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RESEARCH PAPER

Carbon and water relations for developing fruits of *Opuntia ficus-indica* (L.) Miller, including effects of drought and gibberellic acid

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Abstract

Growth, gas exchange rates, and carbohydrate content were studied for developing fruits of the cultivated cactus *Opuntia ficus-indica* (L.) Miller, including effects of drought and exogenous gibberellic acid (GA_3). Fruit development required 110 d from the time of bud differentiation to ripening at 80 d after anthesis, when the fruit mass averaged 67 g. Stomatal conductance and net CO_2 uptake rates for fruits were higher during the night; they were maximal at 7 d before anthesis and decreased as development progressed. Fruits undergoing drought, imposed by detaching terminal stems bearing fruits, were 50% smaller than the control at 80 d after anthesis and did not ripen. Fruits injected with 2 ml of 500 ppm GA_3 were 30% smaller than the control at 80 d after anthesis; they contained a large proportion of aborted seeds that produced a weak sink signal for dry mass accumulation. Gas exchange was higher at 21 d after anthesis for fruits treated with GA_3. Total soluble sugars represented 40% of the fruit's dry mass until 45 d after anthesis, when the sugar content rapidly increased, reaching 90% at 73 d after anthesis. Such an increase was not observed for fruits treated with GA_3, and the sugar content for fruits undergoing drought remained low throughout development. Starch content increased for developing fruits of *O. ficus-indica* until 14 d after anthesis and, except for the fruits undergoing drought, decreased thereafter. Fruit development for *O. ficus-indica* is apparently regulated by water availability as well as hormonal signals originating both within and outside the fruit.

Key words: Abscisic acid, cactus pear, fruit development, fruit photosynthesis.

Introduction

Fruit development requires a major investment of carbon and water (Heim *et al*., 1979; Galen *et al*., 1999; Taiz and Zeiger, 2002). Photosynthetic contributions by young green fruits to their daily carbon budget are considerable for various species, such as apple (*Malus pumila*; Jones, 1981), orange (*Citrus sinensis*; Moreshet and Green, 1980), peach (*Prunus persica*; Pavel and De Jong, 1993), and cactus pear (*Opuntia ficus-indica*; Nobel and De la Barrera, 2000), but the rest of the plant still supplies over 90% of the fruit daily carbon gain via the phloem (Ho *et al*., 1987; Nobel and De la Barrera, 2000). In addition, most, if not all, of the water can be supplied by the phloem, as suggested by the higher (less negative) water potentials observed for developing fruits compared with the adjacent stems of apple (Lang, 1990; Mills *et al*., 1997), avocado (*Persea americana*; Blanke and Whiley, 1995), tomato (*Lycopersicon esculentum*; Mingo *et al*., 2003), some tropical trees (Chapotin *et al*., 2003), and cactus pear (Nobel *et al*., 1994; Nobel and De la Barrera, 2000; De la Barrera and Nobel, 2004). Also, the hydraulic resistance for the xylem of various developing fruits is high (Lovisolo and Schubert, 1998; Nijsse *et al*., 2001; van Ieperen *et al*., 2003).

Opuntia ficus-indica is widely cultivated in arid and semi-arid regions worldwide, mainly for forage, but with increasing importance as a fruit crop (Barbera, 1995; Inglese *et al*., 2002). Its young fruits take up CO_2 primarily at night for this CAM species at a rate equivalent to 35% of

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that for the underlying cladode, with the fruit contribution decreasing as development progresses (Inglese *et al*., 1994; Nobel and De la Barrera, 2000). Similarly, the water vapour conductance for young fruits of *O. ficus-indica* decreases as they ripen (Nerd and Nobel, 2000). The massive succulent stems of *O. ficus-indica* can buffer the plants against prolonged periods of drought by providing considerable quantities of water for fruit development, which, in turn, allows fruit production to occur without irrigation (Pimienta Barrios, 1990; Nerd and Mizrahi, 1995).

Water stress usually reduces fruit yield (Nerd and Mizrahi, 1995; Pessarakli, 1995; van Iersel *et al*., 1994), leading, in some cases, to the abscission of immature fruit (Pessarakli, 1995). By contrast, drought hastens ripening and seed maturation for tomato (García-Martínez and Hedden, 1997) and reduces fruit abscission for lychee trees (*Litchi chinensis*; Batten *et al*., 1994). Responses to drought are often hormonally mediated by the roots rather than by a change in the shoot water status (Davies and Zhang, 1991; Nobel and De la Barrera, 2002; Mingo *et al*., 2003), which can complicate the analysis of water limitations. Detached cladodes of *O. ficus-indica* provide a model system for studying drought responses without the chemical signalling from the roots; their net CO_2 uptake rates are similar to those for cladodes on droughted plants (Raveh and Nobel, 1999) and their succulence allows them to remain alive for many months (Nobel, 1996; Nobel and Castañeda, 1998).

Gibberellins control fruit development in various ways and at different developmental stages. For *O. ficus-indica*, the endogenous level of gibberellins (including GA_3) increases during flower development and becomes maximal during anthesis (Inglese *et al*., 1998). At this stage, GA_3 inhibits the differentiation of new flower buds, as also does its injection into detached cladodes (Nobel, 1996). For many species, GA_3 produced by developing seeds stimulates the growth and maturation of that fruit (Srivastava, 2002; Taiz and Zeiger, 2002). Moreover, treating emasculated flowers of *O. ficus-indica* or seedless grapes with GA_3 leads to the development of normal-sized, though seedless, fruits (Pharis and King, 1985; García-Martínez and Hedden, 1997). However, treatment of flowers at or before anthesis with GA_3 induces seed abortion for pea (*Pisum sativum*) and *O. ficus-indica* (Gil and Espinoza, 1980; García-Martínez and Hedden, 1997). The fruit pulp of *O. ficus-indica* originates from the funicle, which connects the seed to the ovary, indicating that fruit development depends on the presence of seeds (Pimienta Barrios, 1990). Yet the carbon and the water relations of fruits whose seed abortion is induced by gibberellin, compared with those having fertilized seeds, are not known during fruit development for *O. ficus-indica*.

The 24 h patterns of net CO_2 uptake and water vapour conductance at various stages throughout fruit development were therefore studied in the field for *O. ficus-indica* to evaluate the effects of drought and GA_3 on fruit development. The following two hypotheses were tested: (1) drought will negatively affect fruit development for this cactus—in particular, fruits will have a lower stomatal conductance, which will lead to lower rates of CO_2 uptake and reduced growth; and (2) treatment of flowers with GA_3 will induce seed abortion, arresting normal fruit development—in particular, fruits will be smaller and will ripen at a slower pace than the control, and rates of gas exchange will also be reduced by GA_3. In addition, the contents of soluble sugars and starch during fruit development were determined to gain insight into carbohydrate polymer and sugar patterns for *O. ficus-indica*.

Materials and methods

Plant material and treatments

Fruit development was studied for nine-year-old plants of *Opuntia ficus-indica* (L.) Miller (accession number 1279 of Texas A&M University, Kingsville, TX) approximately 2 m in height at the Agricultural Research Station, University of California, Riverside, CA, from April to August 2000 with supplemental data on fruit size from 2001 and 2002. Daily mean air temperature averaged 26.3±1.2 °C, ranging from 20.6 °C at 14 d before anthesis to 31.0 °C at 73 d after anthesis. The total daily photosynthetic photon flux (PPF, wavelengths of 400–700 nm; measured with a LI-188S integrating quantum sensor, Li-Cor, Lincoln, NE) averaged 57±2 mol m^{-2} d^{-1} on a horizontal surface. Fruits of *O. ficus-indica* are unilocular, polyspermic, fleshy berries (Pimienta Barrios, 1990; Nerd and Mizrahi, 1995). At the time of anthesis, 80 flowers growing on 16 terminal cladodes (five flowers per cladode, with all additional flowers removed) were marked for growth measurements (the 'control').

