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The ratio of variable to maximum chlorophyll fluorescence from photosystem II, measured in leaves at ambient temperature and at 77K, as an indicator of the photon yield of photosynthesis

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Abstract. The response of a number of species to high light levels was examined to determine whether chlorophyll fluorescence from photosystem (PS) II measured at ambient temperature could be used quantitatively to estimate the photon yield of O_2 evolution. In many species, the ratio of the yield of the variable (F_V) and the maximum chlorophyll fluorescence (F_M) determined from leaves at ambient temperature matched that from leaves frozen to 77K when reductions in F_V/F_M and the photon yield resulted from exposure of leaves to high light levels under favorable temperatures and water status. Under conditions which were less favorable for photosynthesis, F_V/F_M at ambient temperature often matched the photon yield more closely than F_V/F_M measured at 77K. Exposure of leaves to high light levels in combination with water stress or chilling stress resulted in much greater reductions in the photon yield than in F_V/F_M (at both ambient temperature and 77K) measured in darkness, which would be expected if the site of inhibition was beyond PSII. Following chilling stress, F_V/F_M determined during measurement of the photon yield in the light was depressed to a degree more similar to that of the depression of photon yield, presumably as a result of regulation of PSII in response to greatly reduced electron flow.

Key words: Chilling stress – Chlorophyll fluorescence (ambient and 77K) – Light stress – Photoinhibition of photosynthesis – Photosynthesis (photoinhibition) – Water stress

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Abbreviations and symbols: F_O = yield of instantaneous fluorescence; F_M = yield of maximum fluorescence; F_V = yield of variable fluorescence; PFD = photon flux density (400–700 nm); PSI (II) = photosystem I (II)

Introduction

The ability to characterize rapidly the status of the photochemical system in intact leaves is critical for studies which are directed at evaluating the response of leaves to changes in their environment, and in particular to changes in the photon flux density (PFD) which they absorb. It has recently been shown that a parameter derived from chlorophyll fluorescence, the ratio of variable/maximum fluorescence (F_V/F_M), which is a quantitative measure of the photochemical efficiency of photosystem (PS) II (Kitajima and Butler 1975), is a quantitative indicator of reductions in the photon yield of O_2 evolution from intact leaves when exposure to high light levels under favorable temperatures and water status results in photoinhibition (Björkman and Demmig 1987; Demmig and Björkman 1987). In a few species, particularly *Monstera deliciosa*, the photon yield was reduced slightly more than F_V/F_M for a short period of time immediately following an exposure to excessive light. It was, however, unclear whether this slight discrepancy was the consequence of an additional limitation to electron transport or whether the biochemical fixation of CO_2 had been impaired.

These previous studies relied on the determination of F_V/F_M from PSII measured from leaf discs frozen to 77K in darkness. Demmig et al. (1987) showed that a good correlation also existed between the photon yield of photosynthesis and F_V/F_M from PSII measured at ambient temperature when leaves of *M. deliciosa* were allowed to recover for 3 h following high-PFD treatments for various periods of time. However, we have recently reported that F_V/F_M measured at ambient temperature was more depressed than F_V/F_M measured at 77K in leaves of *Arbutus unedo* upon exposure to peak levels of natural sunlight during a mediterranean summer (Demmig-Adams et al. 1989a), which might result

from a limitation at the PSII donor side (Schreiber and Neubauer 1987). Thus, differences between chlorophyll fluorescence measured from leaves at ambient temperature versus those frozen to 77K may arise depending on the type of changes which occur in the photochemical system.

The present study was undertaken to further evaluate the validity of using chlorophyll fluorescence (F_V/F_M) determined at ambient temperature as a measure of the photon yield of photosynthesis. Additional sources for differences between the two fluorescence techniques may arise from the fact that the optical window for detecting fluorescence from PSII at ambient temperature is at longer wavelengths (largely above 720 nm; PAM 101 fluorometer; Walz, Effeltrich, FRG) than at 77K (699 mm, 3 nm half-bandwidth in our system). For instance, fluorescence measured at ambient temperature declined markedly as chlorophyll content declined during autumnal leaf senescence in *Platanus occidentalis*, whereas fluorescence measured at 77K did not (Adams et al. 1990), presumably due to the fact that chlorophyll efficiently absorbs fluorescence emitted at 699 nm but not above 720 nm (Virgin 1954; Weis 1985).

We have examined a variety of species to compare the changes in the photon yield of photosynthesis and in chlorophyll fluorescence, measured at both 77K and room temperature, that occur in response to an exposure to a high PFD. A high PFD was the primary factor used to inactivate photosynthesis, with an emphasis on conditions excluding temperature or water stress. In contrast, two other treatments, water stress and chilling stress, were used to alter the linear relationship between F_V/F_M and the photon yield of photosynthesis.

