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INVITED REVIEW

Responses of CAM species to increasing atmospheric CO_2 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_2 concentration. Daily net CO_2 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_2 uptake in CAM species to increasing atmospheric CO_2 concentrations are similar to those for C_3 species and much greater than those for C_4 species. Increases in net daily CO_2 uptake by CAM plants under elevated atmospheric CO_2 concentrations reflect increases in both Rubisco-mediated daytime CO_2 uptake and phosphoenolpyruvate carboxylase (PEPCase)-mediated night-time CO_2 uptake, the latter resulting in increased nocturnal malate accumulation. Chlorophyll contents and the activities of Rubisco and PEPCase decrease under elevated atmospheric CO_2, but the activated percentage for Rubisco increases and the $K_M(HCO_3^-)$ 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_2 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_2 concentrations. With increasing temperature and drought duration, the percentage enhancement of daily net CO_2 uptake caused by elevated atmospheric CO_2 concentrations increases. Thus net CO_2 uptake, productivity, and the potential area for cultivation of CAM species will be enhanced by the increasing atmospheric CO_2 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_2 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_2, which is assimilated into carbohydrates using 1,5-ribulosebisphosphate carboxylase/oxygenase (Rubisco) and the C_3 photosynthetic carbon reduction cycle (Winter & Smith 1996a). As is the case for the C_4 type of photosynthesis, primary fixation of CO_2 by PEPCase concentrates CO_2 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_2 leakage from the site of the C_3 cycle and effectively decouples the internal CO_2 pool from that of the atmosphere (Monson 1989).

For both C_4 and CAM plants, the CO_2-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_3 and C_4 plants generally result in a three- to five-fold higher water-use efficiency (WUE) for CAM plants than for C_3 or C_4 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_2 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_2 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_2 involves binding of CO_2 by Rubisco (Winter & Smith 1996a). The high intercellular CO_2 concentrations associated with malate decarboxylation suppress stomatal opening during the middle of the day (phase III, Fig. 1). Most facultative C_3–CAM intermediates switch between C_3 and CAM in response to changes in environmental

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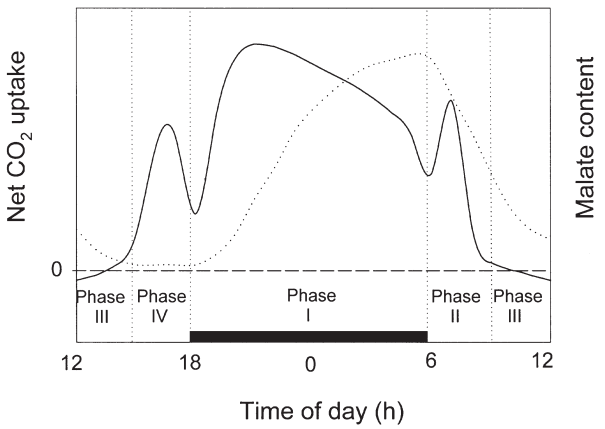

Figure 1. The daily cycle of net CO_2 (solid line) uptake and malic acid accumulation (dotted line) for a typical CAM plant, illustrating the four phases of CAM (Osmond 1978).

factors, especially soil water availability. Reduced water availability favours expression of CAM in the C_3–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_3–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_2 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_2 uptake is accompanied by 'CAM cycling.' This means that, despite nocturnal stomatal closure, malate levels increase during this period as respiratory CO_2 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_2 assimilation. A diel cycle in malic acid accumulation during CAM idling, associated with respiration followed by the re-fixation of respiratory CO_2, 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_3 pathway; about 2–3% are C_4, 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_2 concentration of approximately 280 μmol mol^{-1} 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 (Neftel *et al*. 1985; Schimel *et al*. 1996). Such observations have resulted in extensive research into the effects of atmospheric CO_2 concentration on the photosynthesis of C_3 plants. For most C_3 species, photosynthesis increases at elevated atmospheric CO_2 concentrations and the accompanying increases in intercellular CO_2 concentration, which suppress the oxygenase activity of Rubisco and thereby reduce photorespiratory loss of carbon. Because the specificity of Rubisco for CO_2 relative to O_2 declines with increasing temperature, the stimulation of net CO_2 uptake by elevated atmospheric CO_2 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_2 concentrations have been measured for some C_4 species (Poorter 1993; Lecain & Morgan 1998). However, photosynthesis by C_4 species is more readily saturated as atmospheric CO_2 concentrations rise, reflecting the relative insensitivity of PEPCase to the CO_2:O_2 ratio because of lack of binding of O_2 to the catalytic site (Lawlor & Mitchell 1991).

From a biochemical point of view, obligate CAM species could respond similarly to C_4 species if PEPCase were saturated at close to the current atmospheric CO_2 concentration (Ting 1994; Winter & Smith 1996b). Nonetheless, facultative CAM plants might respond more to elevated atmospheric CO_2 concentrations (Ting 1994). Compared with C_3 and C_4 species, relatively few studies have been conducted on the effects of elevated atmospheric CO_2 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_2 concentrations

Family/species	Productivity	Morphological changes	CO_2 uptake	Enzymes
Monocotyledons				
Agavaceae				
Agave deserti	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 *in vivo* % increased (1)
Agave salmiana	Increased (4)		Afternoon and night-time uptake increased (4, 5)	PEPCase decreased (4), Rubisco decreased but activated *in vivo* % increased (4), PEPCase K_M decreased (4)
Agave vilmoriniana	Increased (6)		Night-time uptake increased (7)	
Yucca schidigera[a]			Increased (8)	
Orchidaceae				
Mokara Yellow[b]	Increased (9–11)	Root : shoot ratio increased (9, 10)	Nocturnal malate accumulation increased (9)	PEPCase decreased (9), Rubisco decreased (9)
Bromeliaceae				
Aechmea magdalenae	Increased (12)			
Ananas comosus	Increased (13–15)	Root : shoot ratio increased (13), leaf thickness increased (13)	Increased morning and night-time uptake (13–16), WUE increased (15)	
Tillandsia ionantha			Nocturnal malate accumulation increased (17)	
Dicotyledons				
Aizoaceae				
Mesembryanthemum crystallinum[c]				
Cactaceae				
Ferocactus acanthodes	Increased (3)		Increased (3)	
Hylocereus undatus		Chlorenchyma cell volume increased (18)	Increased (18)	
Opuntia ficus-indica	Increased (4, 19–23)	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)	Afternoon, night-time and early-morning uptake increased (20–23, 27), WUE increased (21, 23)	PEPCase decreased (4, 27, 28), Rubisco decreased but activated *in vivo* % increased (4, 27, 28), PEPCase K_M decreased (4)
Stenocereus queretaroensis			Afternoon and night-time uptake increased (5)	
Clusiaceae				
Clusia uvitana[c]			Night-time uptake decreased (29)	
Portulacaceae				
Portulacaria afra[c]			Night-time uptake decreased (30)	
Crassulaceae				
Crassula arborescens			Nocturnal malate accumulation increased (17)	
Kalanchoë blossfeldiana[c]	Increased (31)			
Kalanchoë daigremontanum			Unchanged (32, 33)	
Kalanchoë pinnata	Increased (34)		Daytime uptake increased (34)	

[a] Seedlings of *Yucca schidigera* as used in this investigation are possibly facultatively C_3 (Huxman *et al*. 1998).
[b] *Arachnis hookeriana* × *Ascocenda* Madame Kenny.
[c] C_3–CAM intermediate.

References: (1) Graham & Nobel (1996), (2) Drennan & Nobel (1996), (3) Nobel & Hartsock (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_2 on the patterns of gas exchange differ. For most species, nocturnal CO_2 uptake is enhanced, while for others, daytime CO_2 uptake is increased with a concomitant decrease in the proportion of nocturnal CO_2 fixation (Table 1). Additionally, a number of changes occur in the morphology, anatomy, and biochemistry of CAM species under elevated atmospheric CO_2 concentrations (summarized in Table 1), which may contribute to their higher net CO_2 uptake rates and the increased biomass productivity as the atmospheric CO_2 concentration increases.

