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Photosystem II Excitation Pressure and Development of Resistance to Photoinhibition[1]

II. Adjustment of Photosynthetic Capacity in Winter Wheat and Winter Rye

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Winter wheat (*Triticum aestivum* L. cv Monopol), spring wheat (*Triticum aestivum* L. cv Katepwa), and winter rye (*Secale cereale* L. cv Musketeer) grown at 5°C and moderate irradiance (250 μmol m^{-2} s^{-1}) (5/250) exhibit an increased tolerance to photoinhibition at low temperature in comparison to plants grown at 20°C and 250 μmol m^{-2} s^{-1} (20/250). However, 5/250 plants exhibited a higher photosystem II (PSII) excitation pressure (0.32–0.63) than 20/250 plants (0.18–0.21), measured as 1 − q_P, the coefficient of photochemical quenching. Plants grown at 20°C and a high irradiance (800 μmol m^{-2} s^{-1}) (20/800) also exhibited a high PSII excitation pressure (0.32–0.48). Similarly, plants grown at 20/800 exhibited a comparable tolerance to photoinhibition relative to plants grown at 5/250. In contrast to a recent report for *Chlorella vulgaris* (D.P. Maxwell, S. Falk, N.P.A. Huner [1995] Plant Physiol 107: 687–694), this tolerance to photoinhibition occurs in winter rye with minimal adjustment to polypeptides of the PSII light-harvesting complex, chlorophyll *a/b* ratios, or xanthophyll cycle carotenoids. However, Monopol winter wheat exhibited a 2.5-fold stimulation of sucrose-phosphate synthase activity upon growth at 5/250, in comparison to Katepwa spring wheat. We demonstrate that low-temperature-induced tolerance to photoinhibition is not a low-temperature-growth effect per se but, instead, reflects increased photosynthetic capacity in response to elevated PSII excitation pressure, which may be modulated by either temperature or irradiance.

Photoinhibition has been defined as a light-dependent decrease in photosynthetic efficiency that may or may not be associated with a decrease in PS_{max} as a result of the absorption of excess light energy (Powles, 1984; Krause, 1988; Osmond, 1994). Thus, photoinhibition manifests itself in vivo as a decrease in ϕO_2 or ϕCO_2 and a reduction in the photochemical efficiency of PSII, as well as a reduction in PS_{max} (Krause, 1988; Osmond, 1994). PSII appears to be the primary target for photoinhibition and it has been proposed that photoinhibition is the result of an overreduction of PSII, which may result in damage to the 32-kD, PSII reaction center D1 polypeptide (Aro et al., 1993a, 1993b). Alternatively, this laboratory and several others have suggested that photoinhibition may be considered a mechanism to protect PSII from overexcitation through the down-regulation of PSII photochemistry (Krause, 1988; Hurry et al., 1992; Öquist et al., 1992b; Huner et al., 1993; van Wijk and van Hasselt, 1993; Krause, 1994).

It was first proposed by Ögren (1991) and subsequently supported by Öquist et al. (1992a, 1992b) that photoinhibition is related to the redox state of PSII. This relationship was examined for a variety of species, and it was concluded that a fundamental feature of photoinhibition among all taxonomic groups was its dependence on PSII excitation pressure, measured as 1 − q_P $\{(Q_A)_{red}/[(Q_A)_{red} + (Q_A)_{ox}]\}$ (Ögren and Rosenqvist, 1992). Thus, susceptibility to photoinhibition has been shown to be correlated with the redox state of PSII, regardless of the environmental constraints on photosynthesis brought about by low temperature or light acclimation (Ögren and Rosenqvist, 1992; Öquist et al., 1992a, 1992b, 1993b).

