

CO₂ uptake of *Opuntia ficus-indica* (L.) Mill. whole trees and single cladodes, in relation to plant water status and cladode age

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Abstract

Most of net photosynthesis determinations in *Opuntia ficus-indica* come from measurements on individual cladodes. However, they have limitations when used to scale up to whole canopy gas exchange, because a large variability of carbon assimilation may occur within the canopy, due to, among others, differences in cladode age and intercepted radiation or individual cladode response to abiotic stresses. The aim of this work was to evaluate the application of open gas exchange chambers, simultaneously applied around the whole canopy, to measure net CO₂ uptake, continuously over a 24 h period, in single *Opuntia ficus-indica* (L.) Mill. potted trees and in relation with their water status. Net CO₂ uptake was also measured for single cladodes differentiated by age. *O. ficus-indica* trees continued their photosynthetic activity 60 days after the irrigation was stopped, when soil water content was lower than 5%. At this stage, current-year and 1-year-old cladodes had become flaccid but still the daily net CO₂ uptake of non-irrigated trees kept the same rate than at the beginning of the experiment, while watered trees had doubled their net CO₂ uptake. The highest instantaneous rates and total daily net CO₂ uptake for both well-watered and non-irrigated trees occurred 60 days after the onset of the dry period, when maximal instantaneous rates were 11.1 in well-watered trees and 8.4 mol m⁻² s⁻¹ in non-irrigated trees. During the drought period, the chlorenchyma fresh weight decreased by 45% and 30%, in 1- and 2-year-old drought cladodes respectively, and marginally increased in current-year ones (+20%). Net CO₂ uptake for 1-year-old and 2-year-old cladodes changed only at highest photosynthetic photon flux density and temperatures, and average seasonal net CO₂ uptake of 2-year-old cladodes was 15% lower than for 1-year-old ones. Whole-tree gas exchange

measurements applied for the first time to *O. ficus-indica* indicated that whole cactus pear trees maintain similar net CO₂ uptake, contrary to previous evidence gathered on single leaves, even after 60 days of drought. A substantial water loss in the most photosynthetically active cladodes was not sufficient to alter this response. Differences in growth accrued by wet and non-irrigated trees were due to the fact that, as the season progressed, the former were able to increase their total daily CO₂ uptake and the latter were not.

Introduction

Opuntia ficus-indica (L.) Mill. is a drought-tolerant cactus, widely cultivated in arid and semi-arid regions worldwide. As most of species of the Cactaceae family, *O. ficus-indica* exhibits Crassulacean Acid Metabolism (CAM), with nocturnal stomata opening and CO₂ uptake occurring, typically, from dusk to dawn, when, through phosphoenolpyruvate (PEP) carboxylation, malate is formed and stored in vacuoles (Nobel, 1988). Measurements of gas exchange in *O. ficus-indica* began in the early 1980s, when Nobel and Hartsock (1983) measured CO₂ uptake on single cladodes, using portable infrared gas-analyzers (IRGA), with cuvettes adapted to fit cladode morphology. At optimal temperature and intercepted radiation, instantaneous values of net CO₂ uptake of 1-year-old cladodes may reach 18 mol m⁻² s⁻¹, with a total daily CO₂ uptake of 680 mmol m⁻² (Nobel and Bobich, 2002). Although individual-cladode net photosynthesis (Pn) determinations are useful to estimate gas exchange rate per unit area they have limitations when used to scale up to whole canopy gas exchange, because a large variability of carbon assimilation may occur within the canopy, due to differences in cladode age (Samish and Ellern, 1975), intercepted radiation (Nobel, 1988), crop load (Inglese *et al.*, 1994), source-sink relationships (Pimienta-Barrios *et al.*, 2005), response to abiotic stress (Nobel and Bobich, 2002). The use of either portable cuvette systems or whole-tree open chambers in the understanding of plant photosynthesis have been widely reviewed and each of these systems has a particular set of advantages and disadvantages (Medhurst *et al.*, 2006; Saxe *et al.*, 1998). Measurements of carbon uptake of whole individual trees date back to Heinicke and Childers (1937) and have been widely applied to different fruit and forest trees. More recently, inexpensive polyethylene homemade chambers have been developed and successfully used for continuous field measurements of whole-tree photosynthesis (Corelli Grappadelli and Magnanini, 1993, 1997; Francesconi *et al.*, 1997; Mattii and Orlandini, 2005; Miller *et al.*, 1996; Wünsche and Palmer, 1997). The most important problems of whole-tree, open gas-exchange systems are related to the control of temperature rise and the accurate measurement of the air flow rate within the chamber (Alterio *et al.*, 2006), which can be solved by careful setting of the air flow rate as to not affect the photosynthetic processes and the CO₂ between the ambient air and the chamber (Balogh *et al.*, 2007). The response of *O. ficus-indica* to water stress, in term of gas

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exchange rate, has been investigated on individual, terminal or basal cladodes while there are no data integrating whole-tree behaviour where cladode interactions are more complex (Andrade *et al.*, 2009; Nobel and Bobich, 2002).