Gas exchange for detached cladodes of *O. ficus-indica* is similar to that of cladodes growing on droughted plants (Raveh and Nobel, 1999). Therefore, a drought treatment was imposed by detaching eight terminal cladodes, each one with five remaining flowers; the cladodes were placed on a table approximately 1 m above the ground to avoid herbivory by rodents. Also at the time of anthesis, five flowers on each of eight (attached) terminal cladodes were injected with 2 ml of a 500 ppm (w/w) gibberellic acid solution (GA_3; Sigma, St Louis, MO). In all three cases (control, drought, and GA_3-treated), cladodes were selected whose floral buds had differentiated at approximately the same time (within 2–3 d); anthesis occurred within 3–4 d of each other. On each sampling date, five fruits of the control and one of the experimental treatments were randomly selected from different cladodes for destructive analysis.

Length and diameter (cm) of 10 floral buds or the ensuing fruits (hereafter collectively referred to as fruits) were measured weekly with a vernier caliper (gradations of 0.02 mm) for the control and both experimental treatments (10 fruits under each condition); assuming that the fruits were prolate spheroids (Nobel and De la Barrera, 2000), their surface areas were calculated from $0.5\pi d^2+0.5\pi dl[\sin^{-1}(1-d^2/l^2)^{0.5}]/(1-d^2/l^2)^{0.5}$, where d (cm) is the diameter at mid-fruit and l (cm) is the fruit length. Five fruits from the control condition and five from one of the two experimental treatments were collected every 2 weeks. Fruit fresh mass (g) was recorded before removing a sample for carbohydrate determination. Fruits were then dried in a forced-draught oven at 80 °C until no further mass changes occurred, usually within 48 h, to determine dry mass (g). Regression curves for length versus fresh mass and versus

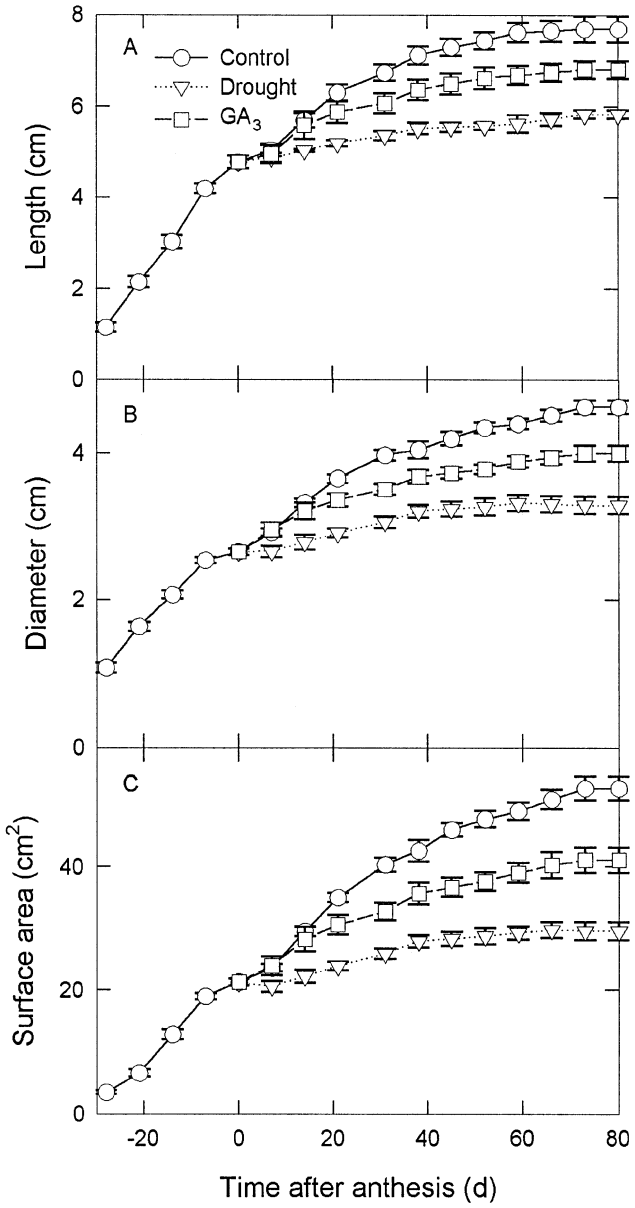

Fig. 1. Time-course for fruit development of *Opuntia ficus-indica* for the control, fruits undergoing drought, and fruits treated with GA_3. Length (A), diameter (B), and surface area (C) were calculated assuming that the fruits were prolate spheroids. Data are means ±SE (n=10 fruits).

dry mass were constructed based on all fruits harvested every 2 weeks; these curves were used to determine fresh and dry mass non-destructively for fruits developing in the field. Gas exchange was measured in the field during the intervening weeks.

Gas exchange

Fruit transpiration (mmol m^{-2} s^{-1}), fruit surface temperature (°C), air temperature (°C), and relative humidity (%) were measured every 2 h over 24 h periods on alternate weeks with a Li-Cor LI-1600 steady-state porometer for which the acrylic top had been removed to allow a tight seal with the fruits using the existing closed-pore foam gasket; water vapour conductance (mmol m^{-2} s^{-1}) was calculated by dividing the transpiration rate by the difference in water vapour mole fraction between the fruit, assumed to be at water vapour saturation, and the air (Nobel, 1999). Concomitantly, net CO_2 uptake (μmol m^{-2} s^{-1}) was measured with a Li-Cor LI-6200 portable photosynthesis system using a 0.25 l cuvette whose lid had been replaced with an acrylic extension with a square opening 1 cm^2 in area that was fitted with closed-pore foam gasket to ensure an air-tight seal when placed in contact with the fruits. Such gas exchange measurements, which were performed on the same fruits throughout their development, were done during clear days, on east-facing unshaded fruits, and avoiding the areoles.

Table 1. *Effect of GA_3 treatment on seeds for fruits of* Opuntia ficus-indica *at 73 d after anthesis*; *data are means ±SE (*n=*5)*

	Control	GA_3	*P* from a *t*-test
Seeds per fruit	246±24	183±43	0.272
Mass per seed (mg)	15.5±0.6	7.4±0.5	0.003
Seed mass per fruit (g)	3.8±0.4	1.3±0.3	0.010

Carbohydrate determination

Tissue samples were obtained with a cork borer 14 mm in diameter, weighed, and ground in 8 ml of boiling ethanol (80% w/w) in a mortar. The fine slurry was transferred to a centrifuge tube together with an additional 6 ml of boiling ethanol used to rinse the mortar and pestle. After centrifuging for 15 min at 1500 *g*, the content of total soluble sugars in the supernatant fluid was analysed with a colorimetric phenol method (Sturgeon, 1990). The pellet obtained after centrifugation was dried at 40 °C for 24 h and then washed three times with methanol:chloroform:water 12:5:3 by vol. and twice with distilled water. Starch from this pellet was hydrolysed with amyloglucosidase followed by an enzymatic glucose determination (Wang and Nobel, 1995).

Statistical analyses

Statistical analyses were performed with SigmaStat (SPSS Science, Chicago, IL, USA). Unless otherwise indicated, *P* values are from pairwise Tukey tests following a Friedman repeated measures ANOVA on ranks. Data are presented as means ±SE.

Results

Length/diameter changes

Fruit growth for *Opuntia ficus-indica* occurred over approximately 110 d (Fig. 1). Three growth phases were observed: (1) relatively rapid growth for small flower buds until anthesis 28 d later, when the experimental treatments of drought and GA_3 were imposed; (2) slow growth for 7 d; and (3) a second period of growth during which the fruits ripened and asymptotically approached their final size at about 80 d after anthesis (Fig. 1). Drought reduced fruit size, and GA_3 induced substantial seed abortion. In particular, the latter seeds were smaller, deformed, and less lignified than viable seeds. At 73 d after anthesis, seed mass was 52% lower and fruit mass was 65% lower following GA_3 treatment compared to the control (Table 1).