Low temperature, in combination with light, increases the susceptibility of photosynthesis to photoinhibition (Powles, 1984; Öquist and Martin, 1986; Greer, 1990; Osmond, 1994). This is thought to occur primarily through a temperature suppression of the PSII repair cycle (Greer et al., 1986, 1991; Kyle, 1987; Chow et al., 1989; Gong and Nilsen, 1989; Aro et al., 1993b). However, it has recently been established that, given a certain excitation pressure on PSII, the susceptibility of photosynthesis to photoinhibition occurs independently of temperature between 0 and 25°C (Öquist et al., 1993b). It was concluded that photoinhibition

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Abbreviations: CABP, 2-carboxyarabinitol 1,5-bisphosphate; CVS, conversion state of the xanthophyll pool; EPS, epoxidation state of the xanthophyll pool; FBPase, Fru-1,6-bisphosphatase; F_M, maximal fluorescence with all PSII reaction centers closed in the dark-adapted state; F_O, minimal fluorescence with all PSII reaction centers open in the dark-adapted state; F_V, variable fluorescence ($F_M - F_O$); F_V/F_M, photochemical yield of PSII in the dark-adapted state; LHCII, light-harvesting complex associated with PSII; ϕCO_2, quantum yield of CO_2 exchange; ϕO_2, quantum yield of O_2 evolution; PS_{max}, maximum light-saturated rate of photosynthesis; PS_{max} O_2, maximum light-saturated rate of O_2 evolution; Q_A, primary stable quinone electron acceptor of PSII; $(Q_A)_{ox}$, oxidized form of Q_A; $(Q_A)_{red}$, reduced form of Q_A; q_P, coefficient of photochemical quenching; 1 − q_P, proportion of reduced Q_A; RuBP, ribulose-1,5-bisP; SPS, Suc-P synthase.

at low temperature is sensitized by a temperature inhibition of photosynthesis and not a low-temperature inhibition of the PSII repair cycle (Hurry and Huner, 1992; Huner et al., 1993; Öquist et al., 1993b).

Winter cultivars of rye (*Secale cereale*), wheat (*Triticum aestivum*), and the herbaceous dicot spinach (*Spinacia oleracea*) grown at low temperature (5°C) and moderate irradiance (250 μmol m^{-2} s^{-1}) exhibit an increased tolerance to photoinhibition as measured by both F_V/F_M and photosynthetic gas exchange (Somersalo and Krause, 1989, 1990; Boese and Huner, 1990; Hurry and Huner, 1992; Huner et al., 1993; Öquist and Huner, 1993). Growth at low temperature is an absolute requirement for acquisition of this tolerance to photoinhibition (Gray et al., 1994). It has been documented that shifts to low temperature do not induce tolerance to photoinhibition, irrespective of either photoperiod or cold-hardening protocol (Öquist and Huner, 1991; Boese and Huner, 1992; Gray et al., 1994). In addition, the degree of tolerance to photoinhibition in wheat is cultivar dependent, with the winter cv Monopol exhibiting a greater tolerance to photoinhibition than the spring cv, Katepwa, after growth at low temperature. However, cold-grown cultivars are more tolerant to photoinhibition than their warm-grown counterparts (Hurry and Huner, 1992; Hurry et al., 1992). Tolerance to photoinhibition is usually associated with the pre-exposure of plants to high irradiance (Powles, 1984; Anderson, 1986; Tyystjärvi et al., 1991; Ögren and Rosenqvist, 1992; Öquist et al., 1992a; Aro et al., 1993a; Osmond, 1994). Thus, low-temperature-induced tolerance to photoinhibition is unique in that 5°C-grown plants require only moderate irradiance (250 μmol m^{-2} s^{-1}) to become tolerant to light 6-fold higher than the growth irradiance.

We have demonstrated previously that the growth of winter cereals at low temperature results in an increased capacity to keep Q_A oxidized under photoinhibitory conditions (Öquist and Huner, 1993; Öquist et al., 1993b). In addition, winter cereals grown at 5°C modulate their rates of photosynthesis so as to increase PS_{max} O_2 with no change in ϕO_2 (Huner et al., 1993). This increase in PS_{max} in cold-grown plants is associated with increased Pi availability, which allows these plants to maintain a larger pool of Q_A in the oxidized state (Huner et al., 1993; Hurry et al., 1993). When the reduction state of Q_A is artificially equalized in winter rye, the sensitivity to photoinhibition is the same for 5- and 20°C-grown leaves (Öquist and Huner, 1993; Öquist et al., 1993b). Furthermore, the 5°C leaves require photon fluxes that are 3-fold greater than that of the 20°C leaves to attain the same redox state of Q_A (Öquist and Huner, 1993). The ability to maintain a greater proportion of Q_A in the oxidized state through increased PS_{max} is thought to account, in part, for the differential tolerance to photoinhibition observed between 5- and 20°C-grown plants (Öquist and Huner, 1993; Öquist et al., 1993b).

