The Nature of Light-Induced Inhibition of Photosystem II in Pumpkin (*Cucurbita pepo* L.) Leaves Depends on Temperature[1]

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ABSTRACT

Attached leaves of pumpkin (*Cucurbita pepo* L.) were treated in high or moderate light at room temperature or a 1°C. The symptoms of photoinhibition appearing during light treatments at room temperature could be attributed to a decrease in the primary activity of PSII. However, when the light treatment was given at 1°C, the quantum yield of photosynthetic oxygen evolution decreased much more than would be expected from the decrease in the ratio of variable to maximum fluorescence at 77°K. Also, light treatment at 1°C lowered the chloroplast wholechain electron transfer capacity much more than it affected PSII electron transport (H_2O to paraphenylbenzoquinone). Light treatments at both room temperature and 1°C led to an increase in B_{max}, which indicates an increase in the proportion of $PSII_\beta$ centers. PSI was not affected by the light treatments, and the treatments in the dark at 1°C caused only minor changes in the measured properties of the leaves. We conclude that high light always inhibits the primary activity of PSII, but at low temperature there is greater inhibition of electron transfer from primary electron accepting plastoquinone of PSII to the plastoquinone pool, which leads to a drastic decrease in the quantum yield of oxygen evolution in the chilling-sensitive pumpkin.

Photoinhibition of photosynthesis is observed when plants are exposed to high photon flux densities. The symptoms of photoinhibition comprise a decline of the quantum yield and the light-saturated rate of photosynthetic oxygen evolution, a decrease in the ratio of variable to maximum fluorescence, and a decrease of PSII electron transfer capacity (for a review, see Powles [23]). Various environmental factors have been reported to accelerate photoinhibition or to induce photoinhibition even in moderate light. These include drought stress (16), high temperature (6) and low temperature (6, 8–12, 15–16, 18, 20; for a review, see Öquist *et al.* [22]).

Two main hypotheses have been presented to explain the accelerating effect of low temperature on photoinhibition. First, low temperature is considered to slow down the enzymatic and diffusion-dependent reactions of photosynthesis, thus decreasing the proportion of absorbed light energy that can be utilized in the photochemical reactions. From this point of view, low temperature acts by limiting dissipation of excitation energy in photochemistry, causing over-excitation of PSII and thus amplifying photoinhibition (20). Second, the

rate of recovery from photoinhibition has been reported to decrease at low temperature (8, 9). As the recovery process has been shown to occur simultaneously with inhibition, the slower rate of recovery could also accelerate photoinhibition at chilling temperatures. However, there is some controversy about the rate of recovery during photoinhibition (10).

The actual nature of the photoinactivation caused by cold temperature and light has not attracted much attention. Ögren *et al.* (20) found that the time-courses of the recovery from chill-induced and high light-induced photoinhibition in *Lemna* were similar, suggesting a common basic phenomenon. However, Sassenrath *et al.* (25) reported that the inhibition of stromal fructose bisphosphatase made a major contribution to the inactivation of the photosynthetic apparatus of tomato leaves in cold temperature and light. The thylakoid electron transfer was still capable of supporting control rates of carbon fixation (13). These results suggest that the photoinactivation at low temperature may differ from high light-induced photoinhibition, at least in chilling-sensitive plants.

The objective of the present study was to distinguish between photoinhibition and chill-induced photoinactivation of photosynthesis in a chilling sensitive plant.

MATERIALS AND METHODS

Plant Material

Pumpkins (*Cucurbita pepo* L.) were grown in a greenhouse at 25 to 30°C and a PPFD of 100 to 200 μmol m^{-2} s^{-1} (16 h light/8 h dark) for 4 weeks. One d before the start of the experiments, the plants were transferred to a PPFD of 20 to 30 μmol m^{-2} s^{-1}, to increase their susceptibility to photoinhibition. Full-grown third, fourth, or fifth leaves were used in the experiments.

Treatments

During the RT[2] photoinhibitory treatments the plants were enclosed in a windowed chamber, and a 80 cm^2 area of one

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[2] Abbreviations: RT, room temperature; F_o, F_v, F_{max}, initial, variable, and maximum fluorescence; k_P and k_{DT}, rate constants for photochemistry and radiationless dissipation of excitation energy; ϕ_{app}, apparent quantum yield of photosynthetic oxygen evolution; PPBQ, paraphenylbenzoquinone; MV, methyl viologen; DCPIP, dichlorophenol-indophenol; WC, whole chain electron transport; Q_B, second electron accepting plastoquinone of PSII; Q_A, primary electron accepting plastoquinone of PSII.

leaf was exposed to the window, which was lighted with a 250 W projector, equipped with a heart-absorbing filter. During the treatment, an air-stream was passed into the chamber and the plant was sprayed with water.

