

Leaf photosynthesis, dark respiration and fluorescence as influenced by leaf age in an evergreen tree, *Prosopis juliflora*

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Abstract

P. juliflora trees produce leaves during two growth periods. The first cohort of leaves is produced during spring in cool conditions, while the second cohort is produced during monsoon under warm conditions. I studied photosynthetic characteristics of young, mature, and old leaves of the previous season (monsoon) in the spring season. Maximum net photosynthetic rate of a young leaf was lower than that of the mature and old leaves. The total CO₂ fixed per day by the young leaves was just 36 % of that in the mature leaves while the old leaves fixed 76 % of that of the mature leaf. The total transpiration rate and water use efficiency (WUE) were similar in the mature and old leaves, while they were much lower in the young leaves. Dark respiration rate was maximal in the young leaves as compared to the mature and old leaves. About 92 % of the total CO₂ fixed per day were respired by the young leaves. The diurnal fluorescence characteristics ($\Delta F/F_m$, q_P , and q_N) of the young, mature, and old leaves showed that photochemical efficiency of photosystem 2 during midday decreased more in the young and old leaves than in the mature ones. However, the fluorescence characteristics showed that in all the three leaf types there was complete recovery of the photochemical efficiency at sunset from the midday depression. F_v/F_m in the young and mature leaves also confirmed this. Hence the young and old leaves were photosynthetically less efficient than mature leaves, but they were well adapted to withstand the harsh environmental conditions.

Additional key words: chlorophyll fluorescence kinetics; photochemical efficiency; photosystem 2; transpiration rate; water use efficiency.

Introduction

During leaf development the net photosynthetic rate (P_N) increases with leaf expansion and reaches maximum usually before maximum leaf area is attained. Decline in P_N occurs after leaf attains maturity and mainly during senescence. The low P_N in newly unfolded leaves is associated with high rates of dark respiration (R_D) and low stomatal conductance (g_s). During further leaf development g_s increases, while R_D declines. During leaf expansion, the pigment contents increase, photosynthetic enzymes are formed, and their activities increase together with the efficiencies of radiant energy utilisation, electron transport chain, and photophosphorylation. The decrease in P_N in senescing leaves is associated with the decrease in g_s , chlorophyll content, enzyme activities, etc. (see Šes-

ták 1985, Čatský and Šesták 1997).

P. juliflora trees, which are well adapted to the extreme environment of Northern India, produce leaves during two growth periods in this region. The first cohort of leaves is produced during spring in cool conditions from February to early April. The second cohort is produced during monsoon under warm conditions from July to September (Goel and Behl 1996). The leaves produced during spring have to face the harsh summer, when PPFD is approximately 2 000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and the temperatures soar to about 45 °C. In the present study I attempted to find new information on the diurnal courses of characteristics of photosynthesis, dark respiration, and fluorescence of young, mature, and old (of the previous

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Abbreviations: C_a , ambient CO₂ concentration; C_i , leaf internal CO₂ concentration; Chl, chlorophyll; E , transpiration rate; ETR, apparent electron transport rate; F , steady state fluorescence; F_0 , minimal fluorescence of a light-adapted leaf; F_m , maximum fluorescence of a dark-adapted leaf; F_m' , maximum fluorescence of a light-adapted leaf; F_v , maximum variable fluorescence; $\Delta F/F_m$, overall photochemical quantum yield of photosystem 2; g_s , stomatal conductance; P_N , net photosynthetic rate; PPFD, photosynthetic photon flux density; PS, photosystem; q_P and q_N , photochemical and non-photochemical quenching coefficient, respectively; R_D , dark respiration rate; T_a , ambient air temperature; VPD, vapour pressure deficit; WUE, water use efficiency.

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soon season) leaves in the spring season.

