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Regulation of Electron Transport in Photosystems I and II in C_3, C_3-C_4, and C_4 Species of *Panicum* in Response to Changing Irradiance and O_2 Levels[1]

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Regulation of the quantum yields of linear electron transport and photosystem II photochemistry (Φ_{II}) with changing irradiance and gas-phase O_2 concentration was studied in leaf tissue from *Panicum bisulcatum* (C_3), *Panicum milioides* (C_3-C_4), and *Panicum antidotale* (C_4) at 200 μbars of CO_2 and 25°C using infrared gas analysis and chlorophyll fluorescence yield measurements. When the O_2 level was increased from 14 to 213 mbars at high irradiance, Φ_{II} increased by as much as 115% in *P. bisulcatum* but by no more than 17% in *P. antidotale*. Under the same conditions Φ_{II} increased to an intermediate degree in *P. milioides*. Measurements of accumulation of the photooxidized form of the photosystem I reaction center ($P700^+$) based on the light-dependent in vivo absorbance change at 830 nm indicate that the steady-state concentration of $P700^+$ varied in an antiparallel manner with Φ_{II} when either the irradiance or O_2 concentration was changed. Hence, O_2-dependent changes in Φ_{II} were indicative of variations in linear photosynthetic electron transport. These experiments revealed, however, that a significant capacity was retained for in vivo regulation of the apparent quantum yield of photosystem I (Φ_I) independently of Φ_{II}. Coordinate regulation of quantum yields of photosystems I and II (expressed as Φ_I:Φ_{II}) in response to changing irradiance and O_2 level differed markedly for the C_3 and C_4 species, and the response for the C_3-C_4 species most closely resembled that observed for the C_4 species. The fraction of total linear electron transport supporting photorespiration at 213 mbars of O_2 was negligible in the C_4 species and was 13% lower in the C_3-C_4 species relative to the C_3 species as calculated from fluorescence and gas-exchange determinations. At high photon-flux rates and high O_2 concentration, the potential benefit to light use for net CO_2 uptake arising from lower photorespiration in *P. milioides* was offset by a reduced capacity for total CO_2- and O_2-dependent noncyclic electron transport in this species compared with *P. bisulcatum*.

During photosynthesis in oxygenic organisms electrons removed from H_2O pass sequentially through PSII and PSI and ultimately to NADP to form NADPH, which is used together with ATP to reduce CO_2 to carbohydrate in the Calvin cycle. Net assimilation of CO_2 in normal air (340 μbars of CO_2, 210 mbars of O_2) by plants possessing the C_3 pathway of carbon metabolism is 30 to 40% lower than it would otherwise be due to photorespiration (Zelitch, 1971). Inhibition of photosynthesis by O_2 (Warburg effect) is associated with refixation of CO_2 and NH_4^+ and with reduction of 3-phosphoglyceric acid to triose phosphate. These compounds arise during metabolism of photorespiratory 2-phosphoglycolate, which is, in turn, produced during oxygenation of RuBP by Rubisco (Jordan and Ogren, 1984; Ogren, 1984). However, partitioning of photosynthetic reductant to photorespiration varies greatly among species (Krall and Edwards, 1990; Krall et al., 1991).

For a given temperature and gas-phase composition with respect to O_2 and CO_2, C_3 leaves have the highest rates of photorespiration (Zelitch, 1971). Inhibition of photosynthesis by O_2 is minimal in C_4 plants, since the primary carboxylation process is catalyzed by O_2-insensitive PEP carboxylase in the leaf MC. The resulting C_4 acids are decarboxylated in the enlarged chlorophyllous cells surrounding the vascular elements (BSC), and the CO_2 is refixed by Rubisco. Photorespiration is suppressed in the BSC because of the very high CO_2 concentration in these cells (Jenkins et al., 1989; Dai et al., 1993). This compartmentation of function in C_4 leaves is associated with clearly identifiable anatomical differences between MC and BSC (Kranz anatomy). An interesting and diverse third class of higher plants (represented by, but not limited to, species in the genera *Panicum*, *Moricandia*, and *Flaveria*) possess properties intermediate to the more common

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Abbreviations: ANOVA, analysis of variance; BSC, bundle sheath cell(s); *CER*, net carbon (CO_2) exchange rate (as μmol m^{-2} s^{-1}, corrected for dark respiratory CO_2 release); $\Delta A830$, in vivo light-dark *A* change at approximately 830 nm; C_i, intercellular CO_2 concentration (μbars); $\Delta A830_m$, maximum in vivo *A* change at 830 nm; Φ_e, quantum yield (mol:mol) of linear photosynthetic electron transport ([4 × *CER*]/incident PPFD); F_m', maximum fluorescence yield (q_P = 0) in light-adapted state; F_s, steady-state fluorescence yield; F_o', minimum fluorescence yield (q_P = 1) in light-adapted state; F_v', variable fluorescence yield (= $F_m' - F_o'$); Hz, hertz (cycles s^{-1}); *J*, rate of linear photosynthetic electron transport (μmol m^{-2} s^{-1}); K_{sp} (in vivo), an in vivo CO_2/O_2 specificity factor; MC, mesophyll cell(s); Φ_e', calculated quantum yield linear electron transport at elevated [O_2]; Φ_I, photochemical quantum yield of PSI ($1 - \Delta A830/\Delta A830_m$); Φ_{II}, photochemical quantum yield of PSII ($[F_m' - F_s]/F_m'$); P_{diss}, fraction of total noncyclic electron transport partitioned to O_2-dependent processes; $P700^+$, photooxidized form of the PSI reaction center; $[P700]_{total}$, [$P700^+$] plus [P700]; q_P, photochemical fluorescence quenching coefficient; RuBP, ribulose bisphosphate.