The chilling treatments were carried out in a windowed growth chamber. Saturated humidity was maintained by constantly spraying water into the chamber. No signs of loss of turgor were allowed. The leaf temperature was measured with a thermocouple inserted into the light-exposed region of the leaf. During the treatment, the variation in the temperature of the light-exposed area of the leaf was less than $\pm 1°C$.

For control experiments, a piece of the leaf was removed and kept in a Petri dish on moist paper in dim laboratory light before the assays.

The combinations of PPFD, duration, and temperature of the treatments were as follows: PPFD 750 μmol m^{-2} s^{-1}: 30, 60, and 120 min at both RT and 1°C; PPFD 1500 μmol m^{-2} s^{-1}: 30, 60, and 120 min at RT, 60 and 120 min at 1°C; PPFD 2500 μmol m^{-2} s^{-1}: 120 and 240 min at RT. The PPFD on the exposed area of the leaf deviated less than 10% from the listed values. The dark chilling treatments at 1°C lasted 120 min. The recovery was tested on plants that had been kept in the growth conditions for 24 h after the treatment.

Fluorescence Measurements

Fluorescence measurements were carried out with a laboratory-constructed fluorometer (18), with some modifications. The detector filter was a 687 nm Schott PIL-1 interference filter, half-width 10 nm, and the PPFD of the exciting light (450 nm broadband) was 80 μmol m^{-2} s^{-1}. The shutter opening time was 2 ms. The spectral overlap of the exciting light and the detected fluorescence was negligible. The AC-induced ripple in the exciting light was compensated by simultaneous measurement of light.

The measurement of the fluorescence parameters and the area integration (19) for the determination of β_{max} were carried out with a microcomputer connected to the oscilloscope. β_{max} Is the maximal area above the fluorescence induction curve accumulated in a first-order reaction. The β_{max} value, obtained by extrapolating the slow, exponential phase of the area growth curve to zero time, gives the proportion of PSIIβ centers, if complementarity between variable fluorescence and photochemistry exists, and if the rate constants for fluorescence and photochemistry are the same for PSIIα and PSIIβ.

The relative rate constants for PSII photochemistry (k_P) and radiationless, nonphotochemical dissipation of excitation energy including energy transfer to PSI (k_{DT}) were calculated from F_o and F_{max} according to Greer *et al.* (11). In the calculation, values of 1 and 70 were assigned to k_F and k_P of control material, respectively. An error in the assumed control value of k_F/k_P would not affect comparisons of k_P values, and would only slightly affect the comparisons of k_{DT}.

Before the fluorescence measurements on the leaves, the leaf discs were dark-adapted for 30 min on moist paper in a Petri dish at RT. A leaf disc was attached to the fluorometer probe in darkness, and the sample was frozen for 1 min in liquid nitrogen. Six leaf discs were measured in every experiment.

The fluorescence induction kinetics of DCMU-poisoned thylakoids at 20°C were measured as in Mäenpää *et al.* (18), using the same equipment as for the leaf discs, except that the actinic light was provided by a 150 W projector fed with a stabilised DC source. The DCMU concentration was 20 μM.

For the 77°K fluorescence measurements on isolated thylakoids, a drop of the thylakoid suspension was inserted into the fluorometer probe, and the suspension was dark-adapted for 3 min before being frozen for 1 min in liquid nitrogen. The light path lengths was 1 mm and the Chl concentration 0.2 mg/mL.

Chloroplast Thylakoids

Chloroplast thylakoids were isolated by first grinding the deveined leaf pieces for 5 s with an Ultra-Turrax (Janke et Kunkel) homogenizer in 50 mM phosphate buffer (pH 7.4) containing 300 mM sucrose, 5 mM MgCl$_2$, and 1 mM EDTA. The homogenate was filtered through one layer of nylon cloth and centrifuged for 5 min at 1000g. The chloroplasts were shocked osmotically by suspending the pellet in buffer solution containing 5 mM sucrose, 10 mM phosphate buffer (pH 7.4), and 5 mM MgCl$_2$, centrifuged for 5 min in 2000g, and finally suspended in 10 mM phosphate buffer (pH 7.4), containing 100 mM sucrose, 5 mM NaCl, and 10 mM MgCl$_2$. Chl was determined according to Arnon (1).