Krause *et al.* (1995) observed that young leaves in a tropical forest trees canopy were more susceptible to high irradiance and showed a higher photoinhibition than mature leaves. However, in low irradiance, fast recovery was observed in both types of leaves. During spring in the tropical region of North India, PPFD and temperatures

are usually moderate. Therefore along with the diurnal fluorescence studies, diurnal F_v/F_m , which is a reliable measure of photoinhibition, was also studied in the young and mature leaves produced during the spring season. My goal was also to assess the ability of young, mature, and old leaves of *P. juliflora* to overcome photoinhibition.

Materials and methods

Plants: Two-year-old *Prosopis juliflora* (Sw.) DC. plants growing in 10 000 cm³ plastic pots on the terrace garden of the Institute under natural conditions throughout the year were used for all the experiments. The plants were watered twice a day and were supplied with Hoagland's solution fortnightly. During all diurnal experiments the plants were watered on the previous evening, after the sunset, and on the actual day of experiments the plants were not watered. The studies were carried with young, mature, or old leaves (monsoon leaves) as mentioned in each experiment.

The young leaves (about two weeks from the time of unfolding) were fully expanded but well distinguished by their light-green colour. The mature leaves (above four weeks from the time of unfolding) were fully expanded, green coloured. The old leaves were from the previous season (monsoon), about 20-24 weeks old, and such leaves were selected which showed no sign of senescence, but were leathery as compared to the young and mature leaves. The three stages of leaves used were on the same plant in all the experiments and at least four plants were studied in each set of experiments. All studies were carried out on clear sunny days. All measurements were performed from pre-dawn till dusk, which was around 06:00 to 18:30.

Photosynthetic parameters: P_N , g_s , and the environmental parameters, viz. photosynthetic photon flux density (PPFD), air temperature, and vapour pressure deficit (VPD) were measured using the Li-Cor model 6200 portable photosynthesis system attached with a 1 000 cm³ chamber (Li-Cor, USA). The leaf used for study was fully exposed and oriented to normal irradiation during measurements to ensure the measurements of gas exchange at the highest possible PPFD.

R_D : The diurnal measurements (in dark) were done simultaneously with the photosynthesis measurements from prior to sunrise around 06:00 to 03:00 of the next morning, using the LI-6200 system. For the R_D studies a 1 000 cm³ leaf chamber of the system was used, which was darkened using a black paper. The values were computed in a similar way as the photosynthesis data.

Diurnal chlorophyll (Chl) fluorescence was measured under natural conditions using portable pulse amplitude modulation fluorometer (PAM-2000, Walz, Effeltrich, Germany), which works on the principle given by Schreiber (1986). The fluorescence parameters determined were $\Delta F/F_m$, where $\Delta F = F_m - F$ represents the overall photochemical quantum yield of PS2 as described by Genty *et al.* (1989). Apparent relative electron transport rate was calculated as $ETR = \Delta F/F_m \times PPFD \times 0.50 \times 0.84$ (Cornic and Briantais 1991), where the factor 0.50 shows that transport of one electron requires absorption of two quanta and two photosystems are involved, and factor 0.84 shows that 84 % of the incident quanta are absorbed by the leaf. Photochemical quenching, which is used as an estimate of the fraction of PS2 open centres, was computed as $q_p = (F_m - F)/(F_m - F_0)$, according to Schreiber *et al.* (1989). Non-photochemical fluorescence quenching was calculated as given by Bilger and Björkman (1990) as $q_N = (F_m - F_m')/(F_m - F_0)$. The calculations of q_N require F_m values; F_m is maximal fluorescence yield of a dark-adapted leaf. During all the diurnal fluorescence studies F_m was determined for each plant before sunrise. All the above fluorescence parameters are displayed or calculated online by the PAM-2000.

The measurement area, depending on the distance of the glass fibre optics, covers only 0.3-0.5 cm² of the leaf. As the *P. juliflora* leaflets are small, 10-15 measurements were made on different leaflets of a single plant, and four different plants were taken for each observation.

