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Fluorescence quenching during photosynthesis and photoinhibition of *Ulva rotundata* Blid.

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Abstract. The relationships between photoinhibition and photoprotection in high and low-light-grown *Ulva* were examined by a combination of chlorophyll-fluorescence-monitoring techniques. Tissues were exposed to a computer-controlled sequence of 5-min exposures to red light, followed by 5-min darkness, with stepwise increases in photon flux. Coefficients of chlorophyll fluorescence quenching ($1-q_P$ and NPQ) were calculated following a saturating pulse of white light near the end of each 5-min light treatment. Dark-adapted chlorophyll fluorescence parameters (F_0 and F_V/F_M) were calculated from a saturating pulse at the end of each 5-min dark period. Low-light-grown *Ulva* showed consistently higher $1-q_P$, i.e. higher reduction status of Q (high primary acceptor of photosystem II), and lower capacity for nonphotochemical quenching (NPQ) at saturating light than did high-light-grown plants. Consequently, low-light plants rapidly displayed photoinhibitory damage (increased F_0) at light saturation in seawater. Removal of dissolved inorganic carbon from seawater also led to photoinhibitory damage of high-light-grown *Ulva* at light saturation, and addition of saturating amounts of dissolved inorganic carbon protected low-light-grown plants against photoinhibitory damage. A large part of NPQ was abolished by treatment with 3 mM dithiothreitol and the processes so inhibited were evidently photoprotective, because dithiothreitol treatment accelerated photoinhibitory damage in both low- and high-light-grown *Ulva*. The extent of photoinhibitory damage in *Ulva* was exacerbated by treatment with chloramphenicol (1 mM) without much effect on chlorophyll-quenching parameters, evidently because this inhibitor of chloroplast protein synthesis reduced the rate of repair processes.

Abbreviations: CAP = chloramphenicol; DIC = dissolved inorganic carbon in seawater (i.e. $CO_2 + HCO_3^- + CO_3^{2-}$); DTT = dithiothreitol; $1-q_P$ = reduction status of Q, the primary acceptor of PSII, measured as described; F_M, F_0, F_V = dark-adapted maximum, minimum and variable fluorescence, respectively; NPQ = nonphotochemical fluorescence quenching, measured as described; PFD = photon flux density

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Introduction

The marine macroalga *Ulva* has been used to understand relationships between chlorophyll fluorescence and photosynthesis since the pioneering studies of Kautsky and Franck (1943). Recently, we have used it as a model system for studies of photoinhibition, investigating both photoprotective processes and photoinhibitory damage in controlled environments and in the field (Henley et al. 1991a, b, 1992; Levavasseur et al. 1991; Franklin et al. 1992). Until now we have used dark-adapted chlorophyll fluorescence parameters and the quantum yield of photosynthetic O_2 evolution as indicators of the efficiency of photosystem II (PSII), photoprotection, and photoinhibitory damage, as described by Björkman (1987) and Krause (1988). In these studies, an increase in the level of minimal fluorescence (F_0) was taken to indicate photoinhibitory damage. Recent studies (Heifetz et al. 1993) show that inhibition of the synthesis of the D–1 protein of PSII reaction centre leads to an increase in F_0, confirming the indicator value of this measurement.

The advent of pulse-modulated fluorescence measurement systems using saturating-pulse techniques (Bradbury and Baker 1981; Schreiber et al. 1986) has permitted mechanistic analysis of chlorophyll fluorescence quenching in vivo during steady-state photosynthetic metabolism under actinic light. A major advance has been the separation of two components of chlorophyll fluorescence quenching during illumination: photochemical quenching (related to the reduction state of Q, the primary electron acceptor of PSII) and nonphotochemical quenching (related to the energization status of thylakoids). Nonphotochemical quenching has been further sub-divided into several components (Krause and Weis 1991), but further partitioning remains

controversial (Havaux et al. 1991). These parameters of chlorophyll fluorescence quenching during illumination are thought to indicate the extent of processes that potentiate photoinhibitory damage ($1-q_P$, the reduction state of Q) on the one hand, and to afford photoprotection on the other (NPQ, nonphotochemical quenching).

In this paper, we have combined the two approaches to examine photoinhibition as photon flux is increased to well above light saturation of photosynthesis. We have measured the parameters of chlorophyll fluorescence quenching during illumination, and assessed the consequences for photoinhibition using chlorophyll fluorescence properties measured after a subsequent period of dark adaptation. We have examined the sensitivity to photoinhibition in plants grown in defined low- and high-light regimes. *Ulva* is not carbon-saturated in seawater (Levavasseur et al. 1991), so we have also assessed the effects of carbon limitation on chlorophyll fluorescence quenching, photosynthesis and photoinhibition. We have also studied the effects of the chloroplast protein synthesis inhibitor chloramphenicol (CAP), which accelerates photoinhibitory damage and slows recovery (Ohad et al. 1984; Samuelsson et al. 1985; Greer et al. 1986; Franklin et al. 1992), and of dithiothreitol (DTT), which prevents photoprotection associated with the transformation of violaxanthin to zeaxanthin (Yamamoto and Kamite 1972; Bilger and Björkman 1990; Franklin et al. 1992).

Materials and methods

Ulva rotundata Blid. (clone 786) was grown at 18° C in controlled environments of the phycotron at Duke University Marine Laboratory, as described previously (Henley et al. 1991a, b). The high-light plants received 11 h at 1750 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and low-light plants received 11 h at 40–80 $\mu mol \cdot m^{-2} \cdot s^{-1}$, from metal-halide lamps, as described earlier (Henley et al. 1991a).

The photosynthetic properties of tissue from these plants were analysed at 18° C in a customized, automated Hansatech O_2-electrode system (Henley et al. 1991a, now available from Hansatech, Kings Lynn, Norfolk, UK). It was fitted with the fibre-optic probe of a Walz PAM fluorimeter (Model 101; Walz, Effeltrich, FRG) described by Schreiber et al. (1986), adjusted to monitor chlorophyll fluorescence during photosynthesis in the presence of a red LED light source (Model LS3/LH36U; Hansatech). When required, the fluorescence detector was replaced by a quantum sensor, in order to measure transmittance of the algal thallus, as described earlier (Franklin et al. 1992).

