Influence of leaf-to-air vapour pressure deficit (VPD)
on the biochemistry and physiology of photosynthesis
in Prosopis juliflora*

Pramod A. Shirke and Uday V. Pathre†
National Botanical Research Institute, Rana Pratap Marg, Lucknow-226 001, India

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Abstract
The effect of leaf-to-air vapour pressure deficit (VPD) was studied in well-watered, potted, 1–2-year-old plants of the leguminous tree P. juliflora grown outside in northern India. The long-term responses to VPD were analysed from diurnal and seasonal variations in gas exchange parameters measured in two cohorts of leaves produced in February and July, respectively. In general, inhibitory effects of high VPD were visible only when the VPD level exceeded a threshold of >3 kPa. There was a substantial decline in net photosynthesis rate and stomatal conductance at high VPD >4 kPa and transpiration showed a decrease in steady-state rate or feedforward response to VPD. The feedforward responses were visible in all seasons, although the plants were exposed to a wide range of VPD during the year and leaf relative water content was constant. The maximum quantum efficiency of PSII measured predawn was constant (around 0.8) in all seasons except summer. Short-term experiments showed that, although gas exchange was severely affected by high VPD in the leaves of both cohorts, the plant maintained a constant, water use efficiency in different seasons. High VPD also caused reductions in Rubisco activity, affecting carboxylation efficiency, and reductions in sucrose and starch content due to a decrease in the activity of sucrose-phosphate synthase. However, the relative quantum yield of PSII and electron transport rates measured at 1500 μmol m⁻² s⁻¹ were unaffected by increasing VPD, indicating the presence of a large alternative sink possibly, photorespiration. The overall results showed that P. juliflora can withstand high VPD by reducing metabolic activity and by effective adjustments in the partitioning of electron flow between assimilation and non-assimilation processes, which, in turn, imposed a strong limitation on the potential carbon gain.

Key words: Feedforward response, stomatal sensitivity, transpiration, VPD.

Introduction
Plants growing in the northern part of India experience major changes in radiation, temperature, and humidity conditions during the year. For example, at Lucknow (latitude 26°30’ N and longitude 80°30’ E) the climate is tropical with high irradiance and high temperature for most of the year, but in winter (November–January) the night temperatures often fall to <5 °C and, during the day, temperatures are moderate (<30 °C). By contrast, in summer (April–June) the temperature ranges from 32–45 °C. The most favourable seasons for growth are considered to be spring (February–March) and monsoon (July–September) when temperatures are 25–35 °C with relative humidity 35–75%. Prosopis juliflora, an exotic species introduced to northern India during afforestation programmes because it grows well in these climatic conditions, shows peculiar patterns of leaf senescence, one after winter and another after the hot and dry summer season. The shedding of leaves is accompanied by the formation of new leaves and the period of senescence and growth of new leaves is very short, giving two different even-aged cohorts of leaves in a year. P. juliflora is highly valued as a multi-purpose resource that can contribute to socioeconomic development in rural communities (Fagg and Stewart, 1994; Goel and Behl, 2000). Previous research on gas exchange has shown
marked variations in response to the diurnal and seasonal environmental changes (Sinha et al., 1997c; Tezara et al., 1998). Pathre et al. (1998) found that VPD was a more important factor than temperature or photosynthetic photon flux density (PPFD) in causing midday depression in net photosynthesis (Pn) and stomatal conductance (gs) in P. juliflora. Numerous studies have documented that high VPD limits Pn in many species under field and laboratory conditions, but there is no consensus as to their mechanism. It could be that evaporation at high VPD causes water stress. Several components of photosynthetic metabolism, electron transport, ATP synthesis, light dissipation, Rubisco, export, and carbohydrate metabolism can be affected by water stress (Lawlor and Cornic, 2002). However, it is difficult to evaluate the effects of water deficit caused by high VPD on overall Pn and productivity, since high VPD often only occurs for a short period around midday and stress effects may not persist during the rest of the day. A direct effect of VPD on stomatal regulation not necessarily related to soil water status has been reported for many woody crops (Franks and Farquhar, 1999; Day, 2000), but it is not known whether the effect is able to affect photosynthetic metabolism. In the present paper, two questions are addressed. Are the effects of high VPD on photosynthetic metabolism the same as those observed under water stress? Second, how does P. juliflora tolerate the wide variation in VPD occurring during the course of the day and between different seasons? To answer these questions an integrated analysis of VPD effects during diurnal and seasonal changes on Pn and related parameters was performed, together with an examination of the short-term effects of VPD. The studies of short-term VPD effects also allowed an evaluation of the acclimation potential of P. juliflora in different seasons.

