High Light-Induced Reduction and Low Light-Enhanced Recovery of Photon Yield in Triazine-Resistant
Brassica napus L.

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ABSTRACT

Triazine-resistant and -susceptible Brassica napus L. plants grown under low photon flux density (PFD) have previously been shown to exhibit a similar photon yield. In contrast, high PFD-grown resistant plants have a lower photon yield than high PFD-grown susceptible plants (JJ Hart, A Stemler [1990] Plant Physiol 94: 1295–1300). In this work we tested the hypothesis that high PFD can induce a differential decrease in photon yield in low PFD-grown plants. We measured photon yield, variable fluorescence/maximum fluorescence, and O₂ flash yield in low PFD-grown resistant and susceptible leaf discs before and after exposure to high PFD exposure. The results demonstrated that high PFD exposure results in a greater decrease in photosystem II (PSII) activity in resistant plants. Characteristics of recovery and other evidence suggest that the differential decrease in PSII efficiency in resistant leaf discs is caused by photoinhibitory damage. We propose that the differential reduction in photon yield and photosynthesis often observed in resistant plants is the result of increased sensitivity to photoinhibition.

The development of triazine resistance in crop plants is an attractive goal because of the potential benefits it offers for weed control. The loss of vigor that accompanies the resistance trait, however, is a serious drawback. Studies with nearly isogenic resistant and susceptible lines of Brassica napus L. revealed that resistant plants grew more slowly (JJ Hart, unpublished data; 5) and had a lower photon yield (15, 18) than susceptible plants. Development of productive resistant crop varieties will depend on our understanding of the molecular mechanism that brings about the loss of photosynthetic performance and growth in plants with the resistance trait.

Jursic and Peacry (18) presented evidence that decreased photosynthesis in resistant plants could be due to the slow Q₂ to Q₁ electron transfer that results from the resistance alteration in the D1 protein of PSII. Recent experiments in our laboratory, however, revealed that resistant plants grown under low PFD do not exhibit the decreased photon yield seen in plants grown under moderate to high PFD conditions (15). A smaller difference in growth is also seen in resistant and susceptible plants grown under low PFD. (JJ Hart, unpublished data; 1). These observations suggest that reduced photon yield and growth in triazine-resistant plants is a consequence of exposure to moderate to high PFD and not simply a result of slowed Q₂ to Q₁ electron transfer per se.

This work was initiated to test the hypothesis that high PFD light causes the differential reduction in photosynthetic yield observed in resistant plants. We exposed low PFD-grown B. napus leaf discs to high PFD and measured photon yield and other photosynthetic traits. We found that exposure to high PFD induces a differential decrease in photon yield in resistant plants. We also observed that the characteristics of recovery from decreased photon yield are consistent with the hypothesis that resistant plants are more sensitive to photoinhibition of PSII than susceptible plants.

MATERIALS AND METHODS

Plant Material

Plants used in these experiments were grown from seeds produced by reciprocal crossing of individuals of the commercially available Brassica napus L. varieties ‘Regent’ and ‘Triton’ (15). All plants were grown in either a high or low light intensity growth chamber as described earlier (15).

High PFD Treatment

A 10 cm² disc was cut from a leaf grown in the low PFD growth chamber. The disc was floated upside down on 25°C water in a glass Petri dish. A floating foam pad with a hole in the center surrounded the disc and held it in position. The edges of the foam in contact with the leaf disc were water-saturated to allow a path for water flow from the bulk water into the cut ends of xylem cells at the periphery of the disc. Leaf discs remained fully turgid even after the highest PFD treatments. Light was provided by a Leitz projector fitted with a 250W Osram HX Xenophot lamp. Light was reflected upward by a mirror onto the adaxial surface of the leaf disc in contact with the water. PFD at the surface of the leaf disc was adjusted to 2000 μmol m⁻² s⁻¹. All subsequent measurements of photon yield, O₂ flash yield and Chl a fluorescence
were made on the same (adaxial) leaf surface that was exposed to high PFD treatment.