GROWTH AND BIOMASS

Biomass accumulation is enhanced by elevated atmospheric CO_2 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_2 concentrations of 650–750 μmol mol^{-1}. These increases are similar to the 33% increase averaged for some 430 C_3 crop species when the atmospheric CO_2 concentration is doubled (Kimball 1983). However, they exceed the approximately 10% increase in dry matter production for C_4 plants with a doubling in the atmospheric CO_2 concentration (Kimball 1983; Lawlor & Mitchell 1991; Poorter 1993), despite the similarity in initial fixation of CO_2 by PEPCase for both C_4 plants during the daytime and CAM plants at night. Thus the response to elevated atmospheric CO_2 concentrations cannot be predicted on the basis of the initial carboxylating enzyme alone, nor is the response saturated at atmospheric CO_2 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_2 saturation of PEPCase.

For *Opuntia ficus-indica*, the most-studied CAM plant with respect to the effect of elevated atmospheric CO_2 concentrations, stimulation of biomass accumulation also occurs as the atmospheric CO_2 concentration is increased from 520 to 720 μmol mol^{-1} (Cui *et al*. 1993). Similarly, increasing the atmospheric CO_2 concentration from 675 to 885 μmol mol^{-1} results in a 13% increase in growth rate for *Agave vilmoriniana* (Idso *et al*. 1986). Although a doubling of the atmospheric CO_2 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_2 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_2 concentrations are attributed to water-logging of the soil (Zhu, Goldstein & Bartholomew 1999).

Acclimatization to elevated atmospheric CO_2 concentrations occurs for some C_3 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_2 concentrations are not different from dry weight gains under the current atmospheric CO_2 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_2 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_2 concentrations as a percentage increase over controls maintained under ambient atmospheric CO_2 concentrations for the same period

Species	Biomass (% increase)	CO_2 (μmol mol^{-1})[a]	Duration (months)	Reference
Agave deserti	30	350–650	12	Nobel & Hartsock (1986)
	31	370–750	17	Graham & Nobel (1996)
Agave salmiana	17	370–730	4	Nobel *et al*. (1996)
Agave vilmoriniana	28	300–600	6	Idso *et al*. (1986)
Orchid 'Mokara Yellow'	80	350–10 000	2	Gouk *et al*. (1997)
	170	350–10 000	3	Gouk *et al*. (1997)
Aechmea magdalenae	36	354–712	3	Ziska *et al*. (1991)
Ananas comosus	–10	354–712	3	Ziska *et al*. (1991)
	23	330–730	4	Zhu *et al*. (1997a)
Ferrocactus acanthodes	30	350–650	12	Nobel & Hartsock (1986)
Opuntia ficus-indica	32	370–750	3	Cui & Nobel (1994)
	21	370–520	4·5	Cui *et al*. (1993)
	55	370–720	4·5	Cui *et al*. (1993)
	40	360–720	12	Nobel & Israel (1994)
Kalanchoë blossfeldiana	37	350–700	1·4	Mortensen & Moe (1992)
Kalanchoë pinnata	51	350/400–700/800	1·9	Winter *et al*. (1997)
	42	350/400–700/800	2·2	Winter *et al*. (1997)

[a]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_2 concentrations decreases with plant age for some C_3 species (Bazzaz *et al*. 1989), and elevated CO_2 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_2 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_2 concentration, but the growth rate subsequently decreases to levels similar to those for cladodes under the current atmospheric CO_2 concentration (Luo & Nobel 1993; North *et al*. 1995). The number of areoles per cladode for *O. ficus-indica* under different atmospheric CO_2 concentrations is similar (North *et al*. 1995), although cladode thickness is greater under elevated atmospheric CO_2 concentrations (Table 1). Nevertheless, the acceleration of development caused by elevated atmospheric CO_2 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_2 concentrations may have important ecosystem and especially agricultural consequences (Nobel 1996). In particular, the CAM plants *Agave mapisaga*, *Agave salmiana*, *Opuntia amyclaea* and *O. ficus-indica* may have an average annual dry mass productivity of 43 tons per hectare per year under the current CO_2 atmospheric concentration (Nobel 1991). This productivity exceeds the average annual above-ground dry-mass productivity of essentially all C_3 plants, and is exceeded by that of only a few C_4 species, notably *Saccharum officinarum* (sugar cane). Based on studies using plants in a field plot that received free-air CO_2 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 μmol mol^{-1}, increasing to 65 tons per hectare per year when the atmospheric CO_2 concentration is doubled (Nobel & Israel 1994), which has major implications for this widely cultivated species as the atmospheric CO_2 concentration increases.

MORPHOLOGY AND ANATOMY

Changes in the morphology and anatomy of photosynthetic organs caused by increasing atmospheric CO_2 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_2 concentrations (Table 1), a consistent effect of the higher atmospheric CO_2 concentrations is an increase in thickness of the photosynthetic organs. For *O. ficus-indica*, exposure to a doubled atmospheric CO_2 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_2 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_2 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_3 species (Thomas & Harvey 1983). An increase in chlorenchyma thickness under elevated atmospheric CO_2 concentrations may be related to a higher CO_2 concentration deeper in the leaf (Powles, Chapman & Osmond 1980). For the CAM species *Crassula argentea*, gradients of increasing $\delta^{13}C$ with increasing distance from the upper epidermis are consistent with a diffusion limitation on CO_2 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_2 concentrations (Zhu *et al*. 1997a). The associated increase in stem dry matter suggests that starch levels are increased under elevated atmospheric CO_2 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_2 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_2 uptake and WUE (Nobel 1999). The uptake of CO_2 and the water vapour conductance are also influenced by stomatal frequency, which decreases by 20% for *O. ficus-indica* under

elevated CO_2 concentrations, although stomatal pore length is unchanged (North *et al*. 1995). Stomatal frequency is lower under elevated CO_2 for some, but not all C_3 and C_4 species (Thomas & Harvey 1983; Rogers *et al*. 1994). A general decrease in stomatal frequency has accompanied the global rise in atmospheric CO_2 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_2 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_2 concentrations (Nobel *et al*. 1994a). On the other hand, epicuticular wax decreases by 40% for *A. deserti* under elevated atmospheric CO_2 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_2 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_2 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_2 concentrations; for example, ratios for *Ferocactus acanthodes*, *A. deserti* and *Agave salmiana* are similar under current and doubled CO_2 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_3 and C_4 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_2 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_2 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_2 concentration increases.

