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Photoabatement by anthocyanin shields photosynthetic systems from light stress

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Abstract

Leaves and other chlorophyllous tissues of plants often show transient or permanent anthocyanin coloration. The question of whether anthocyanins can function as effective light screens to modulate photosynthesis in plants was addressed by comparing photosynthetic responses in reddish-purple pods with those in green pods of the ornamental leguminous tree *Bauhinia variegata*. For these comparisons the actinic radiation employed was either red radiation (RR) which was poorly absorbed by anthocyanin or blue-green radiation (BGR) which was strongly absorbed by anthocyanin. Photon yields of photosystem 2 (PS2) photochemistry and photochemical chlorophyll fluorescence quenching coefficients (q_P), measured over a range of photon flux densities (PFD) up to 1200 μmol m^{-2} s^{-1} at 23 °C and at five temperatures from 8 to 28 °C at a PFD of 260 μmol m^{-2} s^{-1}, were almost identical in green pods irradiated with either RR or BGR and in purple pods irradiated with RR. However, q_P values remained much higher in purple pods irradiated with BGR, *e.g.*, 0.80 in BGR *versus* 0.29 in RR at a PFD of 1200 μmol m^{-2} s^{-1} at 23 °C, and 0.67 in BGR *versus* 0.28 in RR at a PFD of 260 μmol m^{-2} s^{-1} at 8 °C. The higher values of q_P in BGR compared to RR indicated that photoabatement by anthocyanin allowed the first stable acceptor of PS2, Q_A, to be kept in a more oxidized state, thus decreasing the likelihood of photoinhibition. This was confirmed by demonstrating a lower susceptibility to photoinhibition in purple pods than in green pods in the sunlight, either naturally in pods on trees or in detached pods exposed to photoinhibitory conditions. We conclude that photoabatement by anthocyanin is a mechanism for allowing maintenance of higher oxidative levels of PS2 acceptor during episodes of high radiation stress, thereby minimizing photodamage to photosynthetic tissues.

Additional key words: Bauhinia; chlorophyll fluorescence; photoinhibition; radiation colour.

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Abbreviations: BGR = blue-green radiation; Chl = chlorophyll; PFD = photon flux density, 400-700 nm; PS = photosystem; q_P = photochemical chlorophyll fluorescence quenching coefficient; q_N = nonphotochemical chlorophyll fluorescence quenching coefficient; RR = red radiation.

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Introduction

Besides imparting colour to flowers, anthocyanins frequently occur in chlorophyllous tissues. Juvenile leaves of many woody tropical and subtropical species of rainforests (Richards 1952) and open savanah habitats are coloured by anthocyanins. In a number of warm and cool climate species, anthocyanin synthesis may be promoted in leaves of a range of maturities following exposure to adverse environmental factors, especially the interaction of irradiance and low temperature (Mancinelli 1983, Krol *et al.* 1995, Janda *et al.* 1996). Anthocyanins are also found in stems, petioles, leaf veins (*e.g.,* in many species of *Rhododendron*), fruits and even in roots if exposed to light (Tselas *et al.* 1979).

What then is the role, if any, of anthocyanins in tissues containing chlorophyll (Chl)? The function most commonly expounded is one of providing a protective screen against solar UV insolation, although antioxidant (Rice-Evans *et al.* 1995) and antiherbivore (Coley and Kursar 1996) functions have also been proposed. A correspondence between lower reflectance of UV-B radiation and anthocyanin content in the young, expanding leaves of six tropical rainforest species provides an empirical indication that anthocyanins afford protection against solar UV-B radiation (Lee and Lowry 1980). However, the immature rainforest leaves also contain higher levels of strongly UV-absorbing polyphenols (Lee and Lowry 1980), which when located in the epidermis could also protect plant tissues against damage by UV radiation (Robberecht and Caldwell 1978, Shimazaki *et al.* 1988). Finding that anthocyanin accounted for only around 1 % of the total phenolic compounds in young leaves of mango and cocoa, Lee *et al.* (1987) concluded that anthocyanins are relatively unimportant as a screen for solar UV radiation or as a defense against herbivory. It has also been shown that supplementary UV radiation actually decreases the anthocyanin concentration in leaves of the tropical mangrove *Rhizophora apiculata*, while enhancing the concentrations of total phenolic compounds (Moorthy and Kathiresan 1997).