Material and methods

Helianthus annuus L. (sunflower), *Glycine max* (L.) Merrill (soybean), *Gossypium hirsutum* L. var. DP-61 (cotton), *Cucumis sativus* L. (cucumber), *Nerium oleander* L., *Monstera deliciosa* Liebm., and *Schefflera arboricola* (Hayata) Merrill were grown in plastic pots filled with garden soil, watered daily, and received Hewitt's type nutrient solution containing 12 mM NO_3^- once per week (Wong 1979). All were grown in a naturally lit glasshouse (experiments performed from December 1987 to March 1988), but *M. deliciosa* and *S. arboricola* were maintained at 20–30 μmol photons·m^{-2}·s^{-1}, whereas *H. annuus, Glycine max, Gossypium hirsutum,* and *C. sativus* received supplemental light of 400–500 μmol photons·m^{-2}·s^{-1} (12 h photoperiod). *Rhizophora mangle* L. was cultivated in nutrient solution containing artificial sea salt (Sigma, St. Louis, Mo., USA) equivalent to 10% sea water as described previously (Demmig-Adams et al. 1989c). Both *R. mangle* and *N. oleander* received only natural (winter) light of up to 300 μmol photons·m^{-2}·s^{-1}, except for *N. oleander* leaves used in the long-term water stress experiment which received, depending on height of insertion, either 1700 or 2100 μmol photons·m^{-2}·s^{-1} (12 h photoperiod) during development. *Cissus antarctica* Vent. was obtained from a local garden shop and maintained in room light (10 μmol photons·m^{-2}·s^{-1}). Leaves of *Hedera helix* L. were obtained from a natural population growing at a shaded site in the Würzburg Botanical Gardens (FRG).

Leaves were exposed to high PFDs in a ventilated, temperature-controlled gas-exchange chamber as described previously (Demmig et al. 1987). Attached leaves were used in all experiments, except for leaves of *H. helix*, the petioles of which were kept in water, and short-term water-stress treatments of detached *N. oleander* leaves. Long-term (11 d) water stress of *N. oleander* was achieved by withholding water from the potted plant.

Measurements of O_2 exchange at 5% CO_2 and 25° C were determined with a leaf-disc oxygen electrode as described previously (Björkman and Demmig 1987). Discs removed from the leaves during or at the end of a treatment were maintained under low PFD (41 μmol photons·m^{-2}·s^{-1}) with 5% CO_2 in the oxygen electrode chamber for 15 to 25 min prior to the photon-yield determination. Samples for fluorescence analysis were treated similarly by placing them in Petri dishes containing moist filter paper in a growth cabinet at 25° C and 40 μmol photons·m^{-2}·s^{-1}. Chlorophyll fluorescence at ambient temperature was determined with a PAM chlorophyll fluorometer (Walz; Schreiber et al. 1986) on leaf discs after 5 min darkness (Demmig et al. 1987) or in the presence of photosynthetically active radiation (Demmig-Adams et al. 1989b) during the measurement of the photon yield in a leaf-disc oxygen-electrode chamber which had been specially modified for this purpose. Fluorescence from leaf discs predarkened for 5 min and then frozen to 77K (Powles and Björkman 1982) was determined with a laboratory-built system described elsewhere (Demmig-Adams et al. 1989a). Both fluorescence-measuring systems used the same light-emitting diode (H-3000; Stanley, Tokyo, Japan; peak emission at 650 nm) coupled with a short-pass filter (DT Cyan; Balzers, Liechtenstein; λ <680 nm) for excitation of the leaf samples.

All measurements of light, and calibration of the light source used in the photon-yield measurements, were made with a quantum sensor (LI-190SB; Li-Cor, Lincoln, Neb., USA). Leaf temperatures were measured with thermocouples (Omega Engineering, Stamford, Conn., USA) in conjunction with a Thermocouple Thermometer (Model TH-65; Wescor, Logan, Utah, USA).

Results and discussion

Effect of high-PFD treatments on the photon yield of photosynthesis and PSII photochemistry in Rhizophora mangle and Monstera deliciosa. The changes that occurred in PSII fluorescence and in the photon yield of O_2 evolution in shade-grown *R. mangle* leaves during a 7-h exposure to 1530 μmol photons·m^{-2}·s^{-1} are shown in Fig. 1. The ratio F_V/F_M was measured at ambient temperature, either after 5 min dark adaptation or in low light during the photon-yield determination, and at 77K (see *Material and methods* and legend to Fig. 1). All measures of PSII photochemical efficiency (F_V/F_M) declined concomitantly with the decline in photon yield (Fig. 1A). The decline in F_V/F_M resulted from a pronounced reduction in the level of F_M, which was similar at both 77K and ambient temperature, and an increase in the yield of instantaneous fluorescence (F_O), which was slightly greater at 77K (Fig. 1B). These measurements, even those made in the light during the photon-yield determination, do not, however, allow a conclusion as to whether or not F_O had been quenched during the high-PFD treatment (see Demmig-Adams et al. 1989b). There was a close linear relationship between the reductions in the photon yield of O_2 evolution and in F_V/F_M, regardless of how the latter were measured (Fig. 2).

