CO_2 Fluxes of *Opuntia ficus-indica* Mill. Trees in Relation to Water Status

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Abstract

Gas exchange pattern in *O. ficus-indica* (OFI), refers to the Crassulacean Acid Metabolism (CAM); trees have nocturnal stomata opening, so net CO_2 uptake and water loss occur during the cooler part of the 24-hour cycle. Succulent cladodes skip severe periods of drought through their water storer tissue (parenchyma). To study carbon fluxes in stress and no stress conditions, an experiment was carried out on 3-year-old irrigated and non-irrigated OFI potted trees; whole tree gas exchange was measured continuously with a balloon system made up by a portable Infrared Gas Analyzer. Continuous measurements (nighttime) during the summer season were useful to assess differences in carbon uptake under stress and no stress conditions. There was a gradual increment (5 µmol m^2 s^{-1} in June, 7 µmol m^2 s^{-1} in July and 8.8 µmol m^2 s^{-1} in August) in terms of CO_2 uptake in irrigated trees from June to August 2010. The uptake was lower in stressed trees than in irrigated ones in each measurements date. Measurements carried out on non-irrigated trees showed carbon gain even 60 days after irrigation was stopped, with less than 2% of soil water content, far below the wilting point. Considering an average of 6.9 µmol CO_2 m^2 s^{-1}, for well watered trees, from June to August, and a stem area index (SAI) of 2, a daily amount of 21.8 kg ha^{-1} d^{-1} of CO_2 was accumulated in irrigated trees in that period, corresponding to a carbon assimilation of 0.54 T ha^{-1}.

INTRODUCTION

Opuntia ficus-indica (L.) Mill. is a CAM species (Crassulacean Acid Metabolism) belonging to *Cactaceae* family. This group of species shows nocturnal stomata opening and CO_2 uptake. During the nighttime, through phosphoenolpyruvate (PEP) carboxylation, malate is formed and stored in vacuoles, while, during the daytime, stomata are closed, malate is decarboxylated and CO_2 is fixed by Rubisco. In the early 1900s CO_2 uptake was obtained through indirectly measurements; indeed, Richards (1915), monitored gas exchange analyzing day/night fluctuation of acidity in stem tissues sampled from dusk to dawn. Direct measurements of gas exchange in *O. ficus-indica*, however, began in the early 1980s, when Nobel and Hartsock (1984) measured CO_2 uptake on single cladodes grown in growth chambers. From then onward, cladodes gas exchange measurements have been done on single portions of a cladode, at specific intervals (2-4 h) during the night, using a portable system with leaf chambers, adapted to cladode morphology. This system is affected by a large variability of spot measurements, and much depends on operator's skill.

Under optimal conditions (25/15°C day/night) and light saturation, OFI may assimilate 3.44 g m^2 d^{-1} CO_2; three weeks of drought conditions are required for halving net CO_2 uptake over 24-h periods (Nobel, 1988), and net CO_2 uptake over 24-h period is around zero after 50 days of drought (Acevedo et al., 1983). Instantaneous values of net CO_2 uptake of 1-year-old cladodes range from 4.5 to 15 µmol m^2 s^{-1} (Inglese et al., 1994; Pimienta-Barrios et al., 2005). Response of cladodes to drought also depends on newly developing cladodes; indeed, as the number of current-year cladodes increased, total daily

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CO_2 uptake did not change if mother (1-year-old) cladodes were irrigated, while decreased if they were non-irrigated. Anyhow total assimilation was lower in dry rather than in irrigated conditions (Pimienta-Barrios et al., 2005).

The measurements of whole tree gas exchange (Corelli Grappadelli et al., 1993) can very much increase our knowledge on all plant reaction to environmental constraints as well as on interaction between different cladodes of the same tree. Integrating CO_2 uptake at plant level, will also give us a more realistic idea of plant response to environmental conditions and stresses.

In order to investigate this possible system of gas exchange measurement, trees, differently irrigated, were covered with polyethylene balloons and their carbon fluxes were measured continuously during nighttime, with a portable Infrared Gas Analyzer.

