<i>A. salmiana</i>	54	89	Nobel <i>et al.</i> (1996)
<i>O. ficus-indica</i>	53	88	Nobel <i>et al.</i> (1996)
$K_M$ for PEPCase ( $\text{mol m}^{-3} \text{HCO}_3^-$ )			
<i>A. salmiana</i>	0.39	0.33	Nobel <i>et al.</i> (1996)
<i>O. ficus-indica</i>	0.48	0.27	Nobel <i>et al.</i> (1996)
$K_M$ for Rubisco ( $\text{mmol m}^{-3} \text{CO}_2$ )			
<i>A. salmiana</i>	25	26	Nobel <i>et al.</i> (1996)
<i>O. ficus-indica</i>	23	25	Nobel <i>et al.</i> (1996)

carboxylation activity of 15–30% are compensated for by 25–65% increases in the ratio of the activated to total Rubisco (Table 5), leading to increases in Rubisco-mediated daytime  $\text{CO}_2$  uptake.

For *A. deserti* and *O. ficus-indica*, the decreases in PEPCase activity under a doubled atmospheric  $\text{CO}_2$  concentration are greater than those for Rubisco (Table 5; Nobel *et al.* 1994a; Graham & Nobel 1996), and possibly reflect a relatively decreased requirement for this initial carboxylating enzyme as the substrate becomes more abundant. Furthermore, a doubling of the atmospheric  $\text{CO}_2$  concentration results in 15 and 44% decreases in the apparent  $K_M(\text{HCO}_3^-)$  for PEPCase for *A. salmiana* and *O. ficus-indica*, respectively (Table 5). In this regard, the  $K_M(\text{HCO}_3^-)$  for PEPCase from *Zea mays* decreases with decreasing pH (O'Leary 1982).

The  $K_M(\text{CO}_2)$  for Rubisco does not change for *A. salmiana* or *O. ficus-indica* in response to a doubling of the atmospheric  $\text{CO}_2$  concentration (Table 5; Nobel *et al.* 1996). This is consistent with results for *Gossypium hirsutum* ( $\text{C}_3$ ) and *Z. mays* ( $\text{C}_4$ ), whose  $K_M(\text{CO}_2)$  for Rubisco is similar for plants grown from seed at 330 and 640  $\mu\text{mol mol}^{-1} \text{CO}_2$  (Yeoh, Badger & Watson 1981). The  $K_M(\text{CO}_2)$  for Rubisco is unchanged by  $\text{CO}_2$  enrichment for other  $\text{C}_3$  species

as well (Campbell, Allen & Bowes 1988). The  $K_M(\text{CO}_2)$  for Rubisco for *A. salmiana* and *O. ficus-indica* is in the upper part of the range for  $\text{C}_3$  and CAM species (12–25  $\text{mmol m}^{-3}$ ; Yeoh *et al.* 1981), but less than values for  $\text{C}_4$  species (28–34  $\text{mmol m}^{-3}$ ; Yeoh *et al.* 1981). The difference in  $K_M(\text{CO}_2)$  between the  $\text{C}_3$  and  $\text{C}_4$  species may reflect the strong  $\text{CO}_2$ -concentrating mechanism of the  $\text{C}_4$  type (Yeoh *et al.* 1981). The lower  $K_M(\text{CO}_2)$  for Rubisco from CAM species may indicate a lower  $\text{CO}_2$  concentration at the site of the enzyme than for  $\text{C}_4$  species, which may in part explain why CAM species show a greater response to elevated atmospheric  $\text{CO}_2$  concentrations than do  $\text{C}_4$  species. Indeed, *Kalanchoë pinnata*, whose stimulated growth under elevated atmospheric  $\text{CO}_2$  concentrations is attributed to increases in daytime  $\text{CO}_2$  uptake (Winter *et al.* 1997), has a  $K_M(\text{CO}_2)$  for Rubisco of 15  $\text{mmol m}^{-3}$  (Yeoh *et al.* 1981).