Leaves of the shade-tolerant plant *M. deliciosa* exhibited concomitant reductions in F_V/F_M and the photon yield of O_2 evolution upon exposure to 1210 μmol photons·m^{-2}·s^{-1} (Figs. 3, 4), similar to the response of

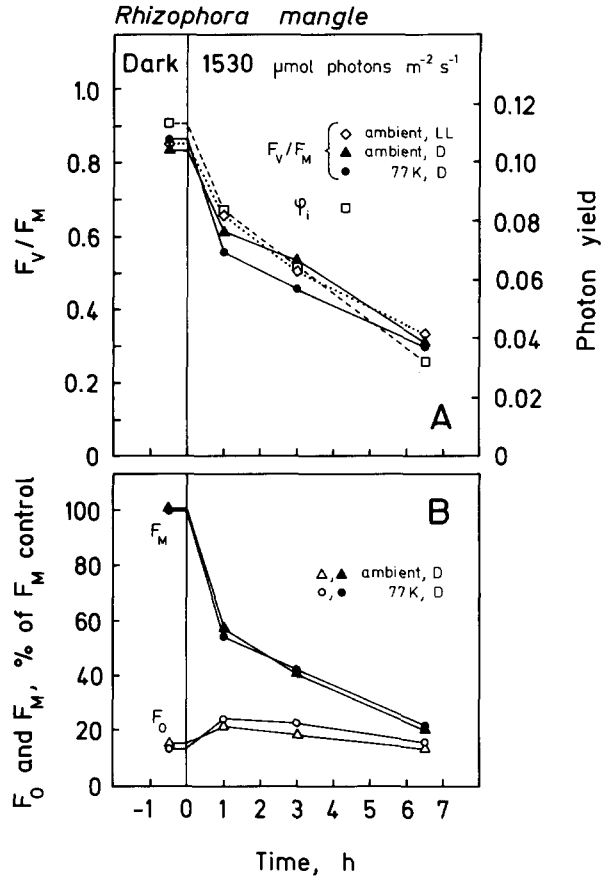

Fig. 1A, B. Time course of changes in (**A**) F_V/F_M from PSII measured at ambient temperature and at 77K, and the apparent photon yield of O_2 evolution (Φ_i) and (**B**) the F_O and F_M levels of PSII fluorescence measured at ambient temperature and at 77K (determined after 5 min darkness) as a percent of the F_M control values from shade leaves of *Rhizophora mangle* during exposure to 1530 μmol photons·m^{-2}·s^{-1} in air at a leaf temperature of 25° C. After removal from the treated leaf, leaf discs for photon-yield determinations were kept at 41 μmol photons·m^{-2}·s^{-1} for 20 min, and measurements of the photon yield were made between 20 and 45 min after removal from the treatment. During the measurement of the photon yield, F_M was determined at 18, 29, and 41 μmol photons·m^{-2}·s^{-1}, and F_O at each of these PFDs was obtained by rapidly darkening the samples. The F_V/F_M ratio in the light was calculated from these values at each PFD and the means of these three determinations (*LL*) are shown in **A**. Measurements of fluorescence from dark-adapted leaves (*D*) were obtained from discs which were kept under 40 μmol photons·m^{-2}·s^{-1} for 30 min plus 5 min in darkness after removal from the treatment. Leaves exhibited light- and CO_2-saturated rates of O_2 evolution = 20.7 ± 1.3 μmol·m^{-2}·s^{-1}, $n = 3$

R. mangle during exposure to high PFD (Figs. 1, 2). The linear relationships between the photon yield of O_2 evolution and all measures of F_V/F_M for both of these species (Figs. 2, 4) are very similar to those observed previously in a number of species where F_V/F_M was examined only at 77K (Björkman and Demmig 1987; Demmig and Björkman 1987).

When an *M. deliciosa* leaf was exposed to an even higher PFD of 1840 μmol photons·m^{-2}·s^{-1}, however, F_V/F_M at 77K was depressed to a lesser extent than the

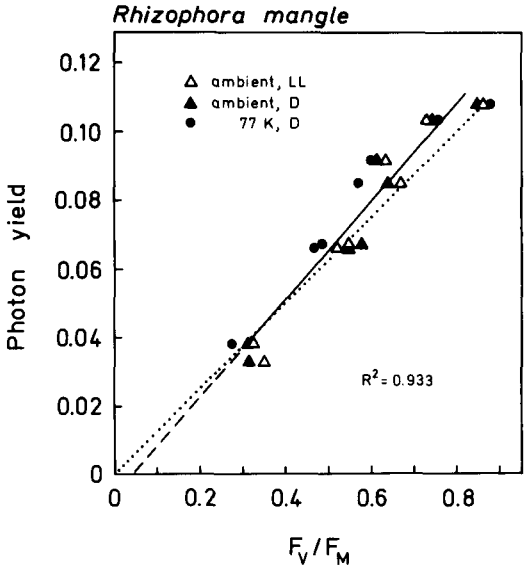

Fig. 2. Changes in the apparent photon yield of O_2 evolution in relationship to changes in F_V/F_M from PSII in leaves of *Rhizophora mangle* following exposure to high PFD for various periods of time. The data were obtained from two different experiments, one of which is shown in Fig. 1A. See legend of Fig. 1 for further details. The *dotted line* is one drawn between the control values and the origin

photon yield of O_2 evolution and F_V/F_M determined at ambient temperature (Figs. 5, 6A). Furthermore, the decrease in F_M at ambient temperature was greater than that at 77K in all measurements, but the increase in F_O was again relatively more pronounced at 77K. In contrast to the previous two experiments (Figs. 2, 4), the relationship between F_V/F_M and the photon yield of O_2 evolution was not a linear one when these parameters were measured immediately after the treatment of *M. deliciosa* at very high PFD, although F_V/F_M determined in low light during the measurement of the photon yield matched the depressions in the photon yield most closely (Fig. 6A). After an additional 5 h of recovery in low light, the reductions in F_V/F_M at both ambient temperature and 77K were quantiatively similar to those of the photon yield of O_2 evolution (Fig. 6B). During this period of recovery there were increases in the photon yield, and increases in F_M at ambient temperature accompanied by decreases in F_O, whereas there were no significant changes in PSII fluorescence determined at 77K (data not shown). Thus, the lower values of F_V/F_M at ambient temperature, relative to 77K, immediately subsequent to the high-PFD treatment were due to a greater quenching of F_M and increase in F_O. These characteristics are identical to those of the fluorescence quenching observed in leaves of *Arbutus unedo* under natural conditions in Portugal (Demmig-Adams et al. 1989a).