A section of *Ulva* thallus (4 cm^2) was placed in the O_2-electrode chamber, and held in the same plane as the red LED light source, with the fluorescence detector behind the tissue. The O_2 concentration of the seawater in the chamber (18 ml) was adjusted to 10–20% air saturation using N_2. The chamber was darkened for 10 min to measure respiratory O_2 uptake and minimal fluorescence (F_O) using a modulated beam of weak red light. Intensity settings were adjusted so as to avoid fluorescence transients during F_O measurements. Maximum fluorescence (F_M) of the dark-adapted tissue was measured with a single saturating pulse (1 s) of white light from a quartz illuminator (KL1500; Schott AG, Mainz, FRG), intensity being adjusted to saturate the fluorescence yield, but to avoid photoinhibition during the pulse.

The tissue was then exposed to a preprogrammed sequence of 5 min light, 5 min dark cycles involving stepwise exposures to photon flux densities (PFD) from 10 to 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of red light. These times were chosen to measure steady states with respect to O_2 exchange and fluorescence (Fig. 1). The rate of O_2 exchange was measured over the last 60 s of each interval. Immediately prior to the end of each light and dark cycle, a single saturating pulse (1 s) of light was given to measure F_M under that condition. Minimal fluorescence after illumination was extremely dynamic, varying with light history of the plant during growth and treatment, and exposure to CO_2 and inhibitors during treatments. Examples of these dynamic responses in F_O, and the timing of data collection for calculation purposes, are shown in Fig. 1. In the presence of saturating dissolved inorganic carbon (DIC) there was little difficulty in identification of F_O, but in the most severe photoinhibitory stress, steady-state levels were not reached, even after 5 min dark adaptation. The behaviour of F_O after illumination was independent of the presence of far red light (715-nm long-pass filter 03FCG111; Melles Griot, Irvine, Calif., USA).

The fluorescence nomenclature used is similar to that of van Kooten and Snel (1990). Thus dark-adapted variable fluorescence, $F_V = F_M - F_O$ [$(F_V)_M$, Schreiber et al. (1986)], and variable fluorescence elicited by a saturating pulse given under a particular actinic light regime, $F_V' = F_M' - F_O'$ [(F_V)s Schreiber et al. (1986)], were used to calculate quenching coefficients for chlorophyll fluorescence according to Bilger and Björkman (1990):

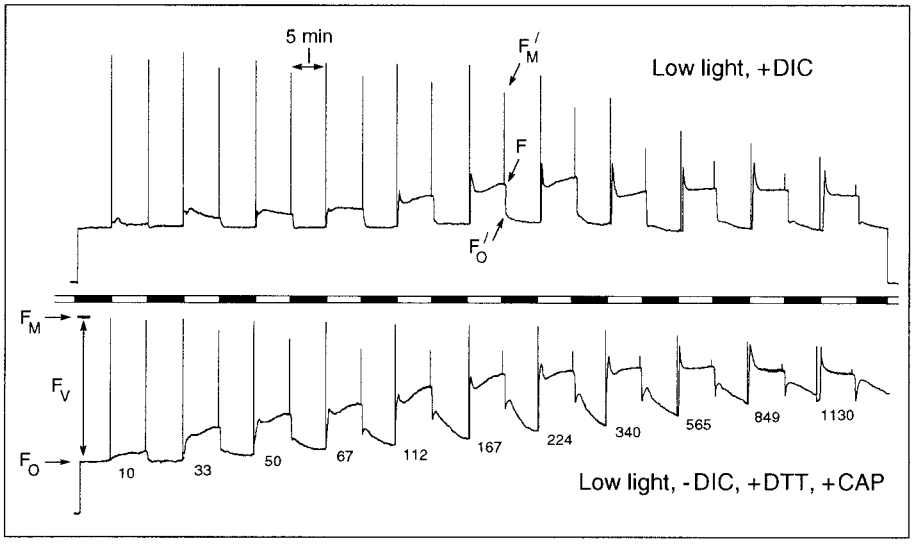

Fig. 1. Primary fluorescence data from two representative experiments with low-light-grown *Ulva*. The *upper panel* shows an experiment with DIC-saturation which protected against photoinhibition and in which significant quenching of variable chlorophyll fluorescence occurred with little change in minimal fluorescence. The *lower panel* shows an experiment with DIC-depletion, in the presence of inhibitors, which led to massive photoinhibition, and in which quenching of variable chlorophyll fluorescence was superimposed on a large rise and dynamic behaviour of minimal fluorescence. Parameters used to calculate fluorescence quenching coefficients are indicated. Numbers below the curves refer to stepwise increases in PFD in $\mu mol \cdot m^{-2} \cdot s^{-1}$, alternating with 5-min dark periods

Reduction status of Q, $1-q_P = 1-\frac{F_M'-F}{F_V'}$

Nonphotochemical quenching, NPQ $= \frac{F_M}{F_M'}-1$

These coefficients were chosen because they are less sensitive to variation in F_O. The value F_O' obtained immediately after turning off the actinic light was used to calculate F_V' from F_M' obtained with the saturating pulse in the preceding light period (Fig. 1).

Tissues were treated with freshly prepared 1 mM CAP or with solid DTT added to a concentration of 3 mM just prior to commencing the experiment. Carbon-dioxide-free seawater was prepared as described previously (Levavasseur et al. 1991) and tissues were rinsed thoroughly with this solution before transfer to the O_2-electrode chamber. It proved impossible to obtain zero photosynthesis with *Ulva* tissue by these means, possibly because of DIC transferred with the tissue, in spite of the washing procedures, and because of respiratory CO_2 generated in the closed system during dark intervals. Thus, these treatments are described as DIC-depleted treatments. Saturated photosynthesis was obtained by addition of 20 mM $NaHCO_3$ to normal seawater (DIC-saturation).