Recently, it has been reported that growth of the green alga *Chlorella vulgaris* at low temperature mimics high-light acclimation (Maxwell et al., 1994). Growth of cells at 5°C resulted in a 2-fold increase in the Chl *a*/*b* ratio and a lower abundance of LHCII polypeptides in comparison to cells grown at 27°C, even though irradiance was kept constant at 150 μmol m^{-2} s^{-1}. However, when 5°C cells were grown at a 30-fold lower irradiance (5 μmol m^{-2} s^{-1}) and hence a lower PSII excitation pressure, these differences were alleviated. In addition, 5°C-grown cells exhibited a greater tolerance to photoinhibition because of reduced absorption of light as a consequence of lower Chl $cell^{-1}$ and increased dissipation of excess light energy through the carotenoid zeaxanthin, resulting in an increased capacity to keep Q_A oxidized (Maxwell et al., 1995a). Therefore, *C. vulgaris* adjusts its photosynthetic apparatus in response to PSII excitation pressure rather than to either low temperature or irradiance per se. Similar trends have been observed for the green alga *Dunaliella salina* (Maxwell et al., 1995b) and the cyanobacterium *Plectonema boryanum* (S. Falk, D.P. Maxwell, N.P.A. Huner, and D.E. Laudenbach, unpublished results).

Excitation pressure reflects the redox poise of intersystem electron transport and carbon metabolism and can be modulated independently by either light or temperature (Maxwell et al., 1994, 1995a, 1995b). How do plants grown at low temperature and moderate irradiance acquire a tolerance to light 6-fold higher than the growth irradiance? In a typical experiment, plants are grown at constant irradiance at either 5 or 20°C. Thus, it has been assumed that the development of low-temperature-induced tolerance to photoinhibition must be a growth temperature effect (Huner et al., 1993). However, we hypothesize that in higher plants, as in green algae (Maxwell et al., 1995a), increased tolerance to photoinhibition observed upon growth at low temperature is not a growth temperature effect per se but, rather, reflects photosynthetic adjustment to high PSII excitation pressure. In this report, we test this hypothesis and show that tolerance to photoinhibition in wheat and rye does indeed represent a response to PSII excitation pressure as reported for *C. vulgaris* (Maxwell et al., 1994, 1995a). However, the photosynthetic adjustment induced by growth under elevated excitation pressure in cereals is distinct from that observed in green algae.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Winter wheat (*Triticum aestivum* L. cv Monopol), spring wheat (*Triticum aestivum* L. cv Katepwa), and winter rye (*Secale cereale* L. cv Musketeer) were germinated from seed in coarse vermiculite either at a temperature of 5/5°C or 20/16°C (day/night) with a 16-h photoperiod in controlled environment growth chambers (Conviron, Winnipeg, Manitoba, Canada). Fluorescent tubes (Cool White, 160 W, F72T12/CW/VHO, Sylvania) provided PAR, which was adjusted to a PPFD of 250 μmol m^{-2} s^{-1} at 5°C (5/250) and either 50, 250, or 800 μmol m^{-2} s^{-1} at 20°C (20/50, 20/250, or 20/800, respectively). Supplemental lighting was provided in the 20/800 growth chamber by a metal halide lamp (MS400-HOR, 400 W; Venture Lighting International, Cleveland, OH). The PPFD was measured at pot height with a Li-Cor (Lincoln, NE) Quantum/Radiometer/Photometer (model LI-189) equipped with a model LI-190SA

quantum sensor (Li-Cor). Hoagland nutrient solution was supplied to all plants as required. Growth kinetic analyses indicated that 6- to 8-week-old leaves from plants germinated and grown at 5°C were of a developmental age comparable to that of 2- to 3-week-old leaves from plants germinated and grown at 20°C (G.R. Gray and N.P.A. Huner, unpublished data). Fully expanded second, third, and fourth leaves were used in all experiments.