It is interesting to note that there was a small but consistent difference between F_O determined at ambient temperature and F_O determined at 77K under control conditions, which became apparent when F_O was expressed as a percentage of the F_M value. In control

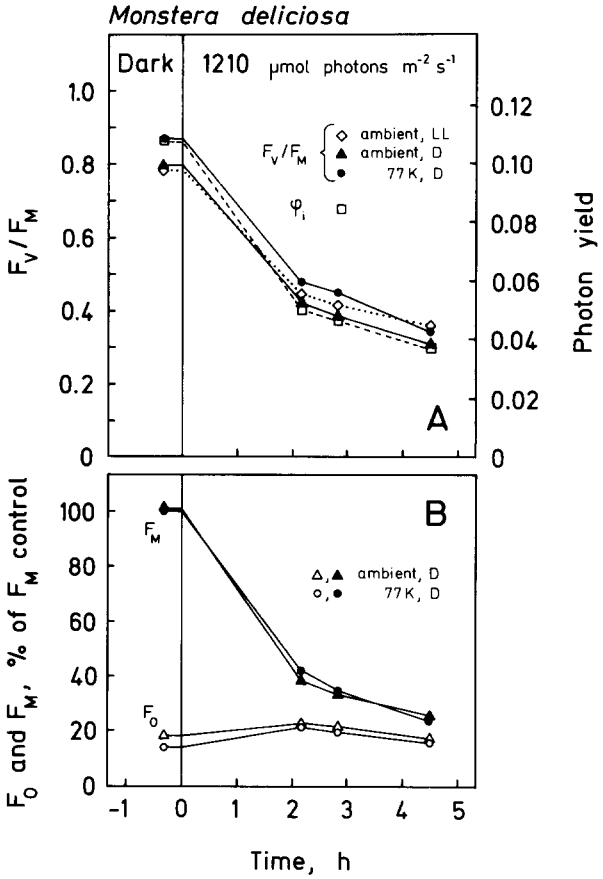

Fig. 3A, B. Time course of changes in (**A**) F_V/F_M from PSII measured at ambient temperature and at 77K, and the apparent photon yield of O_2 evolution and (**B**) the F_O and F_M levels of PSII fluorescence measured at ambient temperature and at 77K (determined after 5 min darkness) as a percent of the F_M control values from a *Monstera deliciosa* leaf during exposure to 1210 μmol photons $\cdot m^{-2} \cdot s^{-1}$ in air at a leaf temperature at 25° C. Other details as in the legend of Fig. 1, except that the photon yield was measured between 15 and 40 min after removal of the disc from the treated leaf, and discs which were darkened for 5 min prior to the measurement of fluorescence received low light for 25 min after removal from the treatment

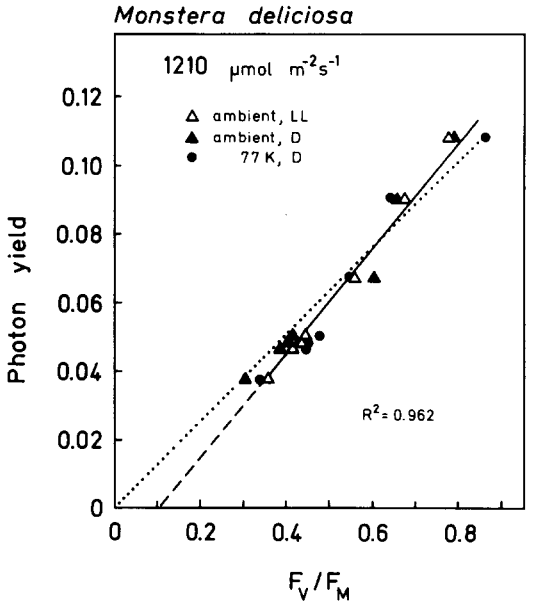

Fig. 4. Changes in the apparent photon yield of O_2 evolution in relationship to changes in F_V/F_M from PSII in leaves of *Monstera deliciosa* following exposure to high PFD for various periods of time. The data were obtained from two different experiments, one of which is shown in Fig. 3A. See legend of Fig. 3 for further details. The *dotted line* is one drawn between the control values and the origin

leaves, F_O was relatively "too high", and thus F_V/F_M was "too low", when determined at ambient temperature as compared to that determined at 77K (Figs. 1, 3, 5; Björkman and Demmig 1987). This difference probably arises from the fact that PSII fluorescence measured from the leaves frozen to 77K is separated from PSI fluorescence by an interference filter with a narrow window centered at 699 nm (Demmig-Adams et al. 1989a), while that measured at ambient temperature is not so narrowly defined. Whereas variable fluorescence at ambient temperature originates exclusively from PSII (Butler 1977), there is a significant contribution of fluorescence emanating from PSI to the F_O level of fluorescence (Wendler and Holzwarth 1987). Therefore, it is not unexpected that F_V/F_M measured at 77K is generally higher compared to that measured at ambient temperature, particularly in *M. deliciosa* which has a relatively high concentration of chlorophyll per unit area. Furthermore, any increase in F_O resulting from changes at the PSII reaction center should be more pronounced in the measurements at 77K, as is actually apparent from the data in Figs. 1, 3, and 5.