Another possible role for anthocyanins is that of shading chloroplasts from visible radiation. Photoabatement of solar visible radiation reaching chloroplasts due to photon absorption by anthocyanins may diminish problems of excess photon energy at critical stages in the development of the chloroplasts or during periods of adverse climatic conditions. For instance, Krol *et al.* (1995) showed a correlation between increased anthocyanin content and increased tolerance to photoinhibition in low-temperature stressed needles of jack pine. However, direct demonstrations of how the presence of anthocyanins can affect photosynthetic function have not been reported thus far. We have addressed this problem in this paper by using as a test system the pods of the ornamental tree *Bauhinia variegata*. Both the flowers and pods of species of this legume show a range of anthocyanin colouration from no colouration to an intense magenta. Cultivars with either only green pods or only reddish-purple pods were selected and photosynthetic activities of the pods were monitored using Chl fluorescence techniques. Direct effects of photoabatement by anthocyanins were investigated by comparing the photosynthetic responses of green pods with those of reddish-purple pods during irradiation with either BGR which was absorbed by

anthocyanin or RR which was not absorbed by anthocyanin. The potential of anthocyanin to decrease the extent of photoinhibition in pods exposed to either artificial or natural photoinhibitory environments was also investigated.

Materials and methods

Plants: Pods were harvested from trees of *B. variegata* L. that flowered in August and developed pods in October. Cultivars used were 'Purpurea' which produces magenta coloured flowers and reddish-purple pods (hereafter referred to as purple pods) and *B. variegata* 'Candida', a white flowering cultivar which produces green pods. Pods were harvested from pairs of 'Purpurea' and 'Candida' trees growing within a few metres of each other at three locations on and around the campus of The University of Queensland (27°28′S). All trees were unshaded by buildings or other trees. Pods, 18-22 cm in length, were harvested in late October (spring) when the seeds were about 80 % developed in size and were placed in a plastic bag containing a few cm^3 of water. The pods were kept in the dark at 23 °C until used.

***In vivo* absorption spectra of pods** were recorded using an *Aminco-Chance* dual-wavelength spectrophotometer, *DW-2A* (*American Instrument Company*). Pods were split in half and the central section of one valve per pod was cut to fit a cuvette and positioned in the spectrophotometer with the exocarp facing the measuring beam.

Chl fluorescence measurements: A pulse modulation Chl fluorometer (*PAM 2000, H. Walz*, Effeltrich, Germany) was used. The maximal photon yield of PS2 photochemistry was measured as F_v/F_m on dark-adapted pods, where the maximal variable fluorescence, F_v, was determined as $F_m - F_0$. The actual photon yield of PS2 photochemistry of irradiated pods was measured as $(F_m' - F)/F_m'$ (Genty *et al.* 1989). The photochemical Chl fluorescence quenching coefficient, q_P, was determined as $(F_m' - F)/(F_m' - F_0')$ and the nonphotochemical Chl fluorescence quenching coefficient as $(F_m - F_m')/(F_m - F_0')$.

Actinic radiation: Filtered radiation from a tungsten-halogen lamp was used to activate photosynthesis in *Bauhinia* pods. BGR was obtained using a *Schott BG-14* glass filter (maximum transmittance 460 nm) and RR using a *Corning 2-61* red cut-off glass filter (transmittance less than 0.5 % at wavelengths below 590 nm). PFD was measured using a Quantum meter *Li-185A* (*Li-Cor*, Lincoln, Nebraska, USA).

PFD response curves using BGR or RR: A disc, 18 mm in diameter, cut from the centre of a valve of either a purple pod or a green pod was placed with the exocarp surface uppermost in the upper chamber of a circular, double chamber made of clear, hard plastic. To circumvent CO_2-limitation of photosynthesis at high PFD, a perforated partition separated the upper chamber from the lower chamber which contained filter paper saturated with 1 M $NaHCO_3$. Chl fluorescence emission from discs was monitored *via* the fluorometer fibreoptics which also provided actinic radiation and radiations used for determination of fluorescence parameters. During the experiment, PFD of the actinic radiation was increased stepwise. At each PFD,

once a steady-state level of fluorescence (F) was reached, after about 15 to 20 min, F was recorded, followed by measurements of F_m' and F_0'. Measurements of F_m' were recorded during brief irradiation with saturating "white light" (PFD of 5000 μmol m^{-2} s^{-1} for 0.8 s) and F_0' after temporarily blocking the actinic radiation and irradiating the disc *via* the fibreoptics with far-red radiation for 5 s. A single disc was used for each response curve and the experiment was repeated. The temperature was 23 °C.