Pigment Extraction and Determination

Pigments were extracted from leaf samples by homogenization in 100% HPLC-grade acetone (OmniSolv; BDH Inc., Toronto, Ontario, Canada) with 0.3 mg mL^{-1} $CaCO_3$ at 4°C in dim light. After the sample was centrifuged at 6000*g* for 5 min, the supernatant was removed and passed through a 0.22-μm syringe filter (Micron Separations Inc., Westborough, MA) prior to storage under nitrogen at −20°C.

HPLC analysis of leaf acetone extracts was performed using a Beckman System Gold Solvent Module equipped with an analytical CSC-Spherisorb ODS-1 reverse-phase column (5-μm particle size, 250 × 4.6 mm i.d.) and an Upchurch Perisorb A guard column (Chromatographic Specialties Inc., Concord, Ontario, Canada). The protocol of Gilmore and Yamamoto (1991) was utilized with minor modifications. The samples were injected using a Beckman 210A sample-injection valve with a 20-μL sample loop. Pigments were then eluted isocratically for 6 min with a solvent system of 100% of acetonitrile:methanol:0.1 M Tris-HCl (pH 8.0) (74:11:3.5, v/v) followed by a 2-min linear gradient to 100% of methanol:ethylacetate (68:32, v/v), which continued isocratically for 4 min. The total run time was 12 min with a flow rate of 2 mL min^{-1}. All solvents were of HPLC grade (OmniSolv).

The A_{440} of the pigments was detected using a diode array detector (System Gold), and peak areas were integrated using the System Gold software (Beckman). Retention times and response factors of β-carotene, lutein, Chl *a*, and Chl *b* were determined by the injection of known quantities of pure standards (Sigma). The retention times of neoxanthin, antheraxanthin, violaxanthin, and zeaxanthin were determined using pigments isolated from barley by TLC (Diaz et al., 1990; Hurry et al., 1992).

The EPS and CVS of the samples were estimated according to the methods of Thayer and Björkman (1992) and Adams and Demmig-Adams (1995), respectively, using the following equations:

$$EPS = (V + 0.5A)/(V + A + Z)$$

$$CVS = (A + Z)/(V + A + Z),$$

where *V*, *A*, and Z correspond to the concentrations of the xanthophyll carotenoids violaxanthin, antheraxanthin, and zeaxanthin, respectively. Xanthophyll pool size was calculated as the sum of violaxanthin, antheraxanthin, and zeaxanthin ($V + A + Z$).

To determine leaf *A*, Chl was extracted in 80% acetone buffered with 25 mM Hepes (pH 7.5) and quantified according to the equations of Porra et al. (1989) on a recording spectrophotometer (model UV-160; Shimadzu Corp., Kyoto, Japan). Leaf absorptance was calculated as described by Öquist et al. (1992b).

Isolation of Thylakoid Membranes

Thylakoids were isolated as described in detail by Huner (1985a) and solubilized in a buffer containing 60 mM Tris-HCl (pH 7.8), 12% (w/v) Suc, 2% (w/v) SDS, 1 mM EDTA, and 58 mM DTT. Samples were heated in a boiling water bath for 90 s prior to electrophoresis.