Results and discussion

Effects of growth in high or low light. Data have been selected from four sets of experiments done in December 1990, January, February and May 1991. Figure 2 shows the PFD-response curves for photosynthetic O_2 evolution in seawater in high-light- and low-light-grown *Ulva* from the phycotron, together with chlorophyll fluorescence quenching responses. As observed previously (Henley et al. 1991a; Levavasseur et al. 1991), low-light plants achieved only about 50% of the photosynthetic rate of high-light plants, although both show the same quantum yields (0.07 mol $O_2 \cdot mol^{-1}$ photons). The coefficients for chlorophyll fluorescence quenching show more extensive reduction of Q, the primary acceptor of PSII, at a given absorbed PFD in low-light plants compared with high-light plants (Fig. 2). In low-light plants, the Q pool is about 80% reduced, whereas in high-light plants it is only about 50% reduced at light saturation.

Inasmuch as high reduction status of Q is believed to predispose PSII to photoinhibitory damage, these data indicate that low-light-grown *Ulva* should be more susceptible to photoinhibition than high-light-grown plants, as observed previously using other criteria (Franklin et al. 1992). Inasmuch as NPQ is believed to indicate the capacity for photoprotective processes, data in Fig. 2 also imply that low-light *Ulva* has much lower capacity for NPQ than high-light *Ulva*. The lower photoprotective capacity of low-light thalli may also predispose these plants to greater photoinhibitory damage.

The protocols used in our experiments serve to apply a steadily increasing light stress to the tissue in the course of each experiment. We arbitrarily chose 5-min dark-adapted F_M and F_O to represent fully relaxed components of chlorophyll fluorescence quenching, but as can be seen in Fig. 1, steady state was not achieved in some treatments. Nevertheless, these data were used to estimate declining photosynthetic efficiency (F_V/F_M) and to indicate the progress of photoinhibitory damage (elevation of F_O) throughout the experiment (Fig. 3). Low- and

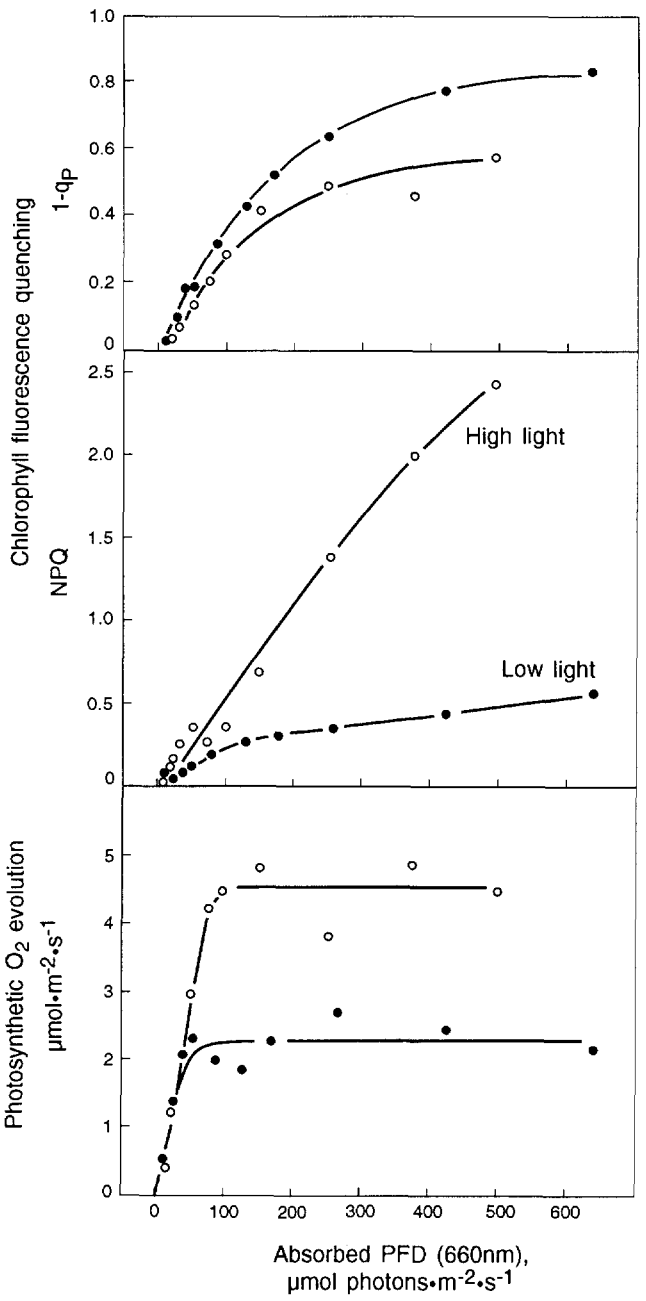

Fig. 2. Chlorophyll fluorescence quenching coefficients and photosynthetic O_2 evolution in low- (●) and high-light-grown (○) *Ulva* in seawater, in response to absorbed PFD

high-light-grown *Ulva* differ markedly in the response of F_O. In high-light plants there was a small initial decline in F_O, which returned to the original level during light-saturated photosynthesis (Fig. 3). In low-light plants, F_O increased rapidly and remained high during exposures to light which saturated photosynthesis.

The rise in F_O, indicative of photoinhibitory damage, (Björkman 1987; Krause 1988) is consistent with the more extensive reduction of Q ($1-q_P$) and limited photoprotective capacity (NPQ) observed in low-light-grown *Ulva*. Interestingly, low- and high-light *Ulva* do not differ