Chl fluorescence measurements on pods at different temperatures: Four discs from purple pods and four discs from green pods, one disc each from the middle of one valve per pod, were arranged in a circle in the circular, double chamber in a high CO_2 atmosphere as described for measurements of PFD response curves. The chamber was sealed and immersed in water at 28 °C. Securely held in place under water, the chamber was irradiated from above with RR or BGR to give a uniform PFD of 260 μmol m^{-2} s^{-1} across the surface of the chamber. Taking care not to block the actinic radiation, the fibreoptic pipe of the fluorometer was fixed above one of the discs. When a steady-state fluorescence level was reached in the disc, F, F_m', and F_0' of the disc were measured. The chamber was then rotated to bring each disc in turn to the position originally occupied by the first disc and fluorescence measurements made as described. The temperature of the water was then decreased step-wise, in 5 °C intervals, and the fluorescence measurements repeated at each temperature. The temperature of the water was controlled by a combination of a heating immersion circulator (model *E, Jubabo Labortechnik*, Germany) and a refrigerated cooler (model *C2G, Grant Instruments*, UK).

Photoinhibition at low temperature: Purple pods and green pods were harvested at 09:00 h, from the southern sides of trees. Pods were dark-adapted for 90 min and F_v/F_m measured. Photoinhibition was induced by placing the pods in sunlight on a rack immersed in circulating water which was maintained at 7 °C. The water was contained in an insulated box covered with a double insulated window through which the sunlight penetrated. The PFD at the level of the pods varied between 750 and 900 μmol m^{-2} s^{-1} during the 1.25-h duration of the experiment. After pods were irradiated, they were dark adapted for 90 min at 23 °C, before remeasuring F_v/F_m values.

In a second experiment, milder photoinhibitory conditions were imposed. Discs (18 mm in diameter) cut from the middle of valves of pods harvested from the southern sides of trees were placed in the circular, double chamber in a high CO_2 atmosphere as described above. The chamber was sealed and immersed in a water bath held at 12 °C. Discs in the chamber were irradiated for 17 h with actinic radiation (PFD, 230 μmol m^{-2} s^{-1}) from a tungsten-halogen lamp filtered through an *80B* filter (*Arrow*, Japan) to produce a blue to red balance closer to that in sunlight. F_v/F_m values were determined as described above on discs before and after treatment after allowing 90 min for dark adaptation.

F_v/F_m of pods exposed to ambient conditions: Pods receiving morning sun on the east to north-east sides of trees were harvested between 09:00 and 09:30 h. After allowing 90 min at 23 °C for dark adaptation, F_v/F_m values were determined on the centre of

the sun-exposed face of each pod and also on the centre of the opposite attached valve, which for most of the day was not exposed to direct sunlight.

Results

Absorption spectra of *Bauhinia* pods: Purple pods and green pods showed almost identical absorption in the red part of the spectrum (maxima at 679 nm), indicating that the pods contained similar Chl concentrations (Fig. 1). However, the purple pods absorbed wavelengths below 600 nm more strongly, giving a ratio of absorbance at 540 nm to that at 679 nm of 0.91 compared with 0.28 for the green pods. Anthocyanins of the purple pods were not located in tissues containing Chl but were confined to the exocarp which could be peeled from pods revealing the underlying green tissues of the mesocarp. The exocarp of green pods was devoid of pigment and was transparent. Fig. 1*c* shows the absorption spectrum of an exocarp peeled from a purple pod. The absorption below 600 nm, due to anthocyanin, showed a broad band with a maximum of 540 nm. The small absorption peak at 679 nm was due to Chl in some mesophyll cells that remained attached to the inner surface of the exocarp.