### Malate

The majority of CAM species subjected to elevated atmospheric  $\text{CO}_2$  concentrations for extended periods show a night-time increase in titratable acidity or malate accumulation (Table 6). This indicates increased night-time  $\text{CO}_2$

fixation (Griffiths 1988) and is consistent with the increase in phase I CO<sub>2</sub> uptake (Fig. 1) and lack of saturation of phase I at the current atmospheric CO<sub>2</sub> concentration. The lack of response of malic acid accumulation in *K. pinnata* to elevated atmospheric CO<sub>2</sub> concentrations possibly reflects the fact that biomass enhancement for this species is due to increased daytime net CO<sub>2</sub> uptake (Winter *et al.* 1997). Although *Kalanchoë daigremontiana* shows similar nocturnal malate accumulation at 300  $\mu\text{mol mol}^{-1}$  CO<sub>2</sub> and in response to a short-term exposure to 1000  $\mu\text{mol mol}^{-1}$  CO<sub>2</sub>, the rate of accumulation is faster at the elevated atmospheric CO<sub>2</sub> concentration. This results in a decreased period of accumulation, suggesting that the extent of malate accumulation is limited by factors other than the atmospheric CO<sub>2</sub> concentration (Holtum *et al.* 1983). Plants subjected to short-term increases in atmospheric CO<sub>2</sub> concentration may lack anatomical and biochemical changes to support increased malate accumulation, e.g. sufficient carbohydrates for increased synthesis of the acceptor. Interestingly, *Tillandsia ionantha* and *Crassula arborescens* both show increased malic acid accumulation in response to short-term exposure to elevated atmospheric CO<sub>2</sub> concentrations, although whether these plants had become acclimatized to the higher CO<sub>2</sub> concentrations in their greenhouse environment is not known (Nowak & Martin 1995).

### Sugars and photosynthate partitioning

Acclimatization to elevated atmospheric CO<sub>2</sub> concentrations, as is observed for many species and especially for C<sub>3</sub> annuals, is usually attributed to altered source–sink relationships, with excess soluble sugars and starch accumulation resulting in feedback inhibition of photosynthesis (Arp 1991; Stitt 1991; Bowes 1993; Moore *et al.* 1999). Feedback inhibition may reflect inhibition of photophosphorylation resulting from depletion of Pi pools, as sugar phosphates build up, reflecting reduced rates of starch and sucrose synthesis (Stitt 1991). Additionally, a build-up of sugars may suppress Rubisco, resulting in a lowered photosynthetic rate (Bowes 1993; Moore *et al.* 1999). For *O. ficus-indica*,

the synthesis of glucomannan, a probable carbon reserve for PEP, increases 170% under a doubled atmospheric CO<sub>2</sub> concentration (Wang & Nobel 1995), which may help explain the large stimulatory effect of a doubled atmospheric CO<sub>2</sub> concentration on its net CO<sub>2</sub> uptake. Increases in soluble sugar and starch content of more than 60% also occur for *O. ficus-indica* in response to long-term exposure to elevated atmospheric CO<sub>2</sub> concentrations, but do not result in down-regulation of photosynthesis (Cui *et al.* 1993; Nobel & Israel 1994).

In the basal cladodes of *O. ficus-indica*, increases in starch accumulation during the daytime and at elevated atmospheric CO<sub>2</sub> concentrations do not lower the photosynthetic rates, as occurs for some C<sub>3</sub> species (Servaites *et al.* 1989), indicating that starch accumulation is not limiting its photosynthesis (Wang & Nobel 1996). Similarly, starch accumulation induced by girdling the petioles of mature leaves of some starch-storing C<sub>3</sub> species also does not directly cause feedback inhibition of photosynthesis (Goldschmidt & Huber 1992). The sucrose content and its daily change in cladodes are similar under current and doubled atmospheric CO<sub>2</sub> concentrations, despite 146% increases in the activity of sucrose-Pi synthase under a doubled atmospheric CO<sub>2</sub> concentration (Wang & Nobel 1996). However, increases in phloem transport to developing daughter cladodes (sinks) under a doubled atmospheric CO<sub>2</sub> concentration ensure that sucrose levels do not increase in the basal cladodes (sources) leading to feedback inhibition (Wang & Nobel 1995, 1996). Sink strength, as evidenced by increases in the activities of sucrose-Pi synthase and soluble starch synthase for daughter cladodes, also increases for *O. ficus-indica* under a doubled atmospheric CO<sub>2</sub> concentration (Black 1993; Jenner & Hawker 1993; Wang & Nobel 1996). Furthermore, the increase in glucose and malate in the sink regions under a doubled atmospheric CO<sub>2</sub> concentration may increase the osmolality of the sink cells and thus decrease the turgor pressure of the phloem in the sink region (Ho 1988), resulting in a more rapid movement of photoassimilate into the daughter cladodes (Wang & Nobel 1996). Thus some of the excess carbohydrate produced in the basal cladodes of *O. ficus-indica* at elevated atmos-

