Diel Patterns of Water Potential Components for the Crassulacean Acid Metabolism Plant Opuntia ficus-indica when Well-Watered or Droughted[1]

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ABSTRACT

Under well-watered conditions, chlorenchyma acidity in cladodes of Opuntia ficus-indica increased substantially at night, fully accounting for the 0.26-megapascal nocturnal increase in osmotic pressure in the outer 2 millimeters. Osmotic pressure in the inner part of the chlorenchyma and in the water-storage parenchyma did not change significantly over 24-hour periods. Three months of drought decreased nocturnal acid accumulation by 73% and essentially abolished transpiration; also, 27% of the chlorenchyma water and 61% of the parenchyma water was lost during such drought, but the average tissue osmotic pressure was little affected. Turgor pressure was maintained in the chlorenchyma after 3 months of drought, although it decreased sevenfold in the water-storage parenchyma compared with the well-watered condition. Moreover, the nocturnal increases in turgor pressure of about 0.08 megapascal in the outer part of the chlorenchyma was also unchanged by such drought. The water potential magnitudes favored water movement from the parenchyma to the chlorenchyma at the end of the night and in the reverse direction during the late afternoon. Experiments with tritiated water support this pattern of water movement, which is also in agreement with predictions based on electric-circuit analog models for Crassulacean acid metabolism plants.

Several morphological and physiological characteristics of CAM plants are advantageous in environments with a limited water supply, such as stomatal opening at night when the evaporative demand is low (5, 18). CAM plants incorporate CO_2 by dark fixation and store it predominantly in the form of malic acid, whose decarboxylation releases CO_2 internally during the day; the stomata tend to remain closed during the daytime, so CAM plants can achieve a high water-use efficiency (11, 12, 26, 29). The assimilatory organs of CAM plants also store large amounts of water per unit surface area. The close proximity of the water storage tissue (parenchyma)

and the photosynthetic tissue (chlorenchyma) reduces the distance for water movement between them, with important consequences for the daily water relations of shoot tissues of CAM plants, the focus of this study.

Certain aspects of the water relations of CAM plants have not been extensively investigated in detail (18). For instance, both solute accumulation and increased tissue elasticity can lower the Ψ[3] at which P becomes zero, a phenomenon that has not been studied in CAM plants. Recently, the effects of acid accumulation rhythms of CAM on individual Ψ components have been evaluated (8, 19, 23, 24), although none of these studies simultaneously measured the diel variations in π and P of different shoot tissues. The increase in the concentration of malic acid and hence in π at night can create a Ψ gradient leading to water acquisition by the chlorenchyma cells (6).

The major objective of the present investigation was to determine the diel patterns of Ψ components in photosynthetic and water storage tissue of cladodes of Opuntia ficus-indica, a CAM plant cultivated worldwide for its fruits and cladodes (11). An understanding of the behavior and interactions of the Ψ components in these tissues may help elucidate the diel pattern of water flow for both well-watered and droughted plants. Transpiration, titratable acidity, π, P, and Ψ therefore were measured over 24-h periods for both well-watered plants and plants subjected to drought for 3 months. A radioactive tracer technique was used to assess the water exchange between the wsp and the chlorenchyma.

MATERIALS AND METHODS

Plant Material

Individual cladodes of Opuntia ficus-indica (L.) Miller were obtained from a commercial plantation in Gilroy, CA, and rooted in a glasshouse at the University of California, Los Angeles, in October 1989. Day/night air temperatures averaged 26°C/16°C, day/night RH averaged 40%/60%, and the total daily PPFD on a horizontal surface averaged 22 mol photons m^{-2} d^{-1}. The plants were maintained in 2-L plastic pots containing sandy soil and were irrigated twice per week with distilled water and once per week with a 0.25-strength Hoagland solution No. 1 supplemented with micronutrients.

[3] Abbreviations: Ψ, water potential; ich, inner chlorenchyma; och, outer chlorenchyma; P, turgor pressure; wsp, water storage parenchyma; ϵ, volumetric elastic modulus; π, osmotic pressure.