Photoinhibition and recovery studies were carried out on the young and mature leaves. Four sets of plants were selected and the leaves were marked on the previous afternoon of the study. After the first set of observation of F_v/F_m under low PPFD (ca. 20-40 $\mu\text{mol m}^{-2} \text{s}^{-1}$), the plants were subjected to the natural PPFD, temperature, and VPD. The degree of photoinhibition was studied by immediately monitoring F_v/F_m of the irradiated leaves under low irradiance. After measuring photoinhibition, the plant was allowed to recover, by transferring it to low PPFD for about 30 min. F_v/F_m was then measured and after the observations the plants were again transferred to the natural conditions, till the next set of diurnal observation of photoinhibition and recovery was monitored.

Results

During the spring season the PPFD was saturating ($>800 \mu\text{mol m}^{-2} \text{s}^{-1}$) for about 7–8 h, and reached more than $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$. The minimum ambient temperatures were around 16°C during the predawn hours, and reached a maximum of around 31°C during the afternoon hours. VPD was around 1.0 kPa during the dark period, while during the afternoon hours it was around 3.5 kPa (Fig. 1).

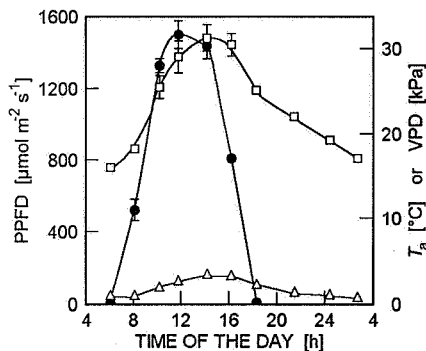


Fig. 1. Diurnal courses of irradiance (PPFD – ●), air temperature (T_a – □), and vapour pressure deficit (VPD – △), as observed on a typical spring day. Each point (bar) represents the mean \pm SE of three replicates.

Photosynthesis: Just after the harsh winter, when the temperatures rise in the early spring, new leaves start emerging. This happens in the first week of February and this study was made from the third week of February up to the second week of March. The photosynthetic and

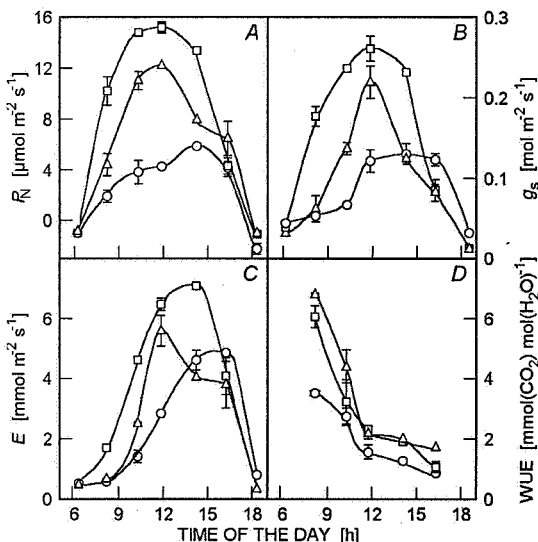


Fig. 2. Diurnal courses of (A) net photosynthetic rate (P_N), (B) stomatal conductance (g_s), (C) transpiration rate (E), and (D) water use efficiency (WUE), measured in leaves of *P. juliflora*. The symbols representing the leaf age are young (○), mature (□), and old (△). Each point (bar) represents the mean \pm SE of three replicates at each diurnal hour.

dark respiration studies were made on three different leaf stages, young fully expanded leaf, fully expanded mature leaf, and on an old leaf of the earlier season.

The diurnal courses of P_N , g_s , E , and WUE in these leaves are shown in Fig. 2. P_N was low in the young leaves, it increased steadily with the PPFD but the maximum rates were just above $5.5 \mu\text{mol m}^{-2} \text{s}^{-1}$. In the mature leaf P_N reached its maximum at 10:00 with a rate of $15.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ and remained steady till 15:00. Later it decreased gradually as PPFD decreased. In the old leaf P_N reached up to $12.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ and was high from 10:00 to 12:00, later it decreased (Fig. 2A). The total CO_2 assimilated, calculated by integrating the P_N observed during the diurnal course, was $0.164 \text{ mol m}^{-2} \text{d}^{-1}$ in the young leaves, while it was 0.460 and $0.348 \text{ mol m}^{-2} \text{d}^{-1}$ in the mature and old leaves, respectively (Fig. 3A).