**Table 6.** Nocturnal acidity increases for CAM species in response to short-term (< 1 week) and long-term (> 1 month) exposure to elevated atmospheric CO<sub>2</sub> concentrations as a percentage increase over controls maintained under the current atmospheric CO<sub>2</sub> concentration for the same period

Species	Elevated CO <sub>2</sub> concentration ( $\mu\text{mol mol}^{-1}$ )	Duration of treatment	Nocturnal acidity (% increase)	Reference
<i>Agave vilmoriniana</i>	752	Long-term	7.5 to 12	Szarek <i>et al.</i> (1987)
<i>Ananas comosus</i>	730	Long-term	34	Zhu <i>et al.</i> (1997a)
Orchid "Mokara Yellow"	10 000	Long-term	23 to 100	Gouk <i>et al.</i> (1997)
<i>Tillandsia ionantha</i>	800	Short-term	50	Nowak & Martin (1995)
<i>Opuntia ficus-indica</i>	750	Long-term	22	Wang & Nobel (1996)
<i>Portulacaria afra</i>	950	Short-term	0	Huerta & Ting (1988)
<i>Crassula arborescens</i>	920	Short-term	50	Nowak & Martin (1995)
<i>Kalanchoë daigremontiana</i>	1000	Short-term	0 to -25	Holtum <i>et al.</i> (1983)
<i>Kalanchoë pinnata</i>	750	Long-term	-8 to -11	Winter <i>et al.</i> (1997)

pheric CO<sub>2</sub> concentrations sustains growth of the daughter cladodes.

Despite a 175% increase in glucose levels in the basal cladodes under a doubled atmospheric CO<sub>2</sub> concentration, the concentrations of glucose (1 mol m<sup>-3</sup>) and sucrose (5 mol m<sup>-3</sup>) in the chlorenchyma of *O. ficus-indica* are 10 and 60 times less, respectively, than those needed to suppress gene expression in annual crops (Sheen 1994). Furthermore, for C<sub>3</sub> species, the occurrence of strong end-product inhibition appears correlated with high acid invertase activity (Goldschmidt & Huber 1992) and an increased hexose flux through hexokinase (Moore *et al.* 1999). In this regard, the activity of invertase is extremely low in basal cladodes of *O. ficus-indica* under both current and elevated atmospheric CO<sub>2</sub> concentrations, and the activity of hexokinase is slightly decreased under a doubled atmospheric CO<sub>2</sub> concentration (Wang & Nobel 1996). The cellular contents of *O. ficus-indica* in particular and CAM species in general are relatively dilute, as indicated by high relative tissue water contents and high water potentials (Nobel 1988). Indeed, the succulence of many CAM species, which buffers fluctuations in the daily supply of water and photoassimilates available for growth (Nobel 1988; Wardlaw 1990), may represent a photosynthetic type that can accommodate increases in carbohydrate levels in response to elevated atmospheric CO<sub>2</sub> concentrations without acclimatization of photosynthesis.