After 3 months, 30 plants with similar cladodes averaging 37 cm in length and 22 cm in width were randomly divided into two groups equal in number. One group was well-watered as before (soil water potential in the root zone >−0.3 MPa, as determined with Wescor PCT 55–05 soil thermocouple psychrometers), and the other was droughted by withholding water for 3 months.

Water Relations Parameters and Chlorenchyma Acidity

Transpiration was determined with a LI-COR LI-1600 steady-state porometer whose cuvette aperture (2 cm^2) was appressed to the cladode surface. For all other parameters, cylindrical cores 13 mm in diameter through the cladodes were removed with a cork borer. Each core was wrapped with parafilm and sealed in a plastic bag to prevent changes in water content. All measurements were repeated at least twice with similar results. The och was arbitrarily defined as the outermost 2 mm of stem tissue, which contains most of the Chl. The ich was the next 3 or 4 mm (about half this distance in plants droughted for 3 months), and the remaining tissue was designated the wsp, which was essentially free of Chl. These three regions were separated with a razor blade and analogous samples from both sides of the cladode were combined. The vascular bundles, which are parallel to the cladode surface and occur between the ich and the wsp, were not included in the samples.

Titratable acidity was determined after macerating the chlorenchyma (och and ich) from a single core in 25 mL of distilled water with a mortar and pestle. The solution was titrated to pH 7.4 using 10 mol m^{-3} NaOH, and data are expressed on the total surface area basis (both sides of the cladode). Cell sap was extruded from all three tissue layers (och, ich, and wsp) using a vise with the samples held between rigid plastic plates. The osmolality of the extruded sap was determined with a Precision Systems μOsmette 5004 freezing-point-depression osmometer and converted to π using the van't Hoff relation (12). The Ψ of the three tissue layers was determined with Wescor C-30 psychrometric sample chambers calibrated daily. Tissue samples of approximately 0.3 to 0.4 g were sealed into the chambers, and Ψ was measured with a Wescor HR-33 dewpoint microvoltmeter in the dewpoint mode after equilibration for 4 h at constant temperature.

Pressure Probe

The P was measured using a pressure probe with a manually adjustable control rod that was used to change the volume in the pressure probe chamber and to control the location of the cell sap/silicon oil interface in the microcapillary tip penetrating a cell (16, 17). The Kulite Semiconductor Products XT-190–300G pressure transducer was calibrated inside the pressure probe with a Heise CMM Bourdon tube pressure gauge. The transducer's output (60.2 mV MPa^{-1}) was measured with a Mercer 9401 digital voltmeter and was recorded with a Cole-Palmer dual-channel flatbed strip-chart recorder. A cylindrical cladode core, wrapped with plastic film to prevent water loss, was examined with a Wild dissecting stereomicroscope at ×100 while the horizontally mounted pressure probe was guided with a Line-Tool ALH three-

dimensional micromanipulator. The outer diameter of the microcapillary tip used to impale the cells was typically about 3 μm, although larger tips (10 μm) were sometimes used to measure the P of the parenchyma cells, as their cell sap more readily blocked the smaller tips.

The ϵ was also estimated with the pressure probe (4, 30). Cell volume was changed in less than 1 s by advancing or retracting the control rod of the pressure probe to move the cell sap/oil interface by a fixed amount, and the associated change in pressure (ΔP) was determined. Using cell dimensions measured with a Zeiss phase-contrast bright-field microscope at ×600, ϵ was calculated from the relationship:

$$\epsilon = \Delta P/(\Delta V/V)$$

where V is cell volume (4, 12, 30). The volume of the closely packed cells was estimated assuming the cells to be rectangular parallelepipeds with a length (ℓ) perpendicular to the cladode surface and a mean distance along the sides (s) in the other two directions ($V = s^2\ell$).