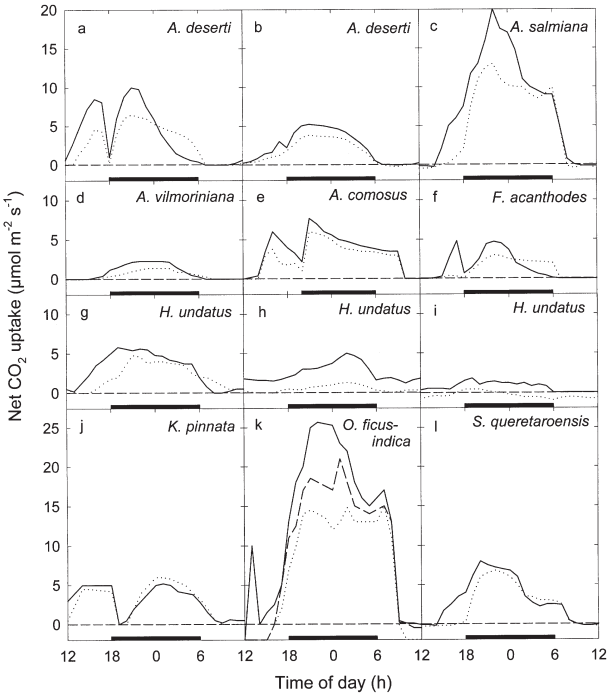

Figure 2. Daily time-courses of the net CO_2 uptake rate under the current atmospheric CO_2 concentration (dotted line) and, unless otherwise indicated, after acclimatization to a doubled atmospheric CO_2 concentration (solid line) under wet conditions for: (a) *Agave deserti*, 350 and 650 μmol mol^{-1} CO_2 (instantaneous increase), day/night air temperatures of 25/15 °C, PPF of 26 mol m^{-2} d^{-1} (Nobel & Hartsock 1986); (b) *A. deserti*, 370 and 750 μmol mol^{-1} CO_2, day/night air temperatures of 25/20 °C, PPF of 15 mol m^{-2} d^{-1} (Graham & Nobel 1996); (c) *Agave salmiana*, 360 and 720 μmol mol^{-1} CO_2, day/night air temperatures of 25/15 °C, PPF of 22 mol m^{-2} d^{-1} (Nobel 1996); (d) *Agave vilmoriniana*, 370 and 750 μmol mol^{-1} CO_2 (Szarek *et al*. 1987); (e) *Ananas comosus*, 350 and 700 μmol mol^{-1} CO_2, day/night air temperatures of 30/20 °C, PPF of 17 mol m^{-2} d^{-1} (Zhu *et al*. 1999); (f) *Ferocactus acanthodes*, 350 and 650 μmol mol^{-1} CO_2 (instantaneous increase), day/night air temperatures of 25/15 °C, PPF of 26 mol m^{-2} d^{-1} (Nobel & Hartsock 1986); (g) *Hylocereus undatus*, 370 and 740 μmol mol^{-1} CO_2, day/night air temperatures of 25/15 °C, PPF of 10 mol m^{-2} d^{-1} (Raveh *et al*. 1995); (h) *H. undatus*, 370 and 740 μmol mol^{-1} CO_2, day/night air temperatures of 25/15 °C, PPF of 10 mol m^{-2} d^{-1}, after 24 d of drought (Raveh *et al*. 1995); (i) *H. undatus*, 370 and 740 μmol mol^{-1} CO_2, day/night air temperatures of 45/35 °C, PPF of 10 mol m^{-2} d^{-1} (Raveh *et al*. 1995); (j) *Kalanchoë pinnata*, 340 and 680 μmol mol^{-1} CO_2 (instantaneous increase), day/night air temperatures of 25/20 °C, PPF of 22 mol m^{-2} d^{-1} (Winter & Engelbrecht 1994); (k) *Opuntia ficus-indica*, 370, 520 (dashed line), and 750 μmol mol^{-1} CO_2 (Cui *et al*. 1993); and (l) *Stenocereus queretaroensis*, 360 and 720 μmol mol^{-1} CO_2, day/night air temperatures of 25/15 °C, PPF of 22 mol m^{-2} d^{-1} (Nobel 1996).

GAS EXCHANGE

The net daily CO_2 uptake for most CAM species is enhanced at elevated atmospheric CO_2 concentrations (Table 1, Fig. 2). The percentage stimulation for plants representing a range of morphologies (i.e. epiphytes, stem succulents and leaf succulents) and maintained at elevated atmospheric CO_2 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_2 concentrations (Table 2). For example,

the biomass enhancement for *Ananas comosus* caused by doubling the atmospheric CO_2 concentration is 23% (Zhu *et al*. 1997b), and the increase in net daily CO_2 uptake is 15% (Fig. 2; Zhu *et al*. 1999). *Agave salmiana* shows a 59% increase in daily net CO_2 uptake under doubled atmospheric CO_2 and a concomitant 55% increase in biomass (Nobel *et al*. 1996).

The enhancement of daily net CO_2 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_2 uptake may occur over 17 months for both current and doubled atmospheric CO_2 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_2 uptake by elevated atmospheric CO_2 concentrations remains high. Similarly, total net daily CO_2 uptake for *O. ficus-indica* is still 35% higher after 1 year under doubled versus the current atmospheric CO_2 concentration (Nobel & Israel 1994). Thus for the species studied, acclimatization of CO_2 uptake to elevated atmospheric CO_2 concentrations does not seem to occur. Immediate exposure to a doubled atmospheric CO_2 concentration results in only a 2% increase in daily net CO_2 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_2 concentrations is maximized once certain physiological and/or morphological changes occur, such as the thickening of the chlorenchyma.

The daily pattern of CO_2 uptake is also altered under elevated atmospheric CO_2 concentrations (Fig. 2). For most species, afternoon (phase IV, Fig. 1) CO_2 uptake increases under elevated atmospheric CO_2 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_2 concentration, a marked increase in the maximum rate of CO_2 uptake occurs during this period. For example, for *A. comosus*, the maximum rate of CO_2 uptake in the afternoon is 85% greater under a doubled atmospheric CO_2 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_2 uptake result in an increase in the percentage contribution of daytime CO_2 uptake to the total daily net CO_2 uptake for CAM species under elevated atmospheric CO_2 concentrations (Table 3). Such responses are consistent with the effect of higher cellular CO_2 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_3 species to elevated atmospheric CO_2 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_2 concentration are indicative of increased Rubisco-mediated CO_2 uptake during the daytime (Zhu *et al*. 1999).

Less expected for CAM species is the increase in PEPCase-mediated uptake of CO_2 at night (phase I, Fig. 2) under elevated atmospheric CO_2 concentrations. PEPCase may be carbon-saturated at current atmospheric CO_2 concentrations (Ting 1994), and elevated atmospheric CO_2 concentrations minimally affect net CO_2 uptake by C_4 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_2 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_2 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_2 uptake under elevated atmospheric CO_2 concentrations and percentage contribution of daytime CO_2 uptake to total daily net CO_2 uptake for CAM plants under ambient (340–370 μmol mol^{-1}) and elevated (680–750 μmol mol^{-1}) atmospheric CO_2 concentrations

Species	Increase in total daily net CO_2 uptake (%)	Daytime net CO_2 uptake (% of total)		Reference
		Ambient CO_2	Elevated CO_2	
Agave deserti	2	22	38	Nobel & Hartsock (1986)
	49	17	24	Graham & Nobel (1996)
Agave salmiana	36	15	23	Nobel (1996), Nobel *et al*. (1996)
Ananas comosus	15	19	33	Zhu *et al*. (1999)
Hylocereus undatus	34	24	35	Raveh *et al*. (1995)
Opuntia ficus-indica				
basal cladodes	41–152	–4	17	Cui *et al*. (1993)
daughter cladodes	41–61	8	15	Cui *et al*. (1993)
Stenocereus queretaroensis	36	7	16	Nobel (1996)