Photon Yield Measurement

A leaf disc was placed in the chamber of a Hansatech LD2 gas phase oxygen electrode. The protocol for photon yield measurement was outlined previously (15). Each photon yield determination required approximately 30 min. In the recovery experiments, the procedure was performed four times over the course of 2 h following high PFD treatment. Results are plotted as the midpoint time of each determination.

Oxygen Flash Yield Measurement

After high PFD treatment, the leaf disc was transferred to the oxygen electrode. The procedure and equipment used in measuring oxygen flash yield were as described previously (15).

Fluorescence Measurements

These measurements were made with a pulse amplitude modulated fluorescence measuring system (H. Walz, Effeltrich, FRG) (29). The protocol for determining $F_v/F_m$ values was described previously (15). For the DCMU-induced fluorescence induction experiment, two leaf discs were cut from a single leaf and floated upside down on 25°C water. One disc was exposed to low PFD (100 μmol m⁻² s⁻¹) and the other to high PFD (2000 μmol m⁻² s⁻¹) PFD for 3 h. A saturating pulse of light was then applied to record $F_{m}$ in each disc. Both discs were then transferred to a solution of $4 \times 10^{-4}$ M DCMU. With the abaxial side of the leaf disk exposed to air and the foam pad providing a path for movement of solution, conditions were favorable for transpiration to assist in drawing solution into the interior of the disk. Complete inhibition as indicated by rapid rise to constant maximal fluorescence was noted about 1 h after introduction of the leaf disc to DCMU solution. To generate a signal, the measuring beam at 1.6 kHz was applied to the leaf disc followed by an actinic light of about 60 μmol m⁻² s⁻¹. The signal was recorded on a Tektronix 2230 Digital Storage Oscilloscope and transferred to a Zenith microcomputer for processing.

Recovery Conditions

To monitor recovery of photon yield, leaf discs were placed in the oxygen electrode chamber immediately following high PFD treatment. Recovery took place under the conditions of photon yield measurements, i.e. 5% CO₂; alternating light/dark cycles with various low light intensities; 28°C; 100% RH. Examination of leaf discs after removal from the electrode chamber revealed no wilting or other visible damage. To measure $F_v/F_m$ recovery after high PFD treatment, the Petri dish containing the leaf disc was simply repositioned over the fluorometer fiber optic probe. Recovery occurred under the conditions of $F_v/F_m$ measurement, i.e. floating upside down on water at room temperature (25°C); air, periodic saturating light pulses. Immediately following high PFD treatment, discs were left in darkness for 10 min. Disks then either remained in darkness for the duration of the experiment or were exposed to low PFD (about 40 μmol m⁻² s⁻¹). Disks treated with 5 and 45 min high PFD received 30 min of low light. Discs treated with 90 min high PFD received continuous low light interrupted by 10 min dark periods prior to saturating pulses applied to record $F_m$.

Figure 1. Photon yield of triazine-resistant and triazine-susceptible B. napus leaves grown in growth chambers at four levels of PFD. Measurements were replicated four times for each leaf disc over the course of 2 h. Each data point represents the mean of two leaf discs. Error bars represent ± 1 se. Some error bars do not extend outside data points.

Figure 2. Time course of change in photon yield and $F_v/F_m$ of low PFD-grown triazine-resistant and -susceptible B. napus leaf discs following exposure to a PFD of 2000 μmol m⁻² s⁻¹ at 25°C. Photon yield measurements were replicated four times for each leaf disc over the course of 2 h. For photon yield, each data point represents the mean of two determinations. For $F_v/F_m$, each data point represents the mean of three determinations. Error bars represent ± 1 se. Some error bars do not extend outside data points.
RESULTS

High PFD-Induced Decrease in Photosynthetic Efficiency

Resistant plants grown under low PFD (about 100 μmol m⁻² s⁻¹) had a photon yield nearly equivalent to susceptible plants (Fig. 1). At higher PFD (450 μmol m⁻² s⁻¹ and above) photon yield decreased in the resistant variety to a greater extent than in the susceptible variety (Fig. 1). Plants grown under low PFD and then exposed to various durations of high PFD showed a decrease in photon yield (Fig. 2). Photon yield was reduced to a greater extent in resistant plants than in susceptible plants following exposure to high PFD.

