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# Effects of Drought on CAM and Water Relations in Plants of *Peperomia carnevalii*

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The mechanisms underlying the drought tolerance of *Peperomia carnevalii* Steyermark (Piperaceae), a succulent herb growing in the understorey of seasonally dry forests, were examined. Crassulacean acid metabolism (CAM) was studied in the field and laboratory, and measurements of water status were made in plants subjected to drought in the greenhouse. Nocturnal acid accumulation and day and night-time $CO_2$ assimilation rates were greatest in watered plants and decreased in drought. The proportion of $CO_2$ recycled through CAM in droughted plants, with nocturnal $CO_2$ uptake close to zero, was higher than in watered plants. Maximum quantum yield of chlorophyll fluorescence remained unchanged during drought, but the PSII quantum yield at the photosynthetic photon flux density at which the plants were grown was significantly decreased. Leaf anatomy consists of a chlorophyll-less hydrenchyma located beneath the upper epidermis, and a two-layered mesophyll. Leaves nearer to the apex are thinner than those nearer to the base of the shoot. Drought caused a reduction in leaf thickness due to shrinkage of the hydrenchyma, but not of the mesophyll. This was associated with the occurrence of a gradient of osmotic potential between these tissues. Comparison of water loss from thin leaves of watered and droughted plants, either partly defoliated at the lower nodes or intact, suggested that water moved from the thick to the thin leaves. This process was related to the occurrence of a gradient of water potential between the thick and the thin leaves. Drought tolerance in *P. carnevalii* is achieved by the operation of CAM and the occurrence of water movement within and between leaves.


**Key words:** Crassulacean acid metabolism, fluorescence, hydrenchyma, mesophyll, *Peperomia carnevalii*, water relations.

## INTRODUCTION

Most species of the genus *Peperomia* are either epiphytic or lithophytic with poorly developed root systems (Ting *et al.*, 1985); consequently, they are frequently subjected to water deficit. Ting *et al.* (1985) proposed that plants of such species would obtain sufficient water only during and just after rains, and could suffer water deficit shortly afterward. Other species of *Peperomia*, although forming roots and growing in soil, may be subjected to seasonal water deficit.

*Peperomia carnevalii* Steyermark is a perennial, evergreen herb endemic to Venezuela, which grows on the floor of sub-montane semi-deciduous, seasonally dry forests. Plants are shallow-rooted and their substrate consists mainly of litter. Plants are 10–30 cm tall, show little branching and each shoot bears, on average, nine nodes with four–five succulent leaves on partly succulent stems (Steyermark, 1984). Leaves nearer to the apex are thinner than those towards the base of the shoot. This dimorphism is not the product of development; rather, leaf thickness remains unchanged as the internodes elongate. This dimorphism may be related to the plant's ecological situation, including its water supply.

The occurrence of Crassulacean acid metabolism (CAM) has been recognized in several species of Piperaceae. Values of $\delta\ ^{13}C$ in 15 species of *Peperomia* suggested that carbon gain occurred mainly through daytime $CO_2$ uptake, although acidity oscillated throughout the day (Ting *et al.*, 1985). After studying 93 species of *Peperomia*, Holthe *et al.* (1992) concluded that water deficit accelerated the developmental induction of CAM. CAM can operate in several modes, including obligate, facultative and variations thereof. In obligate CAM, $CO_2$ assimilation takes place almost exclusively during the night in watered as well as droughted plants; in cacti, up to 99 % of carbon assimilation takes place during the night (Nobel, 1988*a*). In the cycling mode, nocturnal acid accumulation occurs in watered plants without nocturnal $CO_2$ assimilation e.g. *Talinum calycinum* (Martin *et al.*, 1988). In inducible CAM, night-time $CO_2$ assimilation is induced in $C_3$ plants by factors such as drought (Herrera *et al.*, 1991) and salinity (Winter, 1985). In severely stressed plants, the last stage in the operation of CAM, termed idling, consists of daily oscillations in titratable acidity without daytime or night-time $CO_2$ assimilation (Ting, 1985). The operation of CAM and CAM-idling may help plants, such as *P. carnevalii*, tolerate water deficit in their natural habitat.

Another feature of the plant which is probably important in surviving drought is a water-storing tissue beneath the upper epidermis which is common among species of the genus *Peperomia* (Metcalfe and Chalk, 1972). The water movement that takes place from the hydrenchyma to the

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mesophyll of leaves of *P. magnoliaefolia* was interpreted as a mechanism for the maintenance of the photosynthetic capacity under water deficit (Schmidt and Kaiser, 1987).

