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Effects of light and drought on CAM and on-line carbon discrimination in the bromeliad *Neoregelia cruenta* (R. Graham) L.B. Smith.

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Introduction

Different growth-forms and photosynthetic modes are fundamental when Bromeliaceae ecology is under consideration. Varying from water impounding rosettes to atmospheric-plants with holdfast roots (Pittendrigh, 1948), and from C_3 to CAM photosynthesis, bromeliads are found in contrasting habitats (Martin, 1994). The bromeliad *Neoregelia cruenta* occupies mainly terrestrial but occasionally epiphytic habitats in the *restingas* (coastal sand ridge plains) of Rio de Janeiro (Reinert *et al.*, 1996) and show high morphological plasticity under different light levels. Fully exposed plants have a tight impounding rosette of short broad leaves varying from yellow to dark red in colour, whereas full shade plants have a broad open rosette of long narrow dark green leaves.

Studies of the plasticity displayed by bromeliads in response to short and long term acclimation to high light and water stress benefit from on-line carbon isotope discrimination technique (Δ), known as on-line discrimination. This technique is used in association with gas exchange measurements to determine changes in the carbon isotope composition of air passing over a leaf during photosynthesis. The use of Δ to reveal the dynamic balance between PEPC and Rubisco activities in each phase of CAM was first shown in the epiphytic bromeliad *Tillandsia utriculata* (Griffiths et al, 1990). During the transient Phases II and IV of CAM, both carboxylation enzymes were active in different proportions along the phases. Subsequently, Δ has been used to analyse the proportion of PEPC and Rubisco activity in *Kalanchoe, Clusia* and *T. stricta* and to calculate mesophyll conductance in CAM plants (Roberts *et al.*, 1996; Reinert and Griffiths, 1999).

In the work presented here we studied CAM in the field and laboratory using gas exchange, diurnal titratable acids measurements and Δ in order to examine the mechanisms underlying high light and drought resistance of *N. cruenta*.

Materials and methods

Sampling was carried out in two consecutive summers in the *restinga* of Barra de Maricá, Rio de Janeiro. Individuals of *Neoregelia cruenta* were studied under exposed, semi-exposed and shade conditions. Integrated PFD was recorded using a quantum sensor (type QS1, Delta T devices, England). Three leaf discs from 5 plants from each light regime were taken over 24 hours at 2 hourly intervals, kept on ice and deep frozen within a few hours. Freeze-thawed leaf discs were dried in a

microwave for 2-4 minutes prior to titratable acidity (T.A.) analysis using phenolphthalein as an indicator, according to Reinert *et al.* (1998).

Plants were collected from the *restinga* and maintained in a tropical greenhouse at the University of Newcastle, UK. Plants were maintained without soil under natural light, well watered and supplied with standard commercial nutrients. Laboratory gas exchange and on-line carbon isotope discrimination measurements were carried out simultaneously on two lots of plants: well watered and without water for 10 days prior to the measurements. The incident PFD regimes were 450, 300 and 150 μmol photon m^{-2} s^{-1} for a 12 hour photoperiod, corresponding to a total daily incident PFD of 20.0, 13.0 and 6.5 photon m^{-2} day^{-1}, respectively. Gas exchange measurements were made with a Walz mini-cuvette system (CMS 400) with an external IRGA (Binos 100 Walz, Germany). Cuvette day/night temperature was 22°C with the dew point at 19°C. In association with the open gas exchange system, CO_2 was collected in a liquid nitrogen cold trap under partial vacuum from the reference (compressed air tank) and analysis (following passage through the leaf chamber) outflow. Measurement of the ratio of ^{13}C to ^{12}C in the CO_2 was carried out as described by Griffiths *et al.* (1990) and Roberts *et al.* (1996). Instantaneous carbon isotope discrimination (Δ) was derived using the following equation as described in Evans *et al.* (1986),

$$\Delta = \frac{\xi(\delta_o - \delta_e)}{1 + \delta_o - \xi(\delta_o - \delta_e)}$$

Where $\xi = c_e/(c_e - c_o)$. δ and c are the CO_2 isotope composition and CO_2 concentration entering (*e*) and leaving (*o*) the cuvette, respectively. Using a simplified model, it is possible to predict the value of Δ for C_3 and C_4 modes of carbon assimilation (Farquhar *et al.*, 1989), whereby

$$\Delta = a + (b - a)\frac{p_i}{p_a}$$

Where *a* equals 4.4‰, the isotopic fractionation due to diffusion of CO_2 in air and *b* is that due to discrimination during C_3 carboxylation (*b* = 27‰) and C_4 carboxylation (*b* = -5.7‰). p_i and p_a refers to intercellular and ambient concentration of CO_2, respectively, measured during gas exchange.