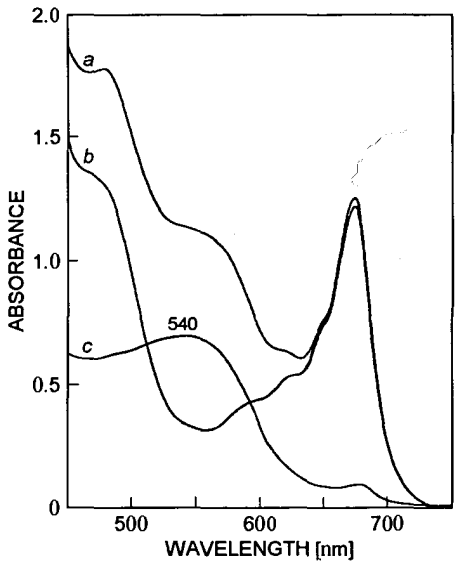

Fig. 1. *In vivo* absorption spectra of *Bauhinia* pods. *a*, purple pod; *b*, green pod; *c*, exocarp peeled from a purple pod.

Photosynthetic responses in BGR or RR: In order to investigate the potential of anthocyanin for photoabatement, photosynthetic parameters were determined using as the actinic radiation either BGR, which is absorbed by both Chl and anthocyanin, or RR, which is absorbed by Chl but only slightly by anthocyanin. Measurements were made over a range of PFD from 0 to 1200 μmol m^{-2} s^{-1}. The choice of actinic radiation made little difference in the case of the green pods (Fig. 2, *left*). Photosynthetic efficiency, indicated by the quantum yield of PS2 photochemistry, and the photochemical Chl fluorescence quenching coefficient, q_P, decreased with

increasing PFD, slightly faster in RR than in BGR. As PFD was increased, there was an increased diversion of absorbed photon energy to nonphotochemical pathways as

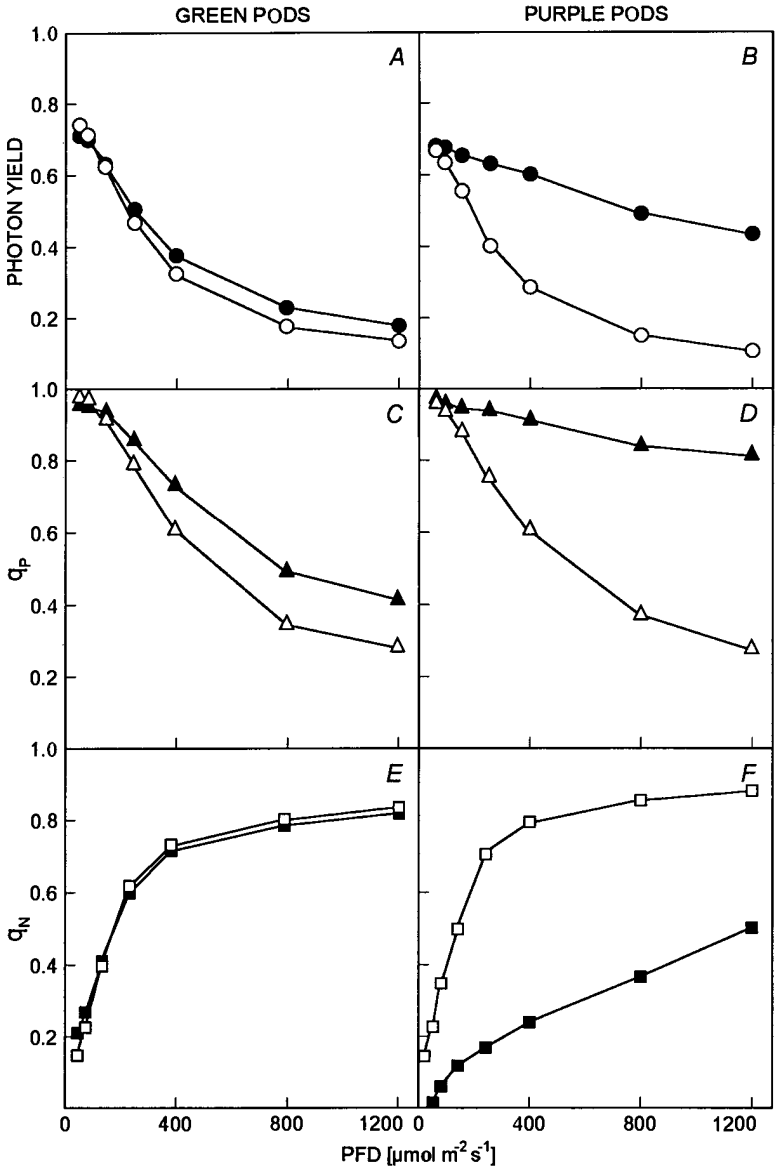

Fig. 2. Photosynthetic responses of green (*A, C, E*) or purple (*B, D, F*) pods of *Bauhinia* irradiated with either blue-green (*filled symbols*) or red radiation (*open symbols*). ●, ○, photon yield of photosystem 2 photochemistry; ▲, △, photochemical quenching coefficient, q_P; ■, □, nonphotochemical quenching coefficient, q_N. The temperature was 23 °C.