Radioisotope

Tritiated water was injected into the chlorenchyma and the wsp to help indicate the direction of water movement between these tissues for well-watered and droughted plants. About 0.5 μL of tritiated water (ICN Radiochemicals; specific activity of 2 × 10^8 Bq cm^{-3}) was injected into the center of the och (1 mm below the stem surface) and the center of the wsp (12 mm below the surface under well-watered conditions) using 1-μL Hamilton microliter syringes. The injections were made simultaneously for locations separated laterally by 80 mm along a cladode surface. To prevent evaporation, the holes were sealed with petrolatum after the needles were carefully withdrawn from a cladode. Two hours after injection of the tritiated water, cores (13 mm diameter) centered on the injection site were removed, divided into chlorenchyma and parenchyma, and placed into scintillation vials. Tissues were solubilized with 2 mL of PROTOSOL (New England Nuclear) for 1 d, then 5 mL of AQUASOL-2 liquid scintillation cocktail (New England Nuclear) was added for counting in a Beckman LS-1801 liquid scintillation counter. The amount of tritium in the chlorenchyma was expressed as a percentage of the tritium recovered from an entire core (4–8% of the injected radioisotope was lost by evaporation from the injection hole or by transpiration). If the tritiated water equilibrated with the tissue water, then the location of the radioisotope indicates the overall movement of water; in any case, the location for the recovered radioisotope indicates the movement of the labeled water pool.

RESULTS

Both the chlorenchyma and the parenchyma of O. ficus-indica were thick (Table I), which greatly facilitated the measurements of tissue acidity and Ψ components. In particular, the chlorenchyma on each side of the cladode averaged 5.5 mm in thickness under wet conditions, decreasing 27% in thickness after 3 months of drought. The parenchyma situated between the two layers of chlorenchyma averaged 13.4 mm

Table I. *Thickness and Water Content of the Chlorenchyma and the wsp of O. ficus-indica When Well-Watered and after a 3-Month Drought*

Values for the chlorenchyma refer to the sum for both sides of a cladode. Data were obtained in the early afternoon and are presented as mean ± SE (n = 6 plants).

	Well-Watered		Droughted	
	Chlorenchyma	Parenchyma	Chlorenchyma	Parenchyma
Total thickness (mm)	11.0 ± 0.2	13.4 ± 0.9	8.0 ± 0.5	4.9 ± 0.2
Total water content (kg m⁻²)	8.6 ± 0.4	11.3 ± 1.1	6.3 ± 0.3	4.4 ± 0.2

in thickness under wet conditions, decreasing 63% in thickness after 3 months of drought (Table I). Similarly, the mass of water per unit surface area of the cladodes during the drought decreased 27% for the chlorenchyma and 61% for the parenchyma. Thus, the cladode water content after 3 months of drought was only 54% of the water content under well-watered conditions (Table I).

Large differences in transpiration rates occurred between wet and dry conditions for *O. ficus-indica* (Fig. 1). For well-watered plants (Fig. 1A), transpiration began to increase about 2 h before sunset and reached a maximum of 1.1 mmol m^{-2} s^{-1} near midnight; water loss was low during the daytime. For plants subjected to a 3-month drought (Fig. 1B), both daytime and nighttime transpiration was low, less than 0.1 mmol m^{-2} s^{-1}.

For both well-watered and droughted plants, the titratable acidity of the chlorenchyma per unit cladode surface area was higher at the end of the night compared with at the end of the day (Fig. 2). However, the nocturnal increase in acidity was 362 mmol m^{-2} for well-watered plants (Fig. 2A) but only

97 mmol m^{-2} after a 3-month drought (Fig. 2B). In both cases, the titratable acidity per unit area became less than 100 mmol m^{-2} at the end of the day. Titratable acidity in the water-storage parenchyma did not change significantly at night under well-watered and drought conditions.

For the well-watered plants, the π of the och increased 0.26 MPa during the nighttime and decreased the same amount during the daytime (Fig. 3A). Little change occurred in π of the ich or the wsp over the 24-h period, although π_{wsp} apparently increased slightly at night. After 3 months of drought, maximum π_{och} was still similar to that of the well-watered plants, but the degree of day/night variations was much less (Fig. 3B). π_{ich} was also little affected by the 3-month drought, whereas π_{wsp} increased an average of 0.10 MPa.