MATERIAL AND METHODS

The aim of the experiment was to measure gas exchange of whole trees of *Opuntia ficus-indica* 'Gialla' in relation to water status; weekly measurements of CO_2 exchange rates of three irrigated and three non-irrigated OFI trees were carried out from 3 June to 6 September 2010 on each of six 3-years-old trees of OFI trained in 150 liters black polyester pots, filled with a sandy-loam soil characterized by a field capacity of 26.3% and a wilting point equal to 12.3%. Trees were placed at the experimental site of the Department, located in Palermo (38°06'N ; 13°21'E, 40 m a.s.l.) in two rows in a random design. Three of the six trees were continuously watered from April throughout the season, to maintain soil water content not lower than 20% (75% of available water). In the remaining trees no irrigation was supplied from 2 June, during the spring flush of flowers and cladodes, and for 10 weeks, when trees were re-watered to assess their recovering capacity. Measurements were carried out using balloons applied to single trees (A), cladodes (B) or branchlets (C) (Corelli Grappadelli and Magnanini, 1993; Francesconi et al., 1997). Balloons were made of low density (95% transparency) polyethylene plastic sheets, closely fixed around OFI canopies. The constant airflow through the chambers was generated by 3 fan-pipes with a capacity of 8-10 L s^{-1}: this ventilation system was formed by centrifugal fans powered by electric motors and PVC pipes of 5.4 cm internal diameter. Air capacity was hourly measured in the middle point of the adductor pipes, using an hot wire digital anemometer (Testo 405-V1, Testo S.p.a., Italy); air velocty 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: multiplying the tube sectional area time the sectional air velocity into the pipe, the fan air capacity was calculated. Air capacity generated by pipe fans, was also verified against the same parameter measured by a venture meter (GMR Strumenti, Italy). CO_2 concentration difference between air inside and outside the balloons, was measured using an Infrared Gas Analyzer (model, CIRAS-1, PP-Systems, Hitchin Herts, UK). All measurements started at 9.00 pm to last until 7 am; differences in CO_2 concentration were alternately taken in each of three balloons with a 20 min interval (in one hour, all of the three balloons were measured) using a "home-made" sampler controlled by a data-logger (model CR1000, Campbell Scientific Ltd., Logan, USA). Whole-tree CO_2 assimilation "A" (photosynthetic rate, μmol s^{-1}) was calculated using following equation (Alterio et al., 2006):

$$A=F*\Delta CO_2 \quad (1)$$

where ΔCO_2 is the difference in CO_2 concentration between the outlet and the inlet (μmol mol^{-1}), and F is air molar flow bowed by the fan. Time reading of the CIRAS-1 was set to one minute: in each 20 min interval, difference in CO_2 concentration was measured 20 times and averaged in order to calculate the hourly concentration (difference between inlet and outlet) for each balloon. In order to determine the amount of light received by the cladodes during the day previous the night campaign, a portable PAR sensor (Li-Cor 190 Quantum Sensor) was used: 11 hourly measurements (from 9.30 am to 19.30 pm)

were carried out on both cladode faces putting the sensor with its axis perpendicular to the face of cladode. Daily received PAR was obtained averaging the 11 daily measurements (PPFD expressed in μmols m^{-2} s^{-1}) taken from both cladode faces. At the beginning and at the end of the season, for each tree, the planar surface (both sides) of 1-year-, 2-year- and 3-year-old cladodes was measured, using a paper-replica of each cladode, successively measured by a leaf area meter (Delta-T Devices LTD, UK). In 1-year-old cladodes, only the spring flush was left, and all the new buds were removed at the time of their appearance. Air temperature, RH and soil moisture were continuously measured using meteorological sensors (HOBO Onset, USA), placed between the row and above the canopies and in each pot at 15 cm below the soil surface. Photosynthetic Active Radiation (PAR) was hourly measured from dawn to dusk preceding nighttime gas exchange measurement, with a LI-189 quantum photometer (LI-COR, LI-189, USA), placed on the planar surfaces of each cladode of each tree. An integrated daily PAR availability for each tree was then calculated. Three current-year, 1-year- and 2-year-old cladodes samples were taken, from each tree, with a 1.4 cm diameter cork borer, from 12 June to 27 August 2010. Fresh weight of parenchyma and chlorenchyma was measured immediately after sampling; samples were, then, oven-dried (marca stufa) at 60°C to constant mass, to measure their dry weight. Data were processed by Systat 10 (Systat, USA).