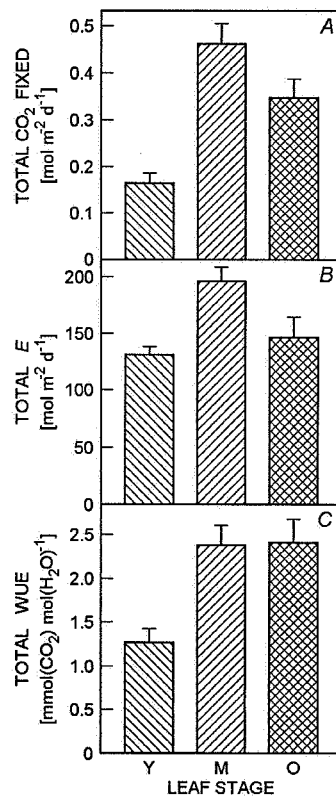


Fig. 3. Total CO_2 fixed (A), total water loss through transpiration (B), and total water use efficiency (C) during the day in young (Y), mature (M), and old (O) leaves of *P. juliflora*. Each bar represents the mean \pm SE of three replicates.

The g_s values were parallel to the P_N ones in all three types of leaves. They were lowest in the young leaf, with a maximum of $0.12 \text{ mol m}^{-2} \text{s}^{-1}$, while in the mature leaf they were 0.26 and in the old leaf $0.22 \text{ mol m}^{-2} \text{s}^{-1}$, respectively (Fig. 2B).

E was lowest in the young leaves, the maximum rate was $4.9 \text{ mmol m}^{-2} \text{ s}^{-1}$ in the afternoon, while it was highest in the mature leaf with $7.1 \text{ mmol m}^{-2} \text{ s}^{-1}$. In the old leaf it was $5.7 \text{ mmol m}^{-2} \text{ s}^{-1}$. There was a slight difference in the time of the peak in the three leaves. In the young leaf E was maximal at 15:00, while in the mature and old leaves it was at 13:00 and 12:00, respectively (Fig. 2C). The total water loss through transpiration was maximum in the mature leaves and minimum in the young leaves. The

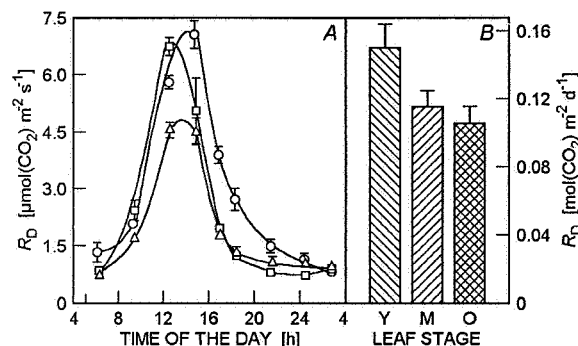


Fig. 4. (A) Diurnal course of dark respiration rate (R_D) in young (○), mature (□), and old (Δ) leaves of *P. juliflora*. (B) Total CO_2 respired during the course of a 24 h period in young (Y), mature (M), and old (O) leaves. Each point (bar) represents the mean \pm SE of three replicates at each diurnal hour.

