American Journal of Botany 82(2): 159–166. 1995.

CLADODE DEVELOPMENT FOR *OPUNTIA FICUS-INDICA* (CACTACEAE) UNDER CURRENT AND DOUBLED CO_2 CONCENTRATIONS[1]

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Morphological and anatomical changes for first-order daughter cladodes (flattened stem segments) of a prickly pear cactus, *Opuntia ficus-indica,* were monitored to determine the effects of a doubled atmospheric CO_2 concentration on their development and mature form. For daughter cladodes developing in controlled environment chambers for 60 d, maximal elongation rates were similar under a photosynthetic photon flux density (PPFD) of 6 mol m^{-2} d^{-1} and a CO_2 concentration of 370 μl $liter^{-1}$, an increased PPFD (10 mol m^{-2} d^{-1}), and an increased PPFD and a doubled CO_2 concentration. These maximal rates, however, occurred at 20, 15, and 12 d, respectively. The maximal relative growth rate under the doubled CO_2 concentration was about twice that under the other conditions. For cladodes at 60 d as well as after 4 and 16 mo in open-top chambers, doubling the CO_2 concentration had no effect on final length or width. At 4 mo, cladodes under doubled CO_2 were 27% thicker, perhaps allowing the earlier production of second-order daughter cladodes. The chlorenchyma was then 31% thicker and composed of longer cells. At 16 mo, the difference in cladode thickness diminished, but the chlorenchyma remained thicker under doubled CO_2, which may contribute to greater net CO_2 uptake for *O. ficus-indica* under elevated CO_2 concentrations. Two other persistent differences were a 20% lower stomatal frequency and a 30% thicker cuticle with more epicuticular wax for cladodes under doubled CO_2, both of which may help reduce transpirational water loss.

Cladodes (flattened stem segments) of opuntias are determinate organs that attain their mature length and width during one season but remain photosynthetically active for many years (Mauseth and Halperin, 1975; Gibson and Nobel, 1986). Cellular properties and developmental events have been investigated for the shoot apical meristem and axillary meristems (areoles) of several opuntias, including *Opuntia cylindrica* (Boke, 1941, 1944), *O. basilaris* (Freeman, 1970), and *O. polyacantha* (Mauseth and Halperin, 1975; Mauseth, 1976, 1984). Much ecophysiological work has also been done on opuntias, ranging from the desert species *O. basilaris* (Szarek, Johnson, and Ting, 1973) to the shade-tolerant arborescent cactus *O. excelsa* (Lerdau et al., 1992) to the widely cultivated prickly pear, *O. ficus-indica* (Nobel, 1988), the species used in the present study. The physiological responses of *O. ficus-indica* under various levels of light, water availability, and atmospheric CO_2 concentration have been characterized (Nobel, 1982, 1991; Cui, Miller, and Nobel, 1993), but the accompanying morphological and anatomical changes have received little attention.

The annual dry weight productivity of *O. ficus-indica* can exceed that of most agricultural crops (Nobel, 1991; Nobel, García-Moya, and Quero, 1992). Its net CO_2 uptake is generally light limited, especially at the plant spacing needed to maximize productivity per unit ground area (Nobel, 1982). Doubling the atmospheric CO_2 concentration from the current level increases daily net CO_2 uptake for *O. ficus-indica* by 47% after 23 wk (Cui, Miller, and Nobel, 1993), leading to a corresponding increase in biomass production (Nobel and Israel, 1994). Increases in plant biomass in elevated CO_2 can be due to increases in the mass of individual organs such as leaves (Thomas and Harvey, 1983; Vu, Allen, and Bowes, 1989), in the number of organs (Oberbauer, Strain, and Fetcher, 1985; Retuerto and Woodward, 1993), or in both, as for *Glycine max* (Cure, Rufty, and Israel, 1989), *Phaseolus vulgaris* (Radoglou and Jarvis, 1992), and *O. ficus-indica* (Luo and Nobel, 1993; Nobel and Israel, 1994). Whether increases in plant biomass are due to accelerated organ maturation or to the larger final mass of individual organs can be investigated through growth analysis combined with morphological and anatomical measurements on mature organs.

The increases in net CO_2 uptake under elevated CO_2 concentrations for *O. ficus-indica,* a species with Crassulacean acid metabolism (CAM), are similar to those for many C_3 species (Bowes, 1991). This occurs despite the greater resemblance of CAM to the C_4 photosynthetic pathway, which is relatively unaffected by CO_2 enrichment (Lawlor and Mitchell, 1991). Under elevated CO_2, several C_3 species also exhibit changes in developmental and anatomical characteristics, such as growth rate (Cure, Rufty, and Israel, 1989; Poorter, 1993), mesophyll thickness (Thomas and Harvey, 1983), epicuticular wax (Thomas and Harvey, 1983), and stomatal frequency (Woodward and Bazzaz, 1988; Eamus, Berryman, and Duff, 1993). These characteristics were examined for cladodes of *O. ficus-indica* to determine whether the morphogenic responses of this CAM plant to elevated CO_2 resemble those of C_3 plants or C_4 plants. Increases in organ size and accompanying changes in areole number and cell size for the epidermis and chlorenchyma were determined for newly initiated cladodes of *O. ficus-indica* growing for 60 d in controlled environment chambers under two levels

[1] Manuscript received 17 February 1994; revision accepted 25 August 1994.