Fruit length for *O. ficus-indica* increased by an average of 1.3 mm d^{-1} for the 28 d before anthesis, when it was

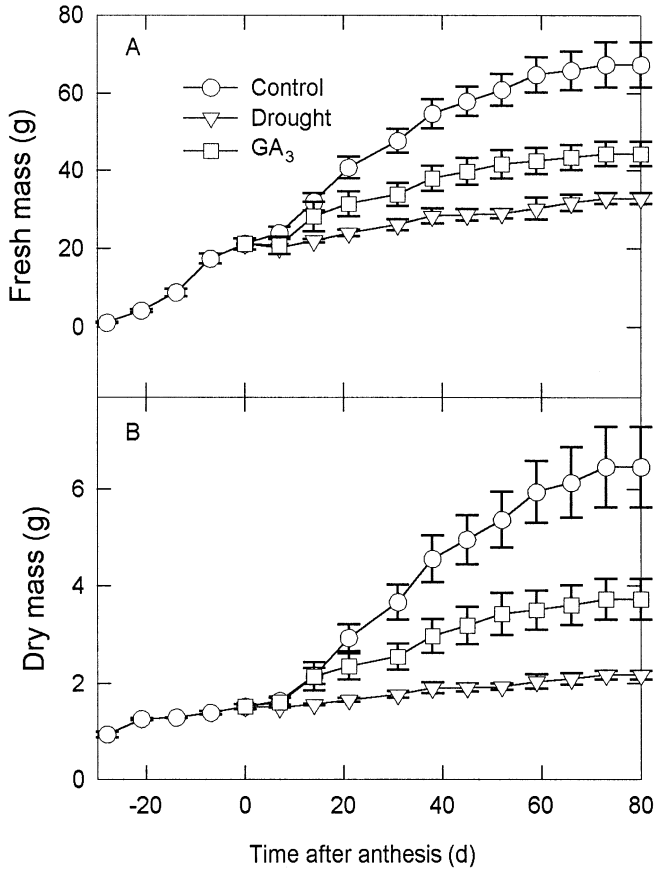

Fig. 2. Time-course for increases in fresh mass (A) and dry mass (B) for developing fruits of *O. ficus-indica*. Masses were calculated from regressions based on length for the control, fruits undergoing drought, and fruits treated with GA_3. Data are mean ±SE (n=10 fruits).

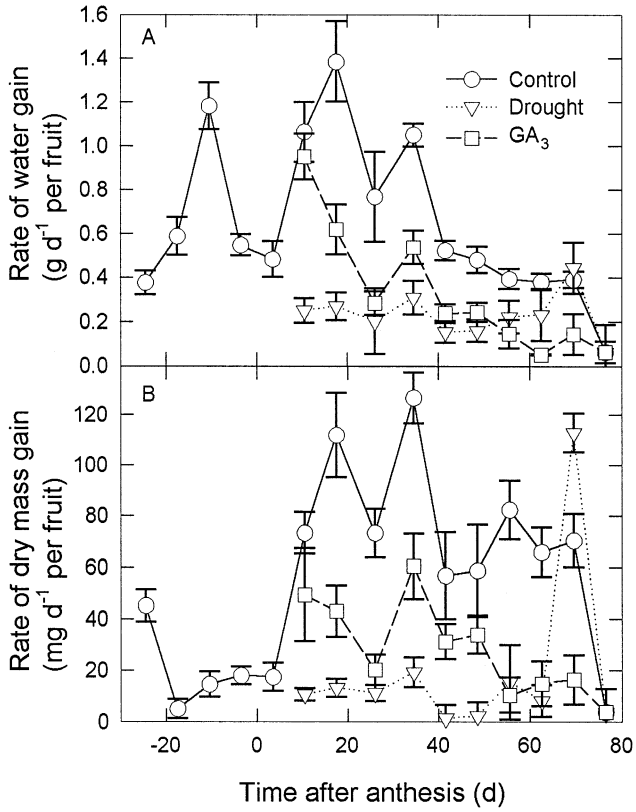

Fig. 3. Rates of water (A) and dry mass (B) gain for developing fruits of *O. ficus-indica* for the control, fruits undergoing drought, and fruits treated with GA_3. Data represent averages between two successive dates of measurement and are means ±SE (n=10 fruits).

4.8 cm. Length for the control then increased by 0.4 mm d^{-1} for 7 d and by 0.9 mm d^{-1} for the next 14 d; thereafter the rate decreased until the final length of 7.7 cm was reached (Fig. 1A). At 80 d after anthesis, fruits undergoing drought were 25% shorter than the control (Fig. 1A; P <0.05) and those treated with GA_3 were 12% shorter (P <0.05). Fruit diameter increased by 0.6 mm d^{-1} for the 28 d just before anthesis, when it was 2.6 cm. Diameter for the control then increased by 0.2 mm d^{-1} for 7 d and by 0.5 mm d^{-1} for the next 14 d; thereafter the rate decreased to zero at 80 d after anthesis (Fig. 1B). At 80 d after anthesis, the diameter was 4.6 cm for ripe control fruits, 29% less for fruits undergoing drought (P <0.05), and 14% less for those treated with GA_3 (P <0.05). Based on the length and diameter, the surface area of fruits of *O. ficus-indica* increased by 0.63 cm^2 d^{-1} for the 28 d before anthesis, when it was 21.3 cm^2 (Fig. 1C). At 80 d after anthesis, the surface area was 52.5 cm^2 for control fruits (Fig. 1C), 44% less for fruits undergoing drought (P <0.05), and 22% less for those treated with GA_3 (P <0.05).

Rate of growth

Based on cubic regressions of fresh mass (*fm*) on fruit length (*l*) [fm=0.352l+0.475l^2+0.0752l^3 (R^2=0.92, P <0.0001)], the fresh mass for control fruits of *O. ficus-indica* was 1.19 g for small buds at 28 d before anthesis, 21.1 g at anthesis, and 67.4 g at 80 d after anthesis (Fig. 2A). At 80 d after anthesis, the fresh mass for fruits undergoing drought was 51% less than the control (P <0.05) and was 34% less for those treated with GA_3 (P <0.05). Similarly, the dry mass (*dm*) for control fruits of *O. ficus-indica*, obtained from cubic regressions on fruit length [dm= –0.106+1.41l–0.459l^2+0.0488l^3 (R^2=0.87, P <0.0001)], was 0.94 g for small buds, 1.52 g at anthesis, and 6.46 g at 80 d after anthesis (Fig. 2B). At 80 d after anthesis, dry mass for fruits undergoing drought was 66% less than the control (P <0.05) and was 42% less (P <0.05) for those treated with GA_3 (Fig. 2B). Although initially the dry mass/fresh mass ratio was higher, beginning at anthesis when it was 0.072, it increased by 3.0×10^{-4} d^{-1} for the control and by 1.5×10^{-4} d^{-1} for fruits treated with GA_3, but decreased by 7.2×10^{-5} d^{-1} for fruits undergoing drought.

The daily rate of water gain (calculated as the daily change in fresh mass minus the daily change in dry mass)