Materials and methods

Plant material and growth conditions
Six-to-eight-months-old, 20 cm high seedlings of Prosopis juliflora (Swartz) DC. were obtained from the National Botanical Research Institute’s Biomass Research Centre, Banthara, situated about 20 km south-west of Lucknow. The plants were transplanted to 10 l earthenware pots containing garden soil and organic manure in early August and placed outdoors, in the terrace garden of NBRI, under natural PPFD, temperature, and humidity conditions. During the experiments, plants were kept well-watered and fertilized fortnightly with Hoagland solution.

Diurnal and seasonal measurements of gas exchange parameters
In P. juliflora new leaves are produced during February (1st cohort) and July (2nd cohort) and it takes about 1 month for complete development of the leaves. Taking into account this seasonal pattern of growth, diurnal cycles were studied in four different months representing the different local climate seasons: 1st cohort, March (spring) and May (summer), 2nd cohort, September (monsoon) and December (winter). All the measurements were made on mature leaves (1–5-months-old) of 1.5–2-year-old plants.

Measurements of diurnal courses of CO2 exchange and water vapour loss were made on leaves with 48–64 leaflets from four different plants with two different, LI-6200 portable photosynthesis systems (Li-Cor Inc., Lincoln, Nebraska, USA). The leaf was kept inside a 1.0 l leaf chamber (Li-Cor, Lincoln, Nebraska, USA) under ambient PPFD until three photosynthetic measurements had been recorded. The measurement process typically took less than 1 min, and no significant increase in the temperature within the chamber was observed during this brief period. The relative humidity during this period was maintained the same as the outside by adjusting the flow-through desiccant. The mean of the three readings was used for comparison. Dark respiration (RD) was measured by covering the chamber completely with black cloth.

Short-term effect of VPD on gas exchange and fluorescence parameters
The short-term effect of VPD was studied by manipulating the VPD in the leaf chamber while PPFD and temperatures were maintained at ambient values. Previous studies determined that leaf gas exchange as well as environmental parameters remained stable between 08.00 h and 11.00 h; therefore, all short-term VPD experiments were completed during this period except those mentioned in Fig. 1 of the Results. On the evening prior to an experiment, the plants were thoroughly watered. The next morning, the plant was exposed to natural conditions for 30 min and a leaf was enclosed in the chamber fully exposed to the sunlight. The desired VPD in the chamber was provided by a dew point generator (LI-610, Li-Cor, Lincoln, Nebraska, USA) and an external fan was used to moderate the temperature rise inside the leaf chamber. The leaf was allowed to acclimatize at low VPD (=2 kPa) initially, and after achieving the steady-state (constant Pn) the photosynthetic parameters were recorded. The leaf was then subjected to the next VPD level by gradually increasing the VPD in the chamber. The time period for reaching a steady-state for a given VPD was usually 30 min and, therefore, the measurements were limited to one leaf per day.