Fᵥ increased and Fₘ decreased in both varieties as exposure time to high PFD increased (Table 1). The rise in Fᵥ in the resistant variety was nearly twice that of the susceptible variety while the decrease in Fₘ was slightly greater in the resistant variety. High PFD also induced a decrease in Fᵥ/Fₘ in plants grown under low PFD (Fig. 2). The decrease was more pronounced in resistant plants than in susceptible plants.

The decrease in photon yield in resistant and susceptible varieties agrees well with the decrease in Fᵥ/Fₘ (Fig. 2). Correlation between photon yield and Fᵥ/Fₘ has been previously discussed (15). Because of the relative ease of measurement, Fᵥ/Fₘ was used as an indicator of photon yield in the recovery experiments described below.

Oxygen flash yield was affected by high PFD exposure in a manner similar to photon yield and Fᵥ/Fₘ. Low PFD-grown plants experienced a decrease in oxygen flash yield after exposure to high PFD, with the resistant variety showing greater sensitivity (Fig. 3).

Because the high PFD exposures described above resulted in relatively small decreases in photon yield and Fᵥ/Fₘ, we tested the response to prolonged high PFD exposure. Leaf discs were given 3 h of high PFD, then fluorescence transients of DCMU-treated leaf discs were recorded. Induction transients of DCMU-treated material can be diagnostic for the cause of photon yield reduction. DCMU-treated discs exhibited the induction transients seen in Figure 4. Fᵥ resulted from turning on the low PFD pulsed measuring light and Fₘ was produced by the white actinic light. In the absence of high PFD treatment, Fᵥ and Fₘ were higher in the resistant variety (Fig. 4A). After 3 h of high PFD, leaf discs exhibited the fluorescence traces seen in Figure 4B. Fᵥ decreased in both susceptible and resistant leaf discs. Fᵥ increased in both resistant and susceptible discs but only slightly in the resistant disc. Fᵥ/Fₘ decreased to a greater extent in resistant discs.

Recovery of Photon Yield following High PFD Treatment

Photon yield of susceptible plants decreased only slightly after exposure to 20 min of high PFD and recovered fully.

Table 1. Percent Increase in Fᵥ and Percent Decrease in Fₘ following High Light Treatment

<table>
<thead>
<tr>
<th>Exposure Time (min)</th>
<th>Fᵥ (% increase)</th>
<th>Fₘ (% decrease)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
<td>Susceptible</td>
</tr>
<tr>
<td>5</td>
<td>4.7 ± 2.2</td>
<td>20.9 ± 1.6</td>
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<tr>
<td>10</td>
<td>3.7 ± 1.3</td>
<td>30.9 ± 2.6</td>
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<td>20</td>
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<td>34.6 ± 5.7</td>
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<tr>
<td>45</td>
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<td>44.2 ± 4.4</td>
</tr>
<tr>
<td>90</td>
<td>10.8 ± 4.7</td>
<td>52.9 ± 3.5</td>
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</tbody>
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Figure 4. Fluorescence induction transients of DCMU-treated triazine-resistant and -susceptible B. napus leaf discs exposed to 3 h of low (A) and high (B) PFD. Discs were incubated on 4 × 10⁻⁴ M DCMU solution for 1 h. Fᵥ was generated by turning on the weak pulsed measuring beam. Transients were produced by exposure of leaf discs to actinic light of about 60 μmol m⁻² s⁻¹.