daytime net CO_2 uptake under the current atmospheric CO_2 concentration (Table 3) and may even have net daytime losses (Cui *et al*. 1993). Under a doubled atmospheric CO_2 concentration, the contribution of daytime to total daily uptake rarely exceeds 17%, whereas this species can show a 100% increase in daily net CO_2 uptake (Table 3) and biomass increases of approximately 40% (Table 2). Indeed, the maximal rate of nocturnal CO_2 uptake for daughter cladodes was 43% higher at an atmospheric CO_2 concentration of 520 μmol mol^{-1} and 96% higher at 720 μmol mol^{-1} CO_2 compared with uptake rates at 360 μmol mol^{-1} CO_2 (Fig. 2), indicating that night-time uptake of CO_2 is far from being saturated at the current atmospheric CO_2 concentration for this CAM species. Lack of saturation of PEPCase at the current atmospheric CO_2 concentration possibly results from low diffusion conductances leading to low internal CO_2 concentrations (Nobel 1999). However, for some CAM species, e.g. *A. comosus*, enhanced night-time CO_2 uptake under a doubled atmospheric CO_2 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_2 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_2 concentration (Fig. 2a,f), which increases the maximum rate of CO_2 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_2 concentration, resulting in only small net gains in total daily net CO_2 uptake. Such patterns are probably associated with the insufficiency of a CO_2 acceptor or a filled malate pool in plants that have not fully adapted to the elevated atmospheric CO_2 concentration through longer-term exposure. For some species, e.g. *Clusia uvitana* and *Portulacaria afra*, decreases in nocturnal net CO_2 uptake occur in response to short-term exposure to elevated atmospheric CO_2 concentrations (Huerta & Ting 1988; Winter *et al*. 1992). Both species are C_3–CAM intermediates that exhibit extensive daytime net CO_2 uptake. For *Mesembryanthemum crystallinum* and *P. afra*, the switch to CAM photosynthesis cannot be induced by manipulating the atmospheric CO_2 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_2 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_2 uptake by elevated atmospheric CO_2 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_2 uptake increases as the PPF increases from 5 to 20 mol m^{-2} d^{-1} under both current and doubled atmospheric CO_2 concentrations. For these three species, the enhancement of CO_2 uptake by elevated atmospheric CO_2 concentrations is greatest for 10–20 mol m^{-2} d^{-1}, a PPF range that may allow maximal response of both daytime and night-time CO_2 uptake to the elevated atmospheric CO_2 concentration. When CAM plants are exposed to water stress or high temperatures, net CO_2 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_2 uptake at only 10–20 mol m^{-2} d^{-1} under the current atmospheric CO_2 concentration, increasing photoinhibition of C_3 photosynthesis with increasing PPF and a concomitant decrease in daytime CO_2 uptake is partially offset by the enhancement of CO_2 uptake by the elevated CO_2 concentration (Raveh *et al*. 1995).

For *A. deserti*, *H. undatus* and *O. ficus-indica*, total daily net CO_2 uptake decreases with increasing duration of drought under both current and elevated atmospheric CO_2 concentrations (Nobel & Israel 1994; Raveh *et al*. 1995; Graham & Nobel 1996). However, the rate of decrease is greater under the current atmospheric CO_2 concentration, e.g. for *O. ficus-indica*, 25, 50 and 75% decreases in total daily net CO_2 uptake occur at 17, 23 and 33 d of drought, respectively, under the current atmospheric CO_2 concentration and at 23, 31 and 40 d, respectively, under a doubled CO_2 concentration. Concomitantly, the percentage enhancement of daily net CO_2 uptake under elevated atmospheric CO_2 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_2 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_2 concentration but still occurs under a doubled atmospheric CO_2 concentration (Nobel & Israel 1994).

The increased ability to withstand drought for CAM species under elevated atmospheric CO_2 concentrations is associated with an increased WUE (Table 4), as has been found for C_3 and C_4 species (Eamus 1991; Ham *et al*. 1995; Jarvis, Mansfield & Davies 1999). The enhancement under a doubled CO_2 concentration occurs despite the greater contribution of daytime CO_2 uptake, which is less water-use efficient, to the total net daily CO_2 uptake. For *A. deserti*, *A. comosus* and *O. ficus-indica*, the increase in WUE is due both to increased daily net CO_2 uptake at elevated atmospheric CO_2 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_2 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_2 uptake to occur for longer periods during drought (Raveh *et al*. 1995; Graham & Nobel 1996).

For both current and elevated atmospheric CO_2 concentrations, maximum daily net CO_2 uptake for many CAM

Table 4. Water-use efficiencies (WUE) for CAM plants under ambient (350–370 μmol mol^{-1}) and elevated (700–750 μmol mol^{-1}) atmospheric CO_2 concentrations

Species	WUE (mmol CO_2 mol^{-1} H_2O)		Reference
	Ambient CO_2	Elevated CO_2	
Agave deserti	20	42	Graham & Nobel (1996)
Ananas comosus	9·5	13	Zhu *et al*. (1999)
Opuntia ficus-indica			
basal cladodes	4	7	Cui *et al*. (1993)
daughter cladodes	10	16	Cui *et al*. (1993)

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_2, but the reduction is generally greater at current than at elevated atmospheric CO_2 concentrations. For *H. undatus* (Fig. 2i), daytime net CO_2 uptake becomes negative at a day/night air temperature of 45/35 °C (Raveh *et al*. 1995), the latter reflecting the sensitivity of C_3 photosynthesis to high temperatures. Increases in leaf temperature increase the O_2:CO_2 ratio at the site of CO_2 fixation via Rubisco, thus increasing photorespiration (Taiz & Zeiger 1998). Elevated atmospheric CO_2 concentrations can offset this increase and may thus contribute to the large percentage enhancement in total daily net CO_2 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_2 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_2 and increases in other greenhouse gases (Kattenberg *et al*. 1996).

For CAM species at the current atmospheric CO_2 concentration, respiration becomes more important at high temperatures (Kluge & Ting 1978). Relatively little is known about the effect of elevated atmospheric CO_2 concentrations on their respiration. In any case, elevated atmospheric CO_2 concentrations may reduce mitochondrial/dark respiration, as occurs for C_3 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_2 concentration (Table 5; Szarek *et al*. 1987; Nobel *et al*. 1994a; Graham & Nobel 1996). Similar decreases occur in C_3 species and suggest increased photosynthetic efficiency (Wullschleger, Norby & Hendrix 1992; Moore *et al*. 1998). For *O. ficus-indica*, the chlorophyll *a*:*b* ratio is 9% lower under a doubled CO_2 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_2 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_2 concentrations. In particular, increased cuticular thickness and decreased transmission of PPF occur for *O. ficus-indica* as the atmospheric CO_2 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_2 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_2 concentrations in photosynthetic shoots and roots (Gouk *et al*. 1997). Many C_3 species show decreases in Rubisco content or activities in response to elevated atmospheric CO_2 concentrations (Drake, Gonzàlez-Meler & Long 1997), potentially resulting in acclimatization to the higher CO_2 concentrations (Bowes 1993; Moore *et al*. 1999). However, transforming the C_3 *Nicotiana tabacum* such that Rubisco production is decreased approximately 15% reduces net carbon uptake at the current but not at a doubled atmospheric CO_2 concentration, indicating a reduced requirement for Rubisco at elevated atmospheric CO_2 concentrations (Masle, Hudson & Badger 1993). Furthermore, as CO_2 is an activator as well as a substrate for Rubisco (Lorimer, Badger & Andrews 1976), increases in internal CO_2 concentrations in response to elevated atmospheric CO_2 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 μmol mol^{-1}) and doubled (720–750 μmol mol^{-1}) atmospheric CO_2 concentrations

Species	Current CO_2	Doubled CO_2	Reference
Chlorophyll content			
Agave deserti (g m^{-2})	0·80	0·64	Graham & Nobel (1996)
Agave vilmoriniana (μg g^{-1} FW)	132	116	Szarek *et al*. (1987)
Opuntia ficus-indica (g m^{-2})	0·65	0·52	Nobel *et al*. (1994a)
O. ficus-indica (μg g^{-1} FW)	320	280	Nobel *et al*. (1994a)
Chlorophyll *a*:*b*			
A. deserti	2·3	2·6	Graham & Nobel (1996)
A. vilmoriniana	2·5	2·8	Szarek *et al*. (1987)
O. ficus-indica	3·2	2·9	Nobel *et al*. (1994a)
PEPCase (μmol m^{-2} s^{-1})			
A. deserti	55	37	Graham & Nobel (1996)
O. ficus-indica	32	20	Nobel *et al*. (1994a)
Rubisco (μmol m^{-2} s^{-1})			
A. deserti	40	35	Graham & Nobel (1996)
Agave salmiana	28	23	Nobel *et al*. (1996)
O. ficus-indica	41	29	Nobel *et al*. (1994a)
O. ficus-indica	34	29	Nobel *et al*. (1996)
Activated:total Rubisco (% of total)			
A. desert	59	74	Graham & Nobel (1996)
A. salmiana	54	89	Nobel *et al*. (1996)
O. ficus-indica	53	88	Nobel *et al*. (1996)
K_M for PEPCase (mol m^{-3} HCO_3^-)			
A. salmiana	0·39	0·33	Nobel *et al*. (1996)
O. ficus-indica	0·48	0·27	Nobel *et al*. (1996)
K_M for Rubisco (mmol m^{-3} CO_2)			
A. salmiana	25	26	Nobel *et al*. (1996)
O. ficus-indica	23	25	Nobel *et al*. (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 CO_2 uptake.