C_3 and C_4 types (Ku et al., 1983; Monson et al., 1984). Such C_3-C_4 intermediate species have been reported to possess a lower CO_2 compensation point, reduced O_2 inhibition of net photosynthesis, and diminished CO_2 evolution into CO_2-free air compared to C_3 species (Brown and Brown, 1975; Krenzer et al., 1975; Brown, 1980; Krall et al., 1991; Ku et al., 1991). It follows that, if photorespiration is lower in C_3-C_4 species compared with C_3 species, then a diminished allocation of linear electron transport to photorespiration should be observed.

This study uses techniques of gas exchange, Chl fluorescence, and in vivo A_{830} as probes of the light use efficiencies of PSII and PSI in response to varying irradiance and $[O_2]$ for C_3, C_3-C_4, and C_4 species of the genus *Panicum*. The objectives of the work are to provide (a) quantitative assessments of O_2-dependent electron transport, (b) insight into the mechanism of regulation of the Warburg effect in the C_3-C_4 intermediate, and (c) a comparison of patterns of coregulation of quantum yields of PSI and PSII with changing irradiance and $[O_2]$ for these species.

MATERIALS AND METHODS

Panicum bisulcatum (C_3), *Panicum milioides* (C_3-C_4), and *Panicum antidotale* (C_4) were grown in a greenhouse in pots containing a commercial potting soil mixture. The plants were watered daily and cultured with nutrient solution weekly.

Measurements of *CER* and transpiration were conducted using an open flow-through system at an external gas-phase $[CO_2]$ of 200 μbars and a leaf temperature of 25°C. The water vapor pressure deficit was maintained at 8 to 11 mbars. Details pertaining to these measurements have been described previously (Long and Hallgren, 1985; Peterson, 1990, 1991). Measurements of *CER* have been corrected for the rate of mitochondrial CO_2 release. The latter was estimated by plotting the rate of net CO_2 uptake versus PPFD at low irradiance levels and extrapolating to darkness. The minimum Φ_e necessary to account for the corrected rate of net CO_2 uptake was calculated as $(4 \times CER)$/(incident PPFD). Actinic white light was supplied by a Schott KL1500 Cold Light Source. Far-red emission present in the actinic light was removed by use of Schott KG1 and heat-reflecting (Optical Coating Laboratory, Inc., Santa Rosa, CA) filters. Irradiance (400–700 nm) was measured using an LI-190SB quantum sensor (Li-Cor Instruments, Lincoln, NE).

Chl fluorescence yield was measured using the Walz pulse amplitude modulation system (H. Walz, Effeltrich, Germany). Following the recording of steady-state gas-exchange parameters at a given irradiance and $[O_2]$, three saturating pulses (7000 μmol photons m^{-2} s^{-1} for 0.7 s) of white light were superimposed on the actinic illumination at intervals of 100 s. F_s was recorded prior to each flash with the modulation frequency of the measuring beam set at 100 kHz. The F_m' level was the maximum signal observed during the saturating pulse. The actinic illumination was extinguished for 3 to 4 s between the flashes to measure F_o' (measuring beam modulation frequency, 1.6 kHz). Weak far-red illumination was superimposed during these measurements to ensure that interphotosystem carriers were oxidized (Weis et al., 1987). q_P indicates the fraction of PSII units currently capable of reducing plastoquinone and was calculated as $(F_m' - F_s)/(F_m' - F_o')$. The efficiency of energy capture by PSII reaction centers was calculated as $F_v'/F_m' = (F_m' - F_o')/F_m'$ (Genty et al., 1989). Φ_{II} is given by $q_P \times (F_v'/F_m') = (F_m' - F_s)/F_m'$ (Genty et al., 1989). Mean values of q_P, F_v'/F_m', and Φ_{II} were calculated from the results of the replicate flashes.

Measurements of light-induced leaf *A* changes in the far-red region followed fluorescence yield determinations. These were conducted to assess steady-state accumulation of $P700^+$, which possesses a broad absorption maximum at approximately 830 nm (Harbinson and Woodward, 1987). The Walz system was adapted so that the same fiberoptic light guide and signal-processing hardware used for fluorescence measurements could be used for the $\Delta A830$ determinations (Schreiber et al., 1988; Peterson, 1991). The rapid (approximately 1 s) light-dark change in amplitude ($\Delta A830$) was assumed to be proportional to the prior steady-state $[P700^+]$. Φ_I was calculated as the fraction of photooxidizable P700 in the nonoxidized state (i.e. $[1 - \Delta A830/\Delta A830_m]$; Harbinson et al., 1990). The $\Delta A830_m$ is the minimal estimate of the signal associated with full photooxidation of P700 as obtained by extrapolation of linear plots of Φ_e versus $\Delta A830$ to $\Phi_e = 0$ at 14 mbars of O_2.

In an experiment with a single leaf sample, measurements were recorded with changing actinic irradiance first at a fixed $[O_2]$ of 14 mbars of O_2 and then similarly at 213 mbars followed by 432 mbars of O_2. Experiments were replicated six times for each species.