Figure 3. Time course of change in O₂ flash yield of low PFD-grown triazine-resistant and -susceptible B. napus leaf discs following exposure to a PFD of 2000 μmol m⁻² s⁻¹ at 25°C. Values for untreated control leaf discs are found in Hart and Stemler (1990). Each data point represents the mean of three determinations. Error bars represent ± 1 so and do not extend outside all data points.
after 105 min (Fig. 5). Susceptible leaf discs exposed to 45 and 90 min of high PFD showed a greater initial drop but recovered to nearly pretreatment level. Photon yield of resistant leaf discs decreased to a greater extent and showed less recovery than similarly treated susceptible leaf discs (Fig. 5).

Figure 6 illustrates recovery of Fv/Fm following 5 min of high PFD (2000 μmol m⁻² s⁻¹). In both resistant and susceptible varieties, there was a rapid recovery of Fv/Fm during the first 10 min in darkness (Fig. 6). In leaf discs maintained in darkness, this rapid increase was followed by a constant level of Fv/Fm. Leaf discs of both susceptible and resistant varieties exposed to 30 min of low PFD following the 10 min dark period showed a further increase in Fv/Fm (Fig. 6). Fv/Fm remained lower in resistant discs. However, the increase in Fv/Fm in the resistant disc following the low PFD treatment was slightly higher than the increase in the susceptible disc.

Recovery of Fv/Fm following 45 min of high PFD showed a slightly different pattern (Fig. 7). In both resistant and susceptible leaf discs maintained in the dark, the rapid increase during the first 10 min was followed by a slow rise. The amplitude of the initial fast rise was larger in susceptible discs. In resistant discs, the low PFD-enhanced increase was greater than in susceptible discs and originated during the low PFD exposure. The final steady state level of Fv/Fm remained lower in resistant as compared with susceptible leaf discs.

Following 90 min of high PFD, Fv/Fm of susceptible leaf discs decreased about 40% (Fig. 8). They then showed the same rapid initial increase in Fv/Fm as seen in discs exposed to shorter durations of high PFD (cf. Figs. 6, 7, and 8). Fv/Fm of susceptible discs maintained in darkness recovered at a slower rate than discs exposed to low PFD during the recovery period.

Fv/Fm of resistant leaf discs also decreased about 40% after exposure to 90 min of high PFD (Fig. 8). In contrast to susceptible leaf discs, resistant discs did not exhibit the rapid initial increase of Fv/Fm during the 10 min dark period following high PFD treatment. A slow increase began several min into the dark period (Fig. 8). The resistant leaf disc treated with low PFD recovered faster than one maintained in darkness.

**DISCUSSION**

Figure 1 demonstrates that photon yield in triazine-resistant *Brassica napus* was dependent on the level of PFD during growth. Resistant plants grown under PFD as low as 400 μmol m⁻² s⁻¹ had a significant reduction in photon yield. In published reports of decreased photon yield in resistant plants,
growth PFD (where specified) was higher than 400 μmol m⁻² s⁻¹ (17, 18, 25). The correlation between growth PFD and photon yield in resistant plants suggests that light was involved in the photon yield depression. The results shown in Figure 2 indicate that exposure of low PFD-grown resistant and susceptible plants to high PFD caused a decrease in photon yield. Clearly, resistant plants are more sensitive to light exposure than susceptible plants. The results also indicate that the lower photon yield reported many times in resistant plants grown under moderate to high PFD conditions may well represent change caused by the light absorption itself.

The decrease in photon yield following exposure to high PFD and the parallel response of F/Fm (Fig. 2) suggest a reduction in the number of active PSI centers. This is supported by the O₂ flash yield response to high PFD exposure (Fig. 3). O₂ flash yield has been used to measure the relative number of active PSII centers in algae (11) and more recently in leaf discs (7, 18). It should be pointed out that even before high PFD exposure, resistant leaf discs had a lower O₂ flash yield (15), probably due to incomplete recovery between flashes of some PSII centers (18). Because the ordinate of Figure 3 represents percentage of pre-high PFD treatment it still reveals a differential reduction in O₂ flash yield in resistant leaf discs. The pattern of decrease of O₂ flash yield in resistant and susceptible leaf discs was similar to that of both photon yield and F/Fm, strongly suggesting a differential decrease in active PSII complexes in resistant leaf discs.