Cladode succulence acts as a buffer to maintain turgescence in the photosynthetic tissue (chlorenchyma), making it possible for the cladode to continue photosynthesizing during dry periods. Small changes in daily net CO₂ uptake of single cladodes occur during the first 7 days of drought (Nobel and Bobich, 2002); after 17 days of drought, cladode net CO₂ uptake over a 24-h period halved, and became almost zero at 50 days (Acevedo *et al.* 1983; Nobel and Hartsock, 1984). Drought combined with an increase of daughter cladodes shortened the period of positive net CO₂ uptake and reduced the maximum rates of CO₂ uptake of 1-year-old basal cladodes, while total daily CO₂ uptake does not change if trees are watered (Pimienta-Barrios *et al.*, 2005). Eventually, detached, unrooted cladodes show a net CO₂ uptake rate similar to that of rooted plants under drought conditions, with a 45% decrease in CO₂ uptake at 4 weeks, 87% at 8 weeks and 93% at 12 weeks after detachment from the mother plant (Raveh and Nobel, 1999).

Nevertheless, all mentioned data have been taken on single individual cladodes and there is no evidence on a whole-tree response to water stress, which could be buffered by the effect of cladode age on CO₂ uptake (Samish and Ellern, 1975). To test this hypothesis, an open system for gas exchange measurements was used to measure CO₂ uptake of (i) single well-watered and non-irrigated *O. ficus-indica* whole-trees, and (ii) individual 1-year- and 2-year-old whole-cladodes.

Materials and methods

The objective of the experiment was to measure CO₂ exchanges in a pot lot made by *Opuntia ficus-indica* 3-year-old trees, cv *Gialla*. Experiments were carried out in: (2.1) whole trees in relation to water status, and (2.2) individual cladodes differentiated by age. All observations were made at the experimental site, located in Palermo (38°06' N, 13°21' E, 40 m a.s.l.), from May to October 2010. Net CO₂ uptake was measured using an open system for gas exchange applied to single trees (2.1) and cladodes (2.2).

Chamber construction and operations

Open chambers were made of low density (95% transparency) polyethylene plastic sheets, closely fixed around the whole canopy or single cladodes of *O. ficus-indica* (chambers were built up 10-15 min before the beginning of the measurement and until the end of them). To keep the CO₂ concentration, between chamber and outside air, much higher than the infrared gas analyzer precision, a constant airflow through the chambers was generated by a ventilation system formed by 6 centrifugal fans powered by electric motors and with PVC pipes of 5.4 cm internal diameter (Miller *et al.*, 1996). Airflow rate within the chambers was 8-10 l s⁻¹ for those built up around the whole trees, and 3-4 l s⁻¹ for those fixed on single cladodes. Differences in the flow rate were due to the different volume of the chambers needed to cover single trees or a single cladode. A foam rubber gasket material sealed the chamber to trunk, or single cladode, and pipes. Air capacity was measured at hour intervals in the middle point of the pipes, using a hot wire digital anemometer (Testo 405-V1, Testo S.p.A., Italy); air velocity was measured positioning inside the pipe the sensing head of the anemometer at two different distances, along its diameter, from the pipe wall, and assuming a velocity of zero at the edge: the fan air capacity was calculated by multiplying the tube sectional area time the sectional air velocity into the pipe. Air capacity generated by pipe fans was also verified

against the same parameter measured by a Venturi meter, built by GMR Strumenti (model FMD-01, Firenze, Italy). CO₂ concentrations of the reference (external) air and the chambers outlet airflow were measured using an IRGA (CIRAS-1, PP-Systems, Hitchin Herts, UK). Temperatures inside the chambers were continuously measured, throughout the sampling periods, being 2.3±0.3°C and 0.2±0.01°C higher than the outside air, respectively during the daytime and at night. Measurements were taken over a 24 h period, starting at 02 p.m. Differences in CO₂ concentration were alternately taken in each of six chambers with a 10' interval (in one hour, all six chambers were measured) using an *home-made* 3-channels solenoid valve controller commanded by a data-logger (model CR1000, Campbell Scientific Ltd., Logan, USA).

Measurements were taken when steady-state conditions were reached, about 10-15 min after the plastic sheets-made chambers were fixed to the trees and the fans were switched on to blow air into the chambers. Whole-tree CO₂ assimilation *A* (photosynthetic rate, μmol s⁻¹) was calculated using following equation (Alterio *et al.*, 2006):

$$A = F * \Delta CO_2$$

where ΔCO₂ is the difference in CO₂ concentration between the outlet and the inlet (μmol mol⁻¹) air flows and *F* is the molar air flow blown by the fan. Time reading of the CIRAS-1 was set to one minute: in each 10 min interval, differences between the external and the outlet air CO₂ concentration were measured 10 times and averaged in order to calculate the hourly concentration for each chamber.