Effect of high-PFD treatments on the photon yield of photosynthesis and PSII photochemistry in a variety of species. The responses of a number of additional species to high light levels, in particular fast-growing mesic species such as soybean, sunflower, cotton, and cucumber, were also determined in order to compare these with the responses of the slower-growing, sclerophyllous species such as *R. mangle* and *M. deliciosa* (Table 1). In general, there was a relatively good agreement between F_V/F_M, determined at either 77K or ambient temperature, and the photon yield of photosynthesis following exposure to high PFD in all species (see especially Response type A). Minor discrepancies between the two measures of F_V/F_M fell into two categories.

In some cases F_V/F_M at 77K declined to a somewhat lesser extent than did F_V/F_M at ambient temperature or the photon yield of photosynthesis (Table 1, Response type B; see also Fig. 6A). This occurred in leaves in which rates of photosynthetic electron transport were very low. These included *N. oleander* which had been subjected to water stress, the chilling-sensitive species *Cucumis sativus* kept at 15° C during the exposure to high PFD, *Hedera helix* when photosynthesis and photorespiration were inhibited, and deep-shade-acclimated *M. deliciosa* and *Cissus antarctica* (see also Adams and Osmond 1988). This response is again similar to that

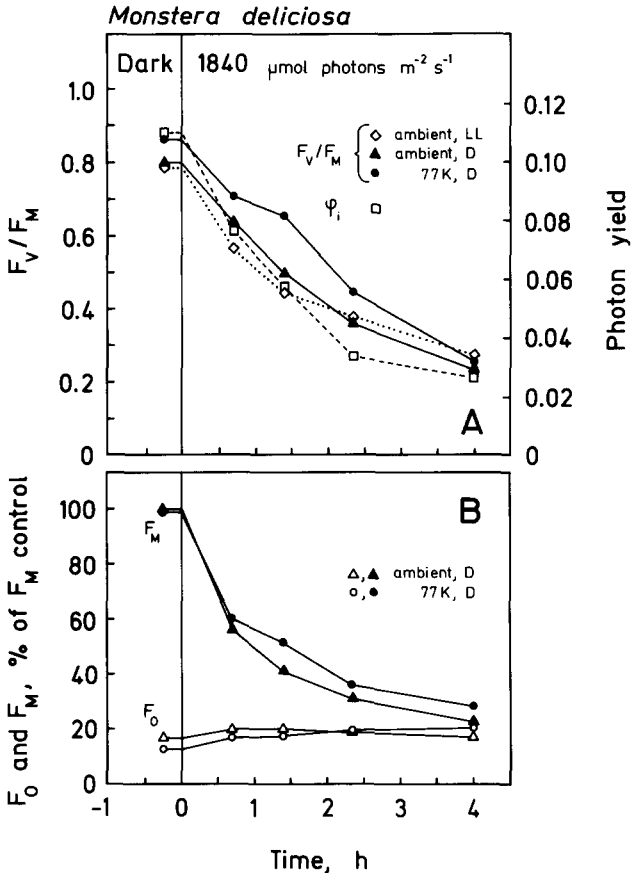

Fig. 5A, B. Time course of changes in (**A**) F_V/F_M from PSII measured at ambient temperature and at 77K, and the apparent photon yield of O_2 evolution and (**B**) the F_O and F_M levels of PSII fluorescence measured at ambient temperature and at 77K (determined after 5 min darkness) as a percent of the F_M control values from a *Monstera deliciosa* leaf during exposure to 1840 μmol photons $\cdot m^{-2} \cdot s^{-1}$ in air at a leaf temperature of 25° C. Other details as in the legend of Fig. 1, except that the photon yield was measured between 15 and 35 min after removal of the discs from the treated leaf, and discs which were darkened for 5 min prior to the measurement of fluorescence received low light for 25 min after removal from the treatment. This leaf possessed a light- and CO_2-saturated rate of O_2 evolution = 21 μmol $\cdot m^{-2} \cdot s^{-1}$