RESULTS AND DISCUSSION

Vegetative Canopy Growth

At the beginning of the experiment, 1-year- and 2-year-old cladodes surface area was statistically similar in irrigated and non-irrigated trees (7974 and 7200 cm^2).

At the end of the experiment (September), current-year vegetation growth in non-irrigated trees was 37% lower than in irrigated ones (4310 vs. 2703 cm^2). The surface area of current-year cladodes was 54 and 37% of 1-year- and 2-year-old cladodes respectively in irrigated and non-irrigated trees (data not shown).

Fresh Weight and Cladode Dry Matter Accumulation

One month after the beginning of measurements (12 July), fresh weight of cork borer cladode samples differed in relation to tissue typology, cladode age and water stress conditions. In 30 days, cladode samples of irrigated trees showed no significant differences in terms of total fresh and dry weight, whilst cladode samples of non-irrigated trees showed a reduction of 24 and 17%, respectively in 1-year- and 2-year-old cladodes (Tables 1 and 2). Parenchyma fresh weight of 1-year- and 2-year-old non-irrigated cladodes in July was 41 and 23% lower than in June, while chlorenchyma fresh weight did not change significantly, due to water migration from the reserve tissue to the photosynthetically active one (Tables 1 and 2). Fifteen days later (29 July), differences between irrigated and non-irrigated conditions were even more evident for each tissue and in relation to cladode age (Table 3). Fresh weight of annual cladodes of non-irrigated trees was 60% lower than irrigated ones (Table 3). This difference is largely due to a reduction of parenchyma (-82%) rather than chlorenchyma (-18%) fresh weight, which, indeed, showed a greater stability than the reserve tissue (Table 3). Fresh weight of 1-year-old cladodes of irrigated trees increased by 11%, while dry weight increased by 52%; 2-year-old cladodes showed a 30% dry weight increments. Otherwise, in non-irrigated trees, fresh weight of 1-year- and 2-year-old cladodes decreased respectively by 25 and 30%, and dry weights marginally increased (Tables 2-3).

Parenchyma and chlorenchyma of 2-year-old cladodes of irrigated trees did not significantly change by date. On the other hand, on non-irrigated trees a reduction of parenchyma fresh weight in 1-year- (-22%) and 2-year-old (-30%) cladodes was measured, with also a reduction of chlorenchyma fresh weight by 24 and 28% respectively (Tables 2-3).

In August (13 August), cladode tissues of irrigated trees continued their growth in terms of fresh and dry weight, while non-irrigated ones showed no growth compared to

previous 15 days measurements (Table 4). Current-year cladodes of non-irrigated trees showed a reduction of 48% of fresh weight, with no apparent parenchyma. 1-year-old cladodes parenchyma and chlorenchyma of non-irrigated trees showed a reduction, respectively, of 67 and 45% in terms of fresh weight, compared to irrigated ones, but there were no differences in terms of dry weight (Table 4); 2-year-old cladodes parenchyma fresh weight of non-irrigated trees was four times lower than in irrigated ones; furthermore dry weight showed a reduction of 40% (Table 4).

Measurements taken 15 days after re-watering showed an increase in terms of cladode fresh weight in non-irrigated trees; irrigation affected current-year cladode fresh and dry weight (Table 5). Moreover, re-watering, increased current-year cladode chlorenchyma dry weight of non-irrigated trees; same trend was measured in 1-year- and 2-year-old cladodes (Table 5).

The percentage distribution of dry and fresh weight during measurements (Tables 3 and 4), clearly showed how the fraction of dry matter invested on chlorenchyma of current-year or 1-year-old stressed cladodes was two times higher than in irrigated ones; moreover, 60 days after the stress condition began, the parenchyma showed a percentage of dry matter four times higher and almost three times higher in irrigated than stressed trees, respectively for 1-year- and 2-year-old cladodes.

CO_2 Uptake of Potted Trees

CO_2 uptake, in both thesis, increased with temperature and along with the season (data not shown), and was lower in non-irrigated trees than in irrigated ones in each measurement date.