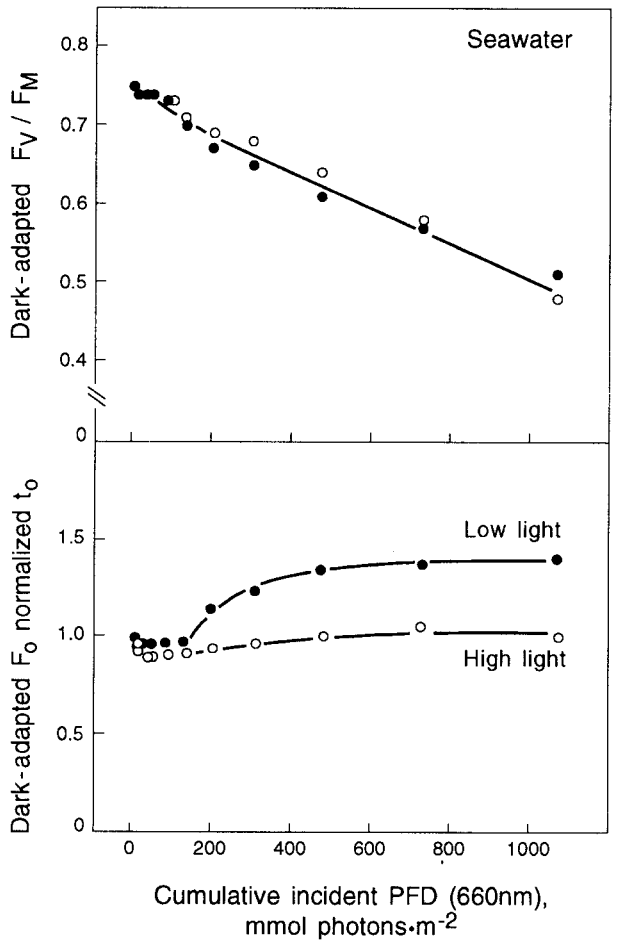

Fig. 3. Dark-adapted chlorophyll fluorescence parameters measured in the course of the PFD-response curve in Fig. 2, and expressed as a function of cumulative incident PFD

much in the changes in F_V/F_M, which is a measure of the quantum efficiency of photochemistry in photosystem II. It is possible that, in low-light plants, the decrease in F_V/F_M was mainly associated with damage to PSII, as also indicated by an increase in F_O. In the high-light plants, on the other hand, the decrease in F_V/F_M could have been associated with increased dissipation of excitation from the PSII antenna, associated with greater NPQ (Fig. 2).

Effects of DIC depletion. We have used the above protocols and data-assessment methods to investigate several other aspects of photoinhibition in *Ulva*. Following early studies in higher plants in which CO_2 deprivation in the light results in massive photoinhibition in vivo, we applied this approach to *Ulva* in which photosynthesis is not carbon saturated in seawater (Levavasseur et al. 1991). Our best efforts to eliminate O_2 evolution with DIC-depleted seawater were unsuccessful. The addition of 20 mM $NaHCO_3$ led to a doubling in light-saturated photosynthetic rate in both low- and high-light plants (cf. Figs. 4e, f with Fig. 2), as observed previously (Levavasseur et al. 1991). In low-light-grown thalli, DIC-depleted conditions led to a much faster rise in $1\text{-}q_P$ and in NPQ than in seawater, but the coefficients were approximately the same at light saturation (cf. Figs. 2 and 4a, c). Addition of 20 mM $NaHCO_3$ drastically slowed the rise of $1\text{-}q_P$ and depressed the slight rise in NPQ with increasing PFD.

In high-light plants, the effects of DIC supply on $1\text{-}q_P$ were similar, but much less pronounced (Fig. 4b). However, in DIC-depleted conditions high-light plants showed the greatest values of NPQ. When high-light plants were DIC-saturated, NPQ was much lower and increased only slowly at higher PFD (Fig. 4d). These changes in $1\text{-}q_P$ and NPQ are similar to those observed in higher C_3-plants, deprived of CO_2 and exposed to 1% O_2 to minimise photorespiratory CO_2/O_2 cycling. Under these conditions, even low PFD is sufficient to generate high values of NPQ and high $1\text{-}q_P$ is common at high irradiance.

These experiments indicated that DIC-depleted conditions might accelerate photoinhibition and DIC-saturated treatments may retard it, as was confirmed by the plots of dark-adapted F_V/F_M and F_O. In Fig. 5, DIC-depleted tissues, both low- and high-light-grown, showed much more rapid decline in F_V/F_M than DIC-saturated treatments. Indeed data points fell below and above, respectively, the seawater data of Fig. 3. The changes in F_O were even more remarkable. In low-light plants, DIC-depletion resulted in a rapid, large increase in F_O which was sustained after exposure to above about 100 mmol · m^{-2}. Evidently the high NPQ attained in high-light-grown *Ulva* under DIC-depleted conditions was not fully effective in photoprotection. The rise of F_O in DIC-depleted treatments was slower and smaller than in low-light plants and declined somewhat at highest PFD exposures (Fig. 5d). Addition of 20 mM $NaHCO_3$ completely abolished the rise in F_O in low- and high-light plants. Responses of both F_V/F_M and F_O in DIC-depleted, high-light plants were consistent with concurrent F_V and F_O quenching associated with photoprotection and elevated NPQ (Fig. 4d). However, the higher, earlier NPQ attained by DIC-depletion evidently did not prevent photoinhibitory damage, which could also have contributed to the accelerated decline in F_V/F_M (Fig. 5).

Effects of DTT. These interactions between chlorophyll fluorescence quenching processes and their relationship to photoinhibition as displayed by changes in dark-adapted F_V/F_M and F_O were examined using DTT, an inhibitor of violaxanthin de-epoxidation (Yamamoto and Kamite 1972). Previous studies have shown that DTT inhibits the in vivo interconversion of violaxanthin to zeaxanthin in *Ulva* (Franklin et al. 1992) as originally established in higher plants (Bilger et al. 1989). This pigment interconversion is well correlated with changes in NPQ (Demmig-Adams and Adams 1992b), and the inhibitor appears to have no other effects of great consequence for the processes under investigation here.

Treatment of low-light-grown *Ulva* with DTT led to an increase in $1\text{-}q_P$ (Fig. 6a) and depressed NPQ dramatically in the presence of DIC (Fig. 6c). The change in dark-adapted F_V/F_M was little affected by DTT (Fig. 7a), but the inhibitor had large effects on dark-adapted F_O