During the night, the P in the och of well-watered plants increased about 0.08 MPa, reaching a maximum at dawn (Fig. 4A). P_{ich} of the well-watered plants exhibited a similar pattern but with approximately half of the change compared with P_{och}, and each tissue had a similar average P over the 24-h period. P_{wsp} was relatively low and the day/night variations were small (Fig. 4A), although a slight decrease (0.02 MPa) occurred during the afternoon and a slight increase during
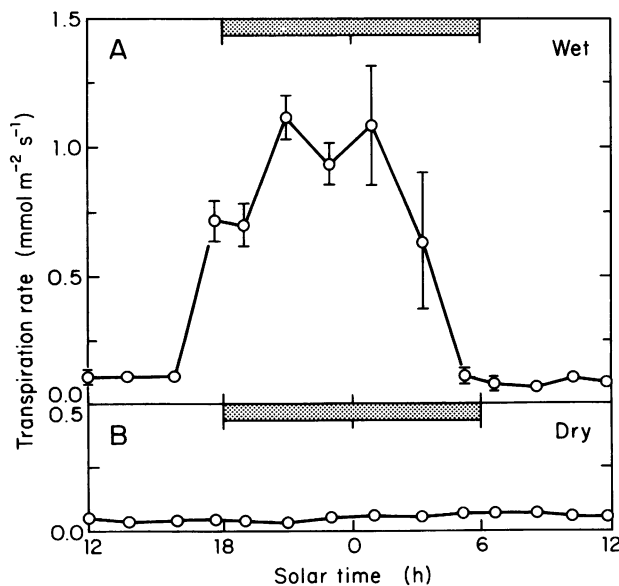

Figure 1. Diel transpiration rates of *O. ficus-indica* for well-watered plants (A) and after droughting for 3 months (B). Vertical bars, SE of the mean for four or five measurements; absence of a bar indicates that the SE was smaller than the symbol. Stippled portions indicate night time.
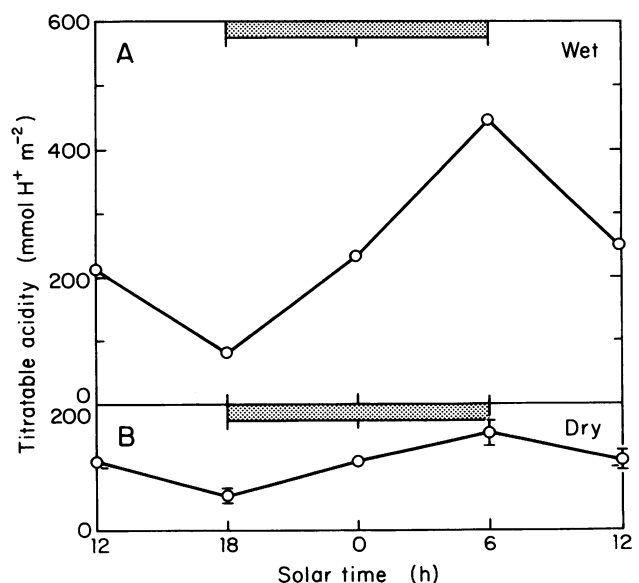