This research was supported by the Environmental Sciences Division, Office of Health and Environmental Research, U.S. Department of Energy Carbon Dioxide Research Program grant DE-FG03-91-ER61252 and Contract DE-FC03-87-ER60615.

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of light and two CO_2 concentrations. Growth responses for these cladodes were compared with measurements on cladodes grown for 4 or 16 mo in open-top chambers to understand the longer-term influence of a doubled CO_2 concentration on the structure of a perennial photosynthetic organ.

MATERIALS AND METHODS

Plant material—Mature cladodes of *Opuntia ficus-indica* (L.) Miller (Cactaceae) averaging 30 cm in length were obtained from the Agricultural Research Station, University of California, Riverside. One group of cladodes was planted in March 1992 in 4-liter pots containing a 1:1 quartz sand : soil mixture (the soil was obtained from the Agricultural Research Station) and maintained in a glasshouse at the University of California, Los Angeles. After 3 mo, the plants were transferred to controlled environment chambers with a 14-hr photoperiod, day/night air temperatures of 25 C/20 C, and day/night relative humidities of about 40%/70%. The plants were watered every 2 to 3 d with 0.1-strength Hoagland's solution, resulting in a soil water potential above −0.1 MPa in the root zone. Twelve control plants were exposed to a total daily photosynthetic photon flux density (PPFD) of 6 mol m^{-2} d^{-1} in the planes of the cladodes (18 mol m^{-2} d^{-1} in a horizontal plane at midcladode) and a mean ambient CO_2 level of 370 μl $liter^{-1}$. Four plants were grown under similar conditions but with a total daily PPFD of 10 mol m^{-2} d^{-1} in the planes of the cladodes, and four plants were grown also at a PPFD of 10 mol m^{-2} d^{-1} but with an elevated CO_2 concentration of 750 μl $liter^{-1}$, adjusted by mass-flow meters and monitored with an AR-5000 infrared gas analyzer (Anarad, Santa Barbara, CA) that was calibrated twice weekly (Cui, Miller, and Nobel, 1993). Plants and CO_2 concentrations were alternated between the two chambers every 2 wk by moving the pots and the CO_2 input system. Measurements were made every 1 to 2 d on first-order daughter cladodes growing on the basal (planted) cladodes.

Another group of cladodes was planted in April 1992 in two open-top chambers with transparent side panels at the Agricultural Research Station (Cui, Miller, and Nobel, 1993), eight basal cladodes per chamber. The chambers were 2.9 m tall and 3.0 m in diameter, with a circular hole 1.8 m in diameter at the top. Local field soil was thoroughly mixed and apportioned equally between the chambers to a depth of 50 cm (deeper than the roots of *O. ficus-indica*; Nobel, 1988). The total daily PPFD on the surfaces of daughter cladodes averaged 17 mol m^{-2} d^{-1} during spring and summer. Temperatures inside the chambers were within 3 C of the outside air during the daytime and within 1 C at night. The soil water potential in the root zone was maintained above −0.2 MPa by watering twice weekly. Ambient or CO_2-enriched air entered through ports in the lower panels at a flow rate ensuring three air exchanges per min in the chambers. The CO_2 concentrations averaged 370 μl $liter^{-1}$ in the current ambient CO_2 chamber and 720 μl $liter^{-1}$ in the doubled CO_2 chamber (Cui, Miller, and Nobel, 1993). Measurements were made in September 1993 on first-order daughter cladodes that were 4 or 16 mo old.

Growth analysis—The initiation of a daughter cladode was determined by a new leaf-bearing shoot becoming visible at an areole of a basal cladode. The elongation rate (cm d^{-1}), dy/dt, of cladode length y (cm) with respect to time t (d) was measured for plants in controlled environment chambers at intervals of 1 to 2 d for 60 d. The data were described by a combination sigmoidal-linear function:

$$y = (K + at)/(1 + e^{b - ct}) \quad (1)$$

where K, a, b, and c are fitted parameters (Luo and Nobel, 1993). Curves were fitted based on Equation 1 using SigmaPlot (Jandel Scientific, Corte Madera, CA). The relative growth rate (d^{-1}), $(1/y)$ (dy/dt), was calculated as:

$$(\ln y_2 - \ln y_1)/(t_2 - t_1) \quad (2)$$

where y_1 and y_2 are cladode lengths at time t_1 and t_2, respectively (Erickson, 1976; Hunt, 1990). Length was the distance from the cladode base to its apex, and width was the longest distance across the obovate cladode. Thickness was determined by inserting a 0.7-mm-thick wire through a cladode at four randomly chosen locations near the midregion. Areoles (or leaves for cladodes ≤ 3 cm long) were counted on both faces and on the margins of cladodes, and the distance between adjacent areoles in a single orthostichy (a helical series of lateral organs originated in sequence) was measured to the nearest 0.2 mm.