The index of stomatal sensitivity to VPD was estimated by measuring steady-state transpiration rates at two VPD levels, 3 kPa and 6 kPa in summer (May) and monsoon (September) representing the two cohorts of leaves (Franks and Farquhar, 1999). Stomatal sensitivity was calculated as ψ = b(a+b) where b is the actual transpiration rate at 6 kPa and a+b is the rate of transpiration that would have occurred had there been no change in steady-state stomatal conductance following VPD change. After Pn measurements the leaf was removed from the leaf chamber, and fluorescence parameters were measured with a portable, pulse-amplitude modulated fluorimeter attached with a leaf–clip (PAM-2000 and 2030-B Walz, Effeltrich, Germany). All fluorescence measurements and calculations of various parameters were done as given by Maxwell and Johnson (2000). Steady-state fluorescence (Fm), and maximal (Fm′) and minimal (F0) fluorescence in the light were measured on 10–12 leaflets of the same leaf after gas exchange measurements. Minimal (Fm) and maximal (Fm′) fluorescence were measured predawn, and the maximum photochemical efficiency of photosystem II (PSII) (Fm′/Fm) was calculated. The effective quantum yield of PSII (Fv/Fm′) was calculated as ((Fm′−F0)/Fm′) and apparent electron transport rates through PSII (ETR) were calculated as 0.5×Fv/Fm′×PPFD. Photochemical quenching (qP) was computed as qP = (Fm′−F0)/(Fm′−F0), and non-photochemical quenching (qN) was calculated as qN = (Fm′−Fm)/(Fm′−F0).

Estimation of photosynthesis (P) was carried out according to Franco and Lüttge (2002) as P = [2L(ETR)]−4[(Pn+RD)/12], where L is the light absorptance assumed to be 0.8.
Estimation of carboxylation efficiency

Carboxylation efficiency (CE) was calculated from the slope value of assimilation rate versus internal CO₂ concentration response curves \((P_{\text{a}}/C_{\text{i}})\). \(P_{\text{a}}/C_{\text{i}}\) response was measured according to McDermitt et al. (1989). A baseline response was measured by placing a fully expanded leaf in a 0.25 l leaf chamber. The leaf was first equilibrated at the desired VPD and after reaching steady-state the system was closed and the draw-down rate of CO₂ was recorded until the CO₂ compensation point \((F)\) was reached. The data for \(P_{\text{a}}\) and \(C_{\text{i}}\) were computed for every 5 \(\mu\)mol mol⁻¹ draw-down of CO₂. Each measurement of the \(P_{\text{a}}/C_{\text{i}}\) change required approximately 30–45 min.

Measurement of export rate

Export of carbon studies were done on the basis of net CO₂ fixed and the difference in the dry weight of the leaves as given by Terry and Mortimer (1972). For each VPD level, \(P_{\text{a}}\) was measured in two leaves of the same plant and one of the leaves was harvested for dry weight determination. The second leaf was then exposed to the desired VPD for 1 h and \(P_{\text{a}}\) was measured every 15 min. After 1 h the leaf was harvested, dried at 70 °C for 24 h, and the dry weight was determined. The amount of carbon fixed was estimated by integration of the measured \(P_{\text{a}}\), and the export rate was then determined as the difference between the mean rate of CO₂ fixation over a 1 h period and the rate of accumulation of dry matter.

Estimation of Rubisco activity

The leaves exposed to different VPD levels were harvested into liquid N and stored either in liquid N or at −70 °C until analyses were made. Frozen leaf material was ground in a mortar with ice-cold 1 M HClO₄ containing 0.1 M MOPS buffer, pH 7.5 containing 10 mM MgCl₂, 1 mM EDTA, 1 mM PMSF, 25 mM DTT, and 5 mM sodium ascorbate. Immediately after extraction the samples were centrifuged to form RuBP after an aliquot of the leaf extract has been preincubated with the assay medium for 10 min at 25 °C to carbamylate the enzyme fully. The ratio of the initial activity to total activity \((\text{Suc6P})\) formed as described earlier (Sinha et al., 1997b).

An aliquot of the sample used for the estimation of Rubisco activity was processed for estimation of protein by the Lowry method as given by Coombs et al. (1985).

Estimation of SPS

The frozen leaves were ground in a mortar with liquid N in a medium containing 0.1 M MOPS buffer, pH 7.5 containing 10 mM MgCl₂, 1 mM EDTA, 1 mM PMSF, 25 mM β-mercaptoethanol, and 0.02% (v/v) Triton-X-100 in the ratio of 1:3 (w/v). The homogenate was quickly centrifuged at 13 000 \(g\) for 1 min and the supernatant was immediately desalted on a Sephadex G-25 column. The column was pre-equilibrated and eluted with the extraction buffer without Triton-X-100. \(V_{\text{max}}\) and \(V_{\text{in}}\) SPS activity in the desalted extract was determined by measuring the sucrose-6-phosphate (Suc6P) formed as described earlier (Sinha et al., 1997b).