Total daily net CO₂ uptake was obtained by integrating (using the hourly average) the instantaneous rates over the 24 h measurement period (Nobel, 1988).

On the dates of gas exchange measurements photosynthetic photon flux density (PPFD) was measured hourly from dawn to dusk on both sides of each sampled cladode, with a LI-190 portable quantum photometer (LI-COR, Lincoln, NE, USA) and then integrated to get the total daily PPFD expressed as mol m⁻² d⁻¹.

Data were submitted to analysis of variance (ANOVA) and means were separated with Tukey test at P≤0.05. The statistical analysis was carried out using Systat 10 (Systat, USA).

CO₂ uptake of potted trees in relation to water status

CO₂ exchange rates of three irrigated and three non-irrigated *O. ficus-indica* trees were measured 0, 1, 3, 6, 8, weeks after the onset of the dry period (started on June 2nd). Measurements were carried out from June 4th until August 2nd 2010. Six 3-year-old trees were used, placed in 250 liters black polyester pots filled with a sandy-loam soil with a field capacity of 26.3% and a wilting point equal to 12.3%. Trees were used as a single replicate.

Three of the six trees were continuously watered throughout the season, starting from 15 April, to maintain soil water content above 20% (75% of available water). In the remaining trees irrigation was suspended on June 2nd, and the trees were re-watered 10 weeks afterwards. To assess their recovering capacity, CO₂ uptake of re-watered and continuously watered trees was measured 2 weeks after the re-watering (29 August 2010).

At the onset of the experiment, all trees had no fruit and 11±1.0 1-year-old mother cladodes; the number of current-year daughter cladodes was set, early in June, to 3±1 and all new buds were removed soon as they appeared. Older cladodes had a similar surface area. At the beginning and at the end of the season, for each tree, the planar surface area of 1-year-, 2-year- and 3-year-old cladodes was measured, using paper silhouettes of each cladode (Garcia de Cortazar and Nobel, 1992), successively measured by a leaf area meter (Delta-T Devices

LTD, Cambridge, UK); cladode thickness was measured at three locations, and total surface area was expressed as $(2 \times \text{projected cladode area}) + (\text{circumference} \times \text{thickness})$ (Wang *et al.*, 1997).

Air temperature and relative humidity (RH) were continuously measured using meteorological sensors (HOBO H-08, Onset, Bourne, MA, USA). Soil moisture was detected, every three days, from soil samples (2 samples in any pot at 15 cm of depth) placed in an air-forced oven (M-250-VF, MPM Instruments srl, Bernareggio, Italy) until constant mass. PPFD was measured as described before.

Three current-year, 1-year- and 2-year-old cladode samples were taken fortnightly, from June 12th, to August 29th 2010, from each tree, between 9.00 a.m. and 10.00 a.m. using a 1.4 cm diameter cork borer. Parenchyma and chlorenchyma were separated, using a sharp scalpel, immediately after sampling to measure their fresh weight; samples were then dried at 80°C to constant mass, in a forced-air oven, to measure their dry weight.

CO₂ uptake of single cladodes differentiated by age

Seven gas exchange measurements were carried out from May 20th to September 17th 2010 on six single, sun-exposed terminal cladodes differentiated by age (three 1-year-old and three 2-year-old cladodes), growing on each of three watered, potted cactus pear trees. The planar surface of the sampled 1- and 2-year-old cladodes was measured and the total cladode area was calculated as previously described. Air temperature and RH were continuously measured using meteorological sensors, placed within and above the canopies and PPFD during the daytime was recorded as previously described.

Results

CO₂ uptake of potted trees in relation to water status

At the beginning of the experiment, 1-year- and 2-year-old cladodes surface area was similar in irrigated and non-irrigated trees (7974 cm² and 7536 cm², respectively). At the end of the drought period (August 27th), the surface area of current-year cladodes was 4644 cm² in well-

watered trees and 2036 cm² in the non-irrigated ones, 37% and 21% of whole-canopy surface area, respectively.

Average day/night temperatures increased from 22/14°C early in June, to 26/19°C in late June, 28/20°C in mid July, and 29/23°C in early August. Soil water content never changed in watered pots and decreased, continuously, in non-watered pots. PPFD also increased gradually from 25 mol m⁻² d⁻¹ early in June, to 27.3, 35.1 and 39.2 mol m⁻² d⁻¹, in July and August. At the first measurement date (June 4th) all trees were at field capacity; mean daily net CO₂ uptake was 138.6±6.57 mmol m⁻² d⁻¹ and a variability between the trees not higher than 15% (data not shown). Maximum instantaneous rates occurred from midnight until 2.00 am with an average value (n=6) of 6.5±0.88 mol m⁻² s⁻¹. One week after the onset of the drought period, when soil moisture in non-water pots was 18%, there still were no differences among the trees.