Leaf and stem succulence is a common feature among species of *Peperomia* and suggests a role for stored water in the water economy of these plants. Water movement from the thick to the thin leaves of succulent plants has been shown to be important in maintaining metabolism (Schäfer and Lüttge, 1987; Tüffers *et al.*, 1995).

Field measurements and laboratory experiments reported here were conducted in order to determine the mechanisms governing drought tolerance in *P. carnevalii*. We determined (1) the occurrence of CAM; (2) the role of the hydrenchyma in leaf water economy; and (3) the water transport from the thick to the thin leaves.

## MATERIALS AND METHODS

### *Plant material and growth conditions*

Field work was carried out at the Henri Pittier National Park, Estado Aragua, Venezuela, between 10°13′N and 67°14′W, at an elevation of approx. 400 m. Monthly precipitation ranges from 6 to 173 mm with an annual mean of 800 mm. The dry season spans from December until May. Plants ($n \geqslant 10$) of *Peperomia carnevalii* Steyermark (Piperaceae) were studied at the field site on three dates, covering the rainy season, the dry season and the transitional period. Microclimatic conditions were: maximum photosynthetic photon flux (PFF), 200 ± 100 μmol $m^{-2}$ $s^{-1}$; maximum/minimum air temperature ($T$), 32·0 ± 2·5/22·0 ± 1·0°C (day/night), and leaf–air vapour-pressure gradient ($\Delta w$), 2·8 ± 0·7/1·5 ± 0·3 kPa.

Plants collected in the field were grown in a greenhouse in Caracas at 1000 m elevation in 4 l pots filled with commercial, fertile soil. Roots penetrated less than 10 cm into the soil. Microclimatic conditions in the greenhouse were: maximum PPF, 500 ± 50 μmol $m^{-2}$ $s^{-1}$; $T$, 33·5 ± 2·0/22·1 ± 0·5°C; $\Delta w$, 2·5/1·1 kPa (day/night). Plants were frequently watered to field capacity for 6 months before the drought experiments. Water deficit was imposed by stopping irrigation. Five independent experiments were carried out.

### *Microclimatic measurements*

Air temperature was measured with thermistors connected to telethermometers (Yellow Springs Instruments Co., Yellow Springs, Ohio, USA). A quantum sensor (LI-190S) connected to an LI-185 meter (LI-COR Inc., Lincoln, Nebraska) was used to measure PPF.

### *Gas exchange*

*In situ* measurements in the field were made with an LCA2 infra-red gas analyser connected to a PLC(B) leaf chamber and a ASU(MF) air supply unit (Analytical Development Co. Ltd., Hoddesdon, UK). Measurements in the laboratory under controlled conditions were made as described by Herrera *et al.* (1991) using the LCA2 infra-red gas analyser connected to an assimilation chamber with temperature control (Heinz-Walz Meß- und Regeltechnik, Effeltrich, Germany). Daily courses of $CO_2$ exchange were measured on the same shoot of each plant for the duration of the drought treatment in each experiment. Leaves enclosed in the assimilation chamber for the $CO_2$ exchange experiments belonged to the first six nodes, which included thin, as well as thick, leaves. Microclimatic conditions during measurements in the laboratory were: PPF, 450 ± 50 μmol $m^{-2}$ $s^{-1}$; $T$, (day/night), 20·0 ± 0·5/18·0 ± 0·5°C; $\Delta w$, 2·2 ± 0·5/1·1 ± 0·6 kPa. In the fifth independent experiment, the instantaneous photosynthetic rate in the greenhouse ($n = 3$) was measured at 1100 h using a CIRAS 1 infra-red gas analyser connected to a PLC(B) leaf chamber (PP Systems plc, Hitchin, UK) with supplementary illumination (PPF, 980 ± 90 μmol $m^{-2}$ $s^{-1}$) provided by a portable 50 W open dichroic halogen lamp (Phillips, Germany).

Chlorophyll *a* fluorescence was measured with a Mini PAM fluorometer (Walz, Effeltrich, Germany). Fluorescence coefficients and related parameters were calculated following Schreiber *et al.* (1995). Maximum quantum yield was determined in dark-adapted leaves before dawn, as $F_V/F_M = (F_M - F_O)/F_M$. Quantum yield during illumination at PPF = 400 μmol $m^{-2}$ $s^{-1}$ at 1100 h was calculated as $\Phi_{PSII} = (F'_M - F_s)/F'_M$. Linear electron transport rate was calculated as $J = \Phi_{PSII} \times 0{\cdot}50 \times 0{\cdot}84 \times PPF$ and non-photochemical quenching as $NPQ = (F_M - F'_M)/F'_M$.