Figure 2. Diel variations in titratable acidity for the chlorenchyma of *O. ficus-indica* for well-watered (A) and droughted (B) plants. Conditions are as for Figure 1.
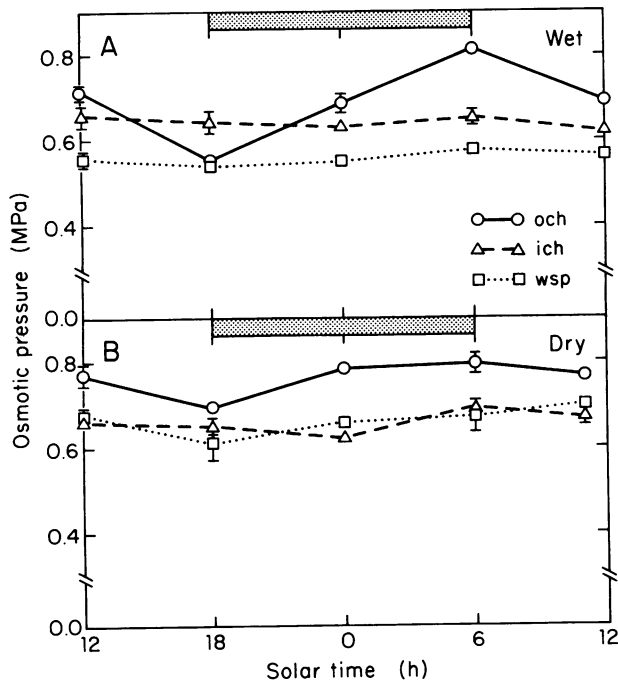
Figure 3. Diel variations in π for the och, ich, and wsp of *O. ficus-indica* for well-watered (A) and droughted (B) plants. Conditions are as for Figure 1.

the morning (Fig. 4A). The diel pattern of P_{och} for droughted plants was similar to that for well-watered plants, except for slightly lower values (Fig. 4B). P_{ich} of droughted plants was relatively low, but substantial increases at night were still observed. For droughted plants, P_{wsp} was very low (≤ 0.01 MPa), and no changes were detected over the 24-h period (Fig. 4B).

The ϵ was calculated for the och and the wsp of well-watered plants. For 25 cells in each of six cladodes, the mean cell length in the och was 103 ± 5 (SE) μm, the mean distance on a side in the och was 57 ± 5 μm, resulting in a mean cell volume in the och of 0.33×10^6 μm^3. For 25 cells in each of six cladodes, the length in the wsp was 358 ± 12 μm, the side in the wsp was 143 ± 7 μm, and volume in the wsp was 7.32×10^6 μm^3. Using these numbers and pressure probe observations, ϵ_{och} was 0.15 ± 0.02 MPa (SE for $n = 10$), and ϵ_{wsp} was 0.05 ± 0.01 ($n = 5$).

Ψ_{och} of well-watered plants decreased an average of 0.3 MPa at night and increased the same amount during the daytime over a 3-d period (Fig. 5A). Ψ_{ich} of the well-watered plants followed a similar pattern but with smaller changes, whereas little variation was observed in Ψ_{wsp}. After 3 months of drought, the average Ψ_{och} and Ψ_{wsp} were only 0.1 MPa less than for the well-watered plants (Fig. 5B), even though nearly half of the cladode water had been lost. For the plants under dry conditions, Ψ_{och} decreased 0.1 MPa during the night and increased a similar amount during the daytime, with little variation in Ψ_{wsp} (reliable Ψ_{ich} measurements could not be obtained after 3 months of drought).

Water movement between the chlorenchyma and the wsp was assessed at dusk and at dawn using injections of tritiated

water 2 h previously (Table II). For injection of tritiated water into the chlorenchyma of both well-watered and droughted plants, a larger fraction of the radioisotope was recovered in the chlorenchyma at dawn compared with at dusk. Consistent with this, a larger fraction of the tritiated water injected into the wsp of both well-watered and droughted plants was also recovered in the chlorenchyma at dawn compared with at dusk (Table II).