Results

Figure 1 shows diurnal cycles of titratable acid (H^+) turnover for the exposed, semi-exposed, and shaded plants of *N. cruenta*. In the first year shade and exposed plants had nearly no water in their tanks. Semi-exposed plants accumulated the highest level of H^+ in comparison to shade and exposed plants. Conversely, in the second year, irrespective of exposure, the three populations showed similar maximum H^+ values.

As was the case in the field, well-watered plants in the laboratory showed larger ΔH^+ values than when subjected to water deficit (Table 1). No significant difference in ΔH^+ was found between PFD treatments. Figure 2 refers to laboratory experiments and shows a representative gas exchange curve and on-line carbon

isotope discrimination for the intermediate light intensity. Table 1 summarises results for all treatments. In plants without water, although all CAM phases were present, there was a substantial decrease in stomatal conductance (g_{H2O}) and CO_2 uptake (J_{CO2}) both at night and day. No consistent differences in g_{H2O} and J_{CO2} were observed between different light treatments. During the transient Phase IV of CAM g_{H2O} and J_{CO2} progressively increased, with a concomitant decrease of Δ, showing a shift from C_3- to C_4-like carboxylation in all six treatments. During the transient period from dark to light carboxylation (Phase II) Δ showed a quick increase, shifting from C_4- to C_3-like carboxylation. The effect of water deficit on Δ could only be investigated in plants under intermediate light, as J_{CO2} was too low in plants under high and low light intensities.

Table 1: Variation on leaf titratable acids (ΔH^+: mmol m^{-2}), integrated carbon assimilation during Phase I (night J_{CO2}: mmol m^{-2}), II and IV of CAM (day J_{CO2}: mmol m^{-2}), percentage of recycled CO_2, measured instantaneous carbon isotope discrimination (Δ) range during Phases I, II and IV of CAM in the six treatments: high (HL), intermediate (IL) and low light (LL), with ($+H_2O$) and without ($-H_2O$) water.

Treatment	ΔH^+	Night J_{CO2}	Day J_{CO2}	Recycled CO_2	Δ Phase I	Δ Phase II	Δ Phase IV
HL+H_2O	80.9	19.6	8.5	48	-1 to 3	11	10 to 20
IL+H_2O	75.1	13.1	20.4	68	-2 to 5	18 to 30	30 to 17
LL+H_2O	69.5	23.7	8.7	32	-3 to 2	-	22 to 7
HL-H_2O	52.6	8.5	6.1	71	0 to 0	-	14 to 17
IL-H_2O	59.2	6.7	15.2	75	0 to 1	10 to 10	24 to 10
LL-H_2O	60.3	7.9	3.0	74	0 to 6	9	7 to 12