SDS-PAGE and Immunoblotting

Solubilized membrane polypeptides were separated by SDS-PAGE using a Mini-Protean II apparatus (Bio-Rad) and the discontinuous buffer system of Laemmli (1970). Electrophoresis was performed using a 5% (w/v) stacking gel and a 15% (w/v) resolving gel prepared according to the procedure of Piccioni et al. (1982). All samples were loaded on an equal Chl basis (3 μg $lane^{-1}$), and a constant current of 15 mA was applied for approximately 1.5 h at 20°C. Polypeptides were either stained with 0.2% (w/v) Coomassie brilliant blue R-250 or electrophoretically transferred (Mini-Trans Blot, Bio-Rad) to nitrocellulose membranes (0.2-μm pore size, Bio-Rad) by applying a constant current of 295 mA for 1 h in transfer buffer as described by Towbin et al. (1979). After blocking the membrane with 4% (w/v) BSA in PBS containing 0.2% (v/v) polyoxyethylene sorbitan monolaurate, the membrane was incubated with a 1:5,000 dilution of a rabbit polyclonal primary antibody raised against the spinach 26-kD Chl *a*/*b*-binding protein of LHCII (Krol et al., 1995). After the samples were washed with the blocking solution, the polypeptide-primary antibody complexes were incubated with a goat anti-rabbit IgG conjugated with horseradish peroxidase (Sigma) as a secondary antibody at a 1:20,000 dilution. The complexes were visualized using a chemiluminescent detection system (ECL, Amersham) and X-Omat RP film (Eastman Kodak).

Photosynthetic O_2 Evolution

CO_2-saturated O_2 evolution of 10-cm^2 discs, composed of segments cut from the middle of several leaves, was measured in the gas phase at 20°C with an O_2 electrode (model LD2; Hansatech Instruments Ltd., King's Lynn, UK) as described in detail previously (Boese and Huner, 1990; Hurry et al., 1992; Gray et al., 1994). Irradiance-response curves were obtained by using 13 irradiance values over the range of 0 to 800 $\mu mol\ m^{-2}\ s^{-1}$ PPFD and were corrected for the levels of dark respiration. The ϕO_2 was calculated by regression analysis of points in the linear, light-limiting range of the irradiance-response curves (0–50 $\mu mol\ m^{-2}\ s^{-1}$ PPFD) and corrected for leaf *A* as described by Öquist et al. (1992b). Values of PS_{max} O_2 were obtained essentially as described above except that saturating white light (3000 $\mu mol\ m^{-2}\ s^{-1}$ PPFD) was supplied from a Hansatech light source (model LS2H).

Photoinhibitory Treatments

Photoinhibition of photosynthesis was induced at 5°C under ambient air conditions using the adaxial sides of leaf segments placed on moist filter paper in aluminum trays as previously described (Hurry and Huner, 1992; Öquist and Huner, 1993). Susceptibility to photoinhibition was quantified by monitoring changes in F_V/F_M as a function of exposure time to an irradiance of 1600 μmol m^{-2} s^{-1} measured at the leaf surface from a bank of three high-pressure sodium pressure lamps (CGE Lucalox, LU-400; Canadian General Electric, Toronto, Ontario, Canada). Temperature at the leaf surface was monitored using a constantan-copper (type T) thermocouple in conjunction with a temperature logger (model MDSS41-TC:G1; Omega Engineering Inc., Stamford, CT) and did not exceed 6°C (data not shown).

Chl *a* Fluorescence Measurements

For all photoinhibition experiments, in vivo fluorescence was measured using a Plant Stress Meter (Biomonitor S.C.I. AB, Umeå, Sweden) as described by Gray et al. (1994). Leaves were sampled 2 h into the photoperiod and dark adapted at room temperature for 1 h prior to measurement of F_V/F_M.

Steady-state fluorescence quenching characteristics were determined in vivo under ambient CO_2 conditions using a PAM 101 Chl fluorometer (Heinz Walz, Effeltrich, Germany) as described in detail by Öquist and Huner (1993). Leaves were dark adapted at the measuring temperature for 1 h prior to the onset of measurement. The q_P was calculated according to the method of van Kooten and Snel (1990) taking into account F_O quenching as described by Bilger and Schreiber (1986). Excitation pressure was calculated as $1 - q_P$ measured at the growth temperature and growth irradiance (Dietz et al., 1985; Demmig-Adams et al., 1990; Ögren, 1991).

Enzyme Extractions and Assays

For all assays, fully expanded third and fourth leaves were harvested 4 h into the photoperiod and quickly frozen at −80°C. Before the assays were performed, leaf tissue was ground in a glass homogenizer with the appropriate extraction buffer and centrifuged at 16,000*g* for 2 min. The supernatant was used immediately for the enzyme assays, which were performed at 25°C. All chemicals were purchased from Sigma.