DISCUSSION

Large seasonal variations in soil water availability and long drought periods occur in regions where platyopuntias such as *O. ficus-indica* are native or are cultivated (3, 11). Prolonged droughts decrease nocturnal stomatal conductance thereby reducing transpirational losses, as has been demonstrated for *O. ficus-indica* (1, 15). After plants of this species were subjected to a 3-month drought, very little transpiration occurred and the water content of the cladodes decreased by 46%, representing water loss mainly from the wsp. For cacti such as *Carnegiea gigantea*, *Ferocactus acanthodes*, and *Opuntia basilaris*, water loss during drought is also preferentially from the wsp rather than from the chlorenchyma (2), as is also the
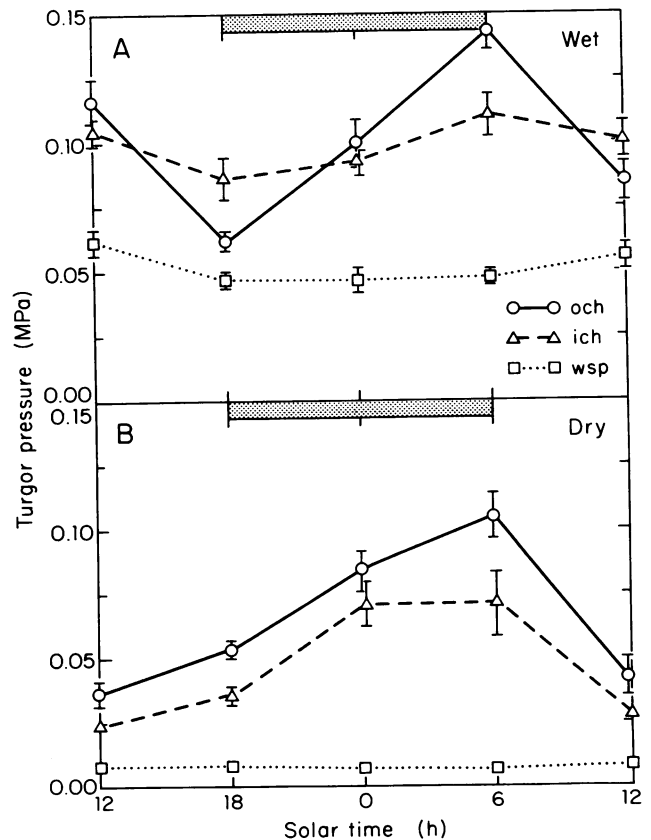

Figure 4. Diel variations in P in the och, ich, and wsp of *O. ficus-indica* for well-watered (A) and droughted (B) plants. Vertical bars, SE for 13 to 22 measurements; absence of a bar indicates that the SE was smaller than the symbol. P was measured at 1.1 mm below the cladode surface for the och, 3.0 mm for the ich, and 9.2 mm for the wsp.
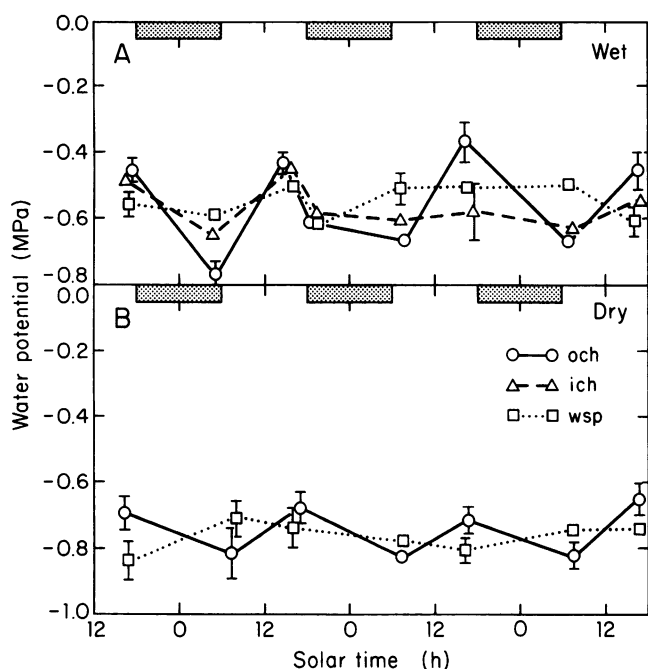
Figure 5. Diel variations in Ψ of the och, ich, and wsp of *O. ficus-indica* for well-watered (A) and droughted (B) plants during four consecutive days. Conditions are as for Figure 1.

case for the CAM species *Peperomia magnoliaefolia* (19). This differential loss can have important consequences for the maintenance of turgor and metabolic activity in the chlorenchyma of CAM plants.