Carbohydrate analysis

Frozen leaf material was ground in a mortar with ice-cold 1 M HClO₄ (usually 1 ml for 10 cm² leaf area) and the extract was centrifuged at 12 000 \(g\) for 2 min at 4 °C. The supernatant was neutralized with 5 M K₂CO₃ and precipitated KClO₃ was removed by centrifugation. The supernatant was kept on ice and used for the estimation of sucrose, while the pellet was used for the determination of starch. Sucrose and starch were estimated enzymatically according to the Jones method as given by Coombs et al. (1985).

An aliquot of the sample used for the estimation of sucrose and starch was extracted with acetone, and the phophothl in the samples was determined according to the method described by Vernon (1960).

Results

To pinpoint the role of VPD during the diurnal course of \(P_{\text{m}}\), \(g_{\text{s}}\) and transpiration \((E)\) in P. juliflora, the gas exchange parameters were compared in two leaves, one exposed to ambient VPD and another to low VPD throughout the day, while the temperature and PPFD were not controlled (Fig. 1). Under ambient VPD conditions the \(P_{\text{m}}\) increased in the morning and reached around 15 \(\mu\)mol m⁻² s⁻¹ at 09.00 h and started decreasing as soon as the VPD exceeded 3.5 kPa (Fig. 1B). The \(P_{\text{m}}\) decreased continuously with the increase in VPD and reached a minimum of 7 \(\mu\)mol m⁻² s⁻¹ at 14.00 h, when VPD was around 6 kPa. The \(P_{\text{m}}\) improved slightly in the afternoon and then declined, presumably due to the sharp decrease in PPFD. The leaves subjected to low VPD (≥2.5 kPa) showed the continued increase of \(P_{\text{m}}\) to 16 \(\mu\)mol m⁻² s⁻¹ until 10.30 h then \(P_{\text{m}}\) decreased slightly to 13 \(\mu\)mol m⁻² s⁻¹, perhaps because of the small increase in VPD, and remained constant until the evening (Fig. 1E). The \(g_{\text{s}}\) under ambient VPD increased until 11.00 h and then decreased continuously at higher VPD (Fig. 1C) while at low VPD it remained more or less constant after an initial increase in the morning (Fig. 1F). The diurnal course of \(E\) at ambient VPD showed a feedforward response (Fig. 1C), with a sharp increase in the morning at around 09.00 h, but then only smaller increases with further increases in VPD. After 12 h when VPD exceeded 5 kPa, \(E\) decreased continuously until evening. Under low VPD conditions, overall \(E\) was low (Fig. 1F).

Diurnal and seasonal variations in VPD could have long-term effects on the photosynthesis and metabolism in P. juliflora (Table 1). The maximum PPFD in different seasons varied only from 1700 to 2000 \(\mu\)mol m⁻² s⁻¹, and, as it was more than twice the photosynthetically saturating irradiance (700 \(\mu\)mol m⁻² s⁻¹) for P. juliflora (Shirke, 2002), probably had little contribution to differences between seasons. The temperature data in different seasons showed that the plants were exposed to >30 °C for most of the day period throughout the year except in winter, when temperatures were moderate (<30 °C). On the other hand, the changes in air vapour pressure deficit (D) ranged from 1.2 to 7.5 kPa during the year. In all seasons the leaves were exposed to relatively high D of >3 kPa during midday and the VPD stress was relieved during the night. The potential quantum efficiency of PSII \((F_{\text{v}}/F_{\text{m}})\), used as
a sensitive indicator of plant performance, remained at an optimum level (≥0.8) in all seasons except in summer. In summer, the minimum $D$ that the plants experienced was around 2 kPa, which rose to 7.5 kPa during midday and this affected several factors indicative of photosynthetic performance. There was a reduction in the amount of carbon fixed per day, and export rates and the activities of Rubisco and SPS were also reduced in the summer season (Table 1).