RESULTS

Figure 1 shows the responses of Φ_e to changing levels of incident irradiance of white light for the three species of *Panicum* studied. Note that electron transport associated with photorespiration, reduction of alternate acceptors (i.e. O_2, NO_2^-), and cyclic processes are not included in measurements of Φ_e. It is assumed, however, that O_2-dependent electron flow is suppressed when the ambient $[O_2]$ is lowered to 14 mbars. Emphasis in these studies was placed on obtaining measurements for irradiance levels >150 μmol photons m^{-2} s^{-1}, since previous work (Peterson, 1990, 1991) showed that enhancement of Φ_{II} caused by increasing the $[O_2]$ to approximately 210 mbars of O_2 from approximately 10 mbars of O_2 was significant only when the PPFD was nonlimiting. Since effects of O_2 on light use and electron transport were of primary interest in this study, a lower than normal external $[CO_2]$ of 200 μbars was used to augment the responses to O_2. Mean values of Φ_e were nearly identical for *P. bisulcatum* (C_3) and *P. milioides* (C_3-C_4) at all irradiance and O_2 levels. For comparison, at 213 mbars of O_2 and 1049 μmol photons m^{-2} s^{-1}, mean (±SE) values of Φ_e were 0.0425 ± 0.0026, 0.0412 ± 0.0023, and 0.0981 ± 0.0062 for the C_3, C_3-C_4, and C_4 species, respectively. Consequently, the extent of inhibition of Φ_e by high $[O_2]$ relative to 14 mbars of O_2 did not differ significantly for the C_3 and C_3-C_4 species over the irradiance range studied (i.e. mean values of 29 and 59% for the C_3 species and 31 and 57% for the C_3-C_4 species at 213 and 432 mbars of O_2, respectively). No significant effect of $[O_2]$ on Φ_e was detected for the C_4 species based on ANOVA (i.e. $P > 0.05$).

At 14 mbars of O_2 values of Φ_{II} were virtually identical at

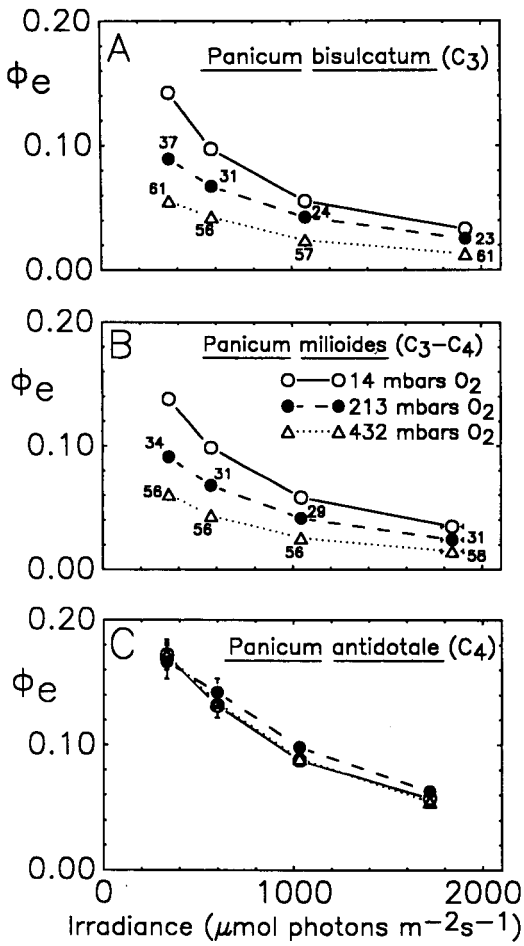

Figure 1. Plots of Φ_e versus incident irradiance for leaf tissue samples of *P. bisulcatum* (A), *P. milioides* (B), and *P. antidotale* (C). Numerical values indicate the percentage of inhibition of Φ_e by high $[O_2]$ relative to 14 mbars of O_2. Each point is a mean of six replicate determinations. Error bars indicate ±SE. In this and subsequent figures, error bars not shown are hidden by the symbols. See "Materials and Methods" for further information concerning experimental conditions and procedures.

each irradiance level for the C_3 and the C_3-C_4 species and yet somewhat lower than corresponding values for the C_4 species at the higher irradiances (Fig. 2). By contrast, at 213 mbars of O_2 the magnitude of Φ_{II} was, on average, 21% higher for *P. bisulcatum* (C_3) compared with *P. milioides* (C_3-C_4) for irradiance levels >150 μmol photons m^{-2} s^{-1}. A small, yet highly significant (indicated by ANOVA, $P<0.001$), enhancement of Φ_{II} by elevated $[O_2]$ was observed for the C_4 species (Fig. 2). Figure 3 shows that at the lower irradiance levels increases in F_v'/F_m' accounted for most of the $[O_2]$-dependent increases in Φ_{II}, whereas increases in q_P became important at the higher irradiances. The relative contributions of q_P and F_v'/F_m' to the increases in Φ_{II} were similar for the C_3 and C_3-C_4 leaves at each irradiance.

The steady-state $[P700^+]/[P700]_{total}$, as indicated by the $\Delta A830/\Delta A830_m$, increased sigmoidally with irradiance for all three species (Fig. 4). At constant irradiance, an increase in $[O_2]$ from 14 mbars to ≥213 mbars was accompanied by a decrease in the $\Delta A830/\Delta A830_m$. Effects of $[O_2]$ on the $\Delta A830/\Delta A830_m$ were highly significant ($P < 0.001$) for all three species and complementary to the respective $[O_2]$-dependent changes in Φ_{II} (Fig. 2).

Figure 5 shows the dependencies of Φ_e on Φ_{II} for the data of Figures 1 and 2. At elevated O_2 levels in the C_3 and C_3-C_4 species a portion of the linear electron flow was diverted to photorespiration so that Φ_e underestimates the actual quantum yield of total noncyclic electron transport. Φ_e' is assumed to be very closely approximated by substitution of the corresponding estimate of Φ_{II} into the regression equation relating the dependence between Φ_e and Φ_{II} at 14 mbars of O_2 (Table I). P_{diss} is given by $(\Phi_e' - \Phi_e)/\Phi_e'$ (Peterson, 1989, 1990). Table I shows mean values of P_{diss} and the dissolved molar $[O_2]/[CO_2]$ based on intercellular gas partial pressures for the three species of *Panicum*. Although the $[O_2]/[CO_2]$ did not differ substantially for *P. bisulcatum* and *P. milioides*, the magnitude of P_{diss} was 13.2 and 8.9% lower in the C_3-C_4 species relative to the C_3 species at 213 and 432 mbars of O_2, respectively. By analogy to the Rubisco enzyme model (Jordan and Ogren, 1984) K_{sp} (in vivo) may be calculated as K_{sp} (in vivo) = $([O_2]/[CO_2]) \times (1.5/P_{diss} - 1)$ (Peterson, 1989,