Measured diel changes in π of CAM plants can be compared with those calculated from diel changes in titratable acidity, assuming a stoichiometry of 2 H^+ per organic acid, as occurs for various CAM species (7, 8, 11, 13, 14). Thus, the nocturnal increase in acidity of 362 mmol H^+ m^{-2} observed for *O. ficus-indica* corresponds to 181 mmol malic acid m^{-2}. With respect to the och (about 2 mm thick), the only tissue in which substantial nocturnal increases in π occurred, such a change in malic acid would raise the π of 2 mm of water at 20°C by 0.22 MPa (12). Because the chlorenchyma was 78% water by volume, the predicted increase in π for the water in such cells is 0.28 MPa, in excellent agreement with the observed dusk-to-dawn π increase (0.26 MPa). A nocturnal increase of 97 mmol H^+ m^{-2} for the droughted plants corresponds to a 0.08-MPa increase in π, also in excellent agreement with the measured π increase (0.09 MPa). The nocturnal increase in acidity for the droughted plants mainly represents fixation of CO_2 released internally by respiration, as the constant and minimal transpiration rate indicated that essentially no stomatal opening occurred under such conditions.

After 3 months of drought, no substantial changes in average daily π_{och} or π_{ich} were observed and only a small increase was observed in π_{wsp} compared with the well-watered condition. The relative constancy of π during drought contrasts with responses observed for other desert succulents, in which doubling of the solute concentration and a consequent doubling of π result from a loss of half of the tissue water during

drought (9–11). Fully three-fourths of the decrease in cladode water content of droughted *O. ficus-indica* resulted from changes in the wsp, which experienced a 61% decrease in water content compared with only a 27% decrease for the chlorenchyma. Such a large decrease in water content of the wsp would be expected to produce a 160% increase in π_{wsp} instead of the 18% observed. Apparently, the amount of osmotically active solutes in the parenchyma decreased substantially, perhaps by the polymerization of sugars to form glucans. The relatively moderate increase in π_{wsp} is important for the water balance of the whole plant, because if π_{wsp} were to increase passively in proportion to the decrease in water content during drought, then the wsp would become a sink instead of a source of water for the photosynthetic tissue.

Diel variations in P represent changes in tissue water content and can affect photosynthetic activity (23). The diel patterns of P in the chlorenchyma of *O. ficus-indica* were similar for well-watered and droughted plants, suggesting that this species maintains high turgor and high water content in the photosynthetic tissue, even during extended periods of decreasing soil water potential. Nocturnal increases in P were greater in the och than in the ich, whereas a slight decrease in P occurred in the wsp at night under well-watered conditions. The decrease in P_{wsp} during the night could correspond to a period of water discharge, and the slight increase during the morning may correspond to a period of refilling of the water-storage cells. The changes in P_{och} generally parallel the time course of changes in π_{och}, suggesting that the increase in osmotically active solutes in the chlorenchyma cells creates a water potential gradient favorable to water uptake by these cells (the reason for the apparent rehydration of chlorenchyma cells in the afternoon under dry conditions is not known). Previous direct measurements of P in the och of two other CAM plants, *Cereus validus* and *Agave deserti* (8), indicate a P increase of similar magnitude to that measured for *O. ficus-indica* during the night. Indirect measurements of bulk P in *Kalanchoë daigremontiana* similarly indicate a nocturnal increase and a daytime decrease in P (23). After a 3-month drought, P_{wsp} averaged sevenfold lower than for the well-watered plants, indicating a nearly complete loss of turgor in the wsp.

Diel changes in P and π are related, but differences between chlorenchyma and parenchyma may be partially accounted for by differences in the ϵ for the two cell types. In the och, ϵ was about 3 times larger than in the wsp of *O. ficus-indica*.

Table II. *Percentage of Recovered Tritiated Water in the Chlorenchyma 2 h after Injection into the Chlorenchyma or into the wsp of Well-Watered and Droughted (3 Months) O. ficus-indica*

Data are presented as mean ± SE ($n = 4$).