indicated by increases in the nonphotochemical Chl fluorescence quenching coefficient, q_N. Differences in the magnitude of changes induced in q_N using RR as opposed to BGR were slight.

With purple pods, PFD response curves were distinctly different depending upon the type of actinic radiation used (Fig. 2, *right*). The decrease in photon yield with RR was similar to that in the green pods, but with BGR the decrease in yield was smaller. Differences were even more marked in the case of q_P. With RR, q_P decreased to below 0.3 at a PFD of 1200 μmol m^{-2} s^{-1} but to only 0.8 in BGR. There was also less diversion of absorbed photon energy to nonphotochemical pathways with BGR.

Temperature effects: Excess photon energy can result in photoinhibition; photoinhibitory pressures can be especially acute at low temperatures as the efficiency and rate of energy usage for CO_2 reductive processes decrease. For this reason the effect of temperature on photon yield and q_P in pods irradiated with either RR or BGR was investigated. The measurements were made on discs cut from pods at five temperatures ranging from 28 °C down to 8 °C. In discs irradiated with RR, photon yield decreased with decreasing temperature. q_P also decreased, from around

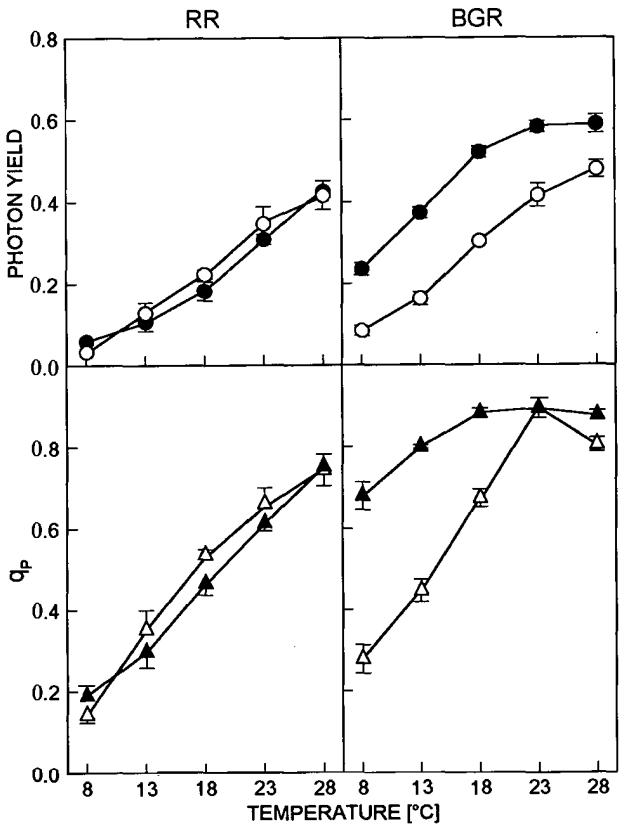

Fig. 3. Photosynthetic responses of *Bauhinia* pod discs irradiated with red (RR) or blue-green (BGR) radiation at different temperatures. ●, ▲, purple discs; ○, △, green discs. Values are the mean ± sem for four discs. The PFD was 260 μmol m^{-2} s^{-1}.

0.74 at 28 °C to less than 0.2 at 8 °C (Fig. 3, *left*). Results obtained with purple discs and green discs were similar. Under BGR, photon yields of the purple discs were higher than those of the green discs at all temperatures (Fig. 3, *right*) and significantly, q_P remained high in the purple discs even at low temperatures, decreasing from 0.89 at 28 °C to only 0.80 at 13 °C and to 0.67 at 8 °C.