Three weeks afterwards, soil moisture in non-irrigated pot was 7.5%. Highest instantaneous rates reached 6.2±0.91 mol m⁻² s⁻¹ in non-irrigated trees and 7.0±0.21 mol m⁻² s⁻¹ in irrigated ones. However, instantaneous CO₂ uptake rates differed during part of the night and the early hours of the morning, which correspond to Phase I and II of the CAM circadian rhythm (Nobel, 1988). As a consequence, daily net CO₂ uptake was 25% lower in non-irrigated than in irrigated trees: 130.7±7.92 mmol m⁻² d⁻¹ and 174.6±8.58 mmol m⁻² d⁻¹, respectively (Figure 1A).

Six weeks after the onset of the drought period, soil moisture in dry pots was 5.5%. The nighttime instantaneous CO₂ assimilation showed a peak of 7.8±1.12 mol m⁻² s⁻¹ and 10±0.48 mol m⁻² s⁻¹ in non-irrigated and irrigated trees, respectively. Significant differences occurred throughout the night (Phase I) and until sunrise. Non-irrigated trees had almost 40% less carbon uptake than irrigated ones, since daily net CO₂ uptake was 146.8±6.12 mmol m⁻² d⁻¹ in non-irrigated and 242.0±8.54 mmol m⁻² d⁻¹ in irrigated trees (Figure 1B).

Eight weeks after the onset of the drought period, at 5% soil moisture, maximum instantaneous CO₂ uptake rates were 11.1±0.94 mol m⁻² s⁻¹ in irrigated trees and 8.2±0.46 mol m⁻² s⁻¹ in the non-irrigated ones. Differences occurred from sunset to sunrise, along Phases IV, I and II of the CAM circadian rhythm (Nobel, 1988). Non-irrigated trees fixed 50% less carbon than irrigated ones, with daily net CO₂ uptake of

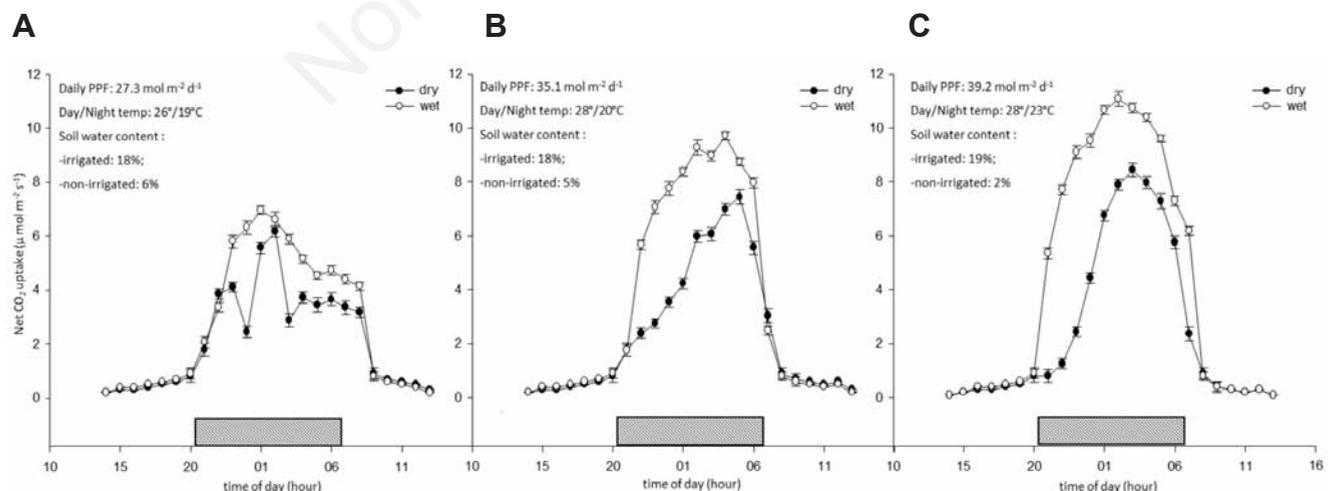


Figure 1. Daily net CO₂ uptake in irrigated and non-irrigated *Opuntia ficus-indica* trees. Data are means (±SE) of 60 min measurements made with open chambers covering three single trees for each treatment (dry and wet), used as single replicates (A: 25 June 2010, B: 16 July 2010, C: 1 August 2010). Dotted bar coincides with the nighttime period.

302.9±9.51 mmol m⁻² d⁻¹ for irrigated trees and 153.8±6.33 mmol m⁻² d⁻¹ for non-irrigated ones (Figure 1C).

During the season, total daily net CO₂ uptake doubled in well-watered trees and did not change in non-irrigated trees (Figure 1A-C). The pattern of net CO₂ uptake did not change with treatments, and well-watered trees had higher assimilation rates during most of the night.