For *A. deserti* and *O. ficus-indica*, the decreases in PEPCase activity under a doubled atmospheric 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 CO_2 concentration results in 15 and 44% decreases in the apparent $K_M(HCO_3^-)$ for PEPCase for *A. salmiana* and *O. ficus-indica*, respectively (Table 5). In this regard, the $K_M(HCO_3^-)$ for PEPCase from *Zea mays* decreases with decreasing pH (O'Leary 1982).

The $K_M(CO_2)$ for Rubisco does not change for *A. salmiana* or *O. ficus-indica* in response to a doubling of the atmospheric CO_2 concentration (Table 5; Nobel *et al*. 1996). This is consistent with results for *Gossypium hirsutum* (C_3) and *Z. mays* (C_4), whose $K_M(CO_2)$ for Rubisco is similar for plants grown from seed at 330 and 640 μmol mol^{-1} CO_2 (Yeoh, Badger & Watson 1981). The $K_M(CO_2)$ for Rubisco is unchanged by CO_2 enrichment for other C_3 species as well (Campbell, Allen & Bowes 1988). The $K_M(CO_2)$ for Rubisco for *A. salmiana* and *O. ficus-indica* is in the upper part of the range for C_3 and CAM species (12–25 mmol m^{-3}; Yeoh *et al*. 1981), but less than values for C_4 species (28–34 mmol m^{-3}; Yeoh *et al*. 1981). The difference in $K_M(CO_2)$ between the C_3 and C_4 species may reflect the strong CO_2-concentrating mechanism of the C_4 type (Yeoh *et al*. 1981). The lower $K_M(CO_2)$ for Rubisco from CAM species may indicate a lower CO_2 concentration at the site of the enzyme than for C_4 species, which may in part explain why CAM species show a greater response to elevated atmospheric CO_2 concentrations than do C_4 species. Indeed, *Kalanchoë pinnata*, whose stimulated growth under elevated atmospheric CO_2 concentrations is attributed to increases in daytime CO_2 uptake (Winter *et al*. 1997), has a $K_M(CO_2)$ for Rubisco of 15 mmol m^{-3} (Yeoh *et al*. 1981).

Malate

The majority of CAM species subjected to elevated atmospheric CO_2 concentrations for extended periods show a night-time increase in titratable acidity or malate accumulation (Table 6). This indicates increased night-time CO_2

fixation (Griffiths 1988) and is consistent with the increase in phase I CO_2 uptake (Fig. 1) and lack of saturation of phase I at the current atmospheric CO_2 concentration. The lack of response of malic acid accumulation in *K. pinnata* to elevated atmospheric CO_2 concentrations possibly reflects the fact that biomass enhancement for this species is due to increased daytime net CO_2 uptake (Winter *et al*. 1997). Although *Kalanchoë daigremontiana* shows similar nocturnal malate accumulation at 300 μmol mol^{-1} CO_2 and in response to a short-term exposure to 1000 μmol mol^{-1} CO_2, the rate of accumulation is faster at the elevated atmospheric CO_2 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_2 concentration (Holtum *et al*. 1983). Plants subjected to short-term increases in atmospheric CO_2 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_2 concentrations, although whether these plants had become acclimatized to the higher CO_2 concentrations in their greenhouse environment is not known (Nowak & Martin 1995).

Sugars and photosynthate partitioning

Acclimatization to elevated atmospheric CO_2 concentrations, as is observed for many species and especially for C_3 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_2 concentration (Wang & Nobel 1995), which may help explain the large stimulatory effect of a doubled atmospheric CO_2 concentration on its net CO_2 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_2 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_2 concentrations do not lower the photosynthetic rates, as occurs for some C_3 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_3 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_2 concentrations, despite 146% increases in the activity of sucrose-Pi synthase under a doubled atmospheric CO_2 concentration (Wang & Nobel 1996). However, increases in phloem transport to developing daughter cladodes (sinks) under a doubled atmospheric CO_2 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_2 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_2 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_2 concentrations as a percentage increase over controls maintained under the current atmospheric CO_2 concentration for the same period

Species	Elevated CO_2 concentration (μmol mol^{-1})	Duration of treatment	Nocturnal acidity (% increase)	Reference
Agave vilmoriniana	752	Long-term	7·5 to 12	Szarek *et al*. (1987)
Ananas comosus	730	Long-term	34	Zhu *et al*. (1997a)
Orchid "Mokara Yellow"	10 000	Long-term	23 to 100	Gouk *et al*. (1997)
Tillandsia ionantha	800	Short-term	50	Nowak & Martin (1995)
Opuntia ficus-indica	750	Long-term	22	Wang & Nobel (1996)
Portulacaria afra	950	Short-term	0	Huerta & Ting (1988)
Crassula arborescens	920	Short-term	50	Nowak & Martin (1995)
Kalanchoë daigremontiana	1000	Short-term	0 to –25	Holtum *et al*. (1983)
Kalanchoë pinnata	750	Long-term	–8 to –11	Winter *et al*. (1997)

pheric CO_2 concentrations sustains growth of the daughter cladodes.

Despite a 175% increase in glucose levels in the basal cladodes under a doubled atmospheric CO_2 concentration, the concentrations of glucose (1 mol m^{-3}) and sucrose (5 mol m^{-3}) 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_3 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_2 concentrations, and the activity of hexokinase is slightly decreased under a doubled atmospheric CO_2 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_2 concentrations without acclimatization of photosynthesis.

CONCLUSIONS

Predicting the response of CAM species to elevated atmospheric CO_2 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_3 and C_4 species, variation of response also occurs for these photosynthetic types (Lawlor & Mitchell 1991; Poorter 1993). Doubling the atmospheric CO_2 concentration greatly stimulates net CO_2 uptake by the CAM species studied (Tables 1 and 3), despite the suggestion that the predominant initial fixation of CO_2 by PEPCase in CAM plants might be saturated at the current atmospheric CO_2 concentration (Ting 1994; Winter & Engelbrecht 1994). The accompanying biomass increase, which reflects increases in both daytime and nighttime net CO_2 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_2 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_3 species. However, unlike some C_3 species, especially annuals, there is little evidence of acclimatization to elevated atmospheric CO_2 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_2 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_2 uptake may contribute to an understanding of why that process is not limited at close to the current atmospheric CO_2 concentration. Whether the $K_M(HCO_3^-)$ for PEPCase decreases in other CAM species exposed to elevated atmospheric CO_2 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_2 concentrations could establish whether acclimatization occurs after years, as well as whether effects on reproduction occur. Furthermore, the response of C_3–CAM intermediates to elevated atmospheric CO_2 concentrations has been little investigated, beyond the apparent insensitivity to atmospheric CO_2 concentrations of the switch from C_3 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_2 uptake and of productivity in response to elevated atmospheric CO_2 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 & Garcia de Cortázar 1991; Nobel 1996), could affect land-use practices with regard to mitigating rising atmospheric CO_2 concentrations. In particular, the international protocol discussed in Kyoto, Japan, in December 1997 pledges countries to work towards a stabilization of atmospheric CO_2 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_2 concentrations, suggests their consideration with regard to terrestrial sequestration of atmospheric CO_2 in arid and semi-arid regions. Such regions, which are poorly suited to C_3 and C_4 crops without irrigation, occupy about 30% of the earth's land area. Moreover, the substantial biomass increases for CAM species under elevated atmospheric CO_2 concentrations could enhance the importance of such native plants in ecosystems worldwide.

REFERENCES

Adams P., Nelson D.E., Shigehiro Y., Chmara W., Jensen R.G., Bohnert H.J. & Griffiths H. (1998) Tansley Review No. 97.

Growth and development of *Mesembryanthemum crystallinum* (Aizoaceae). *New Phytologist* **138,** 171–190.