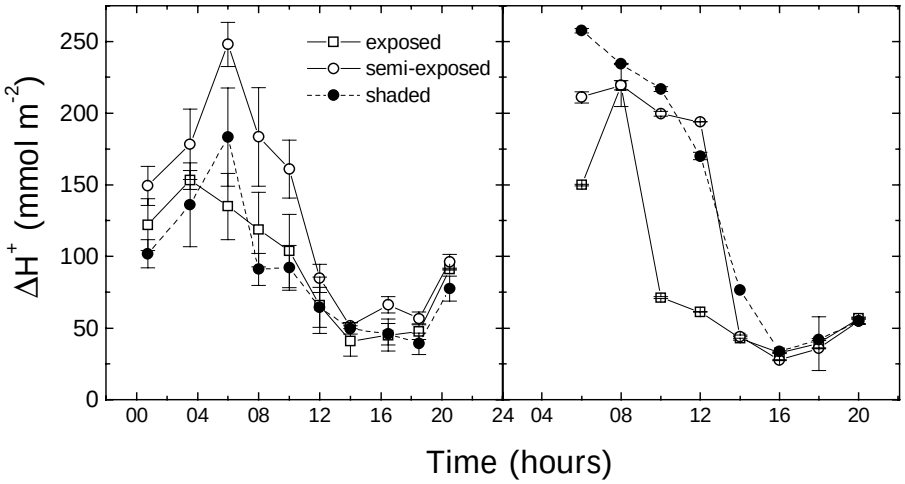

Figure 1: Field leaf titratable acids in exposed, semi-exposed, and the shaded plants of *Neoregelia cruenta* throughout the day in the first (left) and the second year (right). (n=5). Integrated PFD for the sampling day in the first year: 42.5 (exposed); 19.1 (semi-exposed); 3.9 mol photon m^{-2} day^{-1} (shade) and in the second year: 39.7 (exposed); 33.7 (semi-exposed); 17.2 mol photon m^{-2} day^{-1} (shade).

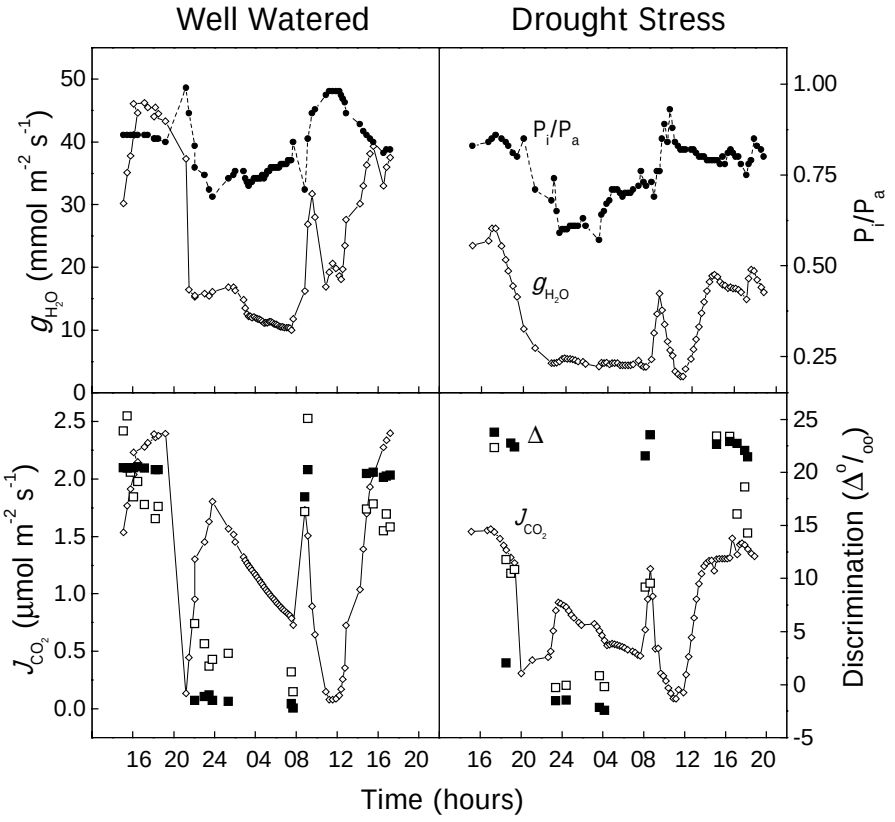

Figure 2: Gas exchange and instantaneous discrimination (Δ) for a single leaf of *Neoregelia cruenta* under 300 µE m^{-2} s^{-1} with (left) and without (right) water in the tank. Closed squares - Δ calculated from *Pi/Pa*, assuming either Rubisco or PEPC dominated carboxylation (see material and methods); open squares - Δ measured instantaneously during photosynthesis.

Discussion

Field measurements of net leaf titratable acids (ΔH^+) in *Neoregelia cruenta* under different light exposure in the first year suggests that CAM is partially inhibited in the species under extreme conditions of incident light and/or water deficit (Fig. 1). In the first year, shade plants received total daily PFD of less than 5 mol photon m^{-2}, whereas fully exposed plants were subjected to more than 40 mol photon m^{-2} day^{-1}, and in both groups plants had nearly empty water reservoirs. Conversely, the semi-exposed population was receiving considerable light (around 20 mol m^{-2} day^{-1}) and plants had adequate water supply in the tank. Low relative water content (data not shown) in the exposed and shaded populations supports the hypothesis that these two populations were under water stress.