Non-irrigated trees were re-watered on August 12; two weeks later (August 27) they had a daily net CO₂ uptake of 263±56.28 mmol m⁻² d⁻¹, while well-watered trees had an assimilation of 314.8±7.41 mmol m⁻² d⁻¹, with an average day/night temperature of 23/16°C, indicating an almost complete recovery of their potential photosynthetic capacity.

Fresh weight and cladode dry matter accumulation

At the beginning of the experiment there were no differences in terms of fresh (fw) and dry (dw) weight of 1-year- and 2-year-old cladodes for both parenchyma and chlorenchyma tissues (Figures 2 and 3). Later in the season, fresh weights of 1-year- and 2-year-old cladodes of watered trees increased respectively by 15% and 50%, while in non-irrigated trees it decreased by 60% and 45% (Figure 2A,B). During the drought period, the parenchyma lost 100%, 67% and 54% fw, respectively in current-year, 1-year and 2-year-old non-irrigated cladodes, while in irrigated trees it increased by 20% in 1-year-old cladodes and by 70% in

2-year-old ones (Figure 2A,B). The fresh weight of the photosynthetic tissue, the chlorenchyma, did not change in well-watered 1-year-old and 2-year-old cladodes, while it increased by 80% in the newly developing current-year cladodes. In trees subjected to drought, the chlorenchyma fw decreased by 35% and 30%, respectively in 1-year- and 2-year-old cladodes and marginally increased in current-year ones (+20%). During the same period, no changes occurred in the watered trees for the dry weight of 1-year old cladode components, while the dry weight of 2-year-old cladodes increased by 60%, and the parenchyma dw doubled (Figure 3A,B). Indeed, in watered trees, at the end of the experiment the dry weight of the parenchyma of 2-year-old cladodes was 2.5 folds higher than for 1-year-old cladodes (Figure 3A,B), while no differences occurred between components of 1- and 2-year-old cladodes of non-irrigated trees (Figure 3A,B). When the experiment began, current-year cladodes were at the earliest stage of their development, and measurements started 30 days afterwards. At the end of the drought period, the fresh weight of current-year cladodes was 2.5 times higher than in non-irrigated trees (Figure 4A). Cladodes, in non-irrigated trees, showed no apparent parenchyma, in terms of fresh and dry weight (Figure 4A,B), while no significant differences occurred in terms of chlorenchyma fresh and dry weight (Figure 4A,B). Measurements taken 15 days after re-watering showed a complete recovering of cladode characteristics. Fresh and dry weight of parenchyma and chlorenchyma of current-year-, 1- and 2-year-old clado-

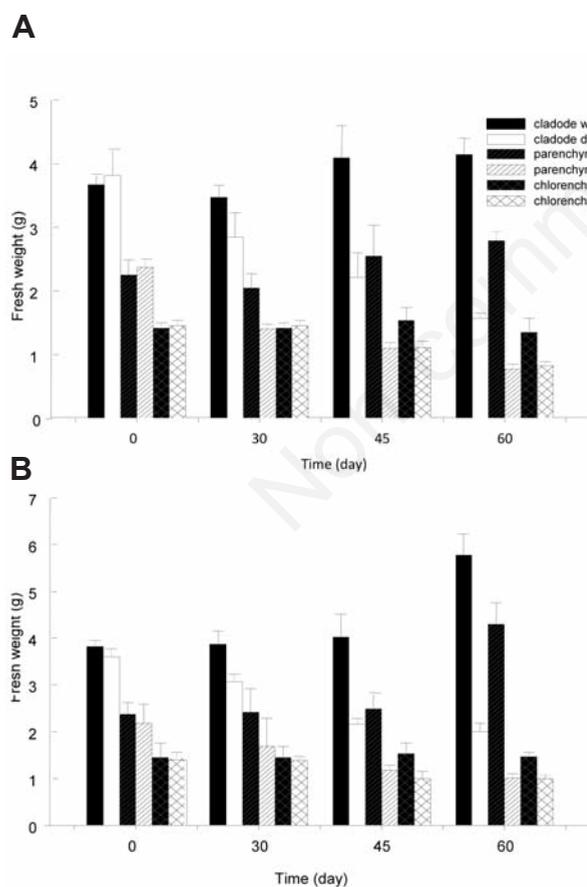


Figure 2. Fresh weight of 1-year-old (A) and 2-year-old cladode (B) components (whole sample, parenchyma and chlorenchyma) taken with cork borer (diameter=1.4 cm) from irrigated (wet) and non-irrigated (dry) *Opuntia ficus-indica* trees at 0, 30, 45 and 60 days after the onset of the dry period.