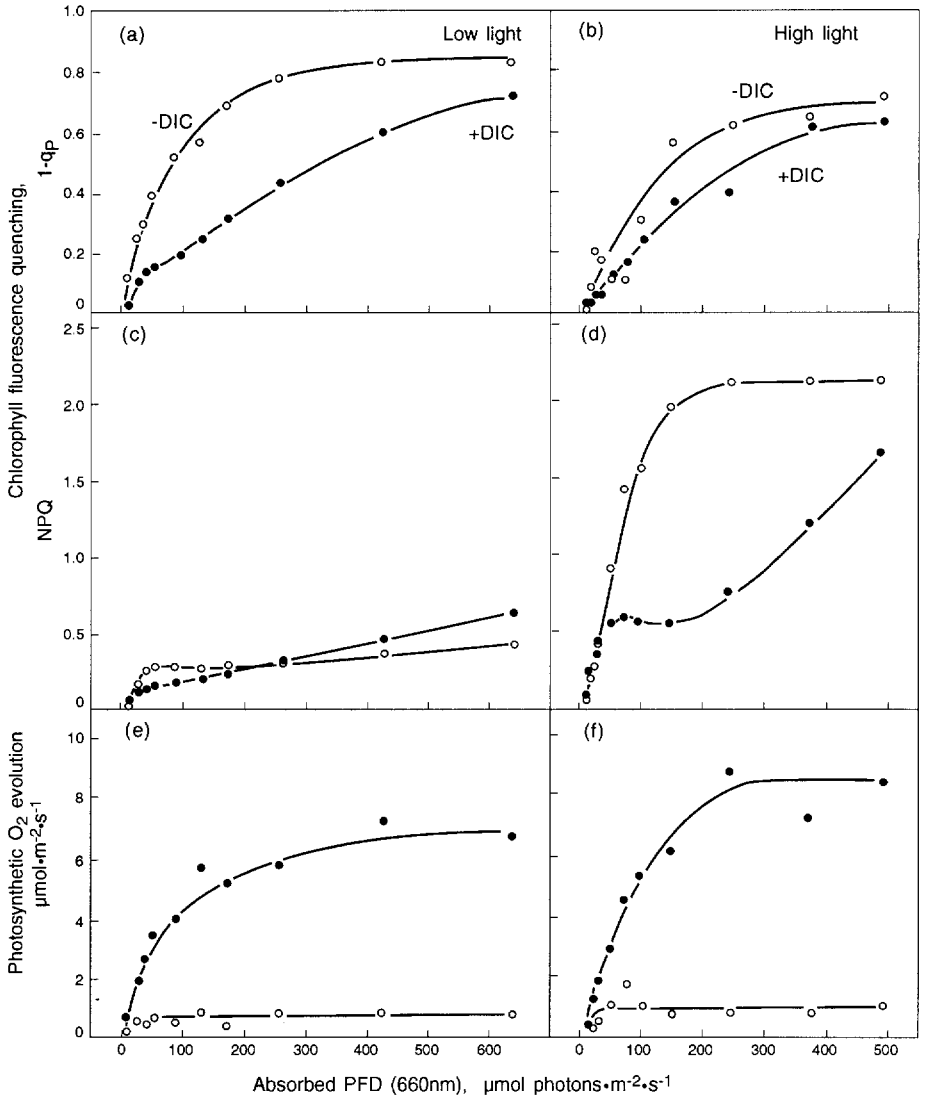

Fig. 4a–f. Effects of DIC-depletion (○) and DIC-saturation (●) on chlorophyll fluorescence quenching coefficients and photosynthetic O_2 evolution in low- (**a, c, e**) and high-light-grown (**b, d, f**) *Ulva* in response to absorbed PFD

(Fig. 7c). The DIC-depleted and DIC-saturated responses of F_O were similar to those in Fig. 5c in which DIC-saturation largely prevented the rise in F_O. However in the presence of 3 mM DTT treatment this protective effect of DIC was abolished, consistent with its effect on NPQ (Fig. 6c). These results are similar to those found in higher plants (Bilger and Björkman 1990; Demmig-Adams et al. 1990).

A similar experiment was done with high-light-grown *Ulva*, but this time 3 mM DTT was added to the sample in DIC-depleted seawater. Figure 6b shows that treatment with 3 mM DTT accelerated the rise in $1\text{-}q_P$ in the DIC-depleted plants, but drastically reduced NPQ (Fig. 6d). These data are consistent with observations in higher plants (Demmig-Adams et al. 1990). The −DIC+DTT treatment markedly accelerated the decline in F_V/F_M (Fig. 7b) and intensified the rise in F_O (Fig. 7d), indicating photoinhibitory damage occurred even in these high-light plants.

Effects of CAP and DTT. These relationships were probed further by means of CAP, an inhibitor of chloroplast-directed protein synthesis which accelerates photoinhibitory damage, possibly by preventing resynthesis of the most rapidly turned over component of PSII, the 33-kDa, D–1 polypeptide (Ohad et al. 1984; Kyle 1987). Figure 8a shows that DIC-depletion led to the expected rapid rise in $1\text{-}q_P$ in plants with low capacity for NPQ (cf. Fig. 4a). Treatment with CAP at DIC-saturation led to a decline in $1\text{-}q_P$ and a rapid rise in NPQ (cf. Fig. 6), indicating no major effect of the inhibitor on chlorophyll fluorescence quenching processes. Addition of DTT inhibited NPQ (Fig. 8c; cf. Fig. 6a, c) and returned the $1\text{-}q_P$ curve to the same as that of the DIC-depleted treatments (Fig. 8a). These severe treatments were expected to maximise photoinhibitory damage, and as observed previously, DIC-depletion in low-light *Ulva* led to rapid initial increase in F_O (Fig. 9c), accounting for the rapid initial decline in F_V/F_M. Addition of CAP and DTT caused a sustained increase in F_O and a sustained decline in F_V/F_M. Most of this photoinhibitory damage was evidently due