Photoinhibition induced at low temperature: Susceptibility to photoinhibition of purple pods and green pods was compared in two experiments. In the first, the degree of photoinhibition induced in intact pods was evaluated following pod exposure to sunlight and a temperature of 7 °C, that is the treatment was a short exposure to severe photoinhibitory conditions. In the second experiment, discs from pods were exposed to "white light" and a temperature of 12 °C: this treatment involved a longer exposure to milder photoinhibitory conditions. The extent of photoinhibition induced was indicated by the extent of the decrease in F_v/F_m. Green pods exposed to sunlight at 7 °C showed considerably more photoinhibition than purple pods (Table 1). A similar difference between green pods and purple pods was found in pod discs exposed to the mild photoinhibitory conditions for 17 h.

Table 1. Photoinhibition of purple pods and green pods of *Bauhinia*. *For experiment 2, pods were harvested from trees at a different location than used in experiment 1.

Photoinhibitory condition	Experimental material	Colour of pods	*n*	F_v/F_m		
				before treatment	after treatment	% decrease
Sunlight (PFD: 750-900 μmol m^{-2} s^{-1}), 7 °C for 1.25 h	Whole pods (exp. 1)	Purple	15	0.785±0.005	0.583±0.025	25.7
		Green	15	0.746±0.010	0.419±0.009	43.8
	Whole pods (exp. 2*)	Purple	17	0.792±0.004	0.532±0.011	32.8
		Green	15	0.791±0.004	0.450±0.012	43.1
White light (PFD: 230 μmol m^{-2} s^{-1}), 12 °C for 17 h	Discs from pods	Purple	6	0.786±0.003	0.589±0.024	25.1
		Green	6	0.793±0.003	0.424±0.040	46.5

Photoinhibition under natural conditions: Experiments were also carried out to determine if the pods on trees became photoinhibited and if so, whether purple pods and green pods had different susceptibilities to photoinhibition. Because the pods generally develop in late October in Brisbane when day temperatures are usually warm, conditions are not conducive to photoinhibition. Hence, measurements were made on pods facing east to north-east, that is these pods were exposed to sunlight during the early, cooler part of the day and were more likely than other pods on trees to become photoinhibited. Control measurements were made on the opposite, mostly shaded valves of the same pods. The exposed pod faces showed evidence of a small degree of photoinhibition when compared with the shaded pod faces, F_v/F_m ratios

Table 2. F_v/F_m of sun-exposed and shaded faces of purple pods and green pods of *Bauhinia*.

Date	Location	Colour of pods	*n*	F_v/F_m Exposed side	Shaded side
26.12.93	1	Purple	16	0.759±0.009	0.785±0.005
		Green	14	0.707±0.019	0.746±0.010
	2	Purple	15	0.759±0.007	0.792±0.002
		Green	16	0.738±0.012	0.791±0.004
	3	Purple	13	0.663±0.022	0.703±0.013
		Green	15	0.625±0.016	0.737±0.012
26.10.94	3	Purple	16	0.652±0.008	-
		Green	16	0.605±0.025	-

were consistently lower on the exposed faces (Table 2). In material collected at three locations on and around the University campus and in the following year at one of these locations, green pods showed a lower mean F_v/F_m than purple pods for the faces exposed to sunlight (Table 2). No differences were found for the shaded faces. The mean F_v/F_m ratio of the shaded side of pods collected from all three locations in the first year was 0.758 ($n = 45$) for green pods and 0.760 ($n = 44$) for purple pods.

Discussion

The purple pods of *Bauhinia* provide an experimental system for investigating how the presence of anthocyanins adjusts photosynthetic activity. Anthocyanin in the exocarp absorbs wavelengths mostly below 600 nm (Fig. 1), thus in effect shielding the chloroplast-containing underlying cells from the short visible wavelengths of sunlight. Fruits of a number of cultivars of mango also form an outer 'coating' of reddish-purple anthocyanin (Schaffer *et al.* 1991) which, as shown by Hetherington (1997), affects photosynthetic efficiency. In young leaves of mango and rainforest species anthocyanin is located in the vacuoles of cells layered above the abaxial epidermis (Lee *et al.* 1987, Woodall *et al.* 1998) and a similar photoabatement effect by the pigment on the PFD reaching the mesophyll chloroplasts might be expected.