The first measurement was made in early June (3 June), when both thesis were treated at field capacity: CO_2 uptake (shown as the mean of 60 min measurements for each time lapse) was 3.5 μmol m^2 s^{-1} (data not shown).

In late June, trees CO_2 uptake values were 5 μmol m^2 s^{-1} in irrigated and 3.9 μmol m^2 s^{-1} in non-irrigated trees, with an average day/night temperature of 29/19°C (Fig. 1).

In July, CO_2 uptake was 7 μmol m^2 s^{-1} for irrigated versus 4.5 μmol m^2 s^{-1} for non-irrigated trees, with an average day/night temperature of 32/24°C (Fig. 2).

Early August measurements showed a similar trend; CO_2 uptake was, in fact, 5 μmol m^2 s^{-1} in non-irrigated trees versus 8.8 μmol m^2 s^{-1} in irrigated ones with an average day/night temperature of 31/25°C (Fig. 3).

After 23 days (6 September) from re-watering of non-irrigated trees, CO_2 uptake was 6.9 μmol m^2 s^{-1} with an average day/night temperature of 23/16°C (data not shown).

CONCLUSIONS

Continuous gas exchange measurements of entire trees, using a methodology never applied to cacti so far, proved that the trees continued their photosynthetic activity 60 days after the irrigation was stopped when soil water content was lower than 2%, far below the wilting point.

Late June measurement did not show a significant difference among the two thesis: both of them improved, in fact, their carbon uptake compared with early June values.

Cladodes water content (Table 2) proved that 40 days with no water supply did not account for any water deficit to chlorenchyma.

June CO_2 uptake in both thesis could be influenced by the spring flush left on 1-year-old cladodes: Acevedo et al. (1983), highlighted, in fact, how current-year cladodes are affected exclusively by C_3 pathway with high respiration rates during their first weeks of development.

The increase of CO_2 uptake during the season may be also related to the activity of current-year cladodes, which was much higher, in irrigated trees, where young cladodes never showed any stress.

Instantaneous (calculated) CO_2 uptake values were lower then those reported by Pimienta-Barrios et al. (2005) on 1-year-old cladodes (15 μmol m^2 s^{-1}), and similar to

other field measurements made on single 1-year-old fruiting cladodes (Inglese et al., 1994).

As a matter of fact, whole tree CO_2 exchange integrates gas exchange activity of cladodes grown in different canopy light environment and of different age.

Considering an average of 6.9 μmol CO_2 m^2 s^{-1}, for well watered trees, from June to August, and a stem area index (SAI) of 2, a daily amount of 21.8 kg ha^{-1} d^{-1} of CO_2 was accumulated in irrigated trees in that period, corresponding to a carbon assimilation of 0.54 T ha^{-1}.

Further research is needed to understand the contribution of single cladodes, differentiated by age, to whole canopy gas exchanges. The reduction of annual growth in non-irrigated plants compared to irrigated ones in terms of fresh and dry weight, resulted from a consistent reduction of parenchymal tissues, while chlorenchyma dry weight of annual non-irrigated cladodes was twice as much as in irrigated ones, though its fresh weight was lower. Cactus pear trees showed a great recovering capacity when re-watered, 70 days after the water stress was imposed. The recovering involved the growth of parenchyma as the major component for cladode water storage.

Literature Cited

Acevedo, E., Badilla, I. and Nobel, P.S. 1983. Diurnal acidity changes, and productivity of a cultivated cactus, *Opuntia ficus-indica*. Plant Physiol. 72:775-780.

Alterio, G., Giorio, P. and Sorrentino, G. 2006. Open-system chamber for measurements of gas exchanges at plant level. Environ. Sci. Technol. 40:1950-1955.

Corelli Grappadelli, L. and Magnanini, E. 1993. A whole tree system for gas-exchanges studies. HortScience 28(1):41-45.

Francesconi, A.H.D., Lakso, A.N. and Denning, S.S. 1997. Light and temperature effects on whole-canopy net carbon dioxide exchange rates of apple trees. Acta Hort. 451:287-2.

Inglese, P., Israel, A.A. and Nobel, P.S. 1994. Growth and CO_2 uptake for cladodes and fruit of the Crassulacean acid metabolism species *Opuntia ficus-indica* during fruit development. Physiol. Plant. 91:708-714.