Anatomical measurements—Tissue samples were removed with a cork borer 13 mm in diameter from five cladodes (on five different plants) for each CO_2 concentration. The thickness of the chlorenchyma was measured under a dissecting microscope with calipers capable of resolving 0.02 mm. Chlorenchyma cell diameter and length were determined from paradermal and transverse sections, respectively, that were cut with a razor blade, stained with 0.05% (w/w) toluidine blue 0 in water, and measured with an ocular micrometer at ×100 on a BH2 microscope (Olympus, Lake Success, NY). The thickness of the cuticle was determined from transverse sections stained with a saturated solution of Sudan III and IV in ethylene glycol and viewed at ×400. The diameter of epidermal cells and the pore length and frequency of stomata were determined from epidermal peels. Cellular dimensions and frequencies were measured from eight randomly chosen microscopic fields from each cladode.

The relative volume of intercellular air space in five cladodes from each open-top chamber was estimated from fresh paradermal sections cut 1 mm deep into the chlorenchyma with a razor blade. Sections were stained with 0.05% toluidine blue 0 and photographed at ×100. Acetate sheets with 400 random points were superimposed on the photographs, and the points over the intercellular air spaces were counted and divided by 400 to estimate the percent volume of air space in the tissue (Parkhurst, 1982; Sajeva and Mauseth, 1991).

To determine the amount of epicuticular wax on the surface of cladodes, the cork borer was used to remove five cylinders from each of five cladodes from the two open-top chambers. The cuticle, along with the underlying epidermis and hypodermis, was peeled from both sides of each cylinder with forceps. The ten discs were then immersed for 15 sec in 15 ml of filtered, distilled chlo-

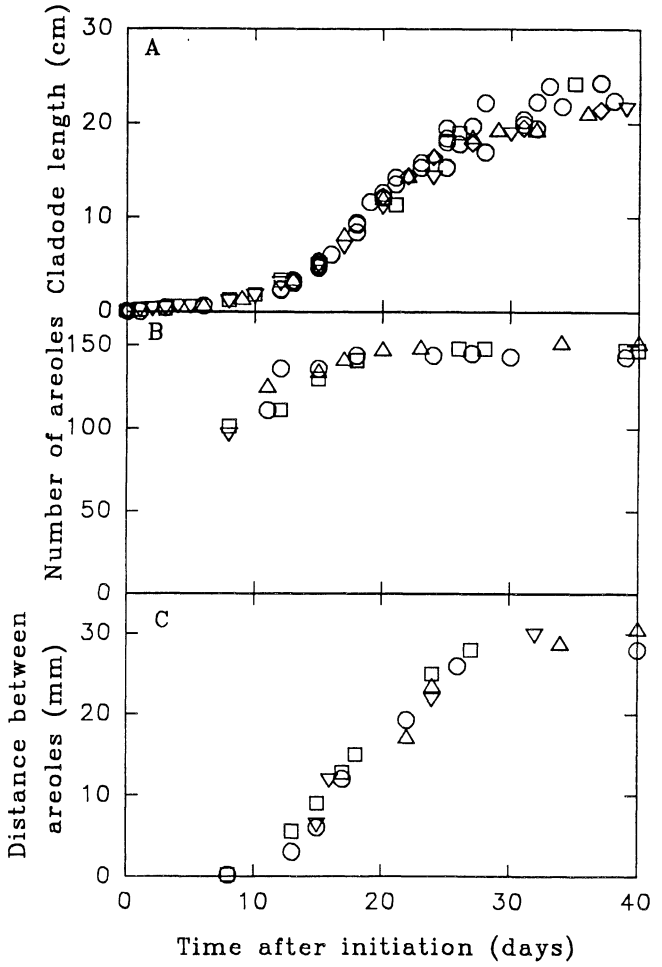


Fig. 1. Morphological changes for four representative first-order daughter cladodes of *Opuntia ficus-indica*: (A) length, (B) areole number, and (C) distance between adjacent areoles at midcladode. The plants were in a controlled environment chamber (PPFD of 6 mol m^{-2} d^{-1}, CO_2 concentration of 370 μl $liter^{-1}$). Different symbols (circle, square, triangle, and inverted triangle) indicate four individual plants in each case.

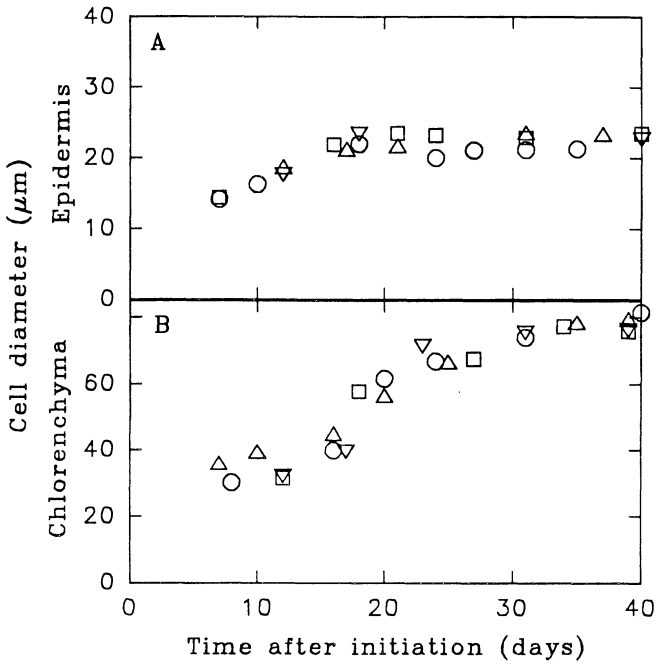


Fig. 2. Anatomical changes for four representative cladodes of *O. ficus-indica*: cell diameter for (A) the epidermis and (B) the chlorenchyma. Symbols and conditions are as for Fig. 1.

roform (Jordan et al., 1984) in preweighed aluminum weighing dishes. The dishes were placed in a desiccator under vacuum for 4 hr until the chloroform evaporated and then dried in the desiccator for 48 hr. The final minus initial weight of the dish, corrected by the weight of the residue after evaporation of 15 ml of chloroform, was used to determine the weight of the wax (Chiu et al., 1992). Differences between means were analyzed by Student's *t*-test.