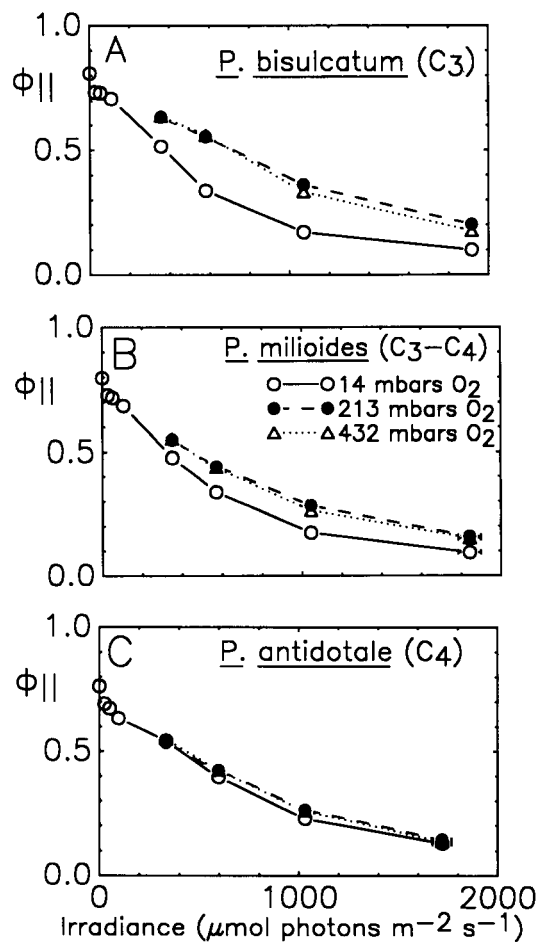

Figure 2. Plots of Φ_{II} versus incident irradiance for the experiments of Figure 1.

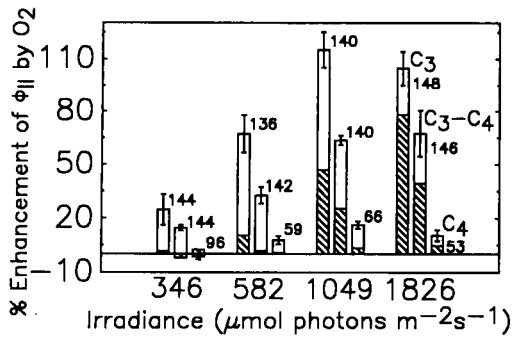

Figure 3. Comparison of the relative increase in Φ_{II} caused by increasing the $[O_2]$ from 14 to 213 mbars for three species of *Panicum*. The percentage increase in Φ_{II} was calculated from data collected at the two O_2 levels for the same leaf sample according to the formula $100 \times \{\Phi_{II}$ (high $[O_2])/\Phi_{II}$ (low $[O_2]) - 1\}$. Error bars indicate ±SE. The shaded portions show the relative contributions of the O_2-dependent increases in q_P to the increases in Φ_{II}. These were calculated as (% increase in $\Phi_{II}) \times$ [(% increase in q_P)/(% increase in q_P + % increase in F_v'/F_m')]. The unshaded portions represent the respective relative contributions of the increases in F_v'/F_m'. Quantities accompanying the bars are associated mean C_i values in μbars at 213 mbars of O_2 (SE values were 5, 5, and 13 μbars for the C_3, C_3-C_4, and C_4 species, respectively). At 14 mbars of O_2 the respective mean C_i values (in μbars, ±SE) were 99 ± 2, 103 ± 3, and 15 ± 9 for the C_3, C_3-C_4, and C_4 species.

1990). Use of this expression with the data of Table I yields K_{sp} (in vivo) (±SE) values of 99.3 ± 2.7 and 124.0 ± 3.7 for *P. bisulcatum* (C_3) and *P. milioides* (C_3-C_4), respectively. Based on the data collected at 432 mbars of O_2 (Table I), it is possible to show that K_{sp} (in vivo) > 9000 for *P. antidotale* (C_4).

Noncyclic electron transport requires the presence of a terminal electron acceptor. At high irradiance Φ_{II} can exhibit a strong dependence on the C_i, indicating that Rubisco is the prime mediator of linear electron flow (Sharkey et al., 1988; Krall et al., 1991). Average C_i values for both the C_3 and C_3-C_4 species increased from 101 to 143 μbars as the $[O_2]$ was increased from 14 to 213 mbars over the irradiance range shown in Figure 3. Thus, increases in Φ_{II} with $[O_2]$ at constant irradiance shown in Figure 3 for the C_3 and C_3-C_4 species were due to increased consumption of NADPH and ATP as determined by the combined rate of oxygenation plus carboxylation of RuBP by Rubisco. Additional analysis of the data was conducted to compare regulation of electron transport in the C_3 and C_3-C_4 species irrespective of changes in C_i.