The diurnal measurements in different seasons showed that the maximum value of $VPD$ varied between 4 kPa (winter) and 9.7 kPa (summer). The leaves from both the cohorts showed a similar response of $P_n$ and $g_s$ to the changes in $VPD$, which increased until a threshold $VPD$ was exceeded and then decreased (Fig. 2A, B). The threshold level $VPD$ for the second cohort leaves was slightly lower (2.3 kPa) than that in the first cohort leaves (3.1 kPa). Similarly, the slope of the decrease in $P_n$ and $g_s$ above the threshold $VPD$ was steeper for the second cohort of leaves compared with the first cohort. In both cohorts the $E$ increased with the increase in $VPD$ until the threshold level and then either remained constant as observed in the monsoon or decreased with a further increase in $VPD$. The threshold $VPD$ for $E$ of the first cohort leaves was 4.5 kPa, but decreased considerably (2.8 kPa) in the second cohort leaves (Fig. 2C) indicating increased stomatal sensitivity at higher $VPD$. The leaves of $P. juliflora$ produced in spring (1st cohort) that were later exposed to dry conditions were less sensitive to $VPD$ compared with the leaves produced in the monsoon (2nd cohort). The maximum $E$ in the monsoon increased to 9.5 mmol $m^{-2} s^{-1}$, but, in summer, it never exceeded 6 mmol $m^{-2} s^{-1}$ (Fig. 2C).

Figure 2 and Table 1 indicate the long-term effects of seasonal changes due to $VPD$ and other environmental parameters on carbon fixation, partitioning, and metabolism in $P. juliflora$ leaves. The short-term effects of high $VPD$ were studied in the summer season by controlling $VPD$ alone under ambient $PPFD$ and temperature. The relative leaf water content ($RWC$) in predawn leaves of $P. juliflora$ was 87 ± 1.6% and changed (1–2%) slightly in some leaves at the highest $VPD$ (6 kPa) level, and $RWC$ did not differ between monsoon and summer leaves (data not shown). Short-term effects of $VPD$ on gas exchange were measured...
CUstomatal sensitivity (VPD) then decreased gradually at higher levels (Fig. 3). The range of PPFD and air temperature are calculated for 08.00 h to 16.00 h for each season from the data obtained from monthly measurements made on clear days (7–10 d). Air vapour pressure deficit (D) values are calculated from air temperatures. Maximum photochemical efficiency of PSII (F_v/F_m) was measured at predawn before the measurements of diurnal gas exchange shown in Fig. 2. The export rate and enzyme activities are shown for the period when P_n was maximum.

<table>
<thead>
<tr>
<th>First cohort</th>
<th>Second cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>Summer</td>
</tr>
<tr>
<td>PPFD (μmol m⁻² s⁻¹)</td>
<td>1210–1930</td>
</tr>
<tr>
<td>Air temperature (°C)</td>
<td>26–34</td>
</tr>
<tr>
<td>D (kPa)</td>
<td>1.7–3.7</td>
</tr>
<tr>
<td>Rubisco activity (μmol CO₂ min⁻¹ mg⁻¹ protein)</td>
<td>Initial 0.64±0.02</td>
</tr>
<tr>
<td></td>
<td>Total 0.81±0.03</td>
</tr>
<tr>
<td>SPS (μmol Suc6P min⁻¹ mg⁻¹ chl)</td>
<td>Vlim 5.12±0.41</td>
</tr>
<tr>
<td></td>
<td>Vmax 15.49±0.93</td>
</tr>
<tr>
<td>Maximum rate of P_n (μmol CO₂ m⁻² s⁻¹)</td>
<td>19±1.6</td>
</tr>
<tr>
<td>Export rate of carbon (μmol C m⁻² s⁻¹)</td>
<td>17.59±2.35</td>
</tr>
<tr>
<td>F_v/F_m (predawn)</td>
<td>0.81±0.01</td>
</tr>
</tbody>
</table>