Amthor J.S. (1991) Respiration in a future, higher CO_2 world. *Plant, Cell and Environment* **14,** 13–20.

Arp W.J. (1991) Effects of source-sink relations on photosynthetic acclimation to elevated carbon dioxide. *Plant, Cell and Environment* **14,** 869–876.

Bartholomew D.P. & Paull R. (1986) Pineapple. In *Handbook of Fruit Set and Development* (ed. S.P. Monselise), pp. 371–388. CRC Press, Boca Raton, Florida.

Bastide B., Sipes D., Hann J. & Ting I.P. (1993) Effect of severe water stress on aspects of Crassulacean acid metabolism in *Xerosicyos*. *Plant Physiology* **103,** 1089–1096.

Bazzaz F.A., Garbutt K., Reekie E.G. & Williams W.E. (1989) Using growth analysis to interpret competition between a C_3 and C_4 annual under ambient and elevated CO_2. *Oecologia* **79,** 223–235.

Beerling D.J. & Woodward F.I. (1993) Ecophysiological responses of plants to global environmental change since the last glacial maximum. *New Phytologist* **125,** 641–648.

Beerling D.J., McElwain J.C. & Osborne C.P. (1998) Stomatal responses of the 'living fossil' *Ginkgo biloba* L. to changes in atmospheric CO_2 concentrations. *Journal of Experimental Botany* **49,** 1603–1607.

Black C.C. (1993) Sink strength: is it real, or measurable? *Plant, Cell and Environment* **16,** 1037–1038.

Bowes G. (1991) Growth at elevated CO_2: photosynthetic responses mediated through Rubisco. *Plant, Cell and Environment* **14,** 795–806.

Bowes G. (1993) Facing the inevitable: plants and increasing atmospheric CO_2. *Annual Review of Plant Physiology and Plant Molecular Biology* **44,** 309–332.

Brulfert J., Müller M., Kluge M. & Queiroz O. (1982) Photoperiodism and Crassulacean acid metabolism. *Planta* **154,** 326–331.

Campbell W.J., Allen L.H. Jr & Bowes G. (1988) Effects of CO_2 concentration on Rubisco activity, amount and photosynthesis in soybean leaves. *Plant Physiology* **88,** 1310–1316.

Crewes C.E., Vines H.M. & Black C.C. (1975) Postillumination burst of carbon dioxide in Crassulacean acid metabolism plants. *Plant Physiology* **55,** 652–657.

Cui M. & Nobel P.S. (1994) Gas exchange and growth responses to elevated CO_2 and light levels in the CAM species *Opuntia ficus-indica*. *Plant, Cell and Environment* **17,** 935–944.

Cui M., Miller P.M. & Nobel P.S. (1993) CO_2 exchange and growth of the Crassulacean acid metabolism plant *Opuntia ficus-indica* under elevated CO_2 in open-top chambers. *Plant Physiology* **103,** 519–524.

Cushman J.C. & Bohnert H.J. (1999) Crassulacean acid metabolism: molecular genetics. *Annual Review of Plant Physiology and Plant Molecular Biology* **50,** 305–332.

Drake B.G., Gonzàlez-Meler M.A. & Long S.P. (1997) More efficient plants: a consequence of rising atmospheric CO_2? *Annual Review of Plant Physiology and Plant Molecular Biology* **48,** 609–639.

Drake B.G., Azcon-Bieto J., Berry J., *et al.* (1999) Does elevated atmospheric CO_2 concentration inhibit mitochondrial respiration in green plants? *Plant, Cell and Environment* **22,** 649–657.

Drennan P.M. & Nobel P.S. (1996) Temperature influences on root growth for *Encelia farinosa* (Asteraceae), *Pleuraphis rigida* (Poaceae), and *Agave deserti* (Agavaceae) under current and doubled CO_2 concentrations. *American Journal of Botany* **83,** 133–139.

Drennan P.M. & Nobel P.S. (1998) Root growth dependence on soil temperature for *Opuntia ficus-indica*: influences of air temperature and a doubled CO_2 concentration. *Functional Ecology* **12,** 959–964.

Eamus D. (1991) The interaction of rising CO_2 and temperatures with water use efficiency. *Plant, Cell and Environment* **14,** 843–852.

Ehleringer J.R. & Monson R.K. (1993) Evolutionary and ecological aspects of photosynthetic pathway variation. *Annual Review of Ecology and Systematics* **24,** 411–439.

Goldschmidt E.E. & Huber S.C. (1992) Regulation of photosynthesis by end-product accumulation in leaves of plants storing starch, sucrose, and hexose sugars. *Plant Physiology* **99,** 1443–1448.

Gouk S.S., Yong J.W.H. & Hew C.S. (1997) Effects of super-elevated CO_2 on the growth and carboxylating enzymes in an epiphytic CAM orchid plantlet. *Journal of Plant Physiology* **151,** 129–136.

Gouk S.S., He J. & Hew C.S. (1999) Changes in photosynthetic capability and carbohydrate production in an epiphytic CAM orchid plantlet exposed to super-elevated CO_2. *Environmental and Experimental Botany* **41,** 219–230.

Graham E.A. & Nobel P.S. (1996) Long-term effects of a doubled atmospheric CO_2 concentration on the CAM species *Agave deserti*. *Journal of Experimental Botany* **47,** 61–69.

Griffiths H. (1988) Crassulacean acid metabolism: a re-appraisal of physiological plasticity in form and function. *Advances in Botanical Research* **15,** 43–92.

Griffiths H. & Smith J.A.C. (1983) Photosynthetic pathways in the Bromeliaceae of Trinidad: relations between life-forms, habitat preference and the occurrence of CAM. *Oecologia* **60,** 176–184.

Guralnick L.J., Rorabaugh P.A. & Hanscom Z. (1984) Seasonal shifts of photosynthesis in *Portulacaria afra* (L.) Jacq. *Plant Physiology* **76,** 643–646.

Ham J.M., Owensby C.E., Coyne P.I. & Bemner D.J. (1995) Fluxes of CO_2 and water vapour from a prairie ecosystem exposed to ambient and elevated atmospheric CO_2. *Agricultural and Forest Meteorology* **77,** 73–93.

Heineke D., Kauder F., Frommer W., Kühn C., Gillissen B., Ludewig F. & Sonnewald U. (1999) Application of transgenic plants in understanding responses to atmospheric change. *Plant, Cell and Environment* **22,** 623–628.

Hew C.S., Hin S.E., Yong J.W.H., Gouk S.S. & Tanaka M. (1995) In vitro CO_2 enrichment of CAM orchid plantlets. *Journal of Horticultural Science* **70,** 721–736.

Ho L.C. (1988) Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. *Annual Review of Plant Physiology and Plant Molecular Biology* **39,** 355–378.

Holtum J.A.M., O'Leary M.H. & Osmond C.B. (1983) Effect of varying CO_2 partial pressure on photosynthesis and on carbon isotope composition of carbon-4 of malate from the Crassulacean acid metabolism plant *Kalanchoë daigremontiana* Hamet et Perr. *Plant Physiology* **71,** 602–609.

Huerta A.J. & Ting I.P. (1988) Effects of various levels of CO_2 on the induction of Crassulacean acid metabolism in *Portulacaria afra* (L.) Jacq. *Plant Physiology* **88,** 183–188.

Huxman T.E., Hamerlynck E.P., Loik M.E. & Smith S.D. (1998) Gas exchange and chlorophyll fluorescence responses of three south-western *Yucca* species to elevated CO_2 and high temperature. *Plant, Cell and Environment* **21,** 1275–1283.

Idso S.B., Kimball B.A., Anderson M.G. & Szarek S.R. (1986) Growth response of a succulent plant, *Agave vilmoriniana*, to elevated CO_2. *Plant Physiology* **80,** 796–797.