Table II shows that at 213 mbars of O_2 and a mean irradiance of 1049 μmol photons m^{-2} s^{-1} the C_i was identical for both the C_3 and the C_3-C_4 species; yet Φ_e' was 18% lower in the latter. J is given by Φ_e (or Φ_e') × PPFD of actinic light. If PSII electron transport were dependent exclusively on availability of CO_2 as $[O_2]$ varied, then an increase in C_i would lead to a proportional increase in J and so that the ratio J:C_i would be constant. For the C_3 species such a strict dependence on availability of CO_2 was not observed, since an increase in $[O_2]$ from 14 to 213 mbars of O_2 resulted in a significant 25% increase in the J:C_i (Table II). In contrast, no significant effect of $[O_2]$ on J:C_i was found for *P. milioides*. Results similar to those of Table II were found at mean irradiance levels of 582 and 1826 μmol photons m^{-2} s^{-1} in that the average J:C_i increased by 17% ($P < 0.05$) and 33% ($P < 0.01$), respectively, for the C_3 species. Changes in J:C_i with $[O_2]$ for the C_3 species at 346 μmol photons m^{-2} s^{-1} or at any irradiance for the C_3-C_4 species were not significant ($P > 0.05$). Mean (±SE) J:C_i values ($n = 18$) averaged over the three highest irradiance levels used were 0.602 ± 0.020 and 0.752 ± 0.028 at 14 and 213 mbars of O_2, respectively, for the C_3 species. Likewise, J:C_i values for the C_3-C_4 species were essentially unaffected by $[O_2]$, i.e. 0.586 ± 0.027 (low $[O_2]$) and 0.602 ± 0.031 μmol m^{-2} s^{-1} $\mu bars^{-1}$ (high $[O_2]$).

Coordinate regulation of PSII and PSI for the three species of *Panicum* was examined as the ratio of Φ_I:Φ_{II} (Fig. 6). Particularly evident is the different response of the Φ_I:Φ_{II} to irradiance and $[O_2]$ for the C_3 species compared with the C_3-C_4 and C_4 species. Elevated $[O_2]$ caused a lowering of the Φ_I:Φ_{II} for the C_3 species at irradiance levels ranging from 600

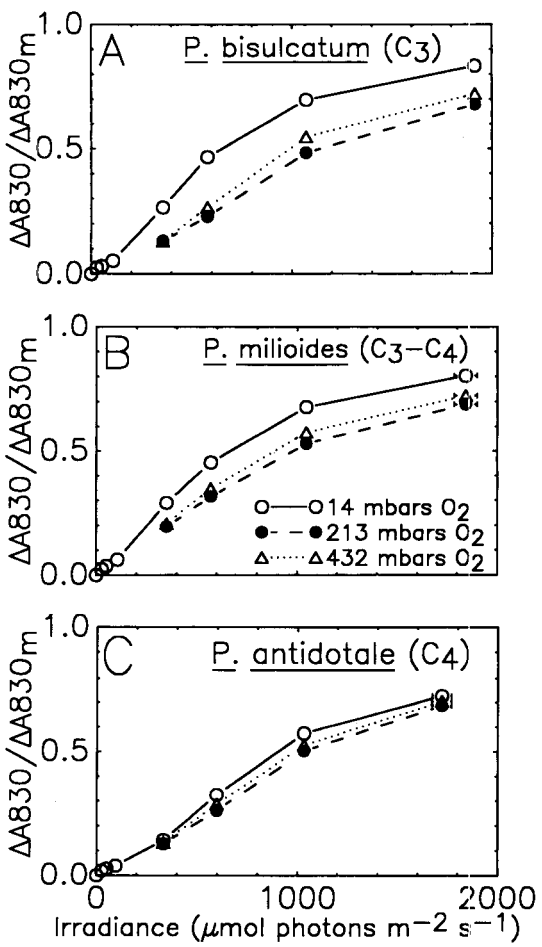

Figure 4. Plots of $\Delta A830/\Delta A830_m$ as an indicator of $[P700^+]/[P700]_{total}$ versus incident PPFD for the experiments of Figure 1. Values of $\Delta A830$ were normalized to the corresponding $\Delta A830_m$ to compensate for extraneous variations in signal amplitude arising from biological variability among leaves and leaf-to-probe geometry.

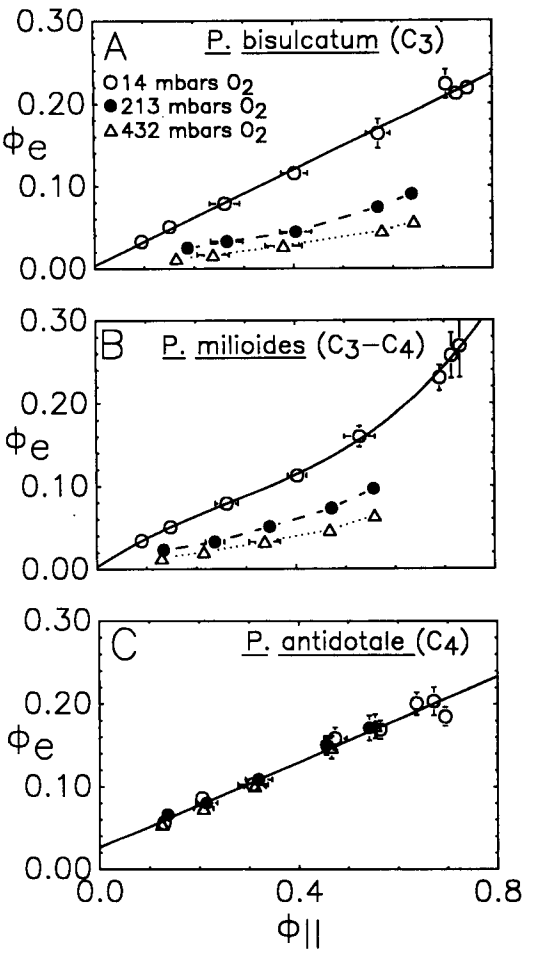

Figure 5. Plots of Φ_e versus Φ_{II} for three species of *Panicum* at three levels of gas-phase O_2 concentration. The solid lines are first-order (A and C) or third-order (B) polynomial regression fits to the data obtained at 14 mbars of O_2 (coefficients of determination ≥ 0.098). The data were obtained from the experiment of Figures 1 through 3. Error bars indicate ±SE.