FBPase was assayed spectrophotometrically according to the procedure of Sharkey et al. (1991b) using an extraction buffer containing 20 mM Hepes-NaOH (pH 7.5), 125 mM NaCl, 400 μM EDTA, 0.02% (w/v) BSA, and 2% (w/v) polyvinylpolypyrrolidone. Cytosolic FBPase activity was determined in an assay buffer containing 100 mM Hepes-NaOH (pH 7.5), 100 mM KCl, 4 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM NADP, 2 units of phosphoglucoisomerase, and 1 unit of Glc-6-P dehydrogenase. The reaction was initiated by the addition of 50 μM Fru-1,6-bisP. Stromal FBPase activity was measured in an assay buffer containing 100 mM Bicine (pH 8.8), 0.5 mM EDTA, 50 mM DTT, 0.5 mM NADP, 2 units of phosphoglucoisomerase, and 1 unit of Glc-6-P dehydrogenase. The reaction was initiated by the addition of 0.4 mM Fru-1,6-bisP and 10 mM $MgCl_2$. The reduction of NADP was monitored at 340 nm and the FBPase rates were determined 5 min after the start of the reaction.

SPS was assayed according to the method of Vassey and Sharkey (1989). Leaf tissue was homogenized in an extraction buffer containing 50 mM Hepes-NaOH (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA, 2.5 mM freshly added DTT, and 0.1% (v/v) Triton X-100. After the sample was centrifuged at 16,000*g* for 2 min, the supernatant was mixed with 250 mg of Sephadex G-25, incubated on ice for 10 min, and subsequently centrifuged at 16,000*g* for 5 min. SPS activity was measured in the supernatant as the time-dependent formation of Suc plus Suc-P from UDP-Glc and Fru-6-P according to the method of Huber et al. (1991). SPS activity was determined with saturating substrate concentrations in the absence of Pi (V_{max} assay).

Rubisco activity was determined according to the method of Sharkey et al. (1991a) using an extraction buffer containing 100 mM Bicine (pH 7.8), 5 mM $MgCl_2$, 1 mM EDTA, 5 mM DTT, 1.5% (w/v) polyvinylpolypyrrolidone, and 0.02% (w/v) BSA. The extract was centrifuged at 16,000*g* for 2 min at 5°C, and 5 μL of the supernatant were immediately used to determine initial Rubisco activity. The total Rubisco activity was determined after the incubation of 1 mL of extract for 10 min with 20 mM $MgCl_2$ and 10 mM $NaHCO_3$. The carbamylation ratio of Rubisco was determined as initial Rubisco activity/total Rubisco activity. Total Rubisco protein was determined by using CABP to partially inhibit Rubisco activity in the leaf extract. Rubisco activity was plotted against the CABP concentration to estimate the total number of Rubisco sites to which CABP was bound. This value was divided by 8 to give the total amount of Rubisco protein.

Rubisco activity was determined in an assay buffer containing 50 mM Bicine (pH 8.0), 15 mM $MgCl_2$, 1 mM EDTA, 10 mM NaCl, 5 mM DTT, 10 mM $NaHCO_3$, 5 mM phosphocreatine, and 5 mM ATP in a final volume of 750 μL. The following were also added: 10 μL of 20 mM RuBP, 10 μL of 10 mM NADH, 10 units of glyceraldehyde-3-phosphate dehydrogenase/3-phosphoglyceric phosphokinase, and 2 units of creatine phosphokinase. The reaction was initiated by the addition of 5 μL of appropriate extract, and the oxidation of NADH was monitored as the difference between A_{334} and A_{405}.