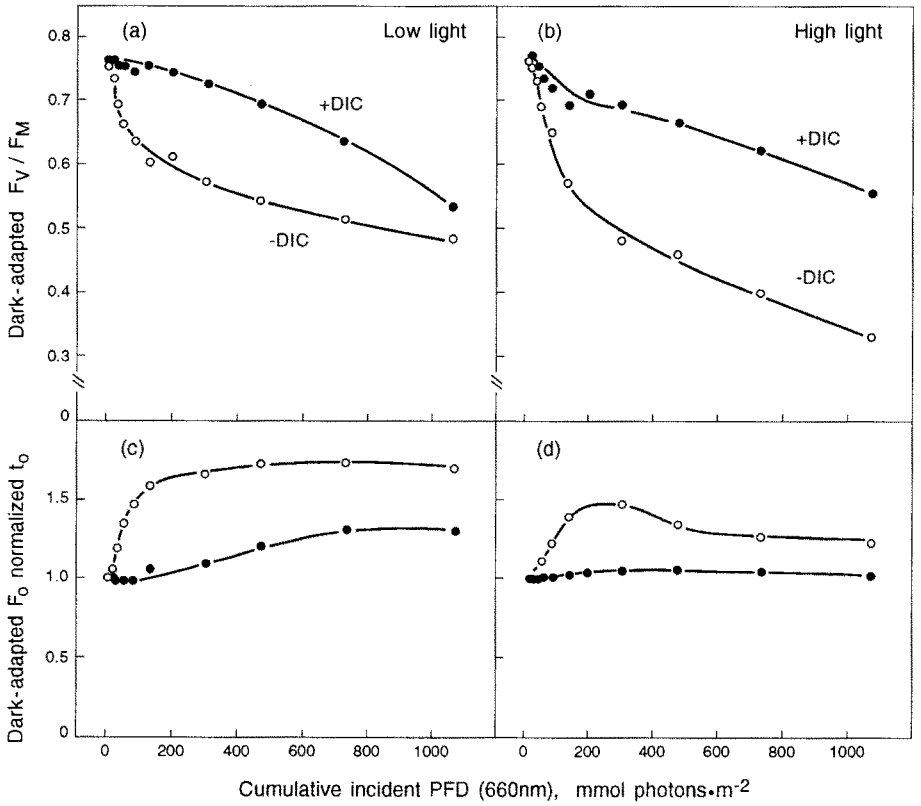

Fig. 5a–d. Dark-adapted chlorophyll fluorescence parameters for low- (**a, c**) and high-light-grown (**b, d**) *Ulva* measured in the course of the PFD-response curves in Fig. 4, and expressed as a function of cumulative incident PFD

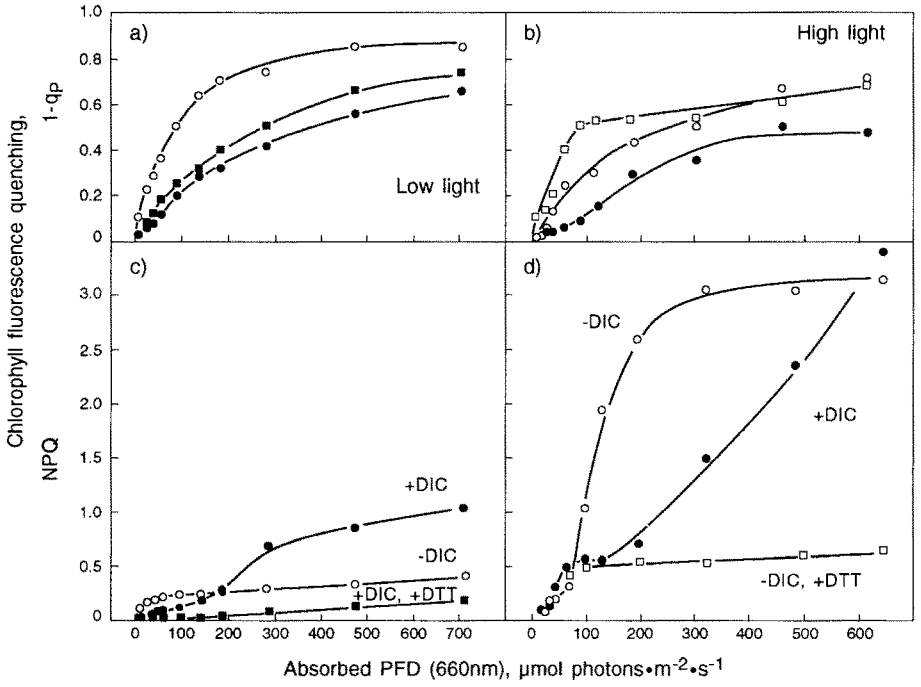

Fig. 6a–d. Effects of DIC-depletion (○) and DIC-saturation (●) on chlorophyll fluorescence quenching coefficients in low- (**a, c**) and high-light-grown (**b, d**) *Ulva* in response to absorbed PFD. Fluorescence quenching was also measured in the presence of 3 mM DTT at DIC-saturation, low-light (■) and DIC-depletion, high-light (□)

to DTT treatment, because in DIC+CAP treatments there was little rise in F_O and a smaller decline in F_V/F_M.

Figure 8b shows that 1 mM CAP had little effect on $1\text{-}q_P$ or NPQ (Fig. 8d) in high-light *Ulva* exposed in DIC-depleted seawater. Similar results were obtained in seawater alone. However, addition of 3 mM DTT impaired NPQ (Fig. 8d). Treatment with CAP or CAP+DTT initially accelerated the decline in dark-adapted F_V/F_M (Fig. 9b) and not surprisingly, CAP accelerated the rise in dark-adapted F_O (Fig. 9d). However,