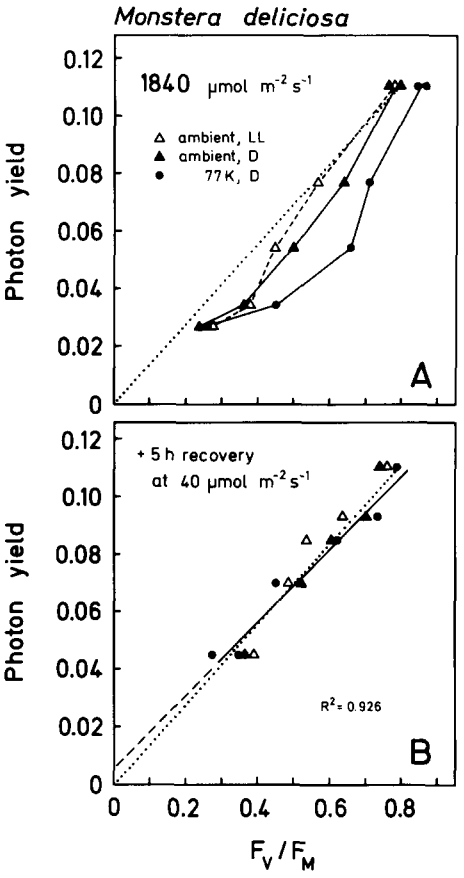

Fig. 6A, B. Changes in the apparent photon yield of O_2 evolution in relationship to changes in F_V/F_M from PSII in a leaf of *Monstera deliciosa* (**A**) immediately following exposure to high PFD for various periods of time (see legend of Fig. 5 for time intervals) and (**B**) subsequent to an additional 5 h of recovery during which the samples were kept at a low PFD. For recovery, leaf discs were placed on moist filter paper in glass Petri plates and maintained in a growth cabinet under 40 μmol photons $\cdot m^{-2} \cdot s^{-1}$ at 25° C. See legend of Fig. 1 for further details. The *dotted line* is one drawn between the control values and the origin

observed in *Arbutus unedo* in the field when stomata closed during midday exposure to maximum PFD and temperature (Demmig-Adams et al. 1989a). At 77K, only one electron is required to reduce the primary electron acceptor for PSII, Q_A, and to obtain F_M, since the reoxidation of Q_A is prevented at these temperatures, whereas at ambient temperature Q_A is rapidly reoxidized and thus higher light fluxes, i.e. considerably more electrons, are required to fully reduce Q_A. Any process which increases the reoxidation rate of Q_A (Horton and Lee 1983) or limits the donation of electrons to Q_A (Schreiber and Neubauer 1987) could be expected to result in F_V/F_M being lower at ambient temperature than at 77K.

The opposite response, in which F_V/F_M at 77K declined to a somewhat greater degree than did F_V/F_M measured at ambient temperature, was observed in a third group of plants (Table 1, Response type C), and has also been observed previously in *Hoya australis* (Adams 1987) and *Opuntia ficus-indica* (data not shown). In the present study this response was observed particularly under conditions that were favorable for photosynthesis, i.e. in well-watered plants with high rates of photosynthesis (photosynthetic capacities, in μmol $O_2 \cdot m^{-2} \cdot s^{-1}$, of 33 ± 10, $n=4$ for *Hedera helix*; 35 ± 11, $n=6$ for *N. oleander*; and 47 ± 5, $n=4$ for *Cucumis sativus*) in the presence of air and a favorable leaf temperature of 25° C. These slightly greater reductions in F_V/F_M at 77K than in F_V/F_M at ambient temperature were due to a relatively greater increase in F_O at 77K than in F_O at ambient temperature, with no apparent differences in the response of F_M between the two measuring techniques (data not shown), which could be related to the PSI contribution to F_O fluorescence at ambient temperature (see above). For the majority of treatments with *H. helix* and *N. oleander*, the photon yield appeared to be depressed in a manner more similar to that of the

Table 1. Changes in PSII photochemical efficiency (F_V/F_M) measured at ambient temperature and at 77K, and in the apparent photon yield of O_2 evolution from several species following exposure to various high-PFD treatments. Leaf temperature was 25° C and exposures took place in air unless otherwise indicated. Water stress was induced in *Nerium oleander* by detaching a leaf and allowing it to desiccate for several hours at 40 μmol photons·m^{-2}·s^{-1}, during which time it lost approx. 16% of its FW, prior to the exposure to high PFD

Response type	Species	Growth light	Treatment			Fluorescence				Apparent photon yield	
			PFD μmol·m^{-2}·s^{-1}	Conditions other than standard	Time (h)	Ambient temperature		77K		mol O_2·(mol photons)$^{-1}$	% control
						F_V/F_M	% control	F_V/F_M	% control		
A	*Helianthus annuus*	high	2060		1.5	0.636	75	0.629	73		
			2060		6.5	0.538	64	0.550	64		
	Glycine max	high	2060		2	0.546	67	0.556	68		
	Gossypium hirsutum	high	2060		3	0.481	60	0.516	65	0.060	65
	Rhizophora mangle	partial shade	1360		2.5	0.464	54	0.449	52	0.057	55
			1520		3	0.575	71	0.558	68	0.065	67
			1520		6.3	0.300	37	0.234	29	0.032	33
	Schefflera arboricola	deep shade	1360		2.5	0.519	64	0.543	65	0.053	49
B	*Nerium oleander*	partial shade	2060	water stress	0.6	0.524	64	0.642	75		
			2060	water stress	1.5	0.402	49	0.510	61		
	Cucumis sativus	high	2060	15° C	1	0.497	61	0.599	74		
			2060	15° C	4.3	0.291	36	0.354	44		
	Hedera helix	shade	1530	2% O_2, 0% CO_2	2.5	0.515	65	0.620	74	0.063	61
	Monstera deliciosa	deep shade	1000	10° C	2.5	0.485	61	0.606	70	0.060	56
			1000		2.5	0.463	58	0.621	71	0.051	47
			1000	30° C	2.5	0.522	68	0.612	72	0.060	56
			1000	35° C	2.5	0.404	51	0.595	70	0.033	31
	Cissus antarctica	deep shade	1210		1.5	0.528	66	0.637	76		
			1210		2.5	0.488	61	0.554	66		
C	*Hedera helix*	shade	1360		2.5	0.637	80	0.557	70	0.086	81
			1530		3	0.691	87	0.641	77	0.083	80
			1530		3	0.647	82	0.567	68	0.070	66
			1530		4	0.652	79	0.603	70	0.079	74
			1530		5	0.598	72	0.595	69	0.063	59
	Nerium oleander	partial shade	1530		2.5	0.672	81	0.585	70	0.060	56
			1530		3.5	0.559	68	0.396	46	0.042	40
			1530		3.5	0.457	55	0.357	42	0.038	35
			2060		3	0.558	67	0.495	58	0.050	48
			2060		3	0.458	55	0.400	47	0.052	50
	Cucumis sativus	high	2060		2.5	0.649	80	0.611	75	0.084	85
			2060		4	0.657	81	0.580	71	0.085	86
			2060	35° C	3	0.732	90	0.726	89	0.093	94