RESULTS

Initial growth characteristics—The length of daughter cladodes increased slowly for about 10 d after initiation and then rapidly from 10 to 30 d, after which little change in length occurred (Fig. 1A). Cladode length was 25.8 ± 1.0 cm (mean ± SE, $N = 10$ cladodes) at 40 d and had further increased only 1.1% ± 0.2% at 60 d. Cladode width averaged 10.3 ± 0.5 cm at 40 d and 11.4 ± 1.1 at 60 d. Cladode thickness was 8.3 ± 0.7 mm at 60 d.

Eight days after initiation, cladodes were approximately 1 cm long and had 97 ± 10 ($N = 10$) leaves subtending an equal number of immature areoles (Fig. 1B). New leaf primordia and areoles were formed at the shoot apical meristem for about 20 d after initiation. After 20 d, areole number remained constant (Fig. 1B). At 40 d, cladodes had 142 ± 5 areoles, with 12% ± 0.3% of them on the margin and 44% ± 1% on each face.

The distance between adjacent areoles in an orthostichy at midcladode was about 0.2 mm at 8 d after initiation (Fig. 1C). Interareolar distance increased steadily until about 30 d, with little increase thereafter. The distance between areoles at 40 d was 29 ± 1 mm (Fig. 1C). Interareolar distance was relatively constant until within 1 cm of the apex, where the distance rapidly decreased. The greatest number of areoles across the width of the cladode occurred at midcladode.

Epidermal cell diameter at midcladode increased by about 60% from 7 to 20 d after cladode initiation, after which it remained relatively constant (Fig. 2A). At 40 d, the diameter of epidermal cells was 23 ± 1 μm ($N = 4$ cladodes). Chlorenchyma cell diameter at midcladode increased about 150% between 7 and 40 d after cladode initiation, with the largest increase occurring between 10 and 20 d (Fig. 2B). At 40 d, the diameter of chlorenchyma cells was 78 ± 1 μm ($N = 4$ cladodes).

Influences of PPFD and CO_2—The elongation rate for cladodes under the control conditions (PPFD of 6 mol m^{-2} d^{-1}, CO_2 concentration of 370 μl $liter^{-1}$) rapidly increased after 10 d. It reached a maximum of 1.4 ± 0.1 cm d^{-1} at 20 d and then decreased 80% by 40 d (Fig. 3A). For cladodes at a higher PPFD of 10 mol m^{-2} d^{-1}, the maximal rate was 1.2 ± 0.1 cm d^{-1}, occurring 15 d after

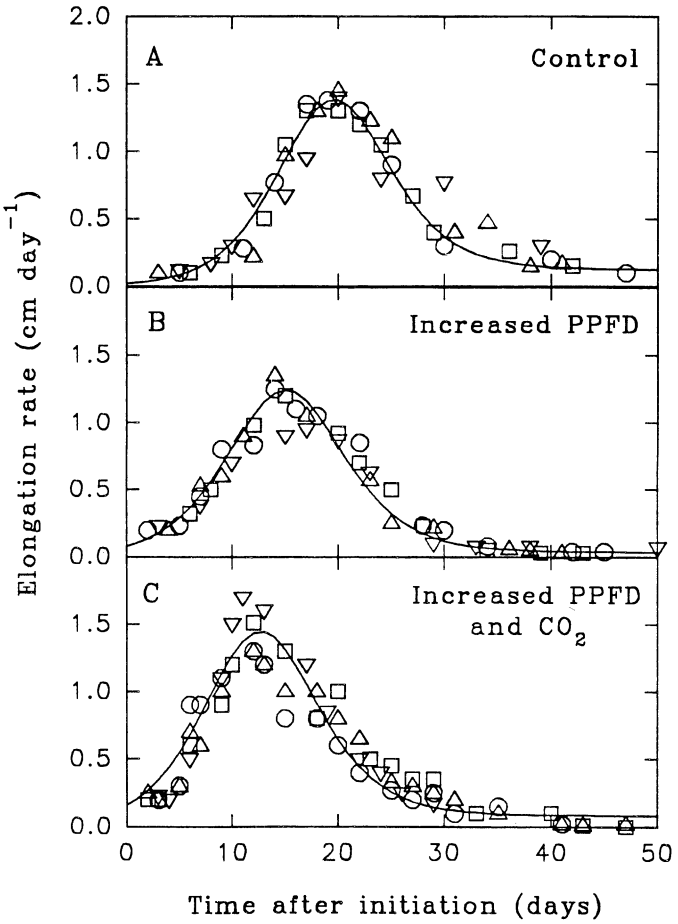


Fig. 3. Elongation rates for four cladodes of *O. ficus-indica*: (A) under control conditions (PPFD of 6 mol d^{-1}, CO_2 concentration of 370 μl $liter^{-1}$); (B) under increased PPFD (10 mol m^{-2} d^{-1}); and (C) under increased PPFD and a doubled CO_2 concentration (750 μl $liter^{-1}$). Different symbols (circle, square, triangle, and inverted triangle) indicate four individual plants in each case. Curves were fitted to the data for each plant based on Equation 1, and the lines are fitted curves based on mean values for the four plants.