The change in fluorescence induction of leaf discs infiltrated with DCMU following high PFD treatment (Fig. 4) is also consistent with loss of active PSII. Reduction in variable fluorescence in thylakoids similar to that exhibited by our leaf discs in Figure 4 has been interpreted as evidence that PSII centers were transformed to fluorescence quenchers (4, 8, 22). The greater reduction in variable fluorescence in resistant discs again suggests a greater loss of active PSII.

The collective responses of photon yield, F/Fm, O₂ flash yield and induction transients of DCMU-treated leaf discs following high PFD treatment strongly suggest that PSII centers were rendered inactive. Several mechanisms can account for loss of photochemical function in PSII. Transfer of absorbed excitation energy away from PSII to PSI in a state I to state II transition can cause a decrease in photon yield of O₂ evolution (12). Nonradiative dissipation of excitation energy within the pigment bed can lower F/Fm and photon yield (9). Photoinhibitory damage to PSI caused by excessive light can also bring about a decrease in photon yield (26) and F/Fm (6, 14).

State I to state II transition is probably not the mechanism responsible for reduced photon yield in the leaves measured in these experiments. The half-time of recovery for state transitions is on the order of 5 min in barley leaves (27) and in leaves of triazine-resistant and -susceptible lines of B. napus similar to those used here (P Jurisinic, personal communication). The time needed for recovery to pre-high PFD treatment levels of photon yield in our experiments was a minimum of 100 min under the most favorable conditions (Fig. 5). Perhaps the most convincing evidence ruling out state transitions is our O₂ flash yield data. State transitions would not cause a decrease in O₂ flash yield. With saturating flashes, every active PSI and PSII center will turn over regardless of the arrangement of antenna LHC. It is therefore unlikely that state transitions contributed much to the long-term decrease in photon yield in our B. napus leaves.

Nonradiative dissipation can probably also be ruled out as the cause of the long-term decrease in photon yield and F/Fm observed in our leaf material. The recovery kinetics of photon yield (Fig. 5) and F/Fm (Figs. 6, 7, and 8) are inconsistent with previous reports of recovery attributed to radiationless dissipation. Recovery half-times of 30 min in soybean (10) and 100 min in cotton (28) contrast with the more than 20 h required for recovery of F/Fm in our high PFD-exposed leaf discs (Fig. 8).

The increase in Fo following high PFD treatment observed in our leaf discs (Table I) is also inconsistent with radiationless dissipation as the cause of decreased photon yield. The model of Kitajima and Butler (20) predicts a decrease in Fo as the result of an increase in the rate constant of nonradiative dissipation. Nonradiative dissipation following high PFD treatment has been experimentally correlated with a decrease in Fm (28).

Finally, our O₂ flash yield data argue against nonradiative dissipation as the cause of reduced photon yield. As pointed out above, saturating flashes will turn over every functioning reaction center regardless of energy diversion in the pigment system. The loss of O₂ flash yield observed in our leaf material following high PFD treatment (Fig. 3) cannot be explained by an increase in nonradiative dissipation.

The relatively long recovery time following high PFD treatment (Fig. 8) is consistent with photoinhibitory damage as the cause of reduced photon yield. Recovery time in darkness requiring hours has been reported in photoinhibited leaves (6, 13, 14, 27). Exposure of leaves to low PFD following photo-

Figure 8. Time course of recovery of F/Fm in triazine-resistant and susceptible B. napus leaf discs following 90 min of exposure to high PFD (2000 μmol m⁻² s⁻¹) at 28°C. Following the 10 min dark period, discs were either exposed to low PFD (40 μmol m⁻² s⁻¹) or remained in darkness. Leaf discs exposed to low PFD were allowed to incubate for 10 min in the dark prior to measurement.
inhibitory treatment has been shown to speed photon yield recovery (13, 14). Faster recovery of \( F_v/F_m \) was observed in our low PFD-exposed discs (Figs. 6, 7, and 8).