depression of F_V/F_M at 77K. In contrast, the depression of the photon yield more closely matched that of F_V/F_M at ambient temperature in *C. sativus*. It must be pointed out, however, that an accurate determination of the photon yield in these species immediately following the high-PFD treatment was difficult because of fluctuations in the rate of respiration. This problem did not arise in species such as *M. deliciosa*, in which respiration rates were much lower.

Monstera deliciosa experienced a very similar and pronounced degree of photoinhibition when exposed to 1000 μmol photons·m^{-2}·s^{-1} for 2.5 h, regardless of the leaf temperature (at least from 10 to 30° C) during the exposure (Table 1), i.e. photoinhibition of *M. deliciosa* was rather temperature-independent over this range. On the other hand, the chilling-sensitive species *C. sativus* experienced little photoinhibition upon exposure to 2060 μmol photons·m^{-2}·s^{-1} at a leaf temperature of 25° C or 35° C, whereas it experienced considerable photoinhibition under the same conditions at a leaf temperature of 15° C (Table 1). The high susceptibility of shade-grown *M. deliciosa* to photoinhibition irrespective of the leaf temperature during the high-PFD exposure is probably related to its relatively low capacity to dissipate excitation energy via electron transport, as its photosynthetic capacity was almost fivefold lower than that of the high-PFD-grown *C. sativus* at 25° C (10.7 ± 0.4 versus 47 ± 5 μmol O_2·m^{-2}·s^{-1}, $n = 5$ and 4, respectively).

Effect of high PFD in combination with long-term water stress in Nerium oleander, and of low to moderate light levels in combination with short-term chilling stress in Cucumis sativus. Exposure of *N. oleander* to a combination of a high light level and water stress over a period of 11 d resulted in a concomitant decrease in the photon yield and the light- und CO_2-saturated rate of O_2 evolution, but F_V/F_M, at both 77K and ambient temperature, declined to a lesser extent than did the photon yield of photosynthesis under these conditions (Fig. 7). The short-term exposure of *C. sativus* leaves to relatively moderate light levels at a leaf temperature of 3.5° C resulted in a massive decline in the photon yield, whereas the photosynthetic capacity and F_V/F_M determined in darkness were affected to a much lesser extent (Fig. 8). Determinations of F_V/F_M in the light during the photon yield measurements resulted in reduced values which matched the reductions in the photon yield more closely (Fig. 8; see also Fig. 6A). This suggests that, during the photon yield determination, there was an increase in thermal dissipation within PSII in response to an impairment of electron flow beyond PSII. Exposure of *C. sativus* to 3.5° C in darkness for 7.5 h had no effect on the photon yield or photosynthetic capacity subsequently measured at 25° C, which is consistent with previous studies which have shown that the inhibition of photosynthesis by chilling temperatures is light-dependent (Powles et al. 1983).

Reductions of the photon yield which are greater than the reductions of F_V/F_M determined in darkness (Figs. 6A, 7, 8) would be expected to result from any