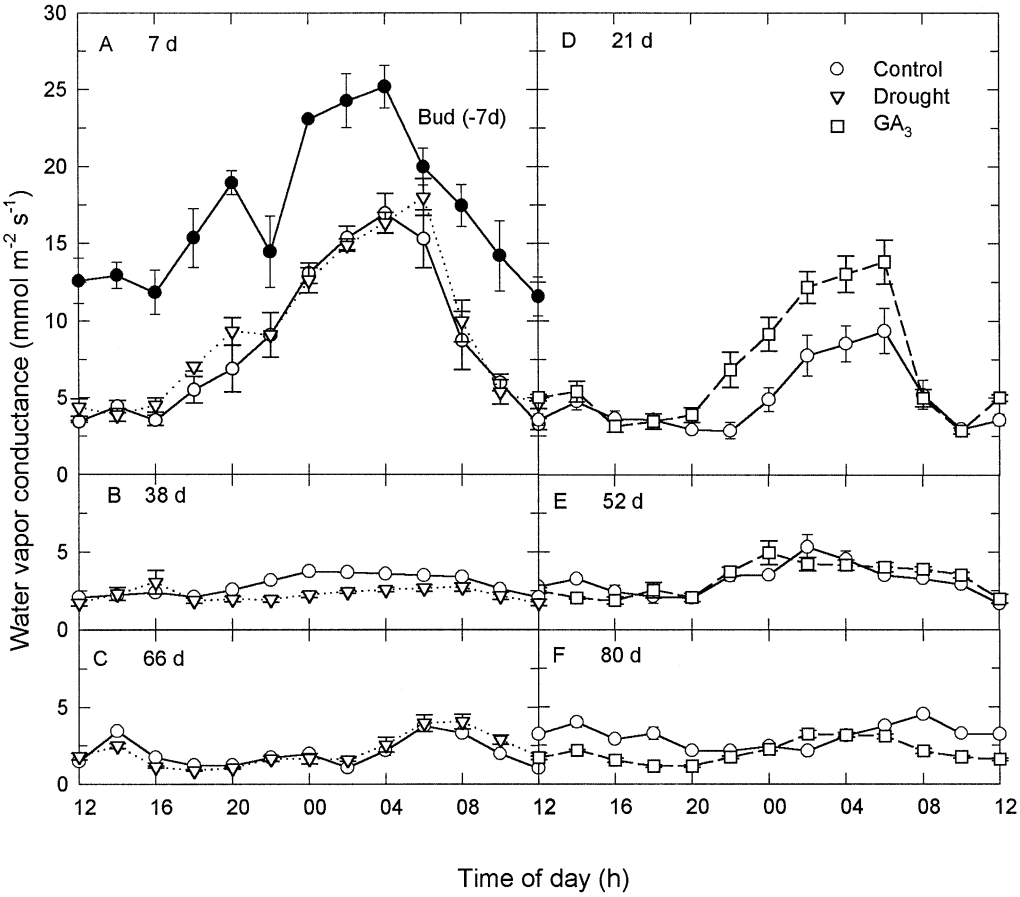

Fig. 4. Daily patterns of water vapour conductance for developing fruits of *O. ficus-indica* at the indicated days relative to anthesis. Measurements over 24 h periods were made for the control, fruits undergoing drought, and fruits treated with GA_3. Data are means ±1 SE (n=5 fruits).

averaged 0.38 g d^{-1} per fruit at 25 d before anthesis, increasing 3-fold at 11 d before anthesis (P <0.05), and then decreasing back to its initial value at 4 d after anthesis (P <0.05; Fig. 3A). A second period of relatively rapid water gain occurred for fruits 7–21 d after anthesis, averaging 0.97 g d^{-1} per fruit, after which it steadily decreased essentially to zero as the fruits ripened (P <0.001 from an ANOVA). For fruits undergoing drought, the rate of water gain was only 0.23 g d^{-1} per fruit from 7–80 d after anthesis. For fruits treated with GA_3, the rate of water gain was maximal at 7 d after anthesis at 0.95 g d^{-1} per fruit and then steadily decreased essentially to zero at 80 d after anthesis (P <0.001 from an ANOVA).

The rate of dry mass gain averaged 45 mg d^{-1} per fruit at 25 d before anthesis, decreasing to 14 mg $fruit^{-1}$ d^{-1} (P <0.05) from 14 d before anthesis to anthesis (Fig. 3B). The rate increased to a maximum of 127 mg d^{-1} per fruit at 31 d after anthesis (P <0.05) and then decreased essentially to zero as the fruits ripened. For fruits undergoing drought, the rate of dry mass gain at 7 d after anthesis was 86% less than for the control (P <0.05) and averaged 10 mg d^{-1} per fruit throughout fruit development. For fruits treated with GA_3, the maximum rate of dry mass gain was 61 mg d^{-1} per fruit at 31 d after anthesis, decreasing to essentially zero at 80 d after anthesis (P <0.01 from an ANOVA).

Transpiration

The water vapour conductance for developing fruits of *O. ficus-indica* was greatest at night, reaching a maximum near 04.00 h, and decreased as ripening progressed (Fig. 4). For flower buds at 7 d before anthesis, the maximal conductance was 25 mmol m^{-2} s^{-1} and the minimum was 13 mmol m^{-2} s^{-1} (Fig. 4A). At 7 d after anthesis, the maximum was 32% lower (P <0.05) and the minimum was 74% lower (P <0.05) and drought had no apparent effect (Fig. 4A). At 38 d after anthesis, the maximal conductance was 3.8 mmol m^{-2} s^{-1} for the control and 30% lower for fruits undergoing drought (P <0.05; Fig. 4B). At 66 d, the maximal conductance for the control was also 3.8 mmol m^{-2} s^{-1} and drought had no apparent effect (Fig. 4C). At 21 d after anthesis, the maximal water vapour conductance for fruits treated with GA_3 was 14 mmol m^{-2} s^{-1}, 56% greater than for the control (P <0.05), and the minimum was 3.0 mmol m^{-2} s^{-1} in both cases (Fig. 4D). At 52 d after

anthesis, the maximal conductance was 4.9 mmol m^{-2} s^{-1} and no effect of GA_3 was apparent (Fig. 4E). At 80 d after anthesis, the maximal water vapour conductance was 4.5 mmol m^{-2} s^{-1} for the control and 28% lower for those treated with GA_3 (P <0.05; Fig. 4F).

Daily transpiration was 24 mol m^{-2} d^{-1} for flower buds 7 d before anthesis and then steadily decreased for fruits, becoming 73% lower at 38 d after anthesis (P <0.05) and averaging 80% lower thereafter (Fig. 5A). Drought had little effect on daily transpiration for developing fruits of *O. ficus-indica* (Fig. 5A). For fruits treated with GA_3, daily transpiration was 28% higher than for the control at 21 d after anthesis (P <0.05), but 38% lower at 80 d (P <0.05; Fig. 5A). Taking into consideration the increase in surface area during fruit development (Fig. 1C), daily transpiration for buds at 7 d before anthesis was 0.77 g d^{-1} per fruit, decreasing by only 33% for fruits at 38 d after anthesis (Fig. 5B) and by 30% thereafter (Fig. 5B). The smaller surface areas for fruits undergoing drought (Fig. 1C) led to a daily transpiration per fruit that averaged 48% lower than for the control at 38 d and 66 d after anthesis (P <0.05; Fig. 5B). For fruits treated with GA_3, daily transpiration per fruit was 22% greater than for the control at 21 d (P <0.05; Fig. 5B), but their smaller surface area (Fig. 1C) and lower transpiration per unit area (Fig. 5A) led to a daily transpiration per fruit that was 57% lower than for the control at 80 d after anthesis (P <0.05; Fig. 5B).

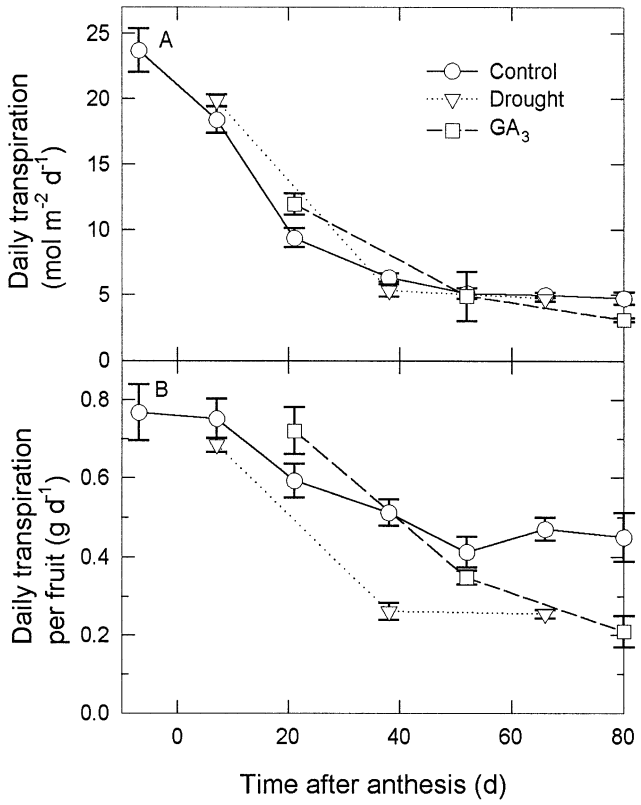

Fig. 5. Daily transpiration for developing fruits of *O. ficus-indica* per unit area (A) and per fruit (B) for the control, fruits undergoing drought, and fruits treated with GA_3. Data are means ±1 SE (n=5 fruits).