Nobel, P.S. and Hartsock, T.L. 1984. Physiological responses of *Opuntia ficus-indica* to growth temperature. Physiol. Plant. 60:98-105.

Nobel, P.S. 1988. Water relation. In: P.S. Nobel (ed.), Environmental biology of Agave and Cacti. Cambridge University Press 85.

Pimienta-Barrios, E., Zañudo-Hernàndez, J. and Nobel, P.S. 2005 Effects of young cladodes on the gas exchange of basal cladodes of *Opuntia ficus-indica* (*Cactaceae*) under wet and dry conditions. Int. J. Plant. Sci. 166(6):961-968.

Richards, H.M. 1915. Acidity and gas exchange in cacti. Washington: Carnegie Institution Publication No. 209.

Tables

Table 1. Fresh and dry weight partition of 1-year- and 2-year-old cladode tissues (chlorenchyma and parenchyma) in irrigated and non-irrigated *Opuntia ficus-indica* trees, differentiated by age (12/06/2010).

	1-year-old cladodes		2-year-old cladodes	
	FW* (g)	DW** (g)	FW (g)	DW (g)
Irrigated	3.67±0.14	0.31±0.02	3.82±0.13	0.35±0.03
Non-irrigated	3.82±0.38	0.31±0.02	3.60±0.17	0.27±0.05
		Parenchyma		
Irrigated	2.25±0.24	0.12±0.02	2.37±0.25	0.16±0.04
Non-irrigated	2.37±0.10	0.11±0.01	2.18±0.41	0.09±0.01
		Chlorenchyma		
Irrigated	1.42±0.08	0.19±0.03	1.45±0.31	0.19±0.03
Non-irrigated	1.45±0.09	0.20±0.01	1.42±0.11	0.18±0.03

* fresh weight; ** dry weight.

Table 2. Fresh and dry weight partition of 1-year- and 2-year-old cladode tissues (chlorenchyma and parenchyma) in irrigated and non-irrigated *Opuntia ficus-indica* trees, differentiated by age (12/07/2010).

	1-year-old cladodes		2-year-old cladodes	
	FW* (g)	DW** (g)	FW (g)	DW (g)
Irrigated	3.47±0.19	0.31±0.02	3.87±0.28	0.35±0.02
Non-irrigated	2.85±0.38	0.28±0.02	3.06±0.17	0.26±0.05
	Parenchyma			
Irrigated	2.05±0.22	0.12±0.02	2.42±0.50	0.16±0.04
Non-irrigated	1.40±0.08	0.08±0.01	1.68±0.61	0.09±0.01
	Chlorenchyma			
Irrigated	1.42±0.08	0.19±0.03	1.45±0.34	0.19±0.03
Non-irrigated	1.45±0.09	0.20±0.01	1.38±0.09	0.17±0.03

* fresh weight; ** dry weight.

Table 3. Fresh and dry weight partition of current-year, 1-year- and 2-year-old cladode tissues (chlorenchyma and parenchyma) in irrigated and non-irrigated *Opuntia ficus-indica* trees, differentiated by age (29/07/2010).

	Current-year cladodes		1-year-old cladodes		2-year-old cladodes	
	FW* (g)	DW** (g)	FW (g)	DW (g)	FW (g)	DW (g)
Irrigated	2.35±0.13	0.10±0.02	4.09±0.51	0.33±0.03	4.32±0.49	0.39±0.05
Non-irrigated	1.20±0.25	0.08±0.00	2.21±0.39	0.25±0.03	2.16±0.12	0.28±0.05
			Parenchyma			
Irrigated	1.15±0.13	0.06±0.00	2.55±0.48	0.14±0.03	2.49±0.30	0.17±0.04
Non-irrigated	0.21±0.10	0.03±0.00	1.10±0.09	0.07±0.01	1.17±0.11	0.15±0.04
			Chlorenchyma			
Irrigated	1.20±0.12	0.04±0.02	1.54±0.20	0.19±0.01	1.83±0.29	0.22±0.03
Non-irrigated	0.99±0.05	0.05±0.00	1.11±0.10	0.18±0.03	0.99±0.17	0.13±0.09

* fresh weight; ** dry weight.