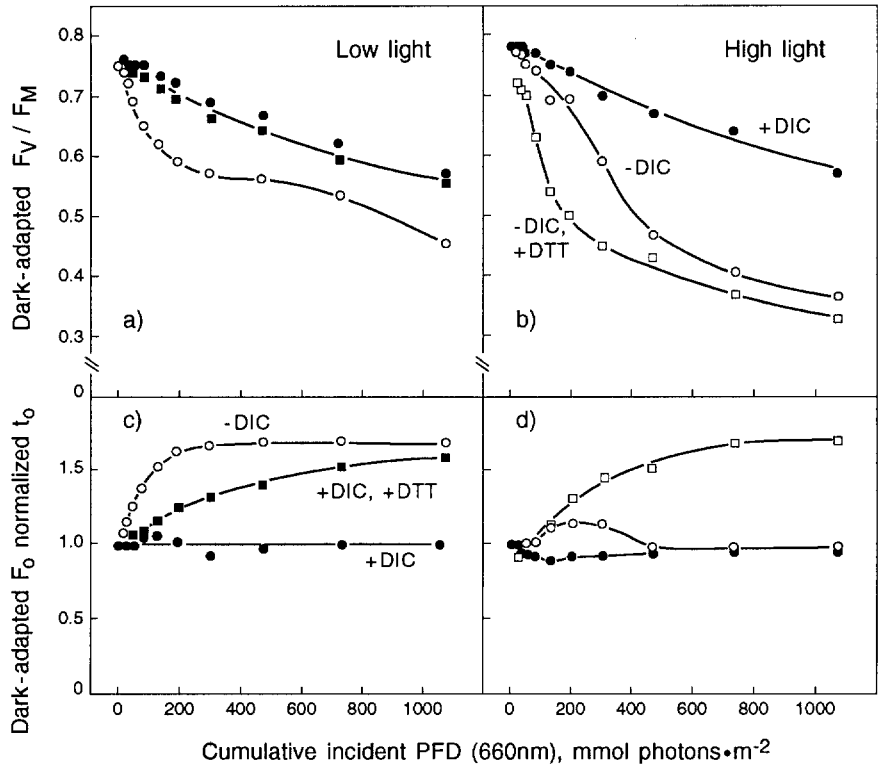

Fig. 7a–d. Dark-adapted chlorophyll fluorescence parameters measured in the course of PFD-response curves shown in Fig. 6, indicating the effects of DIC-depletion, and of DTT in the presence **(a, c)** or absence **(b, d)** of DIC. Data are expressed as a function of cumulative incident PFD

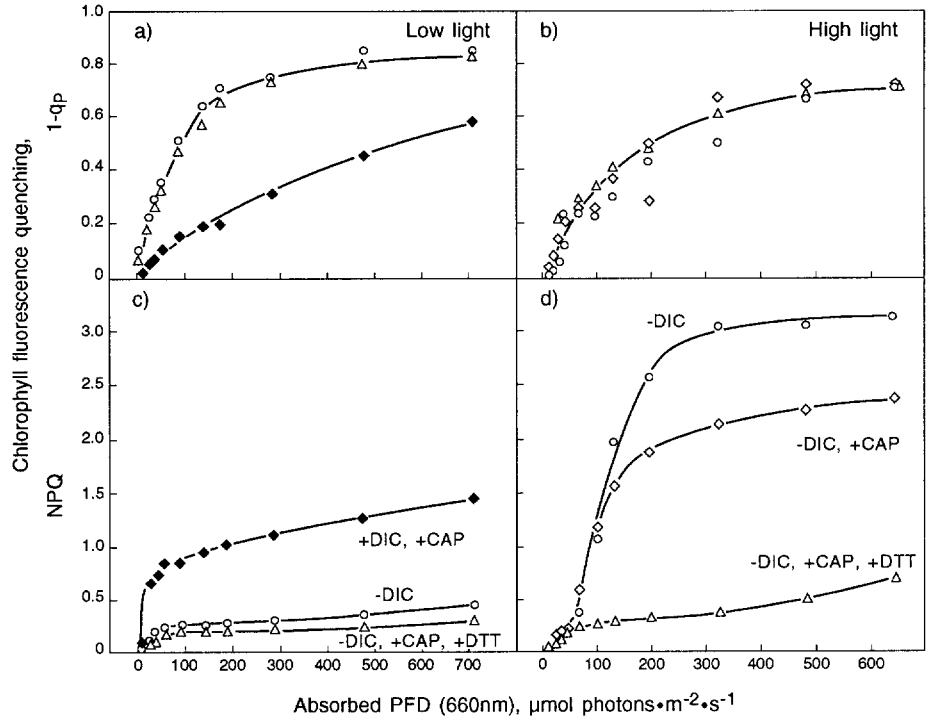

Fig. 8a–d. Effects of DIC-depletion (○), addition of 1 mM CAP in the presence (◆) or absence (◇) of DIC, and addition of 1 mM CAP+3 mM DTT with DIC-depletion (△) on low- **(a, c)** and high-light grown **(b, d)** *Ulva* in response to absorbed PFD

with CAP + DTT an even larger, sustained rise in F_o was found (Fig. 9d). This is consistent with the inhibition of photoprotective functions by DTT and subsequent acceleration of photoinhibitory damage, which was further exacerbated when chloroplast protein synthesis was prevented by CAP.

Conclusions

These experiments confirm an earlier diagnosis of two components of photoinhibition in *Ulva* (Franklin et al. 1992), based on fluorescence properties of dark-adapted tissues, and the effects of DTT and CAP upon these

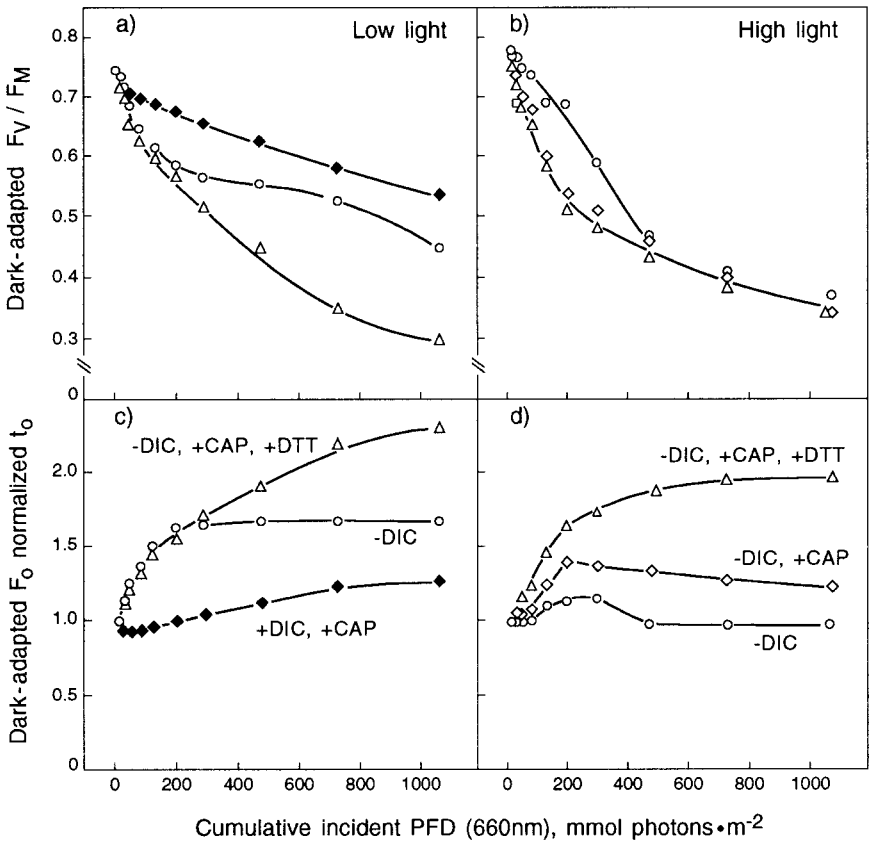

Fig. 9a–d. Dark-adapted chlorophyll fluorescence parameters measured in the course of PFD-reponse curves shown in Fig. 8, indicating the effects of 1 mM CAP, and 1 mM CAP+3 mM DTT added under DIC-depletion. Data are expressed as a function of cumulative incident PFD