### *Defoliation experiment*

Greenhouse-grown plants of similar size ($n = 20$), either intact or with the thick leaves of the lower five nodes removed, were subjected to drought; 20 intact plants were watered every other day as a control.

### *Leaf anatomy, water relations and other parameters*

Free-hand cross-sections of fresh, non-droughted leaves were de-coloured in commercial bleach for 10 min, mounted with glycerin and observed under a light microscope. The thickness of whole leaves and tissues ($n = 10$), separated with the aid of a razor blade and a 10 × magnifying glass, was measured with a vernier caliper with a resolution of 0·1 mm.

Leaf water potential ($\psi$) was determined gravimetrically in leaf disks ($n = 3$) collected at 0600 h and placed in sucrose solutions of increasing osmolality, after Salisbury and Ross (1992). Whole leaves and separated tissues ($n = 3$) were frozen and thawed in disposable syringes, and the osmotic potential of the expressed sap ($\psi_s$) was measured using C52 psychrometric chambers connected to an HR33T microvoltmeter (Wescor Inc., Logan, Utah, USA). Preliminary determinations of $\psi$ on leaf disks of *P. carnevalii* using the psychrometric method gave erratic results due, probably, to the very thick leaves. Turgor potential ($\psi_T$) was calculated as the difference between $\psi_s$ and $\psi$. Leaf water content (water mass/unit leaf area) was determined gravimetrically ($n = 6$).

Acidity was determined in aqueous extracts ($n = 3$) by titration to pH 7·0 with 1 mM KOH (Nobel, 1988*b*). Relative internal $CO_2$ recycling was calculated as $\%R = 100 \times [(0{\cdot}5 \times \Delta H^+) - \int A_N]/(0{\cdot}5 \times \Delta H^+)$ (Griffiths, 1988), where $\Delta H^+$ = nocturnal acid accumulation and $\int A_N$ = integrated night-time assimilation rate.

Chlorophyll content ($n = 3$) was determined after Bruinsma (1963) and soluble sugar content ($n = 3$) after McCready *et al.* (1950) in samples collected at dawn. Succulence index was calculated as water mass/chlorophyll mass after Kluge and Ting (1978). Carbon isotope discrimination ($\delta\ ^{13}C$) was determined in leaves ($n = 6$) of watered plants (Ehleringer and Osmond, 1989).

Results presented are means ± s.e. Significance was assessed through a one-way ANOVA ($P < 0{\cdot}05$).

## RESULTS

### *Gas exchange, nocturnal acid accumulation and fluorescence*

Plants in the field accumulated acid during the night in the rainy season ($\Delta H^+ = 3{\cdot}1 \pm 0{\cdot}3$ μmol cm$^{-2}$), the transitional period ($8{\cdot}3 \pm 1{\cdot}1$ μmol cm$^{-2}$) and the dry season ($0{\cdot}7 \pm 0{\cdot}1$ μmol cm$^{-2}$). The assimilation rate during the rainy season was 1·7 μmol m$^{-2}$ s$^{-1}$ in the day ($A_D$) and 2·0 μmol m$^{-2}$ s$^{-1}$ at night ($A_N$); during the dry season only respiration was detected. Values of $\Delta H^+$, $A_D$, $A_N$ and integrated daytime assimilation rate ($\int A_D$) were very variable among plants measured in greenhouse and controlled conditions. Ranges for these parameters were: $A_D$, 2·5–6·0 μmol m$^{-2}$ s$^{-1}$; $A_N$, 0·5–1·5 μmol m$^{-2}$ s$^{-1}$; $\int A_D$, 82·3–366·3 mmol m$^{-2}$; and $\Delta H^+$, 2·3–6·2 μmol cm$^{-2}$; $\int A_N$ was 22·3–28·2 mmol m$^{-2}$ and the ratio $A_D/A_N$ as well as $\int A_D/\int A_N$ was, on average, 6.

Watered, greenhouse-grown plants measured under controlled conditions showed $CO_2$ assimilation during the day as well as during the night, but rates gradually diminished to almost zero after 30 d of drought, although the peak of $CO_2$ fixation during phase II of CAM (Osmond, 1978) was still significant (Fig. 1).