to 1100 μmol photons m^{-2} s^{-1}. In contrast, the Φ_I:Φ_{II} increased linearly with irradiance for the C_3-C_4 and C_4 species and was independent of $[O_2]$. The Φ_I:Φ_{II} was similar in magnitude for the C_3-C_4 and C_4 species at each irradiance level. Nevertheless, two-way ANOVA using data obtained for only these two species showed that effects of both irradiance and species were significant ($P < 0.002$). For comparison, mean values of Φ_I:Φ_{II} at 213 mbars of O_2 were computed by averaging results obtained at this $[O_2]$ for the four highest irradiance levels shown in Figure 6. The resulting values (±SE) were 1.45 ± 0.02, 1.69 ± 0.06, and 1.87 ± 0.04 for the C_3, C_3-C_4, and C_4 species, respectively (SE values were calculated for each species as $[(\text{error variance})/24]^{0.5}$ after one-way ANOVA for effects of irradiance).

DISCUSSION

The close association between regulation of light utilization in PSII and linear electron transport has been interpreted in terms of a tight coupling between late steps in photosynthesis (consumption of NADPH and ATP) and the primary photochemical processes that follow light absorption by the photosynthetic apparatus so as to avoid potentially harmful overstimulation of reaction centers (Weis et al., 1987; Sharkey et al., 1988; Genty et al., 1989; Weis and Lechtenberg, 1989; Harbinson et al., 1990; Peterson, 1991). Furthermore, the ability to balance carbon-assimilating and photochemical processes enables the chloroplast to maintain key electron transport components in a redox state optimal for photosynthesis as irradiance and acceptor availability vary (Foyer et al., 1990).

A practical application of Chl fluorescence is to measure electron transport in intact systems to supplement information obtained by conventional gas-exchange techniques (Peterson, 1989, 1990; Cornic and Briantais, 1991). Changes in Φ_e that are closely associated with variations in Φ_{II} have been reported for C_4 species and for C_3 and C_3-C_4 species when O_2-dependent electron transport is suppressed (Genty et al., 1989; Krall and Edwards, 1990; Krall et al., 1991; Peterson, 1991). Deviations in this relationship (Fig. 5B) appear to be restricted to conditions of limiting irradiance (Seaton and

Table I. *Inhibition by O_2 of net uptake of CO_2 for species of Panicum at 25°C*

P_{diss}(=[Φ_e' − Φ_e]/Φ_e') is the unbiased estimate of the proportion of linear photosynthetic electron transport partitioned to dissipative O_2-dependent processes. A single determination of P_{diss} was based on CO_2 exchange and Chl fluorescence parameters recorded at a specified irradiance and $[O_2]$. First, Φ_e was calculated as (4 × *CER*)/(incident PPFD). Second, the associated Φ_e' was calculated by substitution of the corresponding Φ_{II} into the first- or second-order regression equation ($r^2 > 0.99$) relating Φ_e to Φ_{II} at 14 mbars of O_2 for the same leaf sample. The dissolved molar $[O_2]/[CO_2]$ was calculated using the intercellular gas partial pressures, leaf temperature, and tabular information on gas solubilities in H_2O versus temperature (Peterson, 1990). Mean values (±SE) shown were averaged across the four irradiance levels shown in Figure 3. The effect of irradiance on P_{diss} was significant ($P < 0.01$) yet weak compared to the effect of species based on ANOVA (0.4 versus 89.9% of total sum-of-squares, respectively). Irradiance did not significantly affect the $[O_2]/[CO_2]$.

Species	213 mbars of O_2		432 mbars of O_2	
	P_{diss}	$[O_2]/[CO_2]$	P_{diss}	$[O_2]/[CO_2]$
	mol:mol		*mol:mol*	
P. bisulcatum (C_3)	0.560 ± 0.012	56.9 ± 1.3	0.732 ± 0.009	97.4 ± 1.1
P. milioides (C_3-C_4)	0.486 ± 0.014	56.3 ± 1.1	0.667 ± 0.008	102.2 ± 2.2
P. antidotale (C_4)	−0.003 ± 0.014	114.5 ± 7.9	0.051 ± 0.015	347.5 ± 67.1

Table II. *Responses of electron transport to $[O_2]$ for P. bisulcatum and P. milioides*

The data were obtained at a mean irradiance of 1049 μmol photons m^{-2} s^{-1} from the experiments of Figures 1 and 2. Values of Φ_e and Φ_e' were calculated as described in Table I. Associated values of *J* were computed as (Φ_e or Φ_e') × incident PPFD. Effects of $[O_2]$ on mean $J:C_i$ for each species were assessed using a *t* test for paired observations (*, $P < 0.05$). Values of SE are also shown ($n = 6$).

Species and $[O_2]$	C_i	Φ_e (low $[O_2]$) or Φ_e' (high $[O_2]$)	$J:C_i$	Increase in mean $J:C_i$
	μbars	mol:mol	μmol m^{-2} s^{-1} ubars^{-1}	%
P. bisulcatum (C_3)				
14 mbars O_2	93 ± 4	0.056 ± 0.003	0.641 ± 0.040	
213 mbars O_2	140 ± 4	0.104 ± 0.004	* 0.800 ± 0.049	25
P. milioides (C_3-C_4)				
14 mbars O_2	106 ± 4	0.058 ± 0.003	0.573 ± 0.032	
213 mbars O_2	140 ± 6	0.085 ± 0.005	0.642 ± 0.061	12

Walker, 1990). With respect to changing $[O_2]$, light reaction products NADPH and ATP are consumed in a relatively fixed proportion by chloroplast carbon metabolism regardless of whether photorespiration is present or absent (Ogren, 1984). Although limited biological variability in the Φ_e versus Φ_{II} relationship can occur even within a species, it is reasonable to assume that the inherent relationship between the quantum yield of linear electron transport and Φ_{II} for a single leaf is preserved as $[O_2]$ changes. This assumption is supported by the responses of Φ_e and Φ_{II} to $[O_2]$ among species known to differ in sensitivity of photosynthesis to O_2.