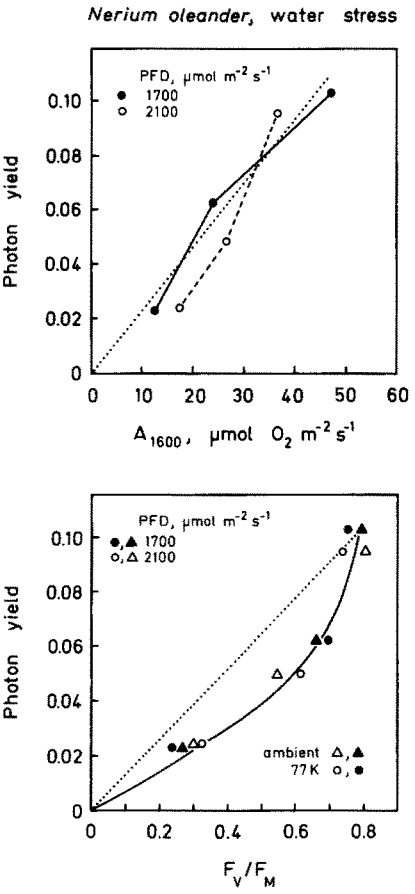

Fig. 7. Changes in the apparent photon yield of O_2 evolution in relationship to changes in the rate of photosynthetic O_2 evolution at 1600 μmol photons·m^{-2}·s^{-1} (*upper panel*) and to changes in F_V/F_M from PSII measured at ambient temperature and at 77K (*lower panel*) from leaves of a *Nerium oleander* plant exposed to water stress and high PFD over a period of 11 d. The leaves had developed under the high-PFD conditions present during the imposition of water stress. In some instances 15% CO_2 was used during the photon-yield measurements, but this did not lead to any increase in the photon yield, indicating that stomatal resistance was not responsible for the decreases in the photon yield. The *dotted line* represents the one-to-one relationship

condition which impairs electron transport beyond the PSII reaction-center complex. This has previously been reported to occur in leaves in response to water stress (Ben et al. 1987) and in response to SO_2 fumigation (Adams et al. 1989). In most instances this type of inhibition reduces photosynthetic capacity to at least the same extent as the photon yield (Ben et al. 1987; Adams et al. 1989), similar to that which was observed during the long-term water-stress treatment of *N. oleander* (Fig. 7). In contrast, the photoinhibition of *C. sativus* resulting from exposure to light at chilling temperatures could be overcome somewhat at high PFD (Fig. 8). In has been suggested that chillings stress results in an inactivation of photophosphorylation (Garber 1977). If inactivation of photophosphorylation was the cause for the specific decrease in the photon yield in *C. sativus*, this inactivation must therefore be reversible at high PFD.

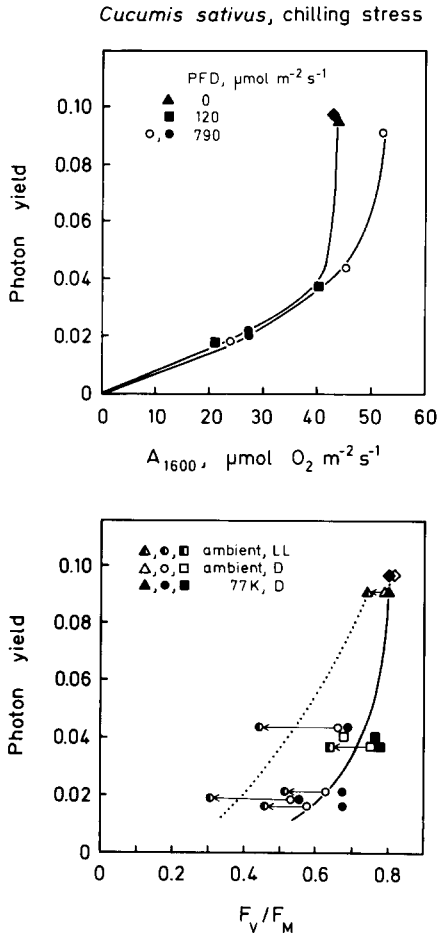

Fig. 8. Changes in the apparent photon yield of O_2 evolution in relationship to changes in the rate of photosynthetic O_2 evolution at 1600 μmol photons·m^{-2}·s^{-1} (*upper panel*) and to changes in F_V/F_M from PSII measured at ambient temperature and at 77K (*lower panel*) from leaves of *Cucumis sativus* exposed to different PFDs for various periods of time (50 min to 2.5 h) at a leaf temperature of 3.5° C in air. The difference between F_V/F_M measured at ambient temperature in darkness, and in the light during the measurement of the photon yield, is indicated by the *arrows*. The *dotted line* estimates the relationship between the photon yield and F_V/F_M determined in the light

Conclusions

It can be concluded that, as was shown previously for F_V/F_M from PSII determined at 77K (Björkman and Demmig 1987; Demmig and Björkman 1987), F_V/F_M determined at ambient temperature is a reliable indicator of the photon yield of photosynthetic O_2 evolution in healthy leaves and in leaves which have been subjected to high light treatments under otherwise favorable conditions. Identical changes in the photon yield of photosynthesis and in F_V/F_M at both ambient temperature and 77K would be expected to result, e.g., from changes in the rate of radiationless energy dissipation in the antenna chlorophyll or in the rate of PSII photochemistry (see Björkman 1987; Kitajima and Butler 1975). The small deviations elucidated in this study suggest the co-occurrence of more than one process, one of which affects F_V/F_M at 77K and at ambient temperature differently (i.e. Response types B and C, Table 1). However, a considerable amount of the quenching of fluorescence that was present at the high PFDs is likely to have relaxed upon removal from the various treatments, and it cannot be excluded that the different response types described above may arise as a result of different relaxation kinetics among the different species and treatments.

When factors other than high light levels alone (e.g. water stress, chilling stress, SO_2 fumigation, etc.) result in a depression of the photon yield due to inhibition at sites other than the PSII reaction-center complex, the photon yield can be much more depressed than F_V/F_M determined from leaf samples predarkened for 5 min. Under such conditions, the determination of F_V/F_M in the light during the measurement of the photon yield is likely to yield a value that more closely approximates that of the photon yield (see also Genty et al. 1989) due to the regulation of PSII (an increase in radiationless energy dissipation) in response to restrictions in electron flow beyond PSII.

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