Table 4. Fresh and dry weight partition of current-year, 1-year- and 2-year-old cladode tissues (chlorenchyma and parenchyma) in irrigated and non-irrigated *Opuntia ficus-indica* trees, differentiated by age (13/08/2010).

	Current-year cladodes		1-year-old cladodes		2-year-old cladodes	
	FW* (g)	DW** (g)	FW (g)	DW (g)	FW (g)	DW (g)
Irrigated	2.31±0.18	0.12±0.02	4.14±0.26	0.30±0.04	5.77±0.52	0.57±0.06
Non-irrigated	1.20±0.05	0.17±0.01	1.67±0.08	0.35±0.01	2.00±0.18	0.38±0.02
			Parenchyma			
Irrigated	1.13±0.11	0.03±0.00	2.79±0.14	0.16±0.03	4.30±0.45	0.32±0.05
Non-irrigated	0.00±0.00	0.00±0.00	0.92±0.08	0.18±0.02	1.01±0.09	0.19±0.10
			Chlorenchyma			
Irrigated	1.18±0.14	0.09±0.01	1.35±0.22	0.14±0.07	1.47±0.09	0.25±0.02
Non-irrigated	1.20±0.05	0.17±0.01	0.75±0.07	0.15±0.01	0.99±0.09	0.19±0.01

* fresh weight; ** dry weight.

Table 5. Fresh and dry weight partition of current-year, 1-year- and 2-year-old cladode tissues (chlorenchyma and parenchyma) in non-irrigated *Opuntia ficus-indica* trees, differentiated by age (27/08/2010).

	Current-year cladodes		1-year-old cladodes		2-year-old cladodes	
	FW* (g)	DW** (g)	FW (g)	DW (g)	FW (g)	DW (g)
Non-irrigated	3.76±0.98	1.26±0.09	6.02±0.65	1.47±0.06	6.63±0.70	1.42±0.02
			Parenchyma			
Non-irrigated	0.55±0.19	0.02±0.01	0.77±0.06	0.11±0.07	0.93±0.11	0.14±0.01
			Chlorenchyma			
Non-irrigated	3.21±0.78	1.24±0.08	5.25±0.01	1.36±0.07	5.70±0.59	1.28±0.02

* fresh weight; ** dry weight.

Figures

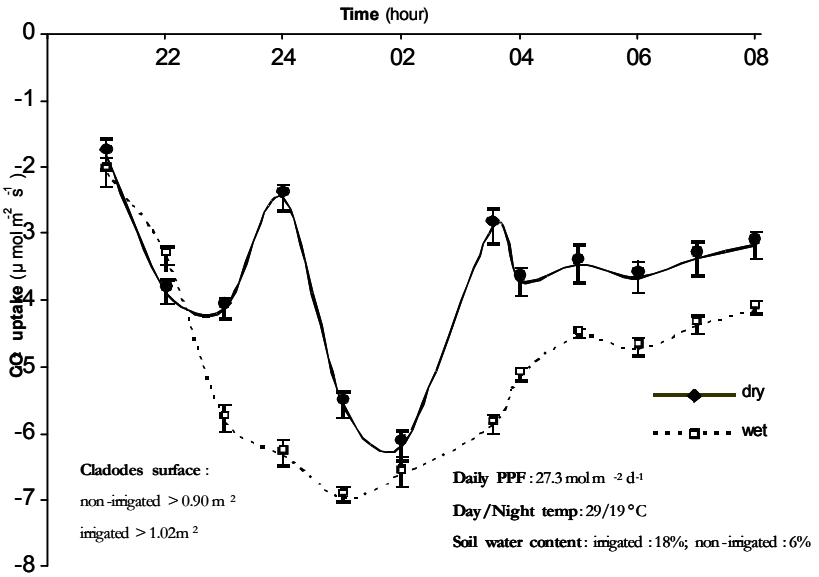

Fig. 1. CO_2 fluxes in irrigated and non-irrigated *Opuntia ficus-indica* trees. Each value is a mean (±SE) of 60 minutes measurements for each time lapse (23-28 June 2010).