Leaves of C_3-C_4 species such as *P. milioides* possess a microanatomy resembling the prominent Kranz syndrome of

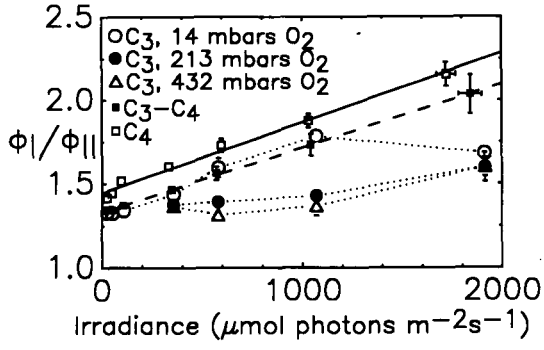

Figure 6. Plots of the mean ratio of $\Phi_I:\Phi_{II}$ (±SE) versus irradiance for three species of *Panicum*. Two-way ANOVA failed to detect a significant effect of $[O_2]$ on $\Phi_I:\Phi_{II}$ for either *P. milioides* (C_3-C_4) or *P. antidotale* (C_4) ($P > 0.2$). Thus, the mean values shown for the C_3-C_4 and C_4 species were computed by pooling measurements obtained at all three O_2 levels ($n = 18$). The effect of irradiance was highly significant for both of these species ($P < 0.001$). Since the effect of $[O_2]$ was clearly significant for *P. bisulcatum* (C_3), data were plotted separately for each O_2 level ($n = 6$). The solid and dashed lines are linear regression fits to the original data. Coefficients of determination were 0.42 and 0.65 for the C_3-C_4 and C_4 species, respectively ($P < 0.001$). The slopes of the lines did not differ significantly. The *y* intercepts (±SE) were significantly different; however, 1.35 ± 0.05 (C_3-C_4) and 1.46 ± 0.03 (C_4).

C_4 leaves (Brown and Hattersley, 1989). *P. milioides* differs from C_4 species in that a functional C_4 cycle is absent so that the path of carbon in photosynthesis and photorespiration is identical with that of C_3 plants (Edwards et al., 1982; Hunt et al., 1987). Rubisco is found in both MC and BSC compartments, and the kinetic properties with respect to carboxylation and oxygenation are typical of those reported for enzymes from C_3 sources (Ku et al., 1976; Holbrook et al., 1985). However, the enzyme catalyzing the key decarboxylation reaction of photorespiration (Gly decarboxylase) is strictly localized in the BSC of C_3-C_4 intermediate species (Hylton et al., 1988). As with C_4 leaves, confinement of decarboxylative processes to the BSC of C_3-C_4 leaves could increase the $[CO_2]$ in these cells so that the oxygenase function of Rubisco is suppressed (von Caemmerer, 1989). This key adaptation prevents merging of CO_2 diffusing to the MC chloroplasts from the external atmosphere with photorespired CO_2 and the consequent re-entry of a portion of the latter into the glycolate pathway (Hunt et al., 1987). In the absence of leakage of CO_2 from the BSC to the MC of C_3-C_4 leaves, futile cycling of CO_2 is eliminated so that the *CER* of the leaf equals the rate of RuBP carboxylation in the MC.

A significant reduction in partitioning of electron transport to O_2-dependent dissipative processes was detected in *P. milioides* relative to that for *P. bisulcatum* in this study (Table I). Based on K_{sp} (in vivo) values a 25% increase in Rubisco specificity for CO_2 versus O_2 in the C_3 plant would be required to match the effect of the physiological modifications associated with C_3-C_4 photosynthesis in *P. milioides* in reducing photorespiration. At a normal atmospheric $[O_2]$ of 213 mbars ≤1% of linear electron transport was consumed by photorespiration in *P. antidotale*, although significant photorespiration was detected at 432 mbars of O_2 (Dai et al., 1993).

At low irradiance levels linear electron transport may be independent of the capacity for carboxylation/oxygenation by Rubisco and strictly dependent on light for photolysis of H_2O. This explains the increase in the degree of O_2-dependent enhancement of Φ_{II} with irradiance (Fig. 3), since effects of increases in C_i and $[O_2]$ on linear electron transport will be maximal only when light is available in excess and the rate of Rubisco turnover limits photosynthesis (Sharkey et al.,

1988). Consistent with this interpretation, effects of $[O_2]$ on Φ_{II} were highest for the C_3 species that also exhibited the greatest capacity for photorespiration among the three species studied (Fig. 3 and Table I). Effects of $[O_2]$ on the C_3 and C_3-C_4 species will include competitive inhibition of Rubisco by O_2 (Jordan and Ogren, 1984), and this could underlie the lack of a further increase in Φ_{II} for these species at 432 mbars of O_2 (Fig. 2). Since photorespiration was negligible in *P. antidotale* at 213 mbars of O_2, this process is not likely to have contributed indirectly to the small, yet detectable, O_2-dependent increases in Φ_{II}. Modest increases in electron transport at elevated $[O_2]$ have been reported for C_4 species, but the mechanism is unclear (Krall and Edwards, 1990; Dai et al., 1993). Intermediate degrees of enhancement of Φ_{II} by O_2 for the C_3-C_4 species (Fig. 3) are not interpreted in terms of photorespiration but rather reflect apparent changes in Rubisco accessibility with varying $[O_2]$.