Israel A.A. & Nobel P.S. (1994) Activities of carboxylating enzymes in the CAM species *Opuntia ficus-indica* grown under current and elevated CO_2 concentrations. *Photosynthesis Research* **40,** 223–229.

Jarvis A.J., Mansfield T.A. & Davies W.J. (1999) Stomatal behaviour, photosynthesis and transpiration under rising CO_2. *Plant, Cell and Environment* **22,** 639–648.

Jenner C.F. & Hawker J.S. (1993) Sink strength: soluble starch synthase as a measure of sink strength in wheat endosperm. *Plant, Cell and Environment* **16,** 1023–1024.

Kattenberg A., Giorgi F., Grassl H., Meehl G.A., Mitchell J.F.B., Stouffer R.J., Tokioka T., Weaver A.J. & Wigley T.M.L. (1996) Climate models – projections of future climate. In *Climate Change 1995* (eds J.T. Houghton, L.G. Meira Filho, B.A. Callender, N. Harris, A. Kattenberg & K. Maskell), pp. 285–357. IPCC, Cambridge University Press, Cambridge.

Kimball B.A. (1983) Carbon dioxide and agricultural yield: an assemblage and analysis of 430 prior observations. *Agronomy Journal* **75,** 779–788.

Kluge M. & Ting I.P. (1978) *Crassulacean Acid Metabolism. Analysis of an Ecological Adaptation*. Springer, Berlin.

Lawlor D.W. & Mitchell R.A.C. (1991) The effects of increasing CO_2 on crop photosynthesis and productivity: a review of field studies. *Plant, Cell and Environment* **14,** 807–818.

Lecain D.R. & Morgan J.A. (1998) Growth, gas exchange, leaf nitrogen and carbohydrate concentrations in NAD-ME and NADP-ME C_4 grasses grown in elevated CO_2. *Physiologia Plantarum* **102,** 297–306.

Loehle C. (1995) Anomalous responses of plants to CO_2 enrichment. *Oikos* **73,** 181–187.

Lorimer G.H., Badger M.R. & Andrews T.J. (1976) The activation of ribulose-1,5-bisphosphate carboxylase by carbon dioxide and magnesium ions. Equlibria, kinetics, a suggested mechanism, and physiological implications. *Biochemistry* **15,** 529–536.

Luo Y. & Nobel P.S. (1993) Growth characteristics of newly initiated cladodes of *Opuntia ficus-indica* as affected by shading, drought and elevated CO_2. *Physiologia Plantarum* **87,** 467–474.

Lüttge U. (1996) *Clusia*: plasticity and diversity in a genus of C_3/CAM intermediate tropical trees. In *Crassulacean Acid Metabolism: Biochemistry, Ecophysiology and Evolution* (eds K. Winter & J.A.C. Smith), pp. 296–311. Springer, Berlin.

Martin C.E., Higley M. & Wang W.-Z. (1988) The ecophysiological significance of CO_2-recycling via crassulacean acid metabolism in *Talinum calycinum* Engelm. (Portulacaceae). *Plant Physiology* **86,** 562–568.

Masle J., Hudson G.S. & Badger M.R. (1993) Effects of ambient CO_2 concentration on growth and nitrogen use in tobacco (*Nicotiana tabacum*) plants transformed with an antisense gene to the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase. *Plant Physiology* **103,** 1075–1088.

Miller A., Tsai C.-H., Hemphill D., Endres M., Rodermel S. & Spalding M. (1997) Elevated CO_2 effects during leaf ontogeny. *Plant Physiology* **115,** 1195–1200.

Monson R.K. (1989) On the evolutionary pathways resulting in C_4 photosynthesis and Crassulacean acid metabolism (CAM). *Advances in Ecological Research* **19,** 57–110.

Moore B.D., Cheng S.H., Rice J. & Seemann J.R. (1998) Sucrose cycling, Rubisco expression, and prediction of photosynthetic acclimation to elevated atmospheric CO_2. *Plant, Cell and Environment* **21,** 905–915.

Moore B.D., Cheng S.H., Sims D. & Seemann J.R. (1999) The biochemical and molecular basis for photosynthetic acclimation to elevated atmospheric CO_2. *Plant, Cell and Environment* **22,** 567–582.

Morison J.I.L. & Lawlor D.W. (1999) Interactions between increasing CO_2 concentration and temperature on plant growth. *Plant, Cell and Environment* **22,** 659–682.

Mortensen L.M. & Moe R. (1992) Effects of CO_2 enrichment and different day/night temperature combinations on growth and flowering of *Rosa* L. & *Kalanchoe blossfeldiana* v. Poelln. *Scientia Horticulturae* **51,** 145–153.

Neftel A., Moor E., Oeschger H. & Stauffer B. (1985) Evidence from polar ice cores for the increase in atmospheric CO_2 in the past two centuries. *Nature* **315,** 45–47.

Nobel P.S. (1988) *Environmental Biology of Agaves and Cacti*. Cambridge University Press, New York.

Nobel P.S. (1991) Tansley Review No. 32. Achievable productivities of CAM plants: basis for high values compared with C_3 and C_4 plants. *New Phytologist* **119,** 183–205.

Nobel P.S. (1996) Responses of some North American CAM plants to freezing temperatures and doubled CO_2 concentrations: implications of global climate change for extending cultivation. *Journal of Arid Environments* **34,** 187–196.

Nobel P.S. (1999) *Physicochemical and Environmental Plant Physiology*, 2nd edn. Academic Press, San Diego.

Nobel P.S. & Garcia de Cortázar V. (1991) Growth and predicted productivity of *Opuntia ficus-indica* for current and elevated carbon dioxide. *Agronomy Journal* **83,** 224–230.

Nobel P.S. & Hartsock T.L. (1986) Short-term and long-term responses of crassulacean acid metabolism plants to elevated CO_2. *Plant Physiology* **82,** 604–606.

Nobel P.S. & Israel A.A. (1994) Cladode development, environmental responses of CO_2 uptake, and productivity for *Opuntia ficus-indica* under elevated CO_2. *Journal of Experimental Botany* **45,** 295–303.

Nobel P.S. & North G.B. (1996) Features of roots of CAM plants. In *Crassulacean Acid Metabolism: Biochemistry, Ecophysiology and Evolution* (eds K. Winter & J.A.C. Smith), pp. 266–280. Springer, Berlin.

Nobel P.S., Cui M. & Israel A.A. (1994a) Light, chlorophyll, carboxylase activity and CO_2 fixation at various depths in the chlorenchyma of *Opuntia ficus-indica* (L.) Miller under current and elevated CO_2. *New Phytologist* **128,** 315–322.

Nobel P.S., Cui M., Miller P.M. & Luo Y. (1994b) Influences of soil volume and an elevated CO_2 level on growth and CO_2 exchange for the Crassulacean acid metabolism plant *Opuntia ficus-indica*. *Physiologia Plantarum* **90,** 173–180.

Nobel P.S., Israel A.A. & Wang N. (1996) Growth, CO_2 uptake, and responses of the carboxylating enzymes to inorganic carbon in two highly productive CAM species at current and doubled CO_2 concentrations. *Plant, Cell and Environment* **19,** 585–592.

North G.B., Moore T.L. & Nobel P.S. (1995) Cladode development for *Opuntia ficus-indica* (Cactaceae) under current and doubled CO_2 concentrations. *American Journal of Botany* **82,** 159–166.

Nowak E.J. & Martin C.E. (1995) Effect of elevated CO_2 on nocturnal malate accumulation in the CAM species *Tillandsia ionantha* and *Crassula arborescens*. *Photosynthetica* **31,** 441–444.

O'Leary M.H. (1982) Phosphoenolpyruvate carboxylase: an enzymologist's view. *Annual Review of Plant Physiology* **33,** 297–315.

Osmond C.B. (1978) Crassulacean acid metabolism: a curiosity in context. *Annual Review of Plant Physiology* **29,** 379–414.