The apparent quantum yield of photosynthetic oxygen evolution at CO$_2$ saturation and the light-saturated activities of PSII (H$_2$O to PPBQ) and whole-chain electron transfer (H$_2$O to MV) were measured with Hansatech oxygen electrodes as in Mäenpää *et al.* (18). Occasionally, DCMU (10 μM) was added to the PSII reaction mixture, and it completely blocked the electron transfer to PPBQ. The PSI electron transfer activity was measured with a Hansatech DW oxygen electrode in saturating red light in 40 mM phosphate buffer (pH 7.4), 1 mM NaCl, 0.6 mM NaN$_3$, 0.12 mM MV, 0.3 mM DCPIP, 32 mM sodium ascorbate, and 0.01 mM DCMU. The Chl concentration was 10 μg/mL. The duroquinol-dependent PSI activity was measured in the same basic medium, from which DCPIP and ascorbate were omitted. The duroquinol was prepared from duroquinone and the measurement was carried out as described previously (24).

RESULTS

The differences between photoinactivation at RT and 1°C were resolved by comparing the effects of high or moderate light on several photosynthetic parameters of pumpkin leaves and thylakoids.

Apparent Quantum Yield and Fluorescence Induction of Pumpkin Leaves

All the light treatments led to a decrease in the variable part of fluorescence measured at 77°K (Table I). This decrease was due both to a decrease in F_{max} and to an increase in F_o. The ratio of variable to maximum fluorescence, F_V/F_{max}, decreased only slightly more at 1°C than at room temperature (Table I).

The apparent quantum yield of photosynthetic oxygen evolution declined during all light treatments except the mild-

Table I. *Effect of Light on 77°K Fluorescence Induction, Rate Constants for PSII Photochemistry (k_p), and Radiationless Dissipation of Excitation Energy in PSII (k_{DT}), and Apparent Quantum Yield of Photosynthetic Oxygen Evolution (ϕ_{app}), of Pumpkin Leaves (% of control)*

Mean and SE of three independent experiments. The plants were treated for 2 h either at RT or at 1°C. The rate constants k_p and k_{DT} were calculated from the fluorescence data.

Para-meter	Control Value	Plants Treated at RT		Plants Treated at 1°C		
		PPFD 750 μmol	PPFD 1500 μmol	PPFD 750 μmol	PPFD 1500 μmol	Darkness
		$m^{-2} s^{-1}$		$m^{-2} s^{-1}$		
F_o		103.2 (3.8)	126.0 (3.6)	108.7 (4.3)	126.7 (3.3)	99.4 (4.0)
F_{max}		76.0 (3.5)	59.6 (2.0)	60.3 (0.7)	51.0 (2.1)	100.4 (3.8)
F_v/F_{max}	0.83 ± 0.001	92.2 (2.2)	77.7 (0.7)	82.1 (1.1)	71.3 (1.2)	100.2 (0.4)
k_P		89.7 (5.6)	62.0 (2.3)	76.0 (3.9)	56.3 (2.0)	101.4 (4.3)
k_{DT}	13.4 ± 0.708	139.6 (7.5)	167.9 (3.7)	186.6 (7.5)	191.8 (2.2)	106.7 (3.7)
ϕ_{app}	0.055 ± 0.002	101.2 (15.3)	73.1 (9.8)	42.1 (9.4)	26.3 (3.6)	86.5 (5.8)

est ones at RT (Table I). At room temperatures, this decline was linearly correlated with the decrease in F_v/F_{max}, but at 1°C, the apparent quantum yield declined much more than F_v/F_{max} (Fig. 1).