cladode initiation (Fig. 3B). Cladodes grown at the higher PPFD and a doubled CO_2 concentration (750 μl $liter^{-1}$) had a maximal elongation rate of 1.4 ± 0.2 cm d^{-1} at about 12 d (Fig. 3C). Maximal elongation rates were not significantly different for cladodes under the three conditions, but they occurred at significantly different times ($P < 0.01$). At 60 d, cladode length, width, and thickness were similar under the three conditions.

Maximal relative growth rates (based on fitted curves and Equation 2) were similar (about 0.27 d^{-1}) for cladodes under control conditions and at the higher PPFD, but occurred at 15 d and 8 d, respectively ($P < 0.05$). For cladodes at the higher PPFD and doubled CO_2 concentration, the maximal relative growth rate occurred at 7 d and was higher (0.42 ± 0.03 d^{-1}) than for the other conditions ($P < 0.05$). From 0 to 20 d after initiation, the mean relative growth rates (based on fitted curves in Fig. 3 and Equation 2) were 0.142 ± 0.003 d^{-1} for cladodes under the control conditions, 0.145 ± 0.004 d^{-1} at the higher PPFD, and 0.192 ± 0.002 d^{-1} when the CO_2 concentration was also increased ($P < 0.05$). From 21 to 40 d after initiation, mean relative growth rates under the same conditions were 0.060 ± 0.002, 0.028 ± 0.006, and 0.022 ± 0.003 d^{-1}, respectively.

TABLE 1. Morphological properties of 4- and 16-mo-old cladodes of *Opuntia ficus-indica* growing in open-top chambers under current and doubled CO_2 concentrations. Data are means ± SE (N = 5 plants).

Cladode age, CO_2 concentration (μl $liter^{-1}$)	Length (cm)	Width (cm)	Thickness (mm)	Areole number
4-mo-old				
370	27.6 ± 1.6	12.1 ± 1.6	18.8 ± 1.4	160 ± 9
720	25.8 ± 1.9	12.3 ± 0.8	24.0 ± 1.6*[a]	166 ± 9
16-mo-old				
370	28.2 ± 1.5	13.4 ± 1.3	25.8 ± 1.2	162 ± 7
720	29.5 ± 1.7	13.9 ± 1.5	27.0 ± 1.1	161 ± 9

[a] *, mean differs significantly for properties between the two CO_2 concentrations ($P < 0.05$).

Long-term effects of CO_2—First-order daughter cladodes of plants growing in open-top chambers were similar in length and width under current and doubled CO_2 concentrations (Table 1). At 16 mo of age, cladodes tended to be longer and wider than at 4 mo, although differences were not significant. At 4 mo, cladodes were 27% thicker in the doubled CO_2 concentration ($P < 0.05$); at 16 mo, such cladodes still tended to be thicker, although not significantly so (Table 1). The number of areoles was about 160, and the distance between adjacent areoles at midcladode was 31 ± 2 mm for both ages and CO_2 concentrations (Table 1).

For first-order daughter cladodes at 4 mo of age, the chlorenchyma was 31% thicker under doubled CO_2 than under the current CO_2 concentration ($P < 0.05$; Table 2). One year later, the chlorenchyma was only 14% thicker for cladodes under the doubled CO_2 concentration ($P < 0.05$). Cell diameter in the chlorenchyma was not significantly different for the two CO_2 levels; cell length at 4 mo was 8% greater under the doubled than under the current CO_2 concentration ($P < 0.05$). The relative volume of intercellular airspace in the chlorenchyma was not

TABLE 2. Chlorenchyma properties for 4- and 16-mo-old cladodes of *Opuntia ficus-indica* growing in open-top chambers under current and doubled CO_2 concentrations. Tissue sections were taken from midcladode. Data are means ± SE (N = 5 plants).

Cladode age, CO_2 concentration (μl $liter^{-1}$)	Tissue thickness (mm)	Cell diameter (μm)	Cell length (μm)	Relative volume of intercellular air space (%)
4-mo-old				
370	26.1 ± 0.9	90 ± 4	189 ± 6	15 ± 1
720	34.1 ± 2.6*[a]	94 ± 5	205 ± 3*	15 ± 2
16-mo-old				
370	33.3 ± 1.3	94 ± 3	213 ± 8	20 ± 2
720	38.1 ± 1.5*	96 ± 4	210 ± 2	21 ± 2

[a] *, mean differs significantly for properties between the two CO_2 concentrations ($P < 0.05$).

TABLE 3. Epidermal properties for 4- and 16-mo-old cladodes of *Opuntia ficus-indica* growing in open-top chambers under current and doubled CO_2 concentrations. Measurements were made at midcladode. Data are means ± SE ($N = 5$ plants).