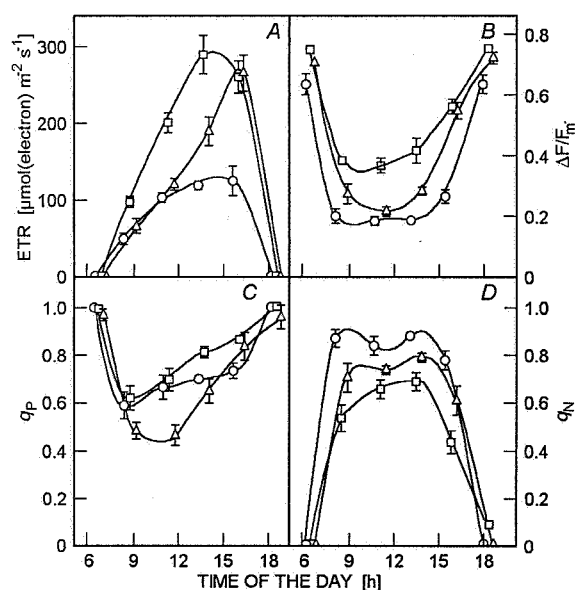


Fig. 5. Diurnal courses of (A) apparent electron transport (ETR), (B) overall photochemical quantum yield of PS2 ($\Delta F/F_m$), (C) photochemical quenching (q_p), and (D) non-photochemical quenching (q_N), measured on *P. juliflora* leaves under natural conditions. The symbols representing the leaf age are young (○), mature (□), and old (Δ). Each point (bar) represents the mean \pm SE of 10-15 replicates at each diurnal hour.

total E was 129, 193, and $144 \text{ mol m}^{-2} \text{ d}^{-1}$ in the young, mature, and old leaves, respectively (Fig. 3B).

WUE was comparable in the mature and old leaves, the maximum was $6.1 \text{ mmol}(\text{CO}_2) \text{ mol}^{-1}(\text{H}_2\text{O})$ in the mature leaves, while in the old leaves it was 6.8 and in the young leaves 3.5. The peak of WUE was found around 08:00 in all leaf types, later on WUE decreased (Fig. 2C). The total WUE per day was lowest in the young leaf with a value of $1.27 \text{ mmol}(\text{CO}_2) \text{ mol}^{-1}(\text{H}_2\text{O}) \text{ d}^{-1}$, while in the mature and old leaves it was 2.38 and 2.42, respectively (Fig. 3C). Thus the water loss was higher in the young leaves than in the mature and old leaves.

R_D was studied simultaneously with photosynthesis in the three leaf types. It was maximum in the young leaves and minimum in the old ones (Fig. 4A). In the young leaves, R_D increased with progress of the day and with leaf temperature. R_D reached its maximum at 15:00 with a rate of $7.0 \mu\text{mol m}^{-2} \text{ s}^{-1}$, which gradually decreased to around $1.6 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and remained constant in the night. In the mature leaves, R_D reached its peak at 12:00 with a rate of $6.7 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and later it decreased to $1.2 \mu\text{mol m}^{-2} \text{ s}^{-1}$. In the old leaves the maximum R_D was $4.5 \mu\text{mol m}^{-2} \text{ s}^{-1}$

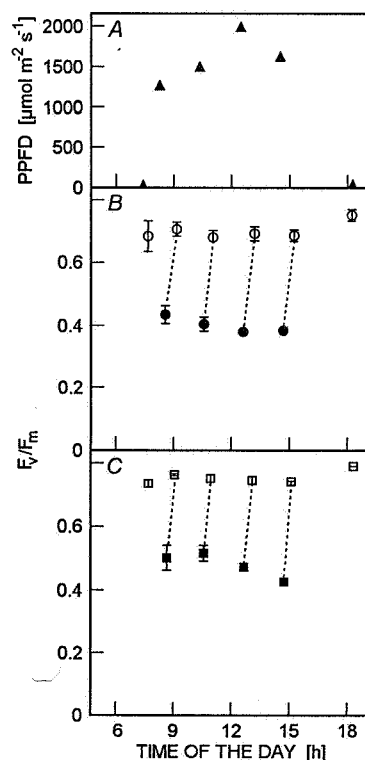


Fig. 6. (A) PPFD during the diurnal course of photoinhibition and recovery experiment. Photoinhibition during the diurnal course (solid symbols) and its recovery (hollow symbols) represented by F_v/F_m in young (B) or mature (C) leaves of *P. juliflora* under natural conditions. Each point (bar) represents the mean \pm SE of three replicates at each diurnal hour.

and it remained high till 15:00, after which it decreased to remain constant at around $1.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the night.