in both summer and monsoon leaves (Fig. 3), since they showed large difference in E and φ to changes in VPD. The PPFD ranged from 1500 to 1700 μmol m⁻² s⁻¹ in summer and 1300–1440 μmol m⁻² s⁻¹ in the monsoon, while the leaf temperature ranged from 36–44 °C in summer and 35–42 °C in the monsoon. The overall rates of P_n, g_s, and E were lower in the summer than those in the monsoon. In both seasons P_n and g_s decreased only when VPD exceeded 3 kPa. The VPD effects were larger in monsoon leaves, with 85% inhibition of P_n and g_s at 6 kPa compared with 2 kPa and only 50% in summer leaves. Similarly, in summer, E increased only up to 3 kPa VPD, while in the monsoon leaves E increased until VPD reached 4 kPa and then decreased gradually at higher VPD levels (Fig. 3). The stomatal sensitivity (φ) changed from 0.40 for summer leaves to 0.57 for monsoon leaves, while the C_i/C_a was approximately 0.6 at all different VPD levels in both seasons.

To evaluate the inhibition of P_n by high VPD, CO₂ response curves were measured on intact leaves under the natural photoperiod, starting from 500 μmol CO₂ mol⁻¹ concentration and an irradiance of 1500–1800 μmol m⁻² s⁻¹ (Fig. 4A). High VPD induced a decrease in the slope (CE) in the leaves and also increased the Γ from 60 μmol mol⁻¹ to 85 μmol mol⁻¹ (Fig. 4C). The measurement of Rubisco activity (Fig. 4B) was consistent with the results obtained from P_e/C_i curves. The total activity of Rubisco was slightly affected by an increase in VPD, but the initial activity declined continuously with an increase in VPD. There was a 50% decrease in initial activity and activation state at 6 kPa compared with that at 2 kPa.

None of the fluorescence derived parameters (ΦPSII, ETR, q_p, or q_N) showed effects of short-term (30–40 min) VPD stress. There was no change in the ΦPSII, ETR, q_p, or q_N except a slight dip in the activity at 3 kPa (Fig. 5). Neither were fluorescence parameters F_0 and F_m' affected at high VPD (data not shown).

The effect of VPD on export rate was examined in June (summer) when daily VPD varied between 3–9 kPa. The export rate was always proportional to the P_n, both increased slightly when VPD increased from 2–3 kPa, but a further increase in VPD caused them to decline sharply to almost 50% at 6 kPa (Fig. 6A). Starch and sucrose content (Fig. 6B) also showed a sharp decline when the leaves were exposed to VPD >3 kPa indicating that high VPD affected starch mobilization and sucrose synthesis. There was a concomitant decrease in extractable SPS activity in P. juliflora leaves with an increase in VPD (Fig. 6C). Both V_max and V_lim activity were inhibited by increased VPD and the reduction in the V_max activity was more than 30% while V_lim activity showed a 35% reduction in the activity as VPD exceeded 3 kPa.

Discussion
Gas exchange in P. juliflora showed an unusual response to VPD changes in the environment. The majority of studies
VPD and either remained constant or decreased at high VPD (Figs 1C, 2C, 3). The model further suggests that, at high VPD, cuticular transpiration from epidermal and guard cells increased substantially. It was not possible to estimate the contribution of cuticular transpiration in amphistomatous leaves of *P. juliflora* and independent measurements of $E$ and $g_s$ are required. The changes in $\varphi$ indicated the acclimation of *P. juliflora* to seasonal changes in humidity. A change in $\varphi$ in wet and dry seasons has been reported for *Acacia auriculiformis*, *Syzygium eucalyptoides*, and *Maranthes corymbos* and were considered to be associated with changes in leaf phenology or anatomy (Thomas et al., 2000). In *P. juliflora* the anatomy was not studied and there were no obvious morphological differences in the leaves of the two cohorts produced in the two different seasons, however, the data indicate that the stomata were efficient enough to maintain a constant instantaneous water-use-efficiency ($P_n/E$) or its complementary measure $C_i/C_a$ in both the summer and the monsoon (Fig. 3).