Responses of the photosynthetic systems of the purple and the green pods of *Bauhinia* to PFD received were monitored in this study using Chl fluorescence techniques to determine photon yields and Chl fluorescence quenching coefficients. Photon yield is a measure of the efficiency with which absorbed photon energy is used to generate electrons *via* PS2 and water splitting. From this, apparent photosynthetic rate in terms of μmol(electron) m^{-2} s^{-1} may be calculated as the product of photon yield and PFD absorbed×0.5 (two photons being required to release one electron). This rate correlates well with photosynthetic rate determined by oxygen evolution (Edwards and Baker 1993) but in C_3 plants the actual rate of CO_2 uptake may be lower depending on the extent to which the electrons generated photochemically are utilized for photorespiration rather than CO_2 fixation.

Photon yield decreases with increasing PFD. In green *Bauhinia* pods the yield decreased from around 0.8 at zero PFD to below 0.2 at a PFD of 1200 μmol m^{-2} s^{-1} (Fig. 2*A*). This happens because as photon energy absorbed by chloroplasts begins to exceed that required for photosynthesis, the energy is increasingly diverted to nonphotochemical pathways, and can be lost as heat *via* the xanthophyll cycle (Demmig-Adams and Adams 1992). Failure to dissipate excess energy can result in photoinhibition and in extreme situations also in photooxidative degeneration of chloroplastic pigments, proteins, and nucleic acids. Energy aportioned to nonphotochemical pathways is indicated by q_N. In the green pods energy was rapidly diverted to nonphotochemical pathways as PFD was progressively increased to 400 μmol m^{-2} s^{-1} ($q_N = 0.7$) and thereafter remained high, with q_N increasing gradually to around 0.8 at a PFD of 1200 μmol m^{-2} s^{-1} (Fig. 2*E*). Consequently at PFD above 400 μmol m^{-2} s^{-1}, if an additional stress is placed on the photosynthetic system of pods, by for instance a reduction in air temperature which correspondingly decreases photosynthetic rate and concommitantly the rate of utilization of photochemical energy, then the capacity of the system to cope with this environmental stress by diverting more energy to nonphotochemical pathways is fairly limited.

The status of actual utilization of absorbed photon energy in PS2 photochemistry is given by q_P. As shown by Fig. 2*C*, q_P decreases with increasing PFD indicating that the steady state level of the primary stable acceptor for PS2, Q_A, becomes more reduced at high PFD ($q_P = 1$ corresponds to the acceptor being fully oxidized and $q_P = 0$ to the acceptor being completely reduced). As the actual acceptor for PS2 is the oxidized form of Q_A, the value of q_P is, within the context of this paper, an important indicator of susceptibility of the photosynthetic system to photoinhibition. By maintaining Q_A mainly in the oxidized form, corresponding to a high q_P, adequate availability of the acceptor is assured and the incidence of photoinhibition is lessened or avoided. However, once nonphotochemical energy processes are saturated (high q_N), any additional PFD or interactive stress will tend to drive q_P lower and increase the likelihood of photoinhibition.

By choosing actinic radiations which were either absorbed or poorly absorbed by anthocyanin, differences in photosynthetic responses by green pods and purple pods were clearly resolved. The choice of actinic radiation made little difference in green pods to the PFD response curves for photon yield, q_P and q_N (Fig. 2). Thus absorption, photochemical utilization, and photon energy dissipation of the BGR and RR were similar. Red wavelengths elicited an equivalent response in purple pods (Fig. 2, *right*), showing that the photosynthetic systems of the two types of pods, in terms of the measurements made, were very similar. A definite effect of photoabatement by anthocyanin on photosynthetic response in purple pods was seen with the BGR (Fig. 2, *right*). The fall in photon yield with increasing PFD was smaller than in green pods and in purple pods irradiated with RR, and q_N values were lower. Significantly, a high q_P was maintained, still around 0.8 at a PFD of 1200 μmol m^{-2} s^{-1} compared with 0.3 to just above 0.4 in purple pods irradiated with RR or in green pods irradiated with either radiation. From these results it can be predicted that the purple pods are better equipped to withstand photoinhibitory pressure from radiation, including sunlight, that contains blue-green wavelengths.