Values of $A_D$ and leaf conductance ($g$) measured *in situ* at 1100 h in the greenhouse were similar for thin and thick leaves of watered plants, and decreased significantly with drought in both thickness classes (Fig. 2A). Nocturnal acid accumulation was greater in watered plants and decreased in drought in both thickness classes; in watered plants $\Delta H^+$ was significantly higher in thick than in thin leaves but differences disappeared after a few days of drought (Fig. 2B).

Internal $CO_2$ recycling was higher in droughted than in watered plants; in two independent experiments it was 15 and 23 % on day 0, increasing to 93 and 69 % after 30 d of drought. Intercellular [$CO_2$] increased with drought from 93 to 150 (thin leaves) and from 150 to 304 (thick leaves) μmol mol$^{-1}$. The ratio of the amount of water saved through recycling to the transpiration rate, calculated as (mass of $CO_2$ recycled)/$\int A_N$ (Fetene and Lüttge, 1991), was seven–20 times higher in plants droughted for 30 d than in watered plants.

Values of $\delta\ ^{13}C$ in watered plants were $-24{\cdot}1 \pm 0{\cdot}8$‰ for thin leaves and $-25{\cdot}2 \pm 0{\cdot}4$‰ for thick leaves; these values are not significantly different ($P < 0{\cdot}05$).

In greenhouse-grown plants, $F_0$ and $F_V/F_M$ were unchanged after 30 d of drought in both leaf types and did not differ significantly between types (Table 1). In contrast, $\Phi_{PSII}$ measured at the growth PPF decreased with drought to approximately 50 % of the control value, whereas NPQ increased by an order of magnitude in both leaf types; NPQ was significantly lower in thin than in thick leaves of watered plants.

### *Water relations*

In leaf cross-sections, there was a clear distinction between the chlorophyll-less hydrenchyma beneath the upper epidermis, and the mesophyll with two well-defined

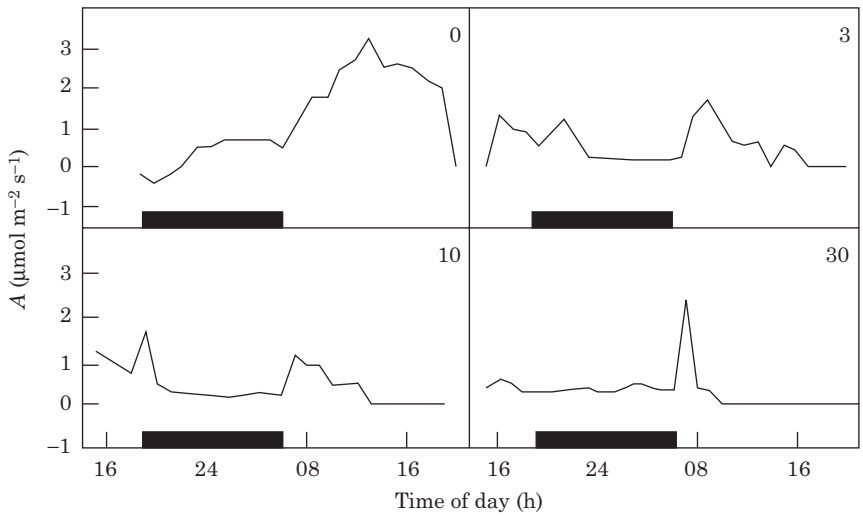


FIG. 1. Changes, caused by drought, in the daily course of $CO_2$ exchange of one watered plant of *Peperomia carnevalii* grown in the greenhouse and measured under controlled conditions. Duration of the drought (d) is indicated in the top right-hand corner of each graph. The dark bar on the abscissa indicates the dark period.

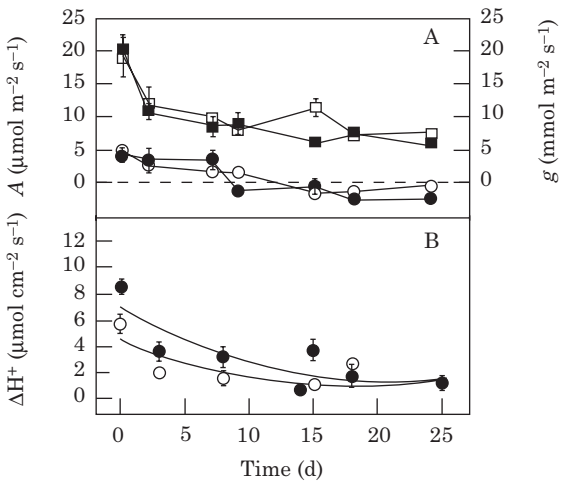


FIG. 2. Changes during drought in: A, photosynthetic rate (○, ●) and leaf conductance (□, ■) of thin (○, □) and thick (●, ■) leaves, and B, nocturnal acid accumulation of thin (○) and thick (●) leaves of greenhouse-grown plants of *Peperomia carnevalii*. Values are means ± s.e. ($n = 3$). Curves in B were fitted to second-order polynomials.