The increase in \( F_v \), seen here in \( B. napus \) leaf discs following high PFD-exposure (Table I) is another indication that photoinhibition was the primary factor in causing reduction of photon yield. An increase in \( F_v \) following photoinhibitory treatment is predicted by the model of Kitajima and Butler (20) and has been demonstrated experimentally (14, 22). The fluorescence induction traces of leaves infiltrated with DCMU shown in Figure 4 are also consistent with photoinhibitory damage. Similar changes in induction transients observed in thylakoids following high PFD treatment were attributed to photo-inhibition (4, 8, 22).

The reduction in \( Q_E \) flash yield following high PFD exposure in low light-grown \( B. napus \) (Fig. 3) is perhaps the most direct evidence of photoinhibitory damage to PSII. The greater degree of apparent photoinhibitory damage seen in the resistant line is consistent with a recent report of increased high light sensitivity in atrazine-resistant mutants of \( Synechocystis \) (19).

The basis for increased sensitivity may lie in the alteration in the D1 protein that confers resistance. Electron transfer from \( Q_A \) to \( Q_B \) has been shown to be slower in resistant \( B. napus \) (15, 18). It is possible that slower electron transfer results in a longer lifetime for \( Q_A \) and \( Q_B \) in the reduced semiquinone state. Interaction of semiquinones with molecular oxygen can lead to reactive species of \( O_2 \) which can cause damage to membrane components (3). Kyle (23) argued that the basis for photoinhibition involves damage to the D1 protein caused by oxygen radicals produced by interaction of \( Q_A^\cdot \) or \( Q_B^\cdot \) with \( O_2 \). If the slow electron transfer in resistant plants increases the lifetime of either semiquinone, there may be a greater opportunity for production of reactive \( O_2 \) species and potential for photoinhibitory damage. Other workers argue that the primary site of photoinhibitory damage is not the D1 protein but rather the reaction center itself (2, 4, 8). Arntz and Trebst (2) concluded that both \( Q_A \) and \( Q_B \) can induce photoinhibition, although they did not offer a mechanism for the role of these quinones in loss of PSII function. Van Miegheem et al. (30) recently proposed that the primary lesion of photoinhibition involves irreversible double reduction of \( Q_A \). Whether the primary damage occurs at the reaction center or the D1 protein, it appears that the slowed electron flow from \( Q_A \) to \( Q_B \) in atrazine-resistant plants contributes to photoinhibitory damage.

Recovery from photoinhibition has been shown to involve removal and replacement of the D1 protein in thylakoid membranes (24). The slopes of plots of recovery of \( F_v/F_m \) (Fig. 8) indicate a faster rate of recovery in resistant leaf discs. Thus, while resistant plants apparently experience more severe levels of photoinhibition, they may be equipped with a greater capacity for repair. Further work will be necessary to determine the basis for this observation.

The rapid time course of recovery of \( F_v/F_m \) immediately following high PFD treatment (Figs. 6, 7, and 8) is suggestive of the relaxation of a fluorescence quenching component related to thylakoid membrane energization (16, 21). The diminishing amplitude of this rapidly relaxing component in resistant discs (Figs. 6, 7, and 8) suggests that the mechanism that causes the relaxation was rendered inactive by high PFD exposure. Again, clarification of the nature of this component will require additional experimentation.

In this work, we have demonstrated that low PFD-grown resistant \( B. napus \) plants experience a differential decrease in efficiency of PSII following high PFD exposure. Based on a number of criteria, we propose that the decrease is due to greater sensitivity to photoinhibition in resistant plants. We suggest that the lower photon yield and diminished photosynthetic capacity often observed in resistant plants are caused by secondary effects of the slow \( Q_A^\cdot \) to \( Q_B^\cdot \) electron transfer that results from the resistance mutation.

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LITERATURE CITED