Krol *et al.* (1995) observed a two-fold increase in q_P in needles of jack pine seedlings after acclimation to low temperature. Acclimation at 5 °C and an 8-h-day PFD of 250 μmol m^{-2} s^{-1} resulted in the synthesis of anthocyanin in the previously green needles. This was accompanied by an increase in tolerance to photoinhibition at 5 °C which was attributed partly to screening by anthocyanin and partly to a higher rate of photosynthesis on a Chl basis that would also tend to increase q_P. While highest photon yields in *Bauhinia* were observed in the purple pods irradiated with BGR, photosynthetic rates were not necessarily higher than in green pods irradiated with the same PFD. This is because the actual PFD absorbed by chloroplast pigments would be considerably lower in purple pods due to competing absorption by anthocyanin. The similar photon yields obtained with RR in purple pods and green pods suggest that, unlike in the low-temperature acclimated jack pine needles, any enhanced tolerance to photoinhibition by purple pods arises as a result of photoabatement by anthocyanin and not from a higher rate of photosynthesis.

If a higher rate of photosynthesis can alleviate photoinhibition, conversely, a lower rate, in the absence of a change in PFD, can increase photoinhibition. This is a consequence of the decrease in rate of utilization of photochemically derived energy for CO_2 fixation. While several environmental factors can depress photosynthetic rate, low temperature is an obvious one, and the interaction of irradiance and low temperature is a common cause of photoinhibition in cultivated plants (Hetherington *et al.* 1989, Baker *et al.* 1994). For this reason the effect of temperature on the photon yield and q_P of *Bauhinia* pods was investigated. Using RR, both photon yield and q_P decreased as temperature was reduced stepwise from 28 to 8 °C, but there were no differences between the purple and the green pods (Fig. 3, *left*). A q_P of less than 0.2 at 8 °C suggested that the pods were especially susceptible to photoinhibition at this temperature. With BGR as the actinic irradiation, the reduction in q_P with decreasing temperature was not as pronounced in the purple pods (Fig. 3, *right*). Photon yield was higher in purple pods than in green pods at all temperatures and with decreasing temperature, the difference between the q_P values of purple and green pods increased. At 8 °C the q_P of the purple pods had fallen to only 0.67 indicating that even at this low temperature, tolerance to photoinhibition was still high at the PFD used. Photoabatement by anthocyanins may also be effective against photoinhibition at high temperatures. Photoinhibition can occur on the exposed surfaces of tropical mango fruit during the summer growing season (Hetherington 1997). Fruit with anthocyanic coloration showed consistently higher photon efficiencies of PS2 photochemistry than green fruit suggesting that the anthocyanin could have a role in amelioration of photoinhibition in this fruit (Hetherington 1997). Even anthocyanic coloration confined to the undersides of leaves, such as that often found on the lower surface of leaves of herbaceous shade plants in tropical rainforests, may fulfill a similar role. This was the conclusion of Gould *et al.* (1995) who found that for the rainforest understory species *Begonia paronia* and *Triolena hirsuta*, photoinhibition as indicated by a lower value of F_v/F_m was significantly higher in green leaves than in leaves with red abaxial surfaces. However, the reduced level of photoinhibition in the red pigmented leaves might also have been attributed to the higher rates of photosynthesis found in the red leaves of both species.

Actual photoinhibition in pods was assessed in two ways. Harvested pods were exposed to photoinhibitory conditions and changes in F_v/F_m ratios were determined as indicators of photoinhibition. The extent to which pods incurred photoinhibition while attached to trees was also determined. Detached purple pods showed much less photoinhibition than green pods following either short-term exposure to sunlight at 7 °C or long-term exposure to a lower intensity of artificial "white light" at 12 °C (Table 1). Although seasonal photoinhibitory pressures during the period of pod ontogeny in *Bauhinia* were slight, the small degree of photoinhibition observed during pod development on trees confirmed the greater susceptibility of green pods over purple pods to photoinhibition (Table 2). These results reinforce the conclusions based on the values for photon yields and Chl fluorescence quenching coefficients about the photoabatory role of anthocyanins when located to the exterior of chloroplast-containing cells. We propose that the production of anthocyanins by many species in tissues overlying photosynthetic tissues is an important mechanism for minimizing photoinhibition in leaves or organs that for some reason are especially vulnerable to light. These reasons could include the early stages of chloroplast development in expanding leaves, changes in sink/source relationships during the transition from photoheterotrophic to photoautotrophic leaf activity and adverse environmental conditions; in essence, any change which leads to a reduction in photosynthetic rate which is not matched by an equivalent reduction in PFD.

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