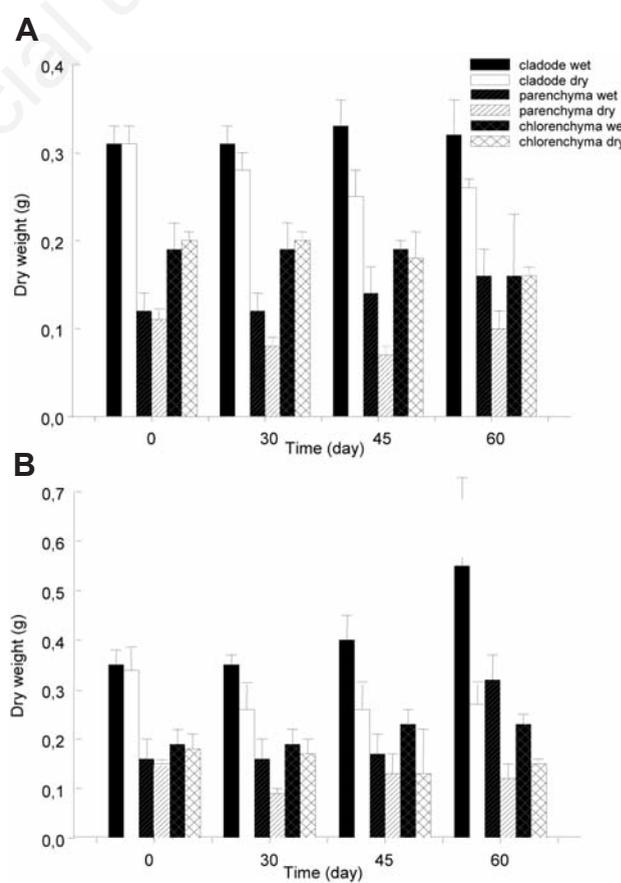


Figure 3. Dry weight of 1-year-old (A) and 2-year-old cladode (B) components (whole sample, parenchyma and chlorenchyma) taken with cork borer (diameter=1.4 cm) from irrigated (wet) and non irrigated (dry) *Opuntia ficus-indica* trees at 0, 30, 45 and 60 days after the onset of the dry period.

des were only 10% lower than for continuously watered trees (*data not shown*).

CO₂ uptake of single cladodes differentiated by age

Net CO₂ uptake of 1- and 2-year-old cladodes averaged, throughout the season, 436.6 mmol m⁻² d⁻¹ and 382.3 mmol m⁻² d⁻¹ respectively (Table 1), accounting for a carbon sequestration of 5.2 g C m⁻² d⁻¹ in 1-year-old cladodes and 4.5 g C m⁻² d⁻¹ in 2-year-old cladodes. Differences related to cladode age occurred only from the second week of July to the first week of August, when net CO₂ uptake of 1-year-old cladodes was almost twice as much than for 2-year-old ones (Table 1). Correlation (regression) between daily net CO₂ uptake and total daily PPFD showed a higher value ($R^2=0.72$) in 1-year-old than in 2-year-old cladodes ($R^2=0.32$) (data not shown), indicating higher photosynthetic efficiency of the former ones at the highest PPFD (Table 1). The nocturnal pattern and the highest instantaneous maximal assimilation rates of net CO₂ did not change with cladode age, but differences occurred at the beginning of the night (Figure 5).

Discussion

A seasonal variation of daily net CO₂ uptake was measured on single cladodes of field grown trees of *O. ficus-indica* with large fluctuations related to alternating dry and wet seasons and different PPFD (Pimienta-Barrios *et al.*, 2000). Most of the data measured on individual, basal, or terminal cladodes and even unrooted ones, indicate a continuous reduction of daily net CO₂ uptake as the water content in the soil declines, until no net uptake is measured after 50 days of drought (Nobel and Bobich, 2002; Raveh and Nobel, 1999). Surprisingly, our measurements integrating whole-tree gas exchange proved that *O. ficus-indica* trees continued their photosynthetic activity 60 days after irrigation was stopped and when soil water content was lower than 5%. At this stage, current-year and 1-year-old cladodes of non-irrigated trees had become flaccid, but, still, daily net CO₂ uptake of the whole tree kept the same rate as at the beginning of the experiment while, along with the season, well-watered trees had doubled their net CO₂ uptake. Eventually, the whole-tree daily CO₂ uptake, which integrates cladodes of different age and photosynthetic capacity, was much lower than values measured for individual cladodes under similar, wet or dry, conditions (Nobel and Bobich, 2002; Pimienta *et al.*, 2000, 2005), indicating the different contribution of single cladodes to whole tree photosynthesis. The highest instantaneous CO₂ uptake rates, 11.1 mol m⁻² s⁻¹ and 8.2 mol m⁻² s⁻¹ for well-watered and non-irrigated trees respectively, occurred in August 2010, 60 days after the onset of the dry period. Similarly, Pimienta-Barrios *et al.* (2000) measured the highest maximal CO₂ assimilation rates in single cladodes of *O. ficus-indica*, grown in a commercial orchard in Mexico, 60 days after the end of the rainy period, when soil moisture was <10%. As a result of a much lower seasonal assimilation rate and a lower surface area development of current-year cladodes, trees subjected to drought had, over the season, a lower P_n and C accumulation than well watered ones. In fact, averaged over the six measurements dates, from June to August, total daily net CO₂ uptake was 275.6 mmol m⁻² d⁻¹ for well-watered trees and 175.6 mmol m⁻² d⁻¹ for non-irrigated trees. Considering a cladode surface area of 12,618 cm² for well-watered and 10,572 cm² for trees under drought, a daily amount of 4.2. g C d⁻¹ was accumulated in well-irrigated trees in that period, while non-irrigated trees had 50% lower values. The capacity of *O. ficus-indica* trees to substantially maintain their photosynthetic activity, though they lost most of the water from the