Net CO_2 uptake

The net CO_2 uptake rate for developing fruits of *O. ficus-indica* was greatest at night, reaching a maximum near 00.00 h, and decreased as ripening progressed (Fig. 6). A maximal rate of 6.1 µmol m^{-2} s^{-1} and a minimal rate of –1.3 µmol m^{-2} s^{-1} were observed for flower buds at 7 d before anthesis (Fig. 6A). At 7 d after anthesis, the maximal rate of net CO_2 uptake decreased by 39% for control fruits (P <0.05) and by 56% for those undergoing drought (P <0.05), while the minimum did not change for either case compared with 7 d before anthesis (Fig. 6A). At 38 d after anthesis, the maximal rate was 1.4 µmol m^{-2} s^{-1} for control fruits and 51% lower for those undergoing drought (P <0.05; Fig. 6B). At 66 d after anthesis, the maximal rate of net CO_2 uptake averaged 0.51 µmol m^{-2} s^{-1} and no effect of drought was apparent (Fig. 6C). At 21 d after anthesis, the maximal rate of net CO_2 uptake was 37% lower than at 7 d before anthesis (P <0.05) and no effect of GA_3 was apparent (Fig. 6D). At 52 d after anthesis, the maximal rate of net CO_2 uptake was 0.78 µmol m^{-2} s^{-1} and also no effect of GA_3 was apparent (Fig. 6E). At 80 d after anthesis, the maximal rate of net CO_2 uptake was 0.78 µmol m^{-2} s^{-1} for the control fruits and 31% lower for those treated with GA_3 (P <0.05; Fig. 6F).

Daily net CO_2 uptake was 168 mmol m^{-2} d^{-1} for flower buds at 7 d before anthesis and then steadily decreased for fruits, becoming 85% lower at 38 d after anthesis (P <0.05) and averaging 88% lower thereafter (Fig. 7A). Daily net CO_2 uptake was lower than for the control for fruits undergoing drought, for example, at 7 d after anthesis it was 37% lower (P <0.05; Fig. 7A). For fruits treated with GA_3, daily net CO_2 uptake was 20% higher than for the control at 21 d after anthesis (P <0.05) and similar thereafter (Fig. 7A). The daily dry mass gain per fruit, based on net CO_2 uptake and including the increasing surface area during development (Fig. 1C), was 13.7 mg d^{-1} per fruit from 7 d before anthesis to 21 d after anthesis; it then decreased by 44% at 38 d after anthesis (P <0.05) and by an average of 68% thereafter (P <0.05; Fig. 7B). Daily dry mass gain for fruits undergoing drought was 47% lower than for the control at 7 d after anthesis (P <0.05) and averaged 67% lower thereafter (P<0.05; Fig. 7B). Treatment with GA_3 had no apparent effect on fruit daily dry mass gain based on net CO_2 uptake (Fig. 7B).

Carbohydrates

The content of soluble sugars steadily increased in developing fruits of *O. ficus-indica*, the rate averaging 43 mg d^{-1} per fruit from 14 d before anthesis to 31 d after anthesis; the rate increased to 264 mg d^{-1} per fruit from 45–59 d after anthesis, leading to 7.2 g of soluble sugars

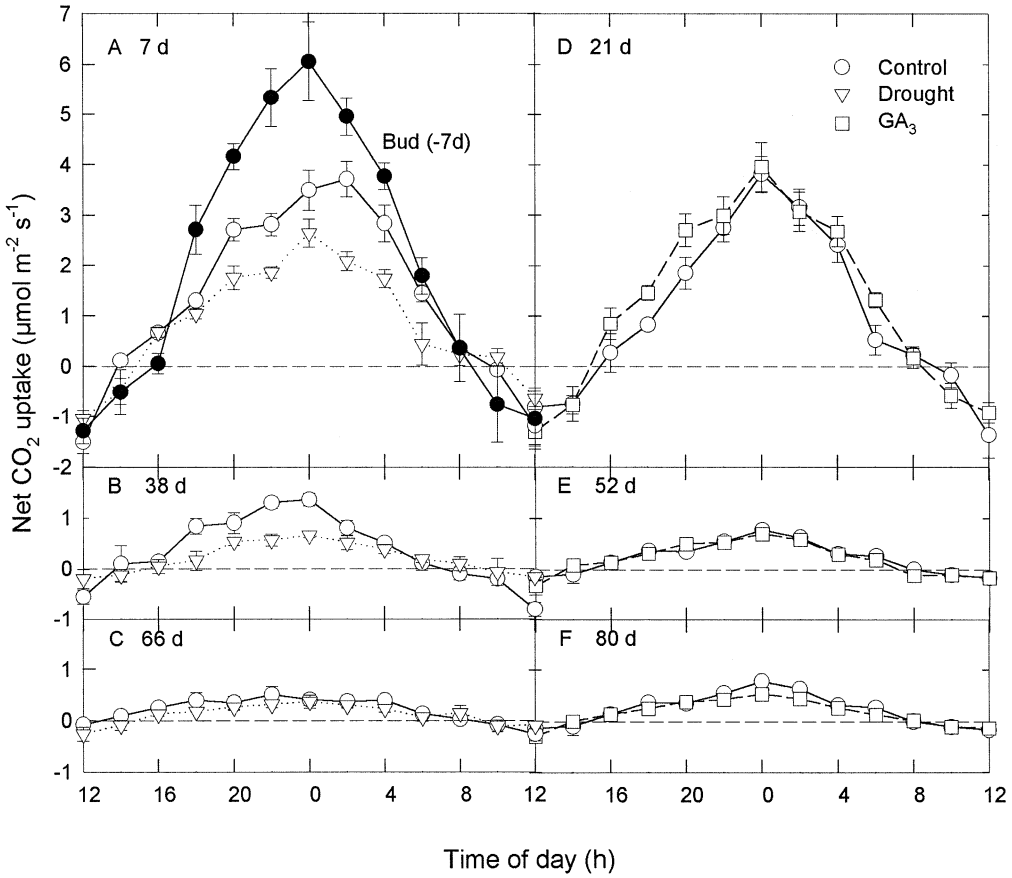

Fig. 6. Daily patterns of the net CO_2 uptake rate for developing fruits of *O. ficus-indica* at the indicated days relative to anthesis. Measurements over 24 h periods were made for the control, fruits undergoing drought, and fruits treated with GA_3. Data are means ±1 SE (n=5).

per fruit at 73 d after anthesis (Fig. 8A). For fruits undergoing drought, the content of soluble sugars was 63% lower than the control at 31 d after anthesis (P <0.05) and was 89% lower at 59 d (P <0.01). For fruits treated with GA_3, an effect on soluble sugar content was apparent only at 73 d after anthesis, when it was 59% lower than for ripe control fruits (Fig. 8A).

The content of starch for young flower buds of *O. ficus-indica* at 28 d before anthesis was 3.1 mg per fruit, steadily increased until a maximum of 9.4 mg fruit^{-1} at 14 d after anthesis, and then decreased to 4.4 mg per fruit at 73 d (Fig. 8B). Decreases in starch content were not observed for fruits undergoing drought, whose starch content at 59 d after anthesis was 2-fold higher than for the control (P <0.05; Fig. 8B). For fruits treated with GA_3, the starch content was similar to that for the control (Fig. 8B).

Discussion

Increases in fruit mass of *Opuntia ficus-indica* during development followed a double sigmoid pattern, consistent with previous observations (Nerd and Mizrahi, 1995). Changes in the growth rate apparently reflected discrete developmental stages. For instance, the first period of rapid growth is presumably due to cell elongation, as suggested by a rapid water gain and a slow dry mass gain that preceded anthesis. During the subsequent period of slow growth near anthesis, dry matter and water supply from the plant was reduced. Following pollination, a third developmental stage was characterized by a relatively rapid growth rate due to a sink signal, such as GA_3 (Grange, 1993; Srivastava, 2002; Taiz and Zeiger, 2002), originated from fertilized ovules. The growth rate then decreased as fruits approached their final size and ripened at 80 d after anthesis. During this latter period, the proportion of the fruit's mass represented by the locule (pulp) increases, while the receptacle (peel) softens and becomes thinner during colour break (Pimienta Barrios, 1990; Nerd and Mizrahi, 1995; Barbera *et al.*, 1994).