RESULTS

Effect of Growth Regime on PSII Excitation Pressure

In experiments designed to elucidate low-temperature-growth effects, one typically compares plants grown at 5°C with control plants grown at 20°C with irradiance held constant. The assumption is that any changes observed must be due to a growth temperature effect. The reduction state of Q_A {i.e. $(Q_A)_{red}/[(Q_A)_{red} + (Q_A)_{ox}]$} can be estimated by steady-state Chl *a* fluorescence as $1 - q_P$ at the growth temperature and growth irradiance, reflecting PSII

excitation pressure (Dietz et al., 1985; Demmig-Adams et al., 1990; Ögren, 1991). The results in Table I indicate that at a constant growth temperature of 20°C, increasing growth irradiance from 50 to 800 μmol m^{-2} s^{-1} resulted in a 4- to 5-fold increase in PSII excitation pressure in both wheat and rye. However, we noted that wheat and rye grown at 5/250 exhibited PSII excitation pressures that were 1.6- to 3.5-fold greater, respectively, than plants grown at 20/250 (Table I). In fact, rye plants grown at 5/250 exhibited a PSII excitation pressure that was comparable to that of plants grown at 20°C but at an irradiance of 800 μmol m^{-2} s^{-1}. Similarly, wheat grown at 5/250 exhibited PSII excitation pressures that were approximately 33% higher than wheat grown at 20/800 (Table I). Although plants grown at 20/250 would normally be considered controls for those grown at 5/250, the latter were grown under significantly higher PSII excitation pressures than the former.

Chl Content, Chl *a/b*, and LHCII Abundance

The results for Monopol and Musketeer indicate that growth at 5/250 resulted in a 1.4- to 1.5-fold increase in Chl leaf $area^{-1}$ compared to plants grown at 20/250, whereas Katepwa exhibited a 17% decrease in Chl leaf $area^{-1}$ when grown under the same conditions (Table II). This is consistent with previous results for wheat and rye (Huner et al., 1993). Although growth temperature and growth irradiance had significant effects on total Chl leaf $area^{-1}$, leaf absorptance changed by less than 10% (data not shown). As expected, the Chl *a*/*b* ratios of wheat and rye leaves developed at 20°C increased by 10 to 20% upon exposure to increasing growth irradiance from 50 to 800 μmol m^{-2} s^{-1} (Table II). However, growth temperature had minimal effects on Chl *a*/*b* (Table II), which is consistent with previous results (Huner, 1985b; Hurry et al., 1992). The results for SDS-PAGE and immunoblotting (Fig. 1) indicated small changes in LHCII abundance as a function of growth irradiance but no apparent effects of growth temperature, which is consistent with the data for Chl *a*/*b* (Table II). Thus, growth of wheat and rye at high excitation pressure appears to have minimal effects on Chl *a*/*b* and LHCII abundance.

Effects of Growth Regime on Photosynthetic Characteristics

Development of wheat and rye at 20°C and a growth irradiance of 50 to 800 μmol m^{-2} s^{-1} resulted in minimal changes in F_V/F_M (Table II). This was consistent with measurements of ϕO_2, which varied less than 10% (0.088 ± 0.004 to 0.097 ± 0.002 mol O_2 mol^{-1} photons) for winter rye exposed to the four different temperature/irradiance growth regimes (data not shown). However, the increase in growth irradiance from 50 to 800 μmol m^{-2} s^{-1} did result in a 3-fold increase in PS_{max} O_2 measured at 20°C in all cultivars examined (Table II). This occurred independently of measuring temperature, since this same trend was observed in winter rye measured at 5°C (data not shown). Furthermore, increasing growth irradiance from 50 to 800 μmol m^{-2} s^{-1} resulted in an increased capacity to keep Q_A oxidized (q_P) regardless of measuring irradiance or measuring temperature (Fig. 2).

Wheat and rye leaves developed at 5/250 exhibited a 5 to 10% lower F_V/F_M ratio in comparison with those developed at 20/250 (Table II). Furthermore, the difference in the ϕO_2 between rye plants grown at 5/250 (0.092 ± 0.003 mol O_2 mol^{-1} photons) and 20/250 (0.088 ± 0.004 mol O_2 mol^{-1} photons) was less than 5%. These results are consistent with a previous report (Öquist and Huner, 1993). However, both winter wheat and rye grown at 5/250 exhibited a PS_{max} O_2 that was 1.2- and 2.1-fold higher, respectively, than that of plants grown at 20/250 (Table II). This same trend was observed in rye whether PS_{max} O_2 was measured at 20°C (Table II) or 5°C (data not shown). In contrast, Katepwa spring wheat grown at 5/250 exhibited a 10% lower PS_{max} O_2 than spring wheat grown at 20/250 (Table II). Furthermore, q_P measured as a function of irradiance for rye grown at 5/250 closely matched that of plants grown at 20/800 regardless of measuring temperature (Fig. 2). Thus, growth of winter wheat and rye at 5/250 appears to stimulate PS_{max} O_2 and increase the capacity to keep Q_A oxidized relative to plants grown at 20/250. However, this is correlated with the fact that winter wheat and winter rye grown at 5/250 were exposed to a high PSII excitation pressure, comparable to that of plants grown at 20/800 (Table I).