Cladode age, CO_2 concentration ($\mu l\ liter^{-1}$)	Stomatal frequency (mm^{-2})	Stomatal pore length (μm)	Epidermal cell diameter (μm)	Cuticle thickness (μm)	Epicuticular wax ($mg\ cm^{-2}$)
4-mo-old					
370	22.8 ± 0.8	52 ± 1	23 ± 1	5.7 ± 0.7	0.21 ± 0.03
720	19.0 ± 1.2*[a]	50 ± 2	22 ± 1	5.9 ± 1.5	0.22 ± 0.04
16-month-old					
370	19.7 ± 1.2	52 ± 0	22 ± 2	9.7 ± 0.6	0.21 ± 0.04
720	15.5 ± 0.7*	51 ± 2	22 ± 4	12.5 ± 0.8*	0.33 ± 0.03*

[a] *, mean differs significantly for properties between the two CO_2 concentrations ($P < 0.05$).

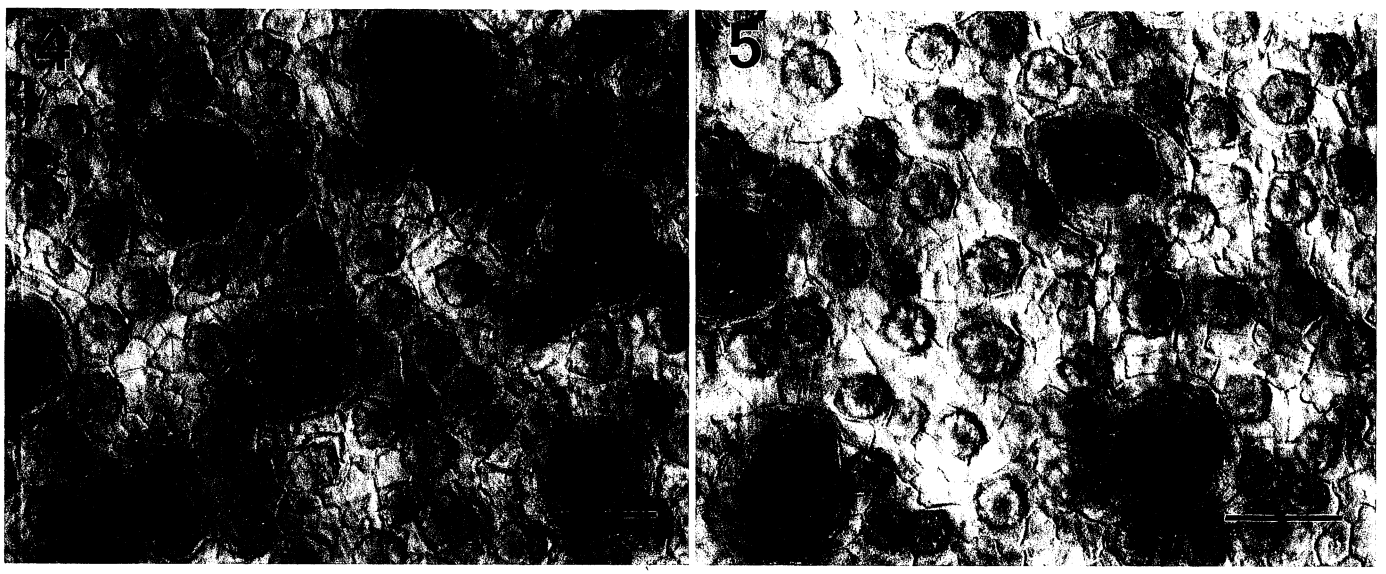
affected by CO_2 concentration but increased by about one-third from 4 to 16 mo of age (Table 2).

At both 4 and 16 mo of age, cladodes had a lower stomatal frequency under doubled CO_2 than under the current CO_2 concentration ($P < 0.05$; Table 3; Figs. 4, 5). In contrast, stomatal pore length was virtually the same for both ages and CO_2 concentrations, as was epidermal cell diameter (Table 3). At 4 mo, the thickness of the cuticle (consisting of the outer tangential epidermal cell wall plus epicuticular wax) was similar for cladodes in both CO_2 concentrations, but at 16 mo the cuticle was 29% thicker for elevated CO_2 ($P < 0.05$; Table 3; Figs. 6, 7). Similarly, the amount of epicuticular wax did not differ with CO_2 concentration at 4 mo but at 16 mo was significantly greater for cladodes in the elevated CO_2 concentration ($P < 0.05$; Table 3).