The water vapour conductance for developing fruits of *O. ficus-indica* was greatest at 7 d before anthesis, when the greatest transpiration and highest net CO_2 uptake rates were also observed. Indeed, at this time, transpiration by fruits was 50% greater than their daily water gain, and CO_2 uptake by fruits contributed about 60% of their daily dry mass gain. After anthesis, fertilization can trigger various

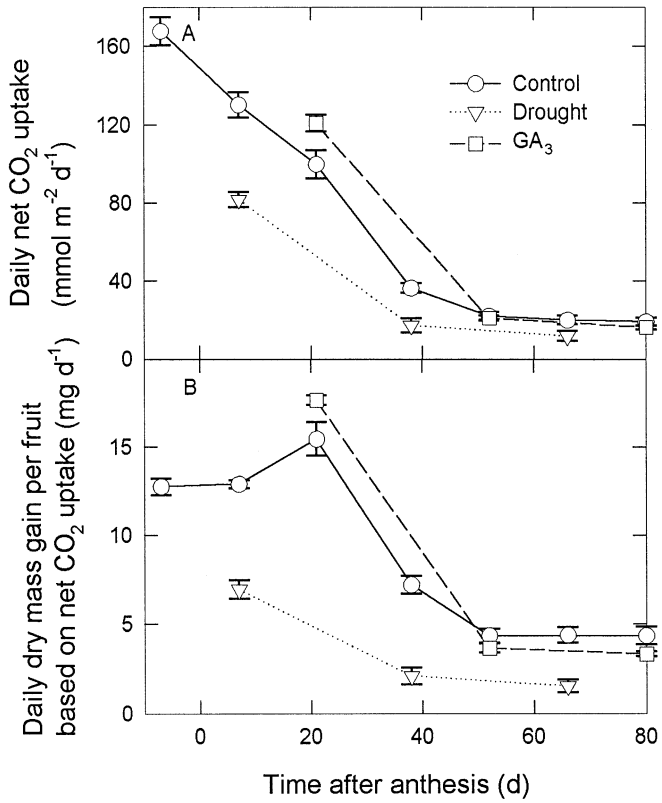

Fig. 7. Daily net CO_2 uptake for developing fruits of *O. ficus-indica* per unit area (A) and per fruit expressed as dry mass gain from net CO_2 uptake (B) for the control, fruits undergoing drought, and fruits treated with GA_3. Data are means ±1 SE (n=5).

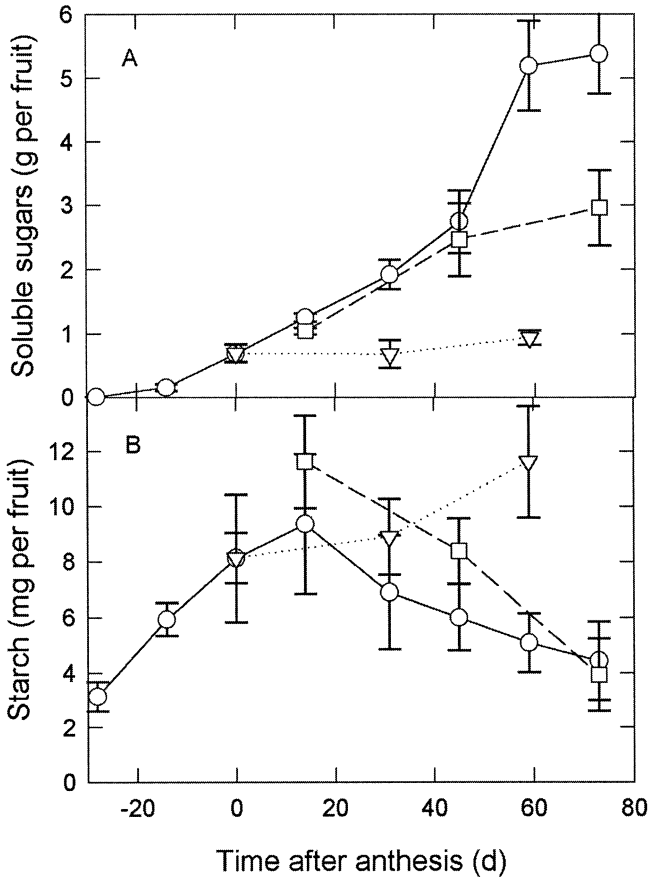

Fig. 8. Total soluble sugars (A) and starch (B) content for developing fruits of *O. ficus-indica*. Bi-weekly carbohydrate determinations were done for the control, fruits undergoing drought, and fruits treated with GA_3. Data are means ±1 SE (n=5).

physiological processes (Grange, 1993; Srivastava, 2002) that, in this case, led to a decrease in water vapour conductance and, consequently, to lower gas exchange rates as development progressed, reflecting the senescence of maternal tissue and the development of embryonic tissue (Haig and Westoby, 1988; Srivastava, 2002). At 20 d after anthesis, transpiration was equivalent to only 50% of the daily water gain, and fruit CO_2 uptake supplied only 10% of the daily dry mass gain. The senescence of maternal tissues became more evident by 70 d after anthesis, as fruits had then essentially ripened and transpiration had increased and was about equivalent to the daily water gain; at this time, CO_2 uptake by fruits had decreased to 5% of the daily dry mass gain.

Fruits growing on detached cladodes of *O. ficus-indica* were considerably smaller than for the control and had not ripened at 80 d after anthesis. Nevertheless, their stomatal conductance and net CO_2 uptake rates were similar to the control throughout development, indicating that the drought treatment did not limit gas exchange. For many plants, ABA from the roots limits transpiration and net CO_2 uptake through stomatal closure in response to water stress (Srivastava, 2002; Taiz and Zeiger, 2002), such as for fruits of *O. ficus-indica* growing on rooted cladodes (Nerd and Nobel, 2000), stems of the hemiepiphytic cactus *Hylocereus undatus* (Nobel and De la Barrera, 2002), and developing fruits of tomato (Mingo *et al.*, 2003). Therefore, the use of detached cladodes of *O. ficus-indica* is a model for studying drought processes, such as fruit development, without the confounding effects of root chemical signalling. Considerable amounts of water were transpired by the control before anthesis, when the stomatal conductance of such fruits was highest, and presumably also by their petals during flower opening (Nobel, 1988; Galen *et al.*, 1999). In addition, total transpiration for the control was 48 g of water per fruit, calculated by integrating the daily transpiration from anthesis to ripening at 80 d after anthesis, when the fruit water content was 61 g. Therefore, the amount of water required for the development of the five fruits per cladode considered here was greater than 45% of the fresh mass for a 1-year-old cladode (Nobel, 1996). Because about 9–15 fruits are allowed to develop out of the 30 or more flower buds that could set fruit for a given cladode in commercial plantations of *O. ficus-indica* (Acevedo *et al.*, 1983;

Inglese, 1995), the water supplied by the plant for fruit development must come from parts of the plant other than the cladode underlying the developing fruits.

Detached cladodes of *O. ficus-indica* can sustain net CO_2 uptake for a period of weeks, the daily amount halving at about 30 d after detachment (Raveh and Nobel, 1999), as it also does for cladodes growing on droughted plants (Acevedo *et al*., 1983; Nobel, 1988). Thus detached cladodes fix limited, but probably sufficient, amounts of carbohydrate to sustain fruit development. However, because the translocation of photosynthates to developing fruits requires considerable amounts of water (Ho, 1988; Nobel and De la Barrera, 2000; Srivastava, 2002), development did not progress for the fruits undergoing drought.