Site of Radioisotope Injection	Tritiated Water Recovered in the Chlorenchyma			
	Well-watered		Droughted	
	Dusk	Dawn	Dusk	Dawn
	% of total recovered			
Chlorenchyma	26 ± 2	58 ± 9	29 ± 3	44 ± 2
wsp	8 ± 1	17 ± 2	19 ± 4	36 ± 3

Thus, the same change in water content will result in a larger change in P for the chlorenchyma cells, assuming equal volumes for the respective cells; because the chlorenchyma cells were over 20-fold smaller in volume than the parenchyma cells, the resulting difference in P is even greater. Therefore, the larger, more elastic cells of the parenchyma can accept more water with smaller changes in P than can the chlorenchyma cells. The mean measured ϵ_{och} (0.15 MPa) is similar to values measured for cells of comparable size in *Capsicum annuum* (4) but much less than values measured for another CAM plant, *K. daigremontiana* (25). Although the use of excised tissue in the present study may lead to underestimations of ϵ for individual cells, the procedure should not affect the relative differences between the chlorenchyma and the wsp.

Measurements with thermocouple psychrometers represent a volume-averaged Ψ for all cells in the tissue sample (28), whereas the pressure probe measures several individual cells near the cut surface of the excised cylindrical cores and the osmometer examines an extruded cell sap, all leading to possible differences in the measurement of Ψ and its components because of the different techniques. Nevertheless, the Ψ of the different tissue layers measured psychrometrically agreed well with those calculated from $P - \pi$, both in average values and in the patterns of diel variation. For instance, psychrometric measurements of Ψ_{och} in the well-watered plants yielded a dusk-dawn decrease of about 0.2 MPa in agreement with the predicted decrease of 0.2 MPa.

Stomata can close when a plant exhausts the water available for transpiration, leading to a feedback response when the cells of the photosynthetic tissue reach zero P (22). Also, stomata may close in drying soil when the P is still positive (27), representing a feedforward mechanism that can enable the plant to avoid desiccation before soil water is completely depleted. *O. ficus-indica* apparently exhibits a seasonal feedforward response, inasmuch as the stomata close at night as drought proceeds but before the P of the photosynthetic tissue decreased significantly. Despite stomatal closure, nocturnal acid accumulation was still occurring at 27% of its maximal rate, recycling CO_2 released by respiration.

The wsp of succulent CAM plants can provide a substantial amount of the water transpired at night (21, 24). The nocturnal increases in transpiration and π decrease Ψ_{och}, thereby inducing water flow from the soil into the transpirational stream and internal water movement from the storage tissue. Therefore, the diel pattern of Ψ_{och} reflects not only diel changes in metabolic activity and transpiration, but also the hydraulic properties of the internal water redistribution pathways. The lowest Ψ_{och} for *O. ficus-indica* occurred at the end of the night, when water movement from the water-storage parenchyma to the chlorenchyma would be most favored. Conditions favoring a reverse flow (och to wsp) occurred during part of the daytime in both the well-watered plants and those droughted for 3 months, because Ψ_{och} was then higher than Ψ_{wsp}. Experiments with tritiated water indicate that water moves more readily toward the wsp at dusk and toward the chlorenchyma at dawn. This is consistent with the expected bidirectional water movement between the photosynthetic and water-storage tissues of *O. ficus-indica* based on diel chlorenchyma Ψ changes and also agrees with the pre-

dicted direction for water movement based on electric circuit analog models for the CAM plants *A. deserti* (20) and *F. acanthodes* (21). Radial water flow, which ensures adequate water supply to the chlorenchyma at night, particularly in droughted plants, may also internally redistribute inorganic and organic solutes, including growth regulators. Although several aspects of the dynamics of water movement between the wsp and the photosynthetic tissue of *O. ficus-indica* are unresolved, both diffusion and mass flow are expected to be involved in such water movement along a short but highly important internal pathway.

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