In *P. juliflora*, the limitations in $P_n$ under high VPD conditions were neither due to light utilization capacity nor to electron transport, as the flux of electrons through PSII was maintained similarly to that in control leaves. This suggests a high capacity of alternative electron acceptors such as photosynthesis. During the diurnal course in the summer, the ratio of $P_n$ in *P. juliflora* often reached 1.8 compared with 0.8 in winter (data not shown). The increase of photosynthesis in response to increasing VPD was also evident from the steady rise of $\Gamma$ (Fig. 4C). In this study’s experimental conditions, $q_P$ and $q_N$ did not change significantly over the range of VPD exposures. The involvement of photoinhibition under water deficit appears to differ between species. In castor bean (Dai et al., 1992) and *Phaseolus vulgaris* L. (Cornic et al., 1989; Brestic et al., 1995) water deficit induced the inhibition of $q_P$ and $\Phi_{PSII}$ but there was no change in $q_N$, $\Phi_{PSII}$, and $\Phi_{RSII}$ in leaves of *Digitalis lantata* (Stuhlfauth et al., 1988), *Solanum tuberosum* L. (Jefferies, 1994), sunflower (Panković et al., 1999), and *Commelina* and *Tradescantia* (Lawson et al., 2002).

Although light energy capture and CO₂ supply were apparently not involved in the VPD-induced inhibition of $P_n$ in *P. juliflora*, other processes of photosynthetic metabolism were found to be affected. There was a significant reduction in initial activity of Rubisco at high VPD, but not in the total activity, suggesting that the catalytic sites were blocked by inhibitors. This may reflect the altered metabolism of inhibitors of importance in the regulation of Rubisco. This agrees with other studies on Rubisco on subterranean clover (Medrano et al., 1997) and sunflower (Tezara et al., 1999) under water-stress conditions. A 50% reduction in the activation state of Rubisco in leaves of *P. juliflora* suggest that high VPD might have affected the activity of Rubisco activate and the supply of ATP. The capacity for RuBP synthesis is a key factor in the

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**Fig. 2.** Relationship between VPD and gas exchange parameters $P_n$, (A), $g_s$, (B), and $E$ (C) in *P. juliflora* growing under natural conditions. The data were obtained from the diurnal measurement of gas exchange parameters in selected months representing different local climate seasons; 1st cohort of leaves (filled symbols) spring (11 March) and summer (6 June), 2nd cohort of leaves (open symbols) monsoon (13 August) and winter (22 December) during the year. Error bars indicate mean ±SE; $n$=4 leaves; each from a different plant.
assimilation of CO2 and depends on the supply of ATP and NADPH and the function of PCR cycle (Lawlor and Cornic, 2002). The determination of the \( P_n/C_i \) curve showed that \( CE \) was affected substantially at high VPD in \( P. \) juliflora, similar to those described under water-deficit conditions in annuals (Panković et al., 1999; Tezara et al., 2002) and tropical species (Bunce and Ziska, 1999).

At ambient CO2 concentrations, there is a strong linear relationship between \( P_n \) and the concurrent export observed in \( C_3 \) species (Grodzinski et al., 1998). Carbon export in \( P. \) juliflora was consistent with this general observation (data not shown), however, the relationship was maintained even under high VPD conditions, since a reduction in export was always proportional to the reduction in \( P_n \). The influence of environmental parameters on export, especially temperature, has been reported in \( Salvia \) splendens (Jiao and Grodzinski, 1996). In \( S. \) splendens a linear relationship between \( P_n \) and export rate was maintained between 15 °C and 35 °C. Above 35 °C, export was inhibited under photorespiratory and non-photorespiratory conditions, although the \( P_n \) was not inhibited under non-photorespiratory conditions. In \( P. \) juliflora an inhibition in \( P_n \) or export rate at 40 °C at low VPD was not observed, perhaps because the plants were already acclimated to the higher temperature regime in the summer. However, the inhibition in export rate may be due to photorespiratory conditions (high \( \Gamma \)) created during exposure to high VPD.