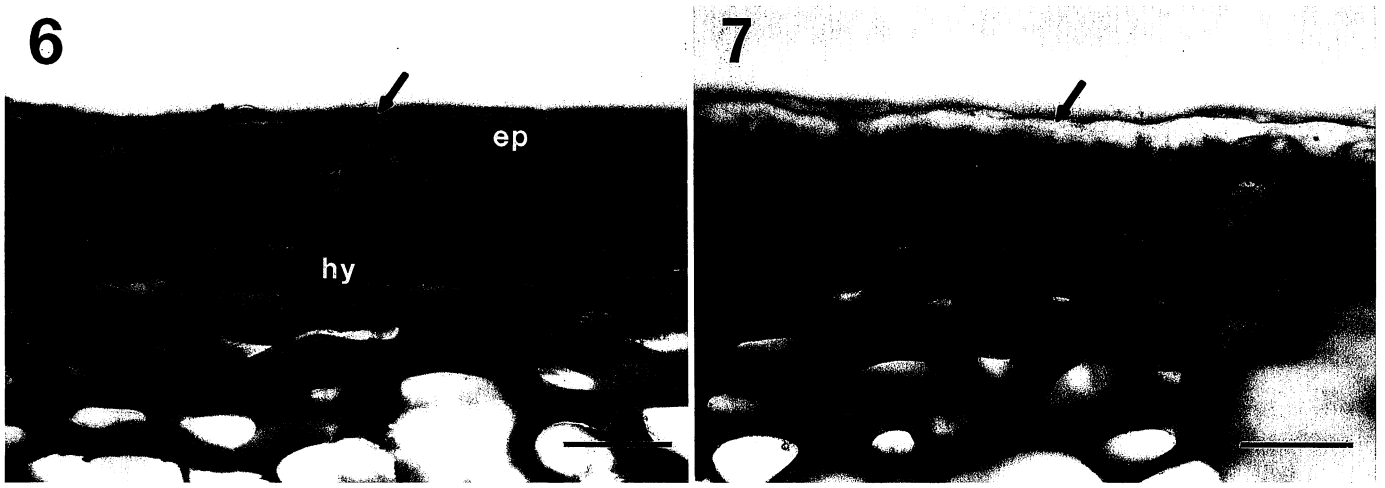
DISCUSSION

Cladodes of *O. ficus-indica* attained their mature length more rapidly when PPFD was increased and more rapidly still when the CO_2 concentration was increased as well. The maximal elongation rate occurred at 20 d under control conditions, 5 d sooner under higher PPFD, and 8 d sooner under a doubled CO_2 concentration with the higher PPFD. Doubling the CO_2 concentration also increased the maximal relative growth rate by about 50% and the mean relative growth rate for the first 20 d after cladode initiation by about 30%. For *O. ficus-indica,* as for several other species, enhancement of the growth rate was limited to the first few weeks of development under an elevated CO_2 concentration (Bazzaz et al., 1989; Johnsen, 1993; Retuerto and Woodward, 1993). From 21 to 40 d after initiation, the mean relative growth rate for cladodes under doubled CO_2 was 10% of the rate for the first 20 d and about 30% of the rate under current CO_2. Two factors may contribute to the diminishing effect of CO_2 on cladode growth over time: 1) the growth rate necessarily slowed as the ultimate size of the determinate organ was approached; and 2) the enhancement of net CO_2 uptake declined with time and consequent aging, as occurs for several other species (DeLucia, Sasek, and Strain, 1985; Johnsen, 1993). However, total net daily CO_2 uptake for *O. ficus-indica* is still 35% higher after 1 yr under the doubled vs. the current CO_2 concentration (Nobel and Israel, 1994), suggesting that other factors may determine maximum cladode length.

Unlike length and width, cladode thickness continued



Figs. 4, 5. Epidermal peels of *O. ficus-indica,* showing stomatal frequencies for cladodes grown for 4 mo in open-top chambers (**4**) under the current CO_2 concentration (370 $\mu l\ liter^{-1}$) and (**5**) under the doubled CO_2 concentration (720 $\mu l\ liter^{-1}$). Calcium oxalate crystals can be seen in the cell layer just beneath the epidermis. Bars = 100 μm.



Figs. 6, 7. Cross sections through the epidermis (ep) and hypodermis (hy) of cladodes of *O. ficus-indica* grown for 16 mo in open-top chambers (**6**) under the current CO_2 concentration and (**7**) under the doubled CO_2 concentration. Sections were stained with Sudan dyes and toluidine blue 0; arrows indicate cuticles with epicuticular wax. Bars = 50 μm.

to increase from 1 to 4 to 16 mo and was greater under the doubled than under the current CO_2 concentration. An increase in stem thickness for cacti over time involves a small amount of wood production plus the enlargement of water- and carbohydrate-storing tissue (Gibson and Nobel, 1986). For *O. ficus-indica,* cladode thickness is directly related to the storage of excess carbohydrates that are drawn upon in the production of fruits and new cladodes (Garcia de Cortázar and Nobel, 1992). Similarly, a critical amount of sucrose is needed in a nutrient medium before areoles of *O. polyacantha* break dormancy and produce leaf-bearing shoots (Mauseth and Halperin, 1975). About 75% more second-order daughter cladodes are produced by first-order daughter cladodes during 3 mo under doubled vs. current CO_2 concentrations (Nobel and Israel, 1994), consistent with their greater thickness attained earlier in development.

For cacti in general, each areole and leaf base has a peripheral meristem responsible for the cell production that gives rise to the ribs, tubercles, or otherwise enlarged photosynthetic stem tissue (Boke, 1980; Gibson and Nobel, 1986). Production of the final number of areoles occurred within about 2 wk of cladode initiation for *O. ficus-indica,* compared to 4 wk for a slower growing platyopuntia, *O. polyacantha* (Mauseth, 1984). The increase in interareolar distance paralleled the increase in cladode length and width, implying that the final size and shape of the cladode are closely related to division and enlargement of cells between areoles. For cladodes in the controlled environment chambers as well as in the open-top chambers, the number of areoles per unit surface area averaged about 0.40 cm^2 under all conditions.