The relative rate constant for PSII photochemistry (k_P) was lowered by the light treatments (Table I). The decrease in k_P was only slightly greater at 1°C than at RT. The rate constant for nonradiative, nonphotochemical dissipation of excitation energy of PSII (k_{DT}), increased more at 1°C than at RT (Table I).
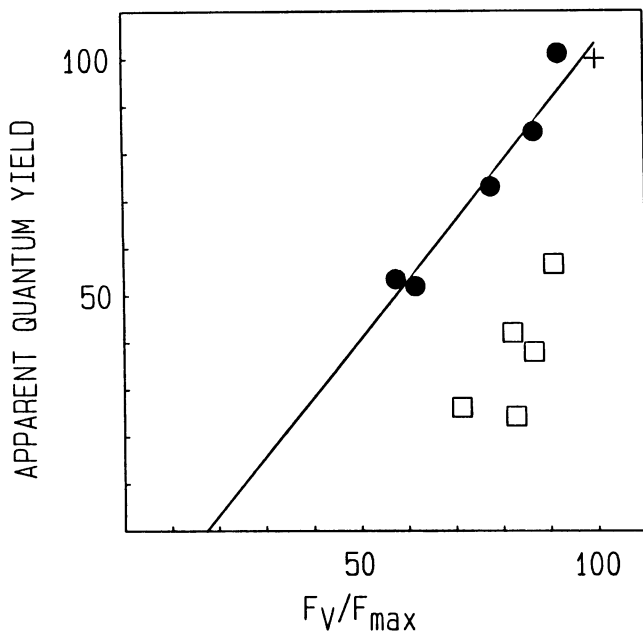

Figure 1. Relationship between the ratio of variable to maximum fluorescence at 77°K and apparent quantum yield of photosynthetic oxygen evolution of pumpkin leaf discs measured after light treatments at RT (●) or 1°C (□). The control value is shown by a cross (+). Each point represents the average of three similar, independent experiments. All values are expressed as percentage of the control. The regression line is calculated from the room temperature data including the control point ($r = 0.978$). The details of the treatments are given in "Materials and Methods."

Electron Transfer Capacity and Fluorescence Induction of Isolated Thylakoids

High light treatment of leaves caused PSII activity of thylakoids to decrease (Fig. 2). In the light treatments at RT, whole-chain electron transfer capacity decreased approximately as much as PSII activity decreased. However, light treatment at 1°C led to a much greater loss of whole-chain electron transfer capacity than would be expected from the decrease in PSII activity. PSI electron transfer capacity was not affected by the treatments (Fig. 2).

The results from fluorescence induction studies of isolated thylakoids paralleled those from intact leaves (Table II). The β_{max} value showed a marked increase during all the light treatments.

Chilling in Darkness

Chilling in darkness (120 min, 1°C) did not affect fluorescence, and caused only a slight decline in ϕ_{app} in pumpkin leaves (Table I). Dark-chilling of leaves did not affect the electron transfer activities of thylakoids (not shown).

Recovery

The light reaction activities and the F_o, F_{max}, and F_v/F_{max} values of the isolated thylakoids returned to control values, when the plants treated either at a PPFD of 2500 μmol m^{-2} s^{-1} at RT for 120 min or at 750 μmol m^{-2} s^{-1} at 1°C for 60 min had been kept for 24 h in the growth conditions. The β_{max} values were still somewhat higher than in the control thylakoids (Table III).