The changes in VPD also affected sucrose metabolism by reducing the activity of SPS. A negative effect of water deficit on SPS has been well documented in leaves of spinach (Quick et al., 1989), \( Phaseolus \) vulgaris (Castillo, 1992), and maize (Pellesciti et al., 1997). In \( P. \) juliflora a sharp drop in sucrose and starch content in the leaves could primarily be due to decreased \( P_n \) and continued respiration (data not shown). Lawlor and Fock (1977) indicated similar effects in stressed leaves at \( RWC < 80\% \). Zrenner and Stitt (1991) suggested that the decreased cell volume during water stress leads to an increased metabolic pool of phosphate (\( P_i \)) and that more \( P_i \) is available for phosphorolytic starch degradation. The increased concentration of \( P_i \) could reduce the activity of SPS in \( P. \) juliflora since the enzyme is deactivated by allosteric inhibition and by phosphorylation (Sinha et al., 1997a, b). High VPD caused a decrease in \( V_{\text{max}} \) and \( V_{\lim} \) compared with leaves.
exposed to low VPD. The decrease in total extractable SPS activity and the increase in SPS phosphorylation state (decreased \( V_{\text{lim}} \) activity) would serve to decrease the flux of C to sucrose in a situation of declining photosynthetic capacity and export.

Previous studies of VPD effects were mostly confined to gas exchange parameters and there are few data on the

Fig. 4. Short-term effect of VPD on carboxylation efficiency (CE) and Rubisco activity in leaves of \( P. \ juliflora \) in May (summer). The response of \( P_n \) to decreasing \( C_i \), subjected to different VPD (kPa) levels as shown in parenthesis (A), initial activity (solid circles), total activity (open circles), and activation state (solid triangles) of Rubisco (B), relationship of CE (solid circles) and CO\(_2\) compensation point, \( \Gamma \) (open circles) derived from the \( P_n/C_i \) curves (C). Error bars indicate mean \( \pm \) SE; \( n=4 \) leaves; each from the different plant.

Fig. 5. Short-term effect of VPD on the fluorescence parameters in \( P. \ juliflora \) leaves in June (summer). Electron transport rates, ETR and relative quantum yield of PSII, \( \Phi_{\text{PSII}} \) (A), photochemical quenching, \( q_P \) and non-photochemical quenching, \( q_N \) (B) at PPFD (\( \geq 1500 \ \mu\text{mol m}^{-2}\ \text{s}^{-1} \)), air temperature (\( \geq 40 \ ^\circ\text{C} \)) and ambient CO\(_2\) level. Error bars indicate mean \( \pm \) SE; \( n=4 \) leaves; each from a different plant.

Fig. 6. Short-term effect of VPD on \( P. \ juliflora \) leaves in June (summer) on rate of carbon fixed, accumulated, and exported (A), starch and sucrose concentration (B), \( V_{\text{max}} \), \( V_{\text{lim}} \) activity of SPS measured in desalted extracts (C). Error bars indicate mean \( \pm \) SE; \( n=4 \) leaves; each from a different plant.
consequences of high VPD to photosynthetic carbon metabolism. These results show that such metabolic effects at large offer deeper insight into the nature of the VPD effect. Apparently, the inhibition of $P_n$ triggered by high VPD leads to the concerted down-regulation of metabolism and export. Secondly the effects produced by short duration (30–40 min) of high VPD were similar to those reported for long duration (days) of water stress, indicating a similar signal transduction cascade for water deficit in both cases. Pankovic et al. (1999) stated that in water-deficient leaves the export of soluble carbohydrates was first affected, which could lead to feedback inhibition of $P_n$, decreased RuBP regeneration, and reduced CE. However, the effects of increasing VPD in $P. juliflora$ indicated that all inhibitory processes were co-ordinated. Although the VPD effects are considered to be short term, and a plant like $P. juliflora$ can withstand them by lowering metabolic activities, consistent exposure to high VPD affected the photosynthetic performance in the summer season. The effects appeared to be highly detrimental, as leaves did not recover, but senesced in the subsequent month. Thus the production of new leaves of $P. juliflora$ in the relatively low VPD period (spring and monsoon) demonstrates an adaptation strategy for survival and growth under tropical conditions.

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