The total R_D calculated by integrating the diurnal R_D curve showed a high value in young leaves, which was above $0.15 \text{ mol}(\text{CO}_2) \text{ m}^{-2} \text{d}^{-1}$. The mature leaves showed a total R_D of $0.116 \text{ mol}(\text{CO}_2) \text{ m}^{-2} \text{d}^{-1}$, while old leaves showed $0.106 \text{ mol}(\text{CO}_2) \text{ m}^{-2} \text{d}^{-1}$ (Fig. 4B). Thus the young leaves showed a 30 % higher R_D than the mature leaves.

Diurnal fluorescence studies (Fig. 5): The ETR rates were very low in the young leaves as compared to the mature and old leaves. The maximum ETR in the young leaves was $111 \mu\text{mol}(\text{electron}) \text{ m}^{-2} \text{s}^{-1}$ as compared to $279 \mu\text{mol}(\text{electron}) \text{ m}^{-2} \text{s}^{-1}$ in the mature leaves (Fig. 5A).

The maximum PS2 yield, $\Delta F/F_m$, was less in the young leaves as compared to the mature and old leaves, and the decrease in $\Delta F/F_m$ with the increase in PPFD was maximal in the young leaves. During midday $\Delta F/F_m$ in the young leaves dropped to 0.17 as compared to 0.35 and 0.22 in the mature and old leaves, respectively. However, the $\Delta F/F_m$ values recovered to predawn values in all the three types of leaves (Fig. 5B).

As compared to the mature leaf, the q_P rates decreased more in the young and old leaves. The lowest value observed in the mature leaf was 0.63 while in the young and old leaf it was 0.53 and 0.44, respectively (Fig. 5C). In

the same manner the non-photochemical quenching (q_N) was maximum in the young leaves with 0.87 while in the old leaves it was 0.81 and in the mature leaves 0.68 (Fig. 5D).

F_v/F_m in young and mature leaves: Just before the plants were subjected to the natural sunlight, the first set of readings was taken on the young and mature leaves. The F_v/F_m value for the young leaves was 0.68, while for the mature leaves it was 0.73 (Fig. 6B,C). The plants were subjected to the natural irradiance to study the degree of photoinhibition. PPFD was above saturation throughout the experiment (Fig. 6A) and it varied from $1\,250 \mu\text{mol m}^{-2} \text{s}^{-1}$ to $2\,000 \mu\text{mol m}^{-2} \text{s}^{-1}$. The drop in F_v/F_m values in young leaves was up to 0.38 during the afternoon hours when the leaves experienced the maximum PPFD. However, the recovery was 100 % when the leaves were allowed to recover under low irradiance (Fig. 6B). In the mature leaves the lowest value of F_v/F_m was 0.42 during the afternoon hours. In the mature leaves the recovery from photoinhibition was 100 % (Fig. 6C).

Though the maximum values of F_v/F_m were low in the young leaves as compared to the mature leaves, the degree of photoinhibition was comparable in the young and mature leaves.

Discussion

During leaf development or leaf life span, generally three phases can be distinguished. Initially the leaf is a net carbon-importing structure and remains so until photosynthetic activities are fully developed and the peak demand of photosynthates for the assembly of cells has subsided (Chaumont *et al.* 1994). This is a period of leaf formation related to increase in leaf area. Leaf maturity begins after maximum leaf area is reached. During photosynthetic maturity peak values of the most important photosynthetic parameters are reached. During the third phase, massive mobilisation and export of carbon and minerals take place. During this period photosynthetic capacity declines (Šesták 1985, Čatský and Šesták 1997). These three stages were confirmed in the present study.