DISCUSSION

Light treatments at RT caused typical symptoms of photoinhibition: apparent quantum yield, PSII electron transfer activity and F_v/F_{max} at 77°K decline concomitantly (Table I, Figs. 1 and 2). When the light treatment was given at 1°C, the correlation between phenomena related to the primary activity of PSII (F_v/F_{max}, electron transfer from H_2O to PPBQ) and the inhibition of ϕ_{app} was broken (Figs. 1 and 2). The more pronounced decrease in ϕ_{app} shows that the photoinac-
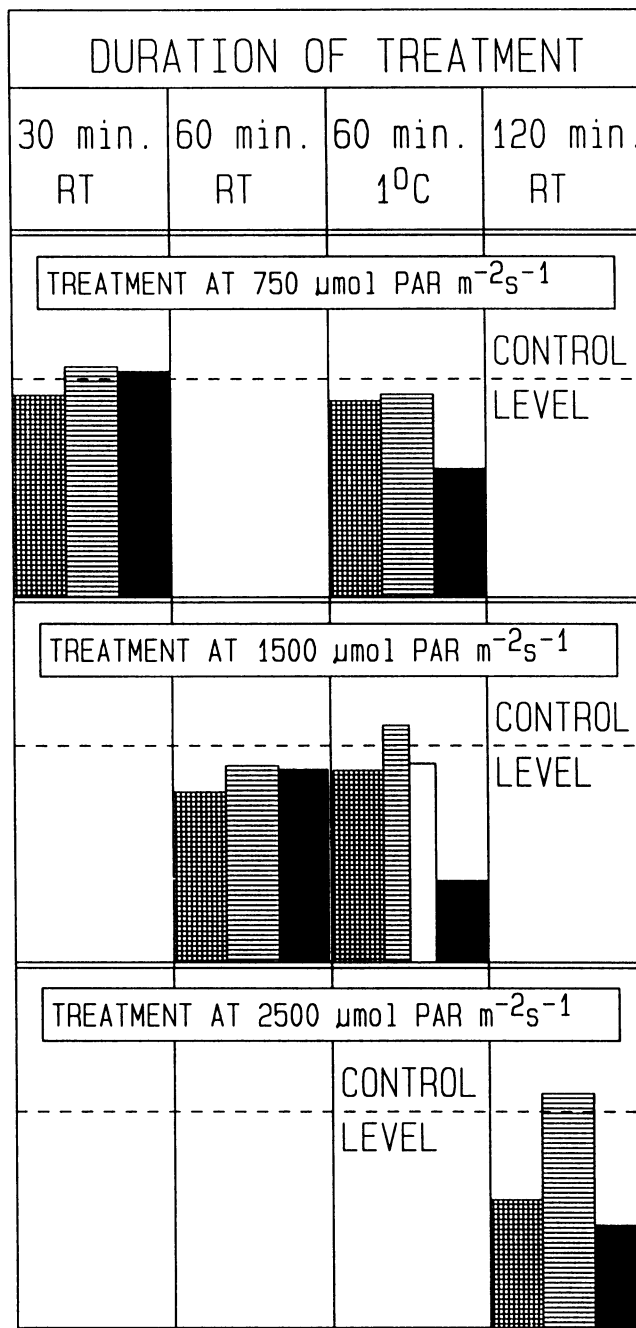
Figure 2. PSII (H_2O to PPBQ, cross-hatched bars), PSI (ascorbate-DCPIP to MV, horizontally hatched bars), PSI (duroquinol to MV, the open bar), and whole-chain (H_2O to MV, black bars) electron transfer capacities of thylakoids isolated from pumpkin leaves after light treatments at a PPFD of 750, 1500, and 2500 μmol m^{-2} s^{-1}, at RT or at 1°C. all values are expressed as percentage of the control. Means of three independent experiments (PSII and WC), or two experiments (PSI). The means of the control values were (n = 8): PSII, 99.9 ± 4.9 μmol O_2 mg Chl^{-1} h^{-1}; WC, 95.9 ± 6.7 μmol O_2 mg Chl^{-1} h^{-1}; PSI (ascorbate-DCPIP to MV), 205.5 ± 29.6 μmol O_2 mg Chl^{-1} h^{-1}; PSI (duroquinol to MV), 143.2 ± 19.0 μmol O_2 mg Chl^{-1} h^{-1}.

tivation at 1°C is not restricted to photoinhibition of the primary activity of PSII. In particular, the relationship between ϕ_{app} and F_V/F_{max} deviates from the straight line obtained after photoinhibition at RT, if the treatment is done at 1°C (Fig. 1). A linear relationship between F_v/F_{max} (5) or F_v (11) and ϕ_{app} has been reported from several plant species after photoinhibitory treatments, and a decline in F_v/F_{max} has often been used to measure photoinhibitory damage.

Photoinactivation is reversible at both 1°C and RT (Table III), and light is an essential factor at 1°C, too (Table I). The main site of inhibition of the electron transfer chain seems to be different at RT at 1°C. Inhibition of electron transfer from H_2O to PPBQ can explain the reduction of ϕ_{app} during treatments at RT. The reduction of the whole-chain electron transfer activity at 1°C in the light (Fig. 2) points to an additional site of inhibition. This additional inhibition is likely to be the reason for the dramatic reduction of ϕ_{app} at 1°C.

The mechanism of the inhibition of the quantum yield and the whole-chain electron transfer capacity was not resolved in this study. Possible inhibition on the reducing side of the PQ pool was ruled out by experiments using duroquinol as the electron donor in PSI activity measurement (Fig. 2). Neither the plastoquinone reduction site at PSII seems to be involved, since the PSII electron transport from H_2O to PPBQ was strictly DCMU-sensitive. However, we suggest that the preferential decline in the WC activity at 1°C was the result of a conformational change that rendered the plastoquinone reduction site ineffective in reducing PQ bud did not affect PPBQ reduction or DCMU binding. A change in the properties of the Q_B site has been reported as one of the first symptoms of photoinhibition of *Chlamydomonas* (21).

Loss of WC activity could also result from a reduction in the diffusion rate of PQ due to changes in membrane lipids during chilling in the light. However, neither saturation of membrane lipids nor segregation of nonbilayer lipids of the thylakoid membrane into other structures were observed in connection with similar reduction of WC activity after 4 d light-chilling of pumpkin (J Ovaska, P Mäenpää, A Nurmi, E-M Aro, unpublished data).