Treatment of fruits of *O. ficus-indica* with GA_3 at anthesis resulted in fewer and smaller (aborted) seeds, and the fruits at 80 d after anthesis were smaller than for the control, consistent with previous studies (Gil and Espinoza, 1980; Pimienta Barrios, 1990). Exogenous GA_3 also inhibits seed development for grape (Agüero *et al*., 2000) and pea (García-Martínez and Hedden, 1997). Moreover, by contrast with many species for which treatment with GA_3 leads to normal-sized or larger fruits (García-Martínez and Hedden, 1997; Taiz and Zeiger, 2002), for some grape cultivars treatment with GA_3 leads to smaller fruits (Agüero *et al*., 2000). In apparent contradiction to the effects of exogenous GA_3, endogenous levels of GA_3 are maximal near anthesis for *O. ficus-indica* (Inglese *et al*., 1998), and this phytohormone is essential for embryo and seed development of grape (Agüero *et al*., 2000) and pea (Swain *et al*., 1997), and for pollen tube elongation of *Arabidopsis thaliana* (Singh *et al*., 2002). Shortly after anthesis, however, endogenous levels of GA_3 are greatly decreased in *O. ficus-indica* (Inglese *et al*., 1998), grape (Agüero *et al*., 2000), and tomato (Srivastava, 2002). Such dual effects of GA_3 have led to fruit development being described using ecological models of kin competition (Haig and Westoby, 1988) in which each developing, or potentially developing, fruit competes for maternal resources with its siblings. The mechanisms for control of fruit development by gibberellins are not fully understood, although phenylalanine ammonia-lyase (PAL; Agüero *et al*., 2000), an enzyme involved in cell-wall development and plant defence against pathogens (Taiz and Zeiger, 2002), and cytokinins have been implicated (Ho, 1988).

For *O. ficus-indica* (Inglese *et al*., 1998), tomato (Srivastava, 2002), and other species (Pharis and King, 1985), a second period of increased levels of GA_3 occurs about half-way between fertilization and ripening. Concurrently, increased levels of ABA occur for tomato (Srivastava, 2002); this phytohormone is involved in seed maturation and in the acquisition of dormancy and acts antagonistically to GA_3 (Srivastava, 2002; Taiz and Zeiger, 2002). As discussed above, ABA leads to stomatal closure in photosynthetic organs, including young fruits (Nerd and Nobel, 2000; Mingo *et al*., 2003). The considerably higher water vapour conductance for fruits treated with GA_3 than for the control at 21 d after anthesis suggests that this phytohormone delayed fruit ripening; whether biosynthesis of ABA was inhibited by GA_3 should be investigated. If such were the case, the biosynthesis of ABA in developing fruits of *O. ficus-indica* probably occurs initially in fertilized seeds. The decrease in water vapour conductance observed at 52 d after anthesis may, in turn, be caused by ABA of funicular origin (Srivastava, 2002). At 80 d after anthesis, the lower water vapour conductance for fruits treated with GA_3 than for the control can reflect the low levels of GA_3 in fruits with aborted seeds and hence a less antagonistic effect with the funicular ABA. Other factors contributing to reduced water vapour conductance at the later stages of fruit development for *O. ficus-indica* are that stomatal frequency decreases as fruit size increases (Nerd and Nobel, 2000) and that stomates become non-functional as ripening approaches (Srivastava, 2002).

While exogenous GA_3 induced seed abortion for developing fruits of *O. ficus-indica*, resulting in smaller fruits and, presumably, in lower levels of endogenous ABA and GA_3 throughout fruit development, the fruits still ripened. Thus fertilized seeds are not necessarily essential for the completion of fruit development in *O. ficus-indica*, similar to the case for various seedless varieties of commercially grown fruits (Pharis and King, 1985; Srivastava, 2002; Taiz and Zeiger, 2002). Moreover, some varieties of *O. ficus-indica* develop with high numbers of apomictic or sterile parthenocarpic seeds (Pimienta Barrios, 1990). Hence, the high level of endogenous GA_3 that occurs near the time of anthesis probably acts as a physiological trigger for the maternal processes leading to fruit development. In such a case, the developmental control may be mediated by carbohydrate metabolism (Ho, 1988), perhaps with the involvement of PAL. Export of auxin from developing fruits has been proposed as a mechanism that inhibits the development of further fruits (‘primigenic dominance’; Bangerth, 1989, 1993). Indeed, the level of auxin increases for tomato shortly after pollination (Srivastava, 2002).

The content of total soluble sugars steadily increased for fruits of *O. ficus-indica* until 45 d after anthesis, but the contribution of sugars to the fruit’s dry mass remained near 40%. After 45 d after anthesis, however, the content of total soluble sugars increased dramatically, becoming 90% of the fruit dry mass at 73 d after anthesis. Indeed, during the final stages of ripening, the locule undergoes rapid growth, while the receptacle, chlorophyll and cell wall components degrade, leading to a thinner and softer fruit peel (Pimienta Barrios, 1990; Gutterman, 1995; Nerd and Mizrahi, 1995). Because the increase in fruit mass had

slowed at this stage, an increase in locular biomass, especially soluble sugars, is mainly due to degradation of cell wall polysaccharides from the receptacle and their mobilization into the locule (Grange, 1993; Nerd and Mizrahi, 1995). This is accompanied by a decrease in starch content observed in the later stages of ripening. For fruits undergoing drought, the contribution of soluble sugars to the fruit's dry mass did not change throughout development, averaging only 25%. At 59 d after anthesis, starch content for fruits undergoing drought was higher than at previous dates, representing 0.6% of the fruit's dry mass, suggesting that the water limitation interrupted starch degradation in addition to limiting growth. For fruits treated with GA_3, the amount of soluble sugars was similar to the control, except it did not increase at 73 d after anthesis, indicating that the aborted seeds induced by this phytohormone were weaker carbohydrate sinks than normal seeds.

In summary, the patterns of gas exchange for green fruits of *O. ficus-indica* were those typical for CAM plants, and the rates decreased as fruit development progressed. Fruits undergoing drought were smaller and did not ripen. This suggests that there are hydraulic constraints for fruit development, which are masked in the field because water for fruit development can be supplied from other parts of the plant during drought. Viable seeds are an important sink signal for driving fruit development. High levels of gibberellins produced by the fruits at the time of anthesis/fertilization (Inglese *et al*., 1998) induce physiological changes in the maternal tissue that ensure the supply of water and dry matter. By contrast, exogenous gibberellins induced seed abortion resulting in a weaker sink signal. During the final stages of fruit development for *O. ficus-indica*, a second pulse of gibberellins, first produced by the developing seeds and then by the funicle, apparently drives a second period of dry matter accumulation and the degradation of locular tissue and remobilization of its carbon compounds to produce the fruit pulp. Hence investments of water and carbon for fruit development are considerable and involve multifaceted interactions between the plant and the developing fruits.

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References

Acevedo E, Badilla I, Nobel PS. 1983. Water relations, diurnal acidity changes, and productivity of a cultivated cactus, *Opuntia ficus-indica*. *Plant Physiology* **72,** 775–780.

Agüero C, Vigliocco A, Abdala G, Tizio R. 2000. Effect of gibberellic acid and uniconazol on embryo abortion in the stenospermocarpic grape cultivars Emperatriz and Perlon. *Plant Growth Regulation* **30,** 9–16.

Bangerth F. 1989. Dominance among fruits/sinks and the search for a correlative signal. *Physiologia Plantarum* **76,** 608–614.

Bangerth F. 1993. Polar auxin transport as a signal in the regulation of tree and fruits development. *Acta Horticulturae* **329,** 70–76.

Barbera G. 1995. History, economic and agro-ecological importance. In: Barbera G, Inglese P, Pimienta-Barrios E, eds. *Agro-ecology, cultivation and uses of cactus pear*. Plant Production and Protection Paper 132. Rome: FAO, 1–11.

Barbera G, Inglese P, La Mantia T. 1994. Influence of seed content on some characteristics of the fruit of cactus pear (*Opuntia ficus-indica* Mill.). *Scientia Horticulturae* **58,** 161–165.

Batten DJ, McConchie CA, Lloyd J. 1994. Effects of soil-water deficit on gas-exchange characteristics and water relations of orchard lychee (*Litchi chinensis* Sonn) trees. *Tree Physiology* **14,** 1177–1189.

Blanke MM, Whiley AW. 1995. Bioenergetics, respiration cost and water relations of developing avocado fruit. *Journal of Plant Physiology* **145,** 87–92.

Davies WJ, Zhang J. 1991. Root signals and the regulation of growth and development of plants in drying soil. *Annual Review of Plant Physiology and Plant Molecular Biology* **42,** 55–76.