A 20% reduction in J:C_i accompanied lowering of the $[O_2]$ for *P. bisulcatum* (Table II). This resulted from suppression of electron transport associated with oxygenation of RuBP by Rubisco. This decline is likely to also include effects of restricted recycling of Pi for photophosphorylation and regeneration of RuBP at the low $[O_2]$ due to suppression of 2-phosphoglycolate synthesis (Harris et al., 1983). The lack of a significant increase in J:C_i with increasing $[O_2]$ for *P. milioides* is consistent with reduced Rubisco capacity at 213 mbars relative to 14 mbars of O_2, thereby limiting the ability of this species to respond to increased availability of substrate and Pi in high $[O_2]$ by increasing linear electron transport. This could result from commitment of a significant fraction of the total Rubisco in the leaf to refixation of CO_2 in the BSC, which would consequently be unavailable for the primary carboxylation reaction occurring in the MC at high $[O_2]$. Thus, sequestration of some Rubisco in the BSC constitutes a cost of reducing photorespiration in C_3-C_4 leaves (von Caemmerer, 1989).

During linear electron transport reducing equivalents are transferred from PSII to the plastoquinone pool. When irradiance levels are high, excitation transfer to PSI exceeds the rate of electron donation (via the intermediary carriers Cyt *f* and plastocyanin) from plastoquinol to $P700^+$. This leads to steady-state accumulation of $P700^+$, which continues to trap energy with production of heat (Weis et al., 1987; Weis and Lechtenberg, 1989). Thus, light use by PSI changes according to the fraction of reaction centers in the nonoxidized state (Harbinson et al., 1990). Because of more effective penetration of leaf tissue by the far-red measuring beam used in $\Delta A830$ measurements, the backscattered radiation carries information from deeper layers of the leaf sample than does the Chl fluorescence measuring beam (Schreiber et al., 1988; Bornmann et al., 1991). Despite the contrasting interactions between the leaf and the individual measuring beams, irradiance- and $[O_2]$-dependent changes in Φ_{II} were accompanied by comparable antiparallel shifts in $\Delta A830/\Delta A830_m$ for all species (Figs. 2 and 4). These findings are consistent with those of Harbinson et al. (1990), who concluded that the tight linkage between Φ_{II} and Φ_I ($= 1 - \Delta A830/\Delta A830_m$) indicates that chloroplast electron transport is primarily linear. Thus, decreases in $[P700^+]/[P700]_{total}$ at elevated $[O_2]$ and constant irradiance are best explained in terms of augmented electron donation from PSII to PSI.

The preceding discussion is consistent with broadly parallel changes in quantum yields of PSI and PSII as irradiance and $[O_2]$ vary. However, the existence of mechanisms to effect modest variations in the relative rates of PSI and PSII photochemistry in response to changes in relative rates of utilization of NADPH and ATP are not precluded. Sigmoidicity in the increase in $\Delta A830/\Delta A830_m$ with irradiance (Fig. 4) is consistent with participation of electron flow to $P700^+$ from the acceptor side of PSI, which would reduce the steady-state accumulation of this cation. This interpretation is supported by the comparisons in Figure 6 in which the apparent quantum yield of PSI always exceeds that of PSII. Coupled cyclic electron flow in PSI could provide a supplemental source of ATP (Harbinson et al., 1990). The results shown in Figure 6 also indicate that regulation of Φ_I:Φ_{II} in *P. milioides* (C_3-C_4) most closely resembles that in *P. antidotale* (C_4) with respect to dependencies on irradiance and $[O_2]$. Interestingly, even limited development of the Kranz syndrome, as exists in *P. milioides*, is associated with a highly C_4-like mechanism of coregulation of quantum yields of PSI and PSII.

Over wide ranges of irradiance and O_2 level, consistent and significant species-dependent differences in Φ_e were not detected for *P. bisulcatum* (C_3) and *P. milioides* (C_3-C_4) (Fig. 1). Paradoxically, a significantly lower proportion of linear electron transport was diverted to photorespiration in C_3-C_4 leaves compared with C_3 tissue (Table I). Assessments of the Warburg effect based solely on gas-exchange measurements at low and high $[O_2]$ tend to assume implicitly that coupling of light absorption to linear electron transport remains constant with $[O_2]$ and that changes occur only in the partitioning of reductant to photorespiration versus fixation of atmospheric CO_2. However, shifts in light utilization with $[O_2]$ did occur to unequal extents in the C_3 and C_3-C_4 species studied here (Figs. 2 and 4). Such changes were not accountable simply in terms of associated fluctuations in the C_i (Table II). Changes in light utilization that compensate for effects of reduced photorespiration may be common among C_3-C_4 species lacking an efficient C_4 cycle (such as *P. milioides* and some species of *Flaveria*). The occurrence of physiological properties associated with reduced photorespiration in these species has not been demonstrated consistently to lead to improved *CER* and growth under unstressed conditions when the C_i is substantially above the compensation point (Brown and Brown, 1975; Ku et al., 1991). Evaluation of the C_3-C_4 syndrome based on comparisons of photosynthesis in C_3 and C_3-C_4 species may be confounded by differing patterns of light use among species for reasons unrelated to O_2 metabolism (Brown, 1980; Ku et al., 1983, 1991; Krall et al., 1991). Nevertheless, results presented here indicate that at high irradiance effects of adaptations that reduced the allocation of photosynthetic energy to photorespiration in *P. milioides* were offset by constraints that limited the $[O_2]$-dependent increase in linear electron transport rate relative to that observed for the C_3 plant *P. bisulcatum*.

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