Osmond C.B. & Björkman, O. (1975) Pathways of CO_2 fixation in the CAM plant *Kalanchoë daigremontiana* II. Effects of O_2 and CO_2 concentration on light and dark CO_2 fixation. *Australian Journal of Plant Physiology* **2,** 155–162.

Poorter H. (1993) Interspecific variation in the growth response of plants to elevated ambient CO_2 concentration. *Vegetatio* **104/105,** 77–97.

Powles S.B., Chapman K.S.R. & Osmond C.B. (1980) Photoinhibition of intact attached leaves of C_4 plants: dependence on CO_2 and O_2 partial pressures. *Australian Journal of Plant Physiology* **7,** 737–747.

Raveh E., Gersani M. & Nobel P.S. (1995) CO_2 uptake and fluorescence for a shade-tolerant cactus *Hylocerus undatus* under

current and doubled CO_2 concentrations. *Physiologia Plantarum* **93,** 505–511.

Robinson S.A., Osmond C.B. & Giles L. (1993) Interpretations of gradients in $\delta^{13}C$ value in thick photosynthetic tissues of plants with Crassulacean acid metabolism. *Planta* **190,** 271–276.

Rogers H.H., Runion G.B. & Krupa S.V. (1994) Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution* **83,** 155–189.

Rogers H.H., Prior S.A., Runion G.B. & Mitchell R.J. (1996) Root to shoot ratio of crops as influenced by CO_2. *Plant and Soil* **187,** 229–248.

Schimel D., Ives D., Enting I., Heimann M., Joos F., Raynaud D. & Wigley T. (1996) CO_2 and the carbon cycle. In *Climate Change 1995* (eds J.T. Houghton, L.G. Meira Filho, B.A. Callendar, N. Harris, A. Kattenberg & K. Maskell), pp. 65–131. IPCC, Cambridge University Press, Cambridge.

Servaites J.C., Geiger D.R., Tucci M.A. & Fondy B.R. (1989) Leaf carbon metabolism and metabolite levels during a period of sinusoidal light. *Plant Physiology* **89,** 403–408.

Sheen J. (1994) Feedback control of gene expression. *Photosynthesis Research* **39,** 427–438.

Smith J.A.C. (1989) Epiphytic bromeliads. In *Vascular Plants as Epiphytes: Evolution and Ecophysiology* (ed. U. Lüttge), pp. 109–138. Springer, Berlin.

Smith J.A.C. & Winter K. (1996) Taxonomic distribution of Crassulacean acid metabolism. In *Crassulacean Acid Metabolism: Biochemistry, Ecophysiology and Evolution* (eds K. Winter & J.A.C. Smith), pp. 427–436. Springer, Berlin.

Stitt M. (1991) Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. *Plant, Cell and Environment* **14,** 741–762.

Stitt M. & Krapp A. (1999) The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant, Cell and Environment* **22,** 583–621.

Szarek S.R., Holthe P.A. & Ting I.P. (1987) Minor physiological response to elevated CO_2 by the CAM plant *Agave vilmoriniana*. *Plant Physiology* **83,** 938–940.

Taiz L. & Zeiger E. (1998). *Plant Physiology*, 2nd edn. Sinauer, Sunderland, Massachusetts.

Teeri J.A., Tonsor S.J. & Turner M. (1981) Leaf thickness and carbon isotope composition in the Crassulaceae. *Oecologia* **50,** 367–369.

Thomas J.F. & Harvey C.N. (1983) Leaf anatomy of four species grown under continuous CO_2 enrichment. *Botanical Gazette* **144,** 303–309.

Ting I.P. (1994) CO_2 and Crassulacean acid metabolism plants: a review. In *Regulation of Atmospheric CO_2 and O_2 by Photosynthetic Carbon Metabolism* (eds N.E. Tolbert & J. Preiss), pp. 176–183. Oxford University Press, New York.

Ting I.P., Patel A., Kauer S., Hann J. & Walling L. (1996) Ontogenetic development of Crassulacean acid metabolism as modified by water stress in *Peperomia*. In *Crassulacean Acid Metabolism: Biochemistry, Ecophysiology and Evolution* (eds K. Winter & J.A.C. Smith), pp. 204–215. Springer, Berlin.

Wang N. & Nobel P.S. (1995) Phloem exudate collected via scale insect stylets for the CAM species *Opuntia ficus-indica* under current and doubled CO_2 concentrations. *Annals of Botany* **75,** 525–532.

Wang N. & Nobel P.S. (1996) Doubling the CO_2 concentration enhanced the activity of carbohydrate-metabolism enzymes, source carbohydrate production, photoassimilate transport, and sink strength for *Opuntia ficus-indica*. *Plant Physiology* **110,** 893–902.

Wardlaw I.F. (1990) The control of carbon partitioning in plants. *New Phytologist* **116,** 341–381.

Winter K. (1979) Effect of different CO_2 regimes on the induction of Crassulacean acid metabolism in *Mesembryanthemum crystallinum* L. *Australian Journal of Plant Physiology* **6,** 589–594.

Winter K. (1985) Crassulacean acid metabolism. In *Photosynthetic Mechanisms and the Environment*, Vol. 6 (eds J. Barber & N.R. Baker), pp. 329–387. Elsevier, Amsterdam.

Winter K. & Engelbrecht B. (1994) Short-term CO_2 responses of light and dark CO_2 fixation in the Crassulacean acid metabolism plant *Kalanchoë pinnata*. *Journal of Plant Physiology* **144,** 462–467.

Winter K. & Smith J.A.C. (1996a) An introduction to Crassulacean acid metabolism. In *Crassulacean Acid Metabolism: Biochemistry, Ecophysiology and Evolution* (eds K. Winter & J.A.C. Smith), pp. 1–13. Springer, Berlin.

Winter K. & Smith J.A.C. (1996b) Crassulacean acid metabolism: current status and perspectives. In *Crassulacean Acid Metabolism: Biochemistry, Ecophysiology and Evolution* (eds K. Winter & J.A.C. Smith), pp. 389–426. Springer, Berlin.

Winter K., Wallace B.J., Stocker G.C. & Roksandic Z. (1983) Crassulacean acid metabolism in Australian vascular epiphytes and some related species. *Oecologia* **57,** 129–141.

Winter K., Zotz G., Baur B. & Dietz K.J. (1992) Light and dark CO_2 fixation in *Clusia uvitana* and the effects of plant water status and CO_2 availability. *Oecologia* **91,** 47–51.

Winter K., Richter A., Engelbrecht B., Posada J., Virgo A. & Popp M. (1997) Effect of elevated CO_2 on growth and crassulacean acid metabolism activity of *Kalanchoe pinnata* under tropical conditions. *Planta* **201,** 389–396.

Wullschleger S.D., Norby R.J. & Hendrix D.L. (1992) Carbon exchange rates, chlorophyll content, and carbohydrate status of two forest tree species exposed to carbon dioxide enrichment. *Tree Physiology* **10,** 21–31.

Yeoh H.-H., Badger M.R. & Watson L. (1981) Variations in kinetic properties of ribulose-1,5-bisphosphate carboxylases among plants. *Plant Physiology* **67,** 1151–1155.

Zhu J., Bartholomew D.P. & Goldstein G. (1997a) Effect of elevated carbon dioxide on the growth and physiological responses of pineapple, a species with crassulacean acid metabolism. *Journal of the American Society of Horticultural Science* **122,** 233–237.

Zhu J., Bartholomew D.P. & Goldstein G. (1997b) Effects of temperature, CO_2, and water stress on leaf gas exchange and biomass accumulation of pineapple. *Acta Horticulturae* **425,** 297–308.

Zhu J., Goldstein G. & Bartholomew D.P. (1999) Gas exchange and carbon isotope composition of *Ananas comosus* in response to elevated CO_2 and temperature. *Plant, Cell and Environment* **22,** 999–1007.

Ziska L.H., Hogan K.P., Smith A.P. & Drake B.G. (1991) Growth and photosynthetic response of nine tropical species with long-term exposure to doubled carbon dioxide. *Oecologia* **86,** 383–389.

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