De la Barrera E, Nobel PS. 2004. Nectar: properties, floral aspects, and speculations on origin. *Trends in Plant Science* **(in press).**

Chapotin SM, Holbrook NM, Morse SR, Gutiérrez MV. 2003. Water relations of tropical dry forest flowers: pathways for water entry and the role of extracellular polysaccharides. *Plant, Cell and Environment* **26,** 623–630.

Galen C, Sherry RA, Carroll AB. 1999. Are flowers physiological sinks or faucets? Costs and correlates of water use by flowers of *Polemonium viscosum*. *Oecologia* **118,** 461–470.

García-Martínez JL, Hedden P. 1997. Gibberellins and fruit development. In: Tomás-Barberán FA, Robins RJ, eds. *Phytochemistry of fruit and vegetables*. Oxford: Oxford University Press, 263–285.

Gil G, Espinoza E. 1980. Desarrollo de frutos de tuna (*Opuntia ficus-indica*, Mill) con aplicación prefloral de giberelina y auxina. *Ciencia e Investigación Agraria* **8,** 141–146.

Grange RI. 1993. Crecimiento del fruto. In: Ascon-Bieto J, Talon M, eds. *Fisiología y bioquímica vegetal*. Madrid: McGraw-Hill-Interamericana, 22–462.

Gutterman Y. 1995. Flower and fruit developmental stages of the xerophyte *Opuntia ficus-indica*. *Israel Journal of Plant Sciences* **43,** 271–280.

Haig D, Westoby M. 1988. Inclusive fitness, seed resources, and maternal care. In: Lovett Doust J, Lovett Doust L, eds. *Plant reproductive ecology: patterns and strategies*. New York: Oxford University Press, 60–79.

Heim G, Landsberg JJ, Watson RL, Brain P. 1979. Ecophysiology of apple trees: dry matter production and partitioning by young Golden Delicious apple trees in France and England. *Journal of Applied Ecology* **16,** 179–194.

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

Ho LC, Grange RI, Picken AJ. 1987. An analysis of the accumulation of water and dry matter in tomato fruit. *Plant, Cell and Environment* **10,** 157–162.

Inglese P. 1995. Orchard planting and management. *Agro-ecology, cultivation and uses of cactus pear*. Plant Production and Protection Paper 132. Rome: FAO, 78–91.

Inglese P, Basile F, Schirra M. 2002. Cactus pear fruit production.

In: Nobel PS, ed. *Cacti: biology and uses*. Berkeley, California: University of California Press, 163–183.

Inglese P, Chessa I, La Mantia T, Nieddu G. 1998. Evolution of endogenous gibberellins at different stages of flowering in relation to return bloom of cactus pear (*Opuntia ficus-indica* L. Miller). *Scientia Horticulturae* **73,** 45–51.

Inglese P, Israel AA, Nobel PS. 1994. Growth and CO_2 uptake for cladodes and fruit of the Crassulacean acid metabolism species *Opuntia ficus-indica* during fruit development. *Physiologia Plantarum* **91,** 708–714.

Jones HG. 1981. Carbon dioxide exchange of developing apple (*Malus pumila* Mill.) fruits. *Journal of Experimental Botany* **131,** 1203–1210.

Lang A. 1990. Xylem, phloem and transpiration flows in developing apple fruits. *Journal of Experimental Botany* **40,** 1069–1078.

Lovisolo C, Schubert A. 1998. Effects of water stress on vessel size and xylem hydraulic conductivity in *Vitis vinifera* L. *Journal of Experimental Botany* **49,** 693–700.

Mills TM, Behboudian MH, Clothier BE. 1997. The diurnal and seasonal water relations, and composition, of 'Braeburn' apple fruit under reduced plant water status. *Plant Science* **126,** 145–154.

Mingo MD, Bacon MA, Davies WJ. 2003. Non-hydraulic regulation of fruit growth in tomato plants (*Lycopersicon esculentum* cv. Solairo) growing on drying soil. *Journal of Experimental Botany* **54,** 1205–1212.

Moreshet S, Green GC. 1980. Photosynthesis and diffusion conductance of the Valencia orange fruit under field conditions. *Journal of Experimental Botany* **120,** 15–27.

Nerd A, Mizrahi Y. 1995. Reproductive biology. In: Barbera G, Inglese P, Pimienta-Barrios E, eds. *Agro-ecology, cultivation and uses of cactus pear*. Plant Production and Protection Paper 132. Rome: FAO, 49–57.

Nerd A, Nobel PS. 2000. Water relations during ripening for fruit of well-watered versus water-stressed *Opuntia ficus-indica*. *Journal of the American Society for Horticultural Sciences* **125,** 653–657.

Nijsse J, van der Heijden GWAM, Van Ieperen W, Keijzer CJ, Van Meetern U. 2001. Xylem hyraulic conductivity related to conduit dimensions along chrysanthemum stems. *Journal of Experimental Botany* **52,** 319–327.

Nobel PS. 1996. Shading, osmoticum, and hormone effects on organ development for detached cladodes of *Opuntia ficus-indica*. *International Journal of Plant Sciences* **157,** 722–728.

Nobel PS. 1988. *Environmental biology of agaves and cacti*. New York: Cambridge University Press.

Nobel PS. 1999. *Physicochemical and environmental plant physiology*, 2nd edn. San Diego: Academic Press.

Nobel PS. 2002. Cactus physiological ecology, emphasizing gas exchange of *Platyopuntia* fruits. *Acta Horticulturae* **581,** 143–150.

Nobel PS, Castañeda MA. 1998. Seasonal, light, and temperature influences on organ initiation for unrooted cladodes of the prickly pear cactus *Opuntia ficus-indica*. *Journal of the American Society for Horticultural Science* **123,** 47–51.

Nobel PS, De la Barrera E. 2000. Carbon and water balances for young fruits of platyopuntias. *Physiologia Plantarum* **109,** 160–166.

Nobel PS, De la Barrera E. 2002. Stem water relations and net CO_2 uptake for a hemiepiphytic cactus during short-term drought. *Environmental and Experimental Botany* **48,** 129–137.

Nobel PS, Andrade JL, Wang N, North GB. 1994. Water potentials for developing cladodes and fruits of a succulent plant, including xylem-versus-phloem implications for water movement. *Journal of Experimental Botany* **45,** 1801–1807.

Pavel EW, DeJong TM. 1993. Estimating the photosynthetic contribution of developing peach (*Prunus persica*) fruits to their growth and maintenance carbohydrate requirements. *Physiologia Plantarum* **88,** 331–338.

Pessarakli M (ed). 1995. *Handbook of plant and crop physiology.* New York: Marcel Dekker.

Pharis RP, King RW. 1985. Gibberellins and reproductive development in seed plants. *Annual Review of Plant Physiology* **36,** 517–568.

Pimienta Barrios E. 1990. *El nopal tunero*. Guadalajara, Jal.: Universidad de Guadalajara.

Raveh E, Nobel PS. 1999. CO_2 uptake and water loss accompanying vernalization for detached cladodes of *Opuntia ficus-indica*. *International Journal of Plant Sciences* **160,** 92–97.

Singh DP, Jermakow AM, Swain SM. 2002. Gibberellins are required for seed development and pollen tube growth in *Arabidopsis*. *The Plant Cell* **14,** 3133–3147.

Srivastava LM. 2002. *Plant growth and development: hormones and environment*. San Diego, CA: Academic Press.

Sturgeon RJ. 1990. Monosaccharides. In: Dey PM, Harborne JB, eds. *Methods in plant biochemistry.* Vol. 2. *Carbohydrates*. San Diego: Academic Press, 1–37.

Swain SM, Reid JB, Kamiya Y. 1997. Gibberellins are required for embryo growth and seed development in pea. *The Plant Journal* **12,** 1329–1338.

Taiz L, Zeiger E. 2002. *Plant physiology*, 3rd edn. Sunderland, MA: Sinauer.

van Ieperen W, Volkov VS, Van Meeteren U. 2003. Distribution of xylem hydraulic resistance in fruiting truss of tomato influenced by water stress. *Journal of Experimental Botany* **54,** 317–324.

van Iersel MW, Oosterhuis DM, Harris WM. 1994. Apoplastic water flow to cotton leaves and fruits during development. *Journal of Experimental Botany* **45,** 163–167.

Wang N, Nobel PS. 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.