In the second year, on the other hand, exposed and shade plants showed higher ΔH^+, in the same range found for the semi-exposed population for both years. It is interesting to note that in the second year there was an unusual rainfall regime in February, which had the lowest levels of rainfall for the last decade (total rainfall of 0.3mm compared to an average rainfall for the month of 92.2mm), causing massive leaf fall of the *restinga* canopy, exposing understory plants to higher PFD. This dry

period was followed by typical high rainfall levels in March, which started two weeks before data collection.

In the laboratory, *Neoregelia cruenta* showed profound decreases in stomatal conductance (g_{H2O}) after 10 days without water in the tanks irrespective of light regime. Plants under water deficit also increased the proportion of refixation of respired CO_2, improving water economy and maintaining minimal CO_2 supply. Similar proportions of refixed to exogenous CO_2 uptake were found in the obligate CAM *Tillandsia utriculata* (Griffiths *et al.*, 1990) and *T. stricta* (Reinert and Griffiths, 1999). Conversely, the facultative CAM *Guzmania monostachia* subjected to high light and drought showed increasing nocturnal CO_2 uptake, suggesting that CAM provides protection under such extreme conditions (Maxwell *et al.*, 1994).

Discrimination against the heavier carbon isotope (Δ) during photosynthesis leads to a depletion of ^{13}C in the leaf while an equivalent enrichment of $^{13}CO_2$ in air occurs if the leaf is enclosed in a cuvette. The progressive decrease in Δ, from a C_3- to C_4-like carboxylation, during Phase IV, indicated a gradual change from Rubisco to PEPC mediated assimilation. Similarly, during Phase II a rapid change from C_4- to C_3-like photosynthesis occurred, indicating that during this Phase PEPC was still active when Rubisco activity was triggered by light. Increase in the malic acid pool after the early morning burst of CO_2 uptake in several CAM plants corroborates these results (e.g. *Mesembryanthemum* and *Aloe*: Willert *et al.*, 1992; *Clusia fluminensis*: Roberts *et al*,.1996).

The ratio of P_i/P_a is related to Δ, to instantaneous CO_2 assimilation rate (J_{CO2}) and to intrinsic WUE, as these processes are independently associated with g_{H2O}. During diffusion limited J_{CO2} most diffused carbon is carboxylated, allowing low discrimination during this process, such that Δ signal mainly reflects the discrimination of ^{13}C during diffusion of CO_2 into stomatal cavity. Diffusion limited C_3 photosynthesis would then show discrimination approaching the theoretical value of +4.4‰ of isotope fractionation (or +3‰ for CAM plants: O'Leary and Osmond, 1980). On the other hand, should J_{CO2} be limited by the rate of carboxylation, the fractionation effect associated with the carboxylation enzyme(s) would determine the final discrimination. Under these conditions discrimination would theoretically approach -7‰ for C_4 carboxylation and +30‰ for C_3 pathway (Osmond and O'Leary, 1980; Farquhar *et al.*, 1989).

Negative Δ values obtained during Phase I have not been shown before for bromeliads and are consistent with the PEPC carboxylation signal for this Phase. The lower range of Δ values observed for Phases II and IV in plants under high or low light and water deficit (lower g_{H2O}) compared to the predicted values could result from two independent processes: 1) diffusional limitations influencing overall discrimination to a greater extent than carboxylation, or 2) higher activity of PEPC in response to water stress. Regarding the first process, two factors should be considered. The higher the stomatal conductance the higher Δ values during C_3-like photosynthesis (discrimination limited by Rubisco), however, during C_4-like, this relationship is inverted (discrimination limited by PEPC), so that differences could partially annul one another. Additionally, it is important to consider that stomatal patchiness is likely to occur during water stress so that calculated P_i could have been overestimated (Brugnoli and Lauteri, 1991). Concerning the second hypothesis, the lower J_{CO2} and g_{H2O} at night in the water stressed plants, and the higher Δ values are entirely consistent with PEPC carboxylation being increasingly limited by closing stomata: thus CAM activity is limited under water deficits, as opposed to the induction found in plants such as *Clusia minor* (Borland, 1993).

In conclusion, the CAM pathway in *Neoregelia cruenta* is an obligate trait, with activity matching the degree of exposure in natural habitats, and activity is reduced under water deficits and exposure, rather than increasing in response to stress as could in *Guzmania monostachia* (Maxwell *et al.*, 1994) and C_3-CAM intermediates.

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