TABLE 1. *Changes in chlorophyll a fluorescence of thin and thick leaves of* Peperomia carnevalii *at the start of the drought period and after 30 d of drought under greenhouse conditions*

| | | Time (d) | |
|---|---|---|---|
| Leaf type | Coefficient | 0 | 30 |
| Thin | $F_0$ | 591[a] | 629[a] |
| | $F_V/F_M$ | 0·818[a] | 0·795[a] |
| | $\Phi_{PSII}$ | 0·753[a] | 0·292[b] |
| | NPQ | 0·1[a]* | 2·24[b] |
| Thick | $F_0$ | 457[a] | 496[a] |
| | $F_V/F_M$ | 0·807[a] | 0·807[a] |
| | $\Phi_{PSII}$ | 0·703[a] | 0·275[b] |
| | NPQ | 0·4[a] | 2·3[b] |

$F_0$ and $F_V/F_M$ were measured in dark-adapted leaves at dawn, and $\Phi_{PSII}$ and NPQ at PPF = 400 μmol $m^{-2}$ $s^{-1}$ at 1100 h in pre-illuminated leaves. Values are means. Values within a row followed by the same superscript indicate no significant differences due to time ($P < 0.05$). * denotes significant differences between thin and thick leaves ($P < 0.05$).

layers of chlorenchyma. Between days 0 and 40 of drought, the succulence index changed from 6·0 to 3·0 g water $mg^{-1}$ chlorophyll for whole thick leaves and from 1·4 to 2·9 g water $mg^{-1}$ chlorophyll for the mesophyll alone. Similar determinations on thin leaves were not carried out during the entire drought treatment because of the difficulty in obtaining uncontaminated sections of tissue in the progressively thinner leaves.

A significant reduction in thickness was found during drought in both leaf types (Fig. 3A, B). The hydrenchyma accounted for 70 and 84 % of the thickness of whole, thin leaves (not shown) and thick leaves (Fig. 3B), respectively, of watered plants. A reduction of 35 % in the thickness of the thick leaves during drought was due to shrinkage of the

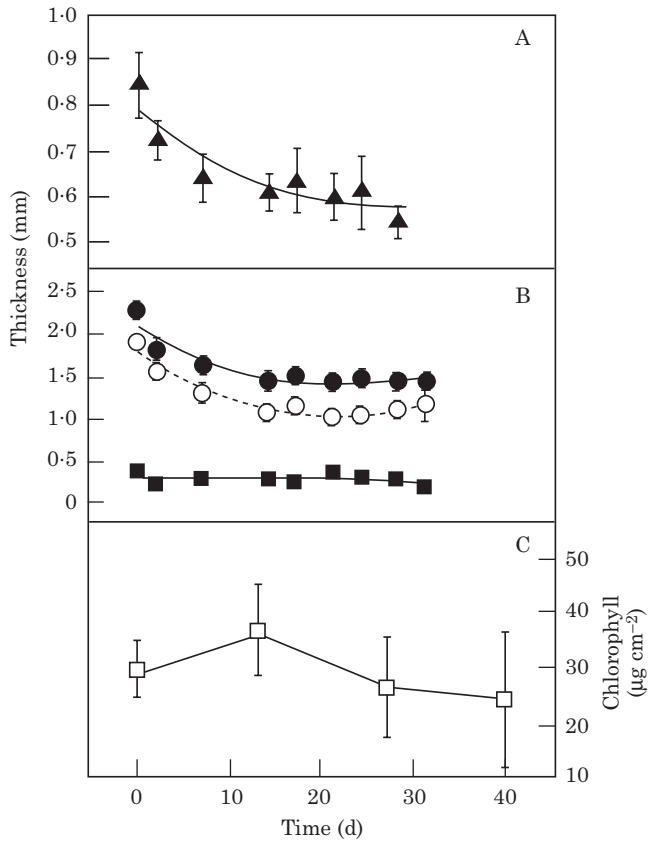


FIG. 3. Changes during drought in: A, the thickness of whole, thin leaves; B, the thickness of whole, thick leaves (○), the mesophyll (■) and the hydrenchyma (○), and C, chlorophyll content of the mesophyll of thick leaves of *Peperomia carnevalii*. Values are means ± s.e. ($n = 10$). Curves were fitted to second-order polynomials.