## CONCLUSIONS

Predicting the response of CAM species to elevated atmospheric CO<sub>2</sub> concentrations on the basis of the initial carboxylating enzyme fails to consider the complexity of this photosynthetic type. Indeed, although generalizations may be more applicable to C<sub>3</sub> and C<sub>4</sub> species, variation of response also occurs for these photosynthetic types (Lawlor & Mitchell 1991; Poorter 1993). Doubling the atmospheric CO<sub>2</sub> concentration greatly stimulates net CO<sub>2</sub> uptake by the CAM species studied (Tables 1 and 3), despite the suggestion that the predominant initial fixation of CO<sub>2</sub> by PEPCase in CAM plants might be saturated at the current atmospheric CO<sub>2</sub> concentration (Ting 1994; Winter & Engelbrecht 1994). The accompanying biomass increase, which reflects increases in both daytime and nighttime net CO<sub>2</sub> uptake, averages about 35% for these CAM species (Table 2), including 40% for the highly productive and widely cultivated *O. ficus-indica* over a one-year period (Nobel & Israel 1994). Decreases in Rubisco activity that occur for CAM species in response to elevated atmospheric CO<sub>2</sub> concentrations are compensated for by increases in the fraction of the enzyme in the activated state (Table 5), thus maintaining photosynthetic performance, as occurs for C<sub>3</sub> species. However, unlike some C<sub>3</sub> species, especially annuals, there is little evidence of acclimatization to elevated atmospheric CO<sub>2</sub> concentrations with time of exposure for most of the CAM species studied. The lack of acclimatization is possibly associated with the succulence of CAM species, which can accommodate large increases in

chlorenchyma thickness and accumulation of photosynthate without feedback inhibition.

Such conclusions, however, are based on the study of relatively few species with no study exceeding 18 months, a relatively short time period in the life span of species such as *A. deserti* and *O. ficus-indica*. Although remarkable similarity in response to elevated atmospheric CO<sub>2</sub> concentrations occurs for the species investigated, only one species, *O. ficus-indica*, has been studied in depth. Thus comprehensive studies of a greater number of CAM species from different families and environments would be valuable. In particular, focusing on PEPCase-mediated night-time CO<sub>2</sub> uptake may contribute to an understanding of why that process is not limited at close to the current atmospheric CO<sub>2</sub> concentration. Whether the  $K_M(\text{HCO}_3^-)$  for PEPCase decreases in other CAM species exposed to elevated atmospheric CO<sub>2</sub> concentrations and the basis for the decrease need to be investigated. Studies of carbohydrate partitioning in CAM species may explain the lack of acclimatization. Longer-term exposure to elevated atmospheric CO<sub>2</sub> concentrations could establish whether acclimatization occurs after years, as well as whether effects on reproduction occur. Furthermore, the response of C<sub>3</sub>-CAM intermediates to elevated atmospheric CO<sub>2</sub> concentrations has been little investigated, beyond the apparent insensitivity to atmospheric CO<sub>2</sub> concentrations of the switch from C<sub>3</sub> to CAM. The variable CAM response of such intermediates may present an opportunity to determine specific contributions, for example of anatomical changes, to the enhancement of net CO<sub>2</sub> uptake and of productivity in response to elevated atmospheric CO<sub>2</sub> concentrations.

Observations of significant biomass increases and lack of acclimatization, coupled with the increasing air temperatures accompanying global climate change that may extend the range of cultivation for freezing-sensitive CAM species (Nobel & García de Cortázar 1991; Nobel 1996), could affect land-use practices with regard to mitigating rising atmospheric CO<sub>2</sub> concentrations. In particular, the international protocol discussed in Kyoto, Japan, in December 1997 pledges countries to work towards a stabilization of atmospheric CO<sub>2</sub> concentrations. The high WUE of CAM plants (Table 4) together with the large biomass productivity of selected CAM crops, both of which should increase under elevated atmospheric CO<sub>2</sub> concentrations, suggests their consideration with regard to terrestrial sequestration of atmospheric CO<sub>2</sub> in arid and semi-arid regions. Such regions, which are poorly suited to C<sub>3</sub> and C<sub>4</sub> crops without irrigation, occupy about 30% of the earth's land area. Moreover, the substantial biomass increases for CAM species under elevated atmospheric CO<sub>2</sub> concentrations could enhance the importance of such native plants in ecosystems worldwide.

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