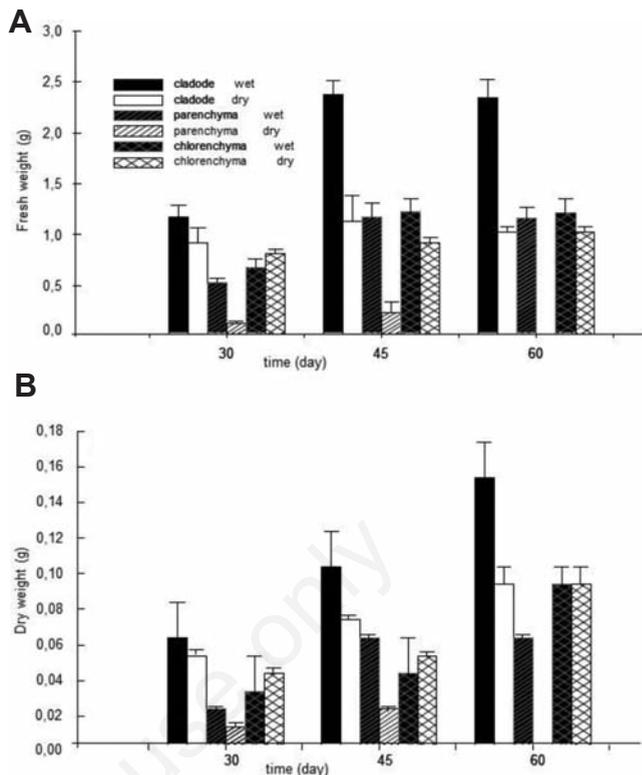


Figure 4. Fresh (A) and dry (B) weight of current-year cladode components (whole sample, parenchyma and chlorenchyma) taken with cork borer (diameter=1.4 cm) from irrigated (wet) and non-irrigated (dry) *Opuntia ficus-indica* trees at 30, 45 and 60 days after the onset of the dry period.

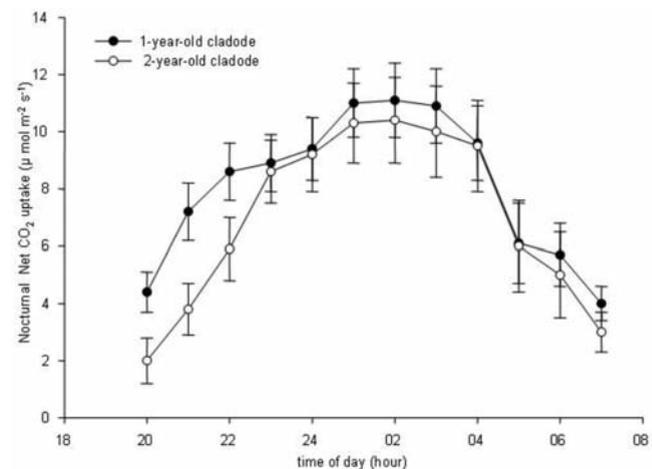


Figure 5. Average nocturnal net CO₂ uptake for 1-year-old and 2-year-old cladodes of *Opuntia ficus-indica* trees. Values are means (\pm standard error) of seven measurements made with open chambers covering three single cladodes for each treatment, used as single replicates. Samplings were taken from May to September 2010.

Table 1. Daily net CO₂ uptake over 24 h period for 1-year- and 2-year-old whole-cladodes of *Opuntia ficus-indica* during summer 2010 (data are means of 3 cladodes).