fluorescence properties. The purpose of the present experiments was to relate chlorophyll fluorescence quenching processes during specific light treatments to the photoinhibitory consequences of these treatments. Thus we have been able to monitor the two main sources of photoinhibitory stress, the reduction status of Q (by the parameter 1-q_P), and nonphotochemical quenching (by the parameter NPQ), which interact to determine the nature and extent of photoinhibition. In all of the above experiments, we conclude that sustained exposure of *Ulva* to PFD treatments which lead to highly reduced Q pools results in an increase in F_O, associated with photoinhibitory damage. Likewise, all treatments which lead to an increase in NPQ are photoprotective, in that they prevent the rise in F_O and prevent photoinhibitory damage. The inhibitor DTT, which impairs the interconversion of violaxanthin and zeaxanthin, always reduces NPQ and usually leads to a simultaneous increase in 1-q_P and to a rise in F_O. The inhibitor CAP, which inhibits the synthesis of chloroplast proteins, usually accelerates photoinhibitory damage.

The interactions among these factors are very susceptible to growth conditions and treatments. Low-light-grown *Ulva* is more susceptible to photoinhibitory damage than high-light-grown *Ulva* because the former has limited capacity for NPQ and sustains a more reduced Q pool at light saturation (Figs. 2, 3). Both low- and high-light-grown *Ulva* experience photoinhibitory damage when exposed under DIC-depleted conditions (Figs. 4, 5). This treatment has little effect on NPQ in low-light-grown *Ulva*, so we conclude that the protection against photoinhibitory damage afforded by DIC-saturation in low-light plants is largely due to photosynthetic electron transport which maintains lower 1-q_P (Figs. 4, 6). However, because the protection against F_O increase afforded by DIC-saturation can be overridden by DTT treatment (Fig. 7c), we conclude that violaxanthin-zeaxanthin interconversion plays a limited role in low-light-grown *Ulva* (Franklin et al. 1992).

In contrast, DIC-depletion in high-light-grown *Ulva* results in a large increase in NPQ which evidently mitigates against photoinhibitory damage, as indicated by the rise and subsequent fall in F_O (Figs. 5d, 7d). Most of the efficacy of NPQ seems to be sensitive to DTT (Figs. 7d, 9d), indicating a major role for xanthophyll interconversions in photoprotection of high-light-grown *Ulva*. This is consistent with the higher capacity for violaxanthin-zeaxanthin interconversion in high-light-grown *Ulva* (Franklin et al. 1992) and in other plants (Demmig-Adams and Adams 1992a).

Our conclusions about the effects of CAP on photoinhibitory damage in the present experiments are less secure. This inhibitor of chloroplast protein synthesis did not have any large effects on NPQ or 1-q_P in high- or low-light-grown *Ulva*. Thus it is unlikely to have altered factors driving photoinhibitory damage. Yet it markedly stimulated F_O in high-light plants in which high NPQ following DIC-depletion was evidently photoprotective (Fig. 9d). In low-light plants, the protection afforded by DIC was not much affected by CAP (cf. Figs. 5c, 9c).

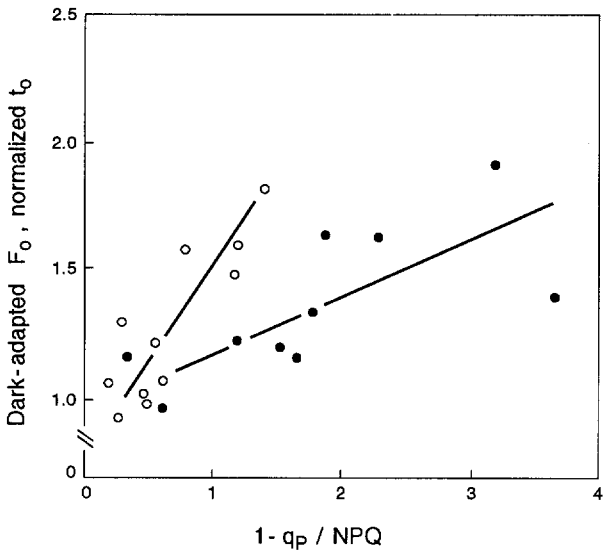

Fig. 10. Correlation between extent of photoinhibitory damage (increase in F_O) and the balance between 1-q_P and NPQ (ratio 1-q_P/NPQ) for low- (●), and high-light-grown (○) *Ulva*

These observations indicate that CAP effects are probably due to inhibition of repair processes, and that the capacity for renewed synthesis of damaged proteins in the PSII reaction centre is greater in high-light-grown *Ulva*.

These conclusions are consistent with a catena of interacting photoinhibitory processes in which excess excitation is first disposed of as heat by some form of NPQ (Demmig-Adams and Adams 1992a), in which zeaxanthin has a particular role or serves as a particular indicator (Horton et al. 1991; Chow 1992). Our DIC-depletion experiments also establish the role for photosynthetic metabolism in this catena of processes. Photon fluxes which exceed the capacity of these two main components of NPQ lead to sustained reduction of Q (high 1-q_P) and to photoinhibitory damage. We have attempted to integrate these opposing processes which promote, and protect against, photoinhibitory damage, by means of the arbitrary ratio 1-q_P/NPQ. Mean values for the parameters 1-q_P, and NPQ at light saturation were calculated in all of the above experiments. These show that the ratio is well-correlated with the increase in F_O (Fig. 10). It is not clear why the data points for high- and low-light-grown plants should be so clearly separated. However, differences between these plants such as lower capacity for NPQ, lower capacity for protein synthesis, and more reduced Q pools in low-light grown plants, may lead to separations in the relationships shown in Fig. 10.

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