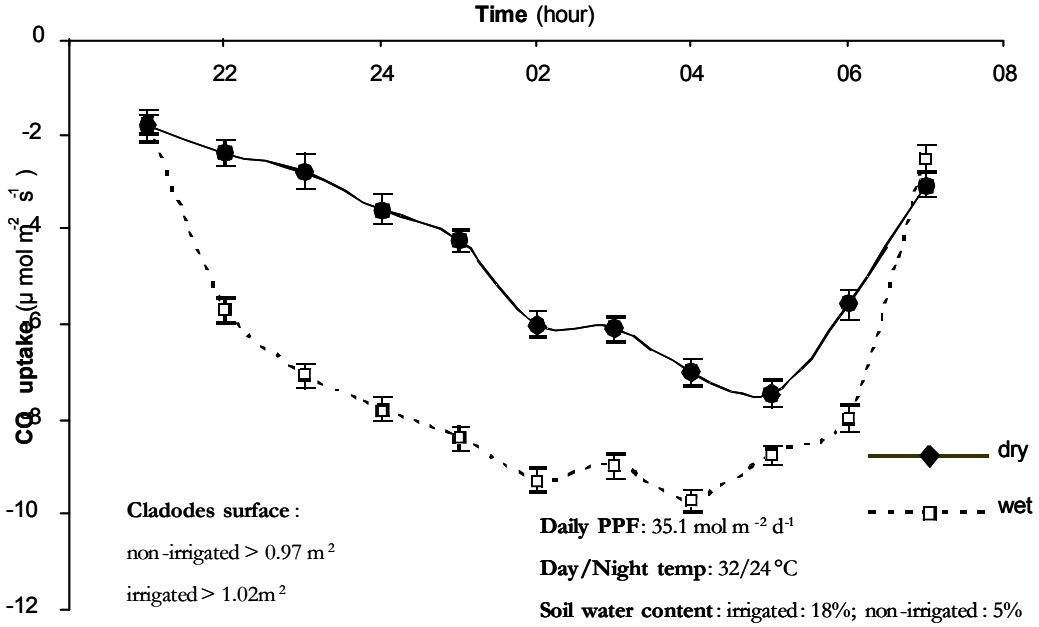

Fig. 2. CO_2 fluxes in irrigated and non-irrigated *Opuntia ficus-indica* trees. Each value is a mean (±SE) of 60 minutes measurements for each time lapse (14-21 July 2010).

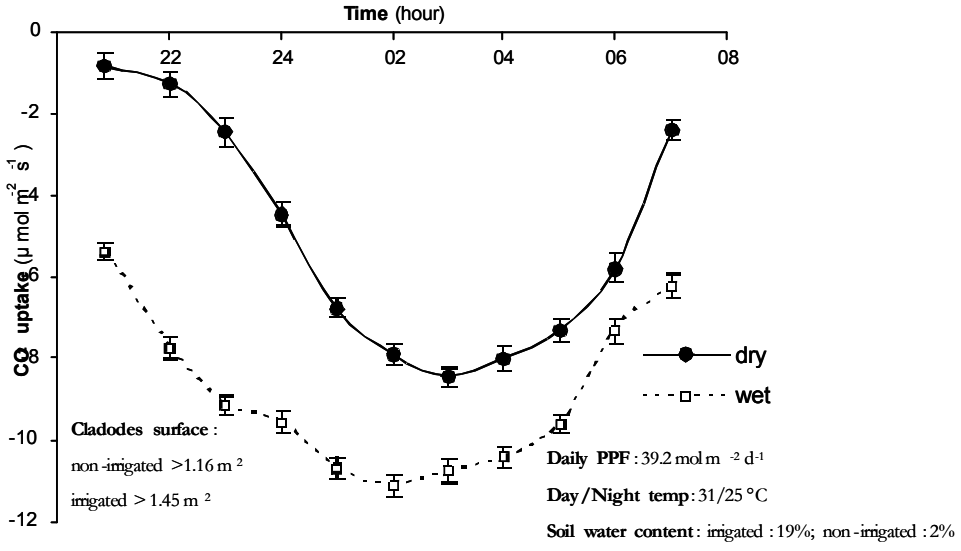

Fig. 3. CO_2 fluxes in irrigated and non-irrigated *Opuntia ficus-indica* trees. Each value is a mean (±SE) of 60 minutes measurements for each time lapse (1-5 August 2010).