The increase in β_{max} during the light treatments (Table II) indicates a change in the ratio of PSII$_\alpha$ to PSII$_\beta$. An increase in β_{max} has earlier been reported after light-chilling treatment of maize (12) and pumpkin (18). Since PSII$_\beta$ is less sensitive to photoinhibition than PSII$_\alpha$ (17), and these two types of PSII are interconvertible (26), it is tempting to consider the increase in β_{max} an indication of protection against photodamage. The proportion of PSII$_\beta$ centers could also increase because mainly the PSII centers are photoinhibited. This, however, does not seem to be the case, as the increase of β_{max} was not accompanied by a similar decrease in the area above the fluorescence curve, which indicates that the total amount of Q_A did not decrease (data not shown). The newly formed PSII$_\beta$ centers may be photochemically less efficient than PSII.

Increased conversion of excitation energy to heat has frequently been suggested to protect plants against high light (4, 7, 14). The increase in k_{DT} (Table I) supports this idea, because an increase in thermal dissipation of excitation energy is the most likely reason to the increase in k_{DT}. The plant seems to

Table II. *Effects of PPFD, Temperature, and Duration of the Treatment on Fluorescence Characteristics Measured on Isolated Thylakoids (in the presence of 20 μM DCMU or at 77°K)*

77°K values of leaves are given for comparative purpose. All values expressed as % of control. Means of three independent experiments. SE parentheses. The control values are shown.

Treatment			DCMU-Poisoned Thylakoids				Thylakoids (77°K)		Leaves (77°K)	
PPFD	Duration	Temperature	F_v/F_{max}		β_{max}		F_v/F_{max}		F_v/F_{max}	
$\mu mol\ m^{-2}\ s^{-1}$	min									
750	30	RT	91.5	(0.6)	110.8	(9.7)	96.4	(1.7)	97.7	(1.3)
1500	60	RT	83.5	(2.1)	247.7	(20.8)	86.6	(2.7)	86.9	(2.6)
2500	120	RT	62.7	(3.1)	173.9	(12.7)	70.5	(1.5)	57.5	(2.7)
750	60	1°C	99.9	(2.0)	170.5	(19.3)	90.9	(0.5)	86.6	(1.5)
1500	60	1°C	79.7	(0.8)	198.2	(5.5)	86.1	(1.2)	82.7	(2.3)
Control value			0.74	(0.01)	0.32	(0.02)	0.79	(0.00)	0.83	(0.001)

Table III. *Fluorescence and Electron Transfer Properties of Thylakoids Isolated from Pumpkin Leaves after 24-h Recovery from Light Treatment at RT or 1°C*

All values expressed as % of control. Mean and SE of three independent experiments.

Treatment Before Recovery		PSII	WC	F_v/F_{max} (77°K)	F_v/F_{max} (DCMU, 20°C)	β_{max}
PPFD	Mean	94.4	108.1	99.3	98.6	126.1
2500 $\mu mol\ m^{-2}\ s^{-1}$ 120 min, RT	SE	10.1	6.3	0.2	0.3	26.1
PPFD 750	Mean	104.7	103.4	100.6	99.0	154.9
$\mu mol\ m^{-2}\ s^{-1}$ 60 min, 1°C	SE	9.1	2.9	2.5	0.4	27.8

regulate k_{DT} in response to the amount of excess excitation energy, as this constant increased more at 1°C than at RT.

A difference in the photoinhibitory quantum flux received by chloroplasts on the two sides of the leaf (15) could complicate the interpretation of the results from isolated thylakoids. However, in pumpkin, the results from fluorescence induction experiments on isolated thylakoids at 77°K are similar to the results for leaf discs at 77°K (Table II).

The responses of chilling sensitive plants to low temperature and light may differ quantitatively from the responses of chilling tolerant plants. In the chilling tolerant *Lemna*, photoinactivation seems to show the same symptoms at warm and cold temperatures (20). In addition to the results obtained in this study, chilling sensitive plants may also show chill-induced inhibition of the enzymes of the Calvin cycle (25) and feedback inhibition of photosynthesis by accumulating photosynthates (2, 3), when translocation is reduced by low temperatures.

Finally, we have shown that fluorescence induction measurements at 77°K, though ideal for monitoring room-temperature photoinhibition, may underestimate the inactivation of the photosynthetic apparatus if the light treatment is given at a low temperature.

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