Date	Total daily net CO ₂ uptake (mmol m ² d ⁻¹)		Total daily PPF (mol m ² d ⁻¹)	Mean daily temperature (°C)
	1-year-old	2-year-old		
21 May	314 ns	349	25.5	17
4 June	510 ns	502	27.3	18
8 June	485 ns	519	31.2	21
16 July	554 a	418b	35.2	23
5 August	554 a	344b	39.0	24
2 September	228 ns	246	28.2	19
17 September	311 ns	298	28.5	18

Different letters between columns indicate significant differences at $P < 0.01$ by Tukey's test; ns=not significant. PPF, photosynthetic photon flux.

parenchyma, is likely to be related to a water movement from mother to daughter cladodes and inter-tissue water transfer from parenchyma to chlorenchyma that reduced water loss of the photosynthetic tissue (Barcikowski and Nobel, 1984; Nobel, 2006; Pimienta-Barrios *et al.*, 2000). Moreover, the chlorenchyma dw of 1- and 2-year-old cladodes did not change significantly along with the season. Similarly, Goldstein *et al.* (1991) and Nerd and Nobel (1991) reported that, after a 15-week drought period, cladode water content decreased by around 60%, and the water-storage parenchyma lost a greater fraction of water (61%) than the chlorenchyma (27%), which most likely helped maintain nocturnal acid accumulation in the latter tissue. The accumulated C, in absence of fruit, was apparently partitioned, in well-watered cladodes, to the parenchyma of 1- and 2-year-old cladodes and to the current-year growth. However more data are needed to assess the extent of dry matter partitioned to the root system and to the older cladodes as well as the nature of carbohydrates stored. Non-irrigated trees showed no apparent growth, in terms of dry matter accumulated in the 1-year and 2-year-old cladodes, and only the chlorenchyma of current-year newly developing cladodes appeared to increase its dry weight during the season. Therefore, apart from diurnal respiration losses, dry matter accumulated during the drought period is likely to be destined to older stems and the root system, which were likely most responsible for plant recovery after the long period of drought (Nobel, 1988). The roots of 18-months-old non-irrigated *O. ficus-indica* trees incorporated 23% more carbon from the basal cladodes than for well-watered ones (Luo and Nobel, 1992), and shifting the carbon allocation pattern in favor of the roots contributes to drought resistance in perennials and woody trees, and facilitates their recovery after long periods of water stress (DaCosta and Huang, 2009; Faust, 1989). Moreover, the soluble sugar content usually decreases with drought, associated with an equivalent increase in polysaccharides, presumably starch, in the water-storage parenchyma, but not in the chlorenchyma (Nerd and Nobel, 1991).

The chlorenchyma of *O. ficus-indica* can reversibly lose 70% of its water content at full turgor and the water storage parenchyma can lose 82% (Andrade *et al.*, 2009) and, indeed, after re-watering cladodes showed a complete recovery in terms of fresh and dry weight as well as gas exchange rate pattern (data not shown). A similar recovery capacity was shown by Pimienta-Barrios *et al.* (2005) after rainfall had occurred following a long period of dry conditions.

One year after the treatments, in June 2011, well-watered trees produced 18 ± 3 new cladodes, 75% on 1-year-old mother cladodes, while trees subjected to drought during the previous season produced 12 ± 2 , new cladodes, 90% on 2-year-old and older mother cladodes. Indeed, the excess dry weight of 1-year-old cladodes, which indicates the capacity of a cladode for the initiation of flower buds when higher than a mini-

mum of 33 g, was 49 g and 23 g, respectively, on irrigated or non-irrigated trees during the previous season.

Few data are available on cladode net CO₂ uptake, according to cladode age. Samish and Ellern (1975) noted that titratable acidity decreased linearly with increasing age, and 1-year-old cladodes had three times higher level of acidity than 2-year-old cladodes, located in a basipetal position. Carbon translocation from 2-year-old cladodes to meet the photo-assimilate demand of 1-year-old fruiting cladodes has been hypothesized (Inglese *et al.*, 1994; De La Barrera and Nobel, 2004), and carbon partitioning between mother and daughter cladodes changes with cladode developmental stages and environmental conditions (shading, water availability) (Luo and Nobel, 1992; Pimienta-Barrios *et al.*, 2005). Our data, taken on sun-exposed, apical 1- and 2-year-old cladodes, show that over seven measurement dates in summer differences between net CO₂ uptake for 1- and 2-year-old cladodes occurred only twice, corresponding to the highest PPFD and temperatures. Nevertheless, net CO₂ uptake of 2-year-old cladodes, averaged throughout the season, was 40% lower than for 1-year-old ones. This value could further decrease, considering their natural basipetal position within the tree that would reduce PPFD availability (Nobel, 1988); seasonal differences of C accumulation, according to cladode age, must be taken into account to set the optimal stem area index (SAI) in cactus pear fruit orchards (Inglese *et al.*, *in litteris*).

In summary, the open gas exchange chamber was successfully applied to measure whole tree or single organ net CO₂ uptake in cactus pear particularly to understand the response of the whole tree to environmental stress. Differently than on single cladode measurements, in our case it was possible to demonstrate how, after 60 days of drought, whole cactus pear trees were still keeping a same level of net CO₂ uptake, though a substantial water loss in the parenchyma of most photosynthetically active cladodes occurred. More investigation is needed to better understand the relationships of cladodes of a different age during a water stress, in terms of water movement and specific contribution to whole-tree photosynthetic activity.

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