hydrenchyma, with mesophyll thickness unchanged. Chlorophyll content of the mesophyll of the thick leaves was constant during 40 d of drought (Fig. 3C); no significant differences were found in the chlorophyll content of whole thin and thick leaves (results not shown), and the mesophyll of the thick leaves.

Parameters of water relations of thin and thick leaves are shown in Table 2. Water potential of the thin as well as the thick leaves decreased after 15 d of drought, values being lower in thin than in thick leaves at the beginning of treatment. The water potential gradient ($\Delta\psi$) between the thin and the thick leaves was higher in watered than in droughted plants. Turgor increased with drought in both leaf types and was higher in thin than in thick leaves at the end of treatment.

Osmotic potential of the mesophyll of the thick leaves decreased with drought whereas little change was observed in the hydrenchyma; this decrease in the $\psi_s$ of the mesophyll was more marked at dawn than at dusk (Table 3). At dawn, the contribution of acids to $\psi_s$ of the mesophyll, calculated assuming that malic acid was the only acid accumulated, was high in watered plants and decreased after 20 d of drought; in contrast, the contribution of sugars remained low and constant throughout the water-deficit treatment (Table 3).

TABLE 2. *Changes with drought in the water, osmotic and turgor potential of thin and thick leaves of* Peperomia carnevalii *collected at dawn, and in the water potential gradient between thin and thick leaves*

| | Time (d) | | |
|---|---|---|---|
| Parameter (MPa) | 0 | 6 | 15 |
| Thin leaves | | | |
| $\psi$ | $-0{\cdot}67^{c*}$ | $-0{\cdot}27^{a*}$ | $-0{\cdot}49^{b}$ |
| $\psi_s$ | $-1{\cdot}04^{a*}$ | — | $-1{\cdot}54^{b}$ |
| $\psi_T$ | $0{\cdot}37^{a}$ | — | $1{\cdot}05^{b}$ |
| Thick leaves | | | |
| $\psi$ | $-0{\cdot}14^{a}$ | $-0{\cdot}17^{a}$ | $-0{\cdot}36^{b}$ |
| $\psi_s$ | $-0{\cdot}47^{a}$ | — | $-1{\cdot}14^{b}$ |
| $\psi_T$ | $0{\cdot}33^{a}$ | — | $0{\cdot}78^{b}$ |
| $\Delta\psi$ | $0{\cdot}53^{b}$ | $0{\cdot}11^{a}$ | $0{\cdot}12^{a}$ |

Values are means; those followed by the same superscript within a row indicate no significant differences due to time ($P < 0{\cdot}05$). * denotes significant differences between thin and thick leaves ($P < 0{\cdot}05$).

TABLE 3. *Changes with drought in the osmotic potential of the tissue sap of thick leaves collected at dawn and dusk, and sugar and acid concentrations in the mesophyll sap at dawn*

| | Time (d) | | |
|---|---|---|---|
| Parameter | 0 | 13 | 20 |
| Dawn | | | |
| $\psi_s$ (MPa) hydrenchyma | $-0{\cdot}57^{a}$ | $-0{\cdot}56^{a}$ | $-0{\cdot}72^{b}$ |
| $\psi_s$ (MPa) mesophyll | $-1{\cdot}22^{c*}$ | $-0{\cdot}78^{a*}$ | $-0{\cdot}96^{b*}$ |
| $\Delta\psi_s$ (MPa) | 0·65 | 0·22 | 0·24 |
| [Sugars] (mmol $l^{-1}$) | $86{\cdot}6^{b}$ | — | $54{\cdot}4^{a}$ |
| $\%\psi_s$ (sugars) | $17{\cdot}5^{a}$ | — | $14{\cdot}0^{a}$ |
| [acids] (mmol $l^{-1}$) | $193{\cdot}4^{b}$ | $147{\cdot}9^{b}$ | $99{\cdot}7^{a}$ |
| $\%\psi_s$ (acids) | $39{\cdot}0^{b}$ | $46{\cdot}6^{b}$ | $25{\cdot}3^{a}$ |
| Dusk | | | |
| $\psi_s$ (MPa) hydrenchyma | $-0{\cdot}58^{a}$ | $-0{\cdot}61^{a}$ | $-0{\cdot}55^{a}$ |
| $\psi_s$ (MPa) mesophyll | $-0{\cdot}82^{c*}$ | $-0{\cdot}59^{a}$ | $-0{\cdot}67^{b*}$ |
| $\Delta\psi_s$ (MPa) | 0·24 | 0·02 | 0·12 |
| [acids] (mmol $l^{-1}$) | 77·5 | 53·3 | 59·9 |
| $\%\psi_s$ (acids) | 23·2 | 22·2 | 22·0 |