Epidermal cells attained their final diameter in less than 3 wk after cladode initiation, whereas chlorenchyma cells enlarged more gradually. Unlike epidermal cell diameter, chlorenchyma cell size differed according to growth conditions. Chlorenchyma cell diameter was about 15% larger for cladodes in open-top chambers than for cladodes in the lower PPFD controlled environment chambers, and chlorenchyma cell length for 4-mo-old cladodes was 8% greater under doubled CO_2. By 16 mo, chlorenchyma cell length for cladodes under the current CO_2 concentration had increased, eliminating the difference under the two CO_2 concentrations. Environmental conditions such as light and CO_2 concentration thus influence the rate at which chlorenchyma cells develop but not necessarily their maximal size. The 30% greater thickness of the chlorenchyma tissue in cladodes under doubled vs. current CO_2 at 4 mo was due to increases both in cell size and in the number of cell layers. At 16 mo, however, the difference in tissue thickness was reduced to 14%, reflecting a difference only in the number of cell layers. Similarly, the number of mesophyll layers but not the mean cell diameter increases under elevated CO_2 for *Glycine max* (Thomas and Harvey, 1983; Vu, Allen, and Bowes, 1989), *Pinus taeda* (Thomas and Harvey, 1983), and *Phaseolus vulgaris* (Radoglou and Jarvis, 1992).

The 30% increase in chlorenchyma thickness for 4-mo-old cladodes is accompanied by a 49% increase in daily net CO_2 uptake for first-order daughter cladodes under doubled vs. current CO_2 concentrations (Cui, Miller, and Nobel, 1993). The greater number of cell layers in the chlorenchyma could lead to greater CO_2 fixation if other key structural and chemical components are unchanged. For example, a reduction in the relative volume of intercellular air space in the chlorenchyma would increase the intercellular diffusion resistance for CO_2, thereby diminishing the effect of increased numbers of chlorenchyma cells (Parkhurst and Mott, 1990). However, the relative volume of intercellular air space for cladodes of *O. ficus-indica* was about 20% at 16 mo, comparable to that for the leaf mesophyll of many C_3 species (Sajeva and Mauseth, 1991), and was unaffected by CO_2 concentration. Although the attenuation of light in the chlorenchyma may limit the photosynthetic capacity of the deeper layers, malate and certain products of photosynthesis may still accumulate in such cells.

Both CO_2 uptake and the conductance to water vapor can be influenced by the frequency of stomata. Stomatal frequency for *O. ficus-indica* was about 20% lower under the doubled than under the current CO_2 concentration, although stomatal pore length was unchanged. Stomatal

frequency is lower under elevated CO_2 for a number of species, including *Pentaclethra macroloba* (Oberbauer, Strain, and Fetcher, 1985), *Nardus stricta* (Woodward and Bazzaz, 1988), and *Maranthes corymbosa* (Eamus, Berryman, and Duff, 1993), but not for others, including *Glycine max, Liquidambar styraciflua,* and *Zea mays* (Thomas and Harvey, 1983). Despite such variable responses to elevated CO_2, a general decrease in stomatal frequency has accompanied the global rise in CO_2 from preindustrial concentrations to the current value, perhaps leading to increased plant water-use efficiency (Beerling and Woodward, 1993). For *O. ficus-indica,* water vapor conductance is 9% to 15% lower under a doubled than under the current CO_2 concentration (Cui, Miller, and Nobel, 1993; Cui and Nobel, 1994) despite increased net CO_2 uptake. The resulting approximate doubling in water-use efficiency may partially reflect the lower frequency of stomata.

In addition to a decrease in stomatal frequency, another long-term effect for *O. ficus-indica* under the doubled CO_2 concentration was an increase in epicuticular wax. By 16 mo, cladodes had accumulated about 60% more epicuticular wax, and the cuticle was about 30% thicker under the doubled than under the current CO_2 concentration. An increase in epicuticular wax is also observed for leaves of *Liquidambar styraciflua* and *Glycine max* grown under elevated CO_2, but not for leaves of *Zea mays* (Thomas and Harvey, 1983). The deposition of epicuticular wax for *Opuntia engelmannii* increases with age (Wilkinson and Mayeux, 1990); thus the increase in wax for *O. ficus-indica* under doubled CO_2 could be due to accelerated maturation. The waterproofing conferred by greater amounts of epicuticular wax can reduce cuticular transpiration (Jordan et al., 1984), which could lead to even greater drought tolerance and higher water-use efficiency for cladodes of *O. ficus-indica* under elevated CO_2 concentrations. A thicker layer of wax could also reduce tissue temperatures, and thereby transpiration, by increasing the reflectance of infrared radiation (Ebercon, Blum, and Jordan, 1977).

In summary, during the first few weeks after initiation, cladodes of *O. ficus-indica* apparently matured more rapidly under the doubled than under the current CO_2 concentration. Although the ultimate sizes of the cladode and of epidermal and chlorenchyma cells were not apparently affected by CO_2 concentration, the acceleration of development can increase biomass productivity by allowing the earlier formation of second-order daughter cladodes (Nobel and Israel, 1994). Three structural differences persisted after 16 mo under doubled CO_2: a thicker cuticle with more epicuticular wax, a lower frequency of stomata, and a thicker chlorenchyma. These could all contribute to increased water-use efficiency for cladodes under elevated CO_2 concentrations, the first two by reducing water loss and the third by expanding the tissue volume available for CO_2 fixation and the storage of photosynthetic products.

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