The low P_N , ETR, $\Delta F/F_m$, and F_v/F_m values observed in the young *P. juliflora* leaves must be due to low Chl content or physiological immaturity as was shown by Krause *et al.* (1995), Sobrado (1996), and Ishida *et al.* (1999). The C_i/C_a ratio observed during the photoperiod in the present study was higher in the young and old leaves than in the mature leaves. This indicates that the leaf age effect on photosynthesis is due to a greater decline of carbon fixation capacity in the mesophyll tissue than is the decline in g_s in young and old leaves (Sobrado

1994, Rajendrudu and Naidu 1997). A decrease in quantum yield would also result in low P_N (Šiffel *et al.* 1993).

WUE was much lower in young leaves than in mature and old leaves. Pathre *et al.* (1990) also observed higher WUE in mature *Acacia auriculiformis* phyllode than in young phyllode. Sobrado (1994) observed higher WUE in mature adult leaves than in young expanding leaves in deciduous and evergreen leaves of a tropical dry forest.

The very high R_D in newly unfolded leaves (Fig. 4A) can be attributed to the low photosynthetic activity and large relative amount of achlorophyllous tissues rather than to high activities of respiratory enzymes or large numbers and dimensions of mitochondria (Čatský and Šesták 1997).

The calculated ETR were fairly high and decreased only with irradiance (Fig. 5A). The strong increase in ETR might be explained by a shift to relatively faster rates of photorespiration caused by the high PPFD and high leaf temperature. Due to photorespiration the electron requirement per CO_2 fixed was higher (Krall and Edwards 1992, Häusler *et al.* 1994) in all the three leaf stages. Photorespiration is an effective means of preventing photoinhibition (Wu *et al.* 1991).

The general decline in the steady state of $\Delta F/F_m$ with increasing PPFD reflects decrease in the fraction of absorbed radiant energy utilised in photosynthesis. Particularly at high PPFD, less radiant energy is utilised in young leaves than in mature leaves; this results in lower $\Delta F/F_m$ in young leaves (Fig. 5B). This effect probably results from both the lower photosynthetic capacity and the higher degree of photoinhibition in the young leaves. Krause *et al.* (1995) also observed in several tropical leaves more susceptibility to photoinhibition in young leaves than in the mature ones; they explained it by lower Chl content in young leaves (50 % less than in the mature leaves). However, the F_v/F_m studies with young and mature leaves showed a similar susceptibility to photoinhibition and also a complete recovery under low irradiance.

In *Eucalyptus* sun leaves, Ögren and Evans (1992) observed exponential recovery phase with a halftime of 45 min. In tropical plant leaves, Krause *et al.* (1995) observed a two-phase recovery in the young leaves of several trees. In the first phase they observed a fast recovery with halftime of about 30 min, which accounted for about 50 % reversion of photoinhibition. The second phase was much slower with halftime of several hours,

and it was apparent towards sunset, and often a complete recovery was not observed. In *P. juliflora*, the F_v/F_m studies showed that the recovery from photoinhibition was very fast in both young and mature leaves, and almost 100 % recovery was observed in 30 min.

Leitsch *et al.* (1994) showed on detached spinach leaves, using streptomycin as an inhibitor of chloroplast protein synthesis, that the fast recovery was independent of turnover of the PS2 reaction centre protein, D1 (cf. also Singh 2000). In contrast, the slow phase depended on protein synthesis in the chloroplast and thus can be attributed to degradation and replacement of photo-inactivated D1 protein. The recovery may be independent of the turnover of PS2 reaction centre protein, and hence the recovery observed was complete and photoinhibition was reversible. The fast recovery from photoinhibition may be related to xanthophyll-cycle activity as observed by Krause *et al.* (1995).

The high degree of reversible photoinhibition observed in all the three leaf types probably represents a dynamic regulatory process protecting them from major photodamage. The study thus shows that the leaves of *P. juliflora* of all ages are well adapted and prepared to face the harsh summer ahead.

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