The inter-tissue osmotic potential gradient and the proportion contributed by sugars and acids to the osmotic potential of the mesophyll are included. Values are means. Different superscripts within a row indicate significant differences ($P < 0{\cdot}05$). * indicates significant differences ($P < 0{\cdot}05$) between hydrenchyma and mesophyll.

In the defoliation experiment, leaf-water content was twice as high in the thick as in the thin leaves of intact, watered plants and decreased significantly with drought: by 40 % in thick leaves, and by 24 % in the thin leaves of intact plants, and by 61 % in the thin leaves of partly defoliated plants (Fig. 4).

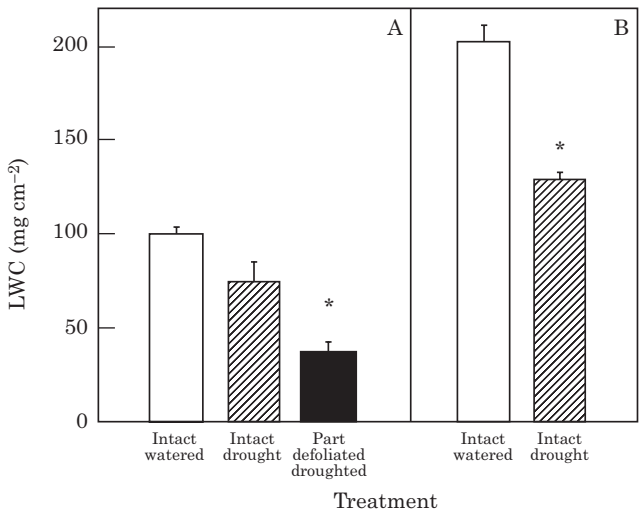


FIG. 4. Water content of leaves of intact and partly defoliated plants of *Peperomia carnevalii* maintained under either frequent watering or drought for 35 d in the greenhouse. A, Thin leaves; B, thick leaves. (□): intact watered plants; (▨), intact droughted plants; (■), partly defoliated, droughted plants. Values are means ± s.e. *indicates a significant difference ($P < 0{\cdot}05$) due to treatment within each leaf type.

## DISCUSSION

In plants of *Peperomia carnevalii*, growing under field and laboratory conditions, CAM operates in the obligate mode, watered plants having the greatest values of night-time assimilation rate and $\Delta H^+$.

Values of $\Delta H^+$ in the laboratory were within the range of those measured in the field. Daytime and night-time assimilation rates were similar to values found in five other species of *Peperomia*, which ranged from 0·3 to 6·9 (daytime) and from 0·0 to 2·7 (night-time) μmol $m^{-2}$ $s^{-1}$ (Ting *et al.*, 1985). The magnitude of $\Delta H^+$ was approximately twice as large in the thick compared to the thin leaves of watered plants, the differences gradually disappearing after 30 d of drought. This is in contrast to the report by Ting *et al.* (1996) on several species of *Peperomia* showing that leaf age determines the appearance of CAM, whereas water deficit only accelerates it.

The relatively low values of $\delta\ ^{13}C$ in plants of *P. carnevalii*, similar to those found in other species of *Peperomia* (Ting *et al.*, 1985), indicate that dark $CO_2$ assimilation is not the main route of carbon acquisition in this species, as supported by the fact that carbon balance of watered plants was higher during the day than during the night.

In plants of *P. carnevalii*, CAM idling was observed after 30 d of drought and the proportion of internally produced $CO_2$ recycled through CAM was large, similarly to other Piperaceae (Sipes and Ting, 1985; Ting, 1985; Ting *et al.*, 1985). In contrast, in other CAM plants, such as *T. calycinum* (Martin *et al.*, 1988), recycling is greatest in watered plants. Recycling in *P. carnevalii* was associated with water saving, since the ratio of water saved by recycling/transpiration rate was much higher in droughted than in watered plants. Internal $CO_2$ recycling in droughted CAM plants has been attributed a role in the prevention of photoinhibition (Osmond *et al.*, 1982) by providing a sink for electron transport. The relationship between recycling and photoprotection in *P. carnevalii* may be inferred from the observation that parameters associated with the

intactness of the photosystems, such as $F_0$ and $F_V/F_M$, remained unchanged. The value of $\Phi_{PSII}$ at the growth PPF decreased significantly with drought in both leaf types in spite of an increase in intercellular [$CO_2$]. This, together with the observed increase in NPQ, suggests that higher values of intercellular [$CO_2$] in droughted plants did not guarantee a sufficient sink for electrons, thereby increasing the need for non-photochemical mechanisms of energy dissipation. These other mechanisms may occur in droughted plants of *P. carnevalii* and must be evaluated. Results suggest that the inability of the photosynthetic machinery to maintain a high $\Phi_{PSII}$ during drought resides in some other mesophyll limitation, such as the Calvin cycle.

The succulence index for whole thick leaves and their mesophyll was typical of CAM plants (Kluge and Ting, 1978). In plants of *P. carnevalii*, the hydrenchyma accounted for nearly half of the thickness of whole leaves of both types, in contrast with plants of *P. columella*, in which the proportion was 12 and 58 % in young and mature leaves, respectively, and increased with drought in young leaves (Christensen-Dean and Morse, 1993). Water content in the mesophyll of both leaf types during drought was maintained at the expense of water lost from the hydrenchyma. This re-distribution of water between tissues was determined by the gradient of osmotic potential between the hydrenchyma and the mesophyll of the thick leaves. Such a gradient was demonstrated in leaves of plants of *Agave deserti* subjected to drought (Schulte and Nobel, 1989) whereby, it was proposed, water re-distribution from the hydrenchyma to the mesophyll would be facilitated in order to protect the photosynthetic tissue from desiccation. Water movement from older to younger leaves was shown in the CAM plants *Delosperma tradescantioides* and *Prenia sladeniana*, in which all cells are succulent (Tüffers *et al.*, 1995).

In our study, the minimum value of $\psi_s$ of whole leaves and of the mesophyll of thick leaves corresponded to the maximum $\Delta H^+$. A diel water movement between tissues was demonstrated in green organs of *A. deserti* and *Ferocactus acanthodes* which was faster during the night, when $\psi_s$ decreased in the mesophyll due to the increase in titratable acidity (Tissue *et al.*, 1991). In leaves of *P. carnevalii*, the dawn values of acid and sugar concentration in watered plants contributed more than half of the value of $\psi_s$. The results of our experiment with partly defoliated plants suggested the occurrence of water movement from the thick to the thin leaves which may have been aided by the increase in the gradient of water potential between them. Additionally, $\psi_T$ in *P. carnevalii* was maintained during drought in both types of leaves, despite a significant decrease in leaf $\psi$. In *Tillandsia recurvata*, high values of $\psi$ and $\psi_s$ and low values of the bulk elasticity modulus measured in plants water-stressed for 2 months, were taken as evidence of the capability of cells with very elastic walls to maintain a high water status in spite of a reduction in water content (Stiles and Martin, 1996). Indeed, maintenance, and possibly increase, in $\psi_T$ during drought occurs in leaves of the CAM plant, *Aptenia cordifolia* (Herppich and Peckmann, 1997).

Chlorophyll content of both leaf types and water content of thin leaves of *P. carnevalii* were maintained during 35–40 d of drought, in contrast with plants of *D. tradescantioides* and *P. sladeniana*, in which loss of chlorophyll and turgor was observed under drought in older leaves (Tüffers *et al.*, 1995). Maintenance of an intact photosynthetic apparatus and turgor would have ecological significance for *P. carnevalii* suffering short-term water deficit even during the rainy season, due to their shallow root system, and long-term water deficit during the dry season.

Drought tolerance in *P. carnevalii* is based on three mechanisms: (1) the operation of CAM, which allows a substantial carbon gain as well as water saving in watered plants during the night, when evaporative demand is low, and, possibly, photoprotection of the mesophyll through internal $CO_2$ recycling in droughted plants; (2) the transport of water from the hydrenchyma to the mesophyll of leaves during drought, which maintains photosynthetic capacity during drought; and (3) the transport of water from the thick, more succulent, leaves to the thin leaves during drought.

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