Effect of PSII Excitation Pressure on Enzymes of Carbon Metabolism

Since the photosynthetic capacity of Katepwa spring wheat was depressed upon growth at 5/250 compared to Monopol winter wheat, we examined four important regulatory enzymes of photosynthetic carbon metabolism in both winter (Table III) and spring (Table IV) cultivars. At 20°C, increasing the growth irradiance from 50 to 250 μmol m^{-2} s^{-1} resulted in a 2- to 4-fold increase in total Rubisco activity and total amount of Rubisco protein in both culti-

Table I. *Effect of growth regime on PSII excitation pressure in winter wheat (T. aestivum L. cv Monopol), spring wheat (T. aestivum L. cv Katepwa), and winter rye (S. cereale L. cv Musketeer)*

PSII excitation pressure is expressed as 1 - q_p and was determined at the growth temperature and growth irradiance. All values present means ± SE; n = 3.

Cultivar	1 - q_p at Growth Regime (°C μmol^{-1} m^{-2} s^{-1})			
	20/50	20/250	20/800	5/250
Monopol	0.097 ± 0.003	0.181 ± 0.006	0.465 ± 0.031	0.617 ± 0.051
Katepwa	0.087 ± 0.008	0.205 ± 0.018	0.476 ± 0.054	0.625 ± 0.010
Musketeer	0.074 ± 0.013	0.196 ± 0.017	0.318 ± 0.034	0.321 ± 0.047

Table II. *Photosynthetic characteristics of winter wheat (T. aestivum L. cv Monopol), spring wheat (T. aestivum L. cv Katepwa), and winter rye (S. cereale L. cv Musketeer) as a result of growth at various temperature and irradiance regimes*

Total Chl and Chl *a/b* ratios were determined by HPLC. PS_{max} O_2 was determined at 20°C with a saturating irradiance of 3000 μmol m^{-2} s^{-1}. The F_V/F_M ratio was measured using the Plant Stress Meter at room temperature following dark adaptation. All values represent means ± SE; $n = 3$.

Cultivar	Characteristic	Growth Regime (°C μmol^{-1} m^{-2} s^{-1})			
		20/50	20/250	20/800	5/250
Monopol	μg Chl cm^{-2}	240 ± 2	338 ± 3	269 ± 2	469 ± 2
	Chl *a/b*	3.14 ± 0.03	3.24 ± 0.4	3.57 ± 0.1	3.13 ± 0.09
	PS_{max} O_2[a]	9.8 ± 0.2	22.3 ± 0.7	31.5 ± 2.0	27.2 ± 0.6
	F_V/F_M	0.76 ± 0.03	0.79 ± 0.01	0.76 ± 0.01	0.71 ± 0.01
Katepwa	μg Chl cm^{-2}	161 ± 4	335 ± 2	318 ± 3	278 ± 1
	Chl *a/b*	3.21 ± 0.2	3.33 ± 0.05	3.88 ± 0.03	3.36 ± 0.02
	PS_{max} O_2[a]	9.7 ± 0.1	18.8 ± 0.7	27.1 ± 2.0	16.9 ± 0.4
	F_V/F_M	0.78 ± 0.01	0.78 ± 0.01	0.76 ± 0.02	0.70 ± 0.02
Musketeer	μg Chl cm^{-2}	275 ± 18	267 ± 14	388 ± 6	412 ± 51
	Chl *a/b*	2.86 ± 0.01	3.17 ± 0.07	3.57 ± 0.04	3.25 ± 0.01
	PS_{max} O_2[a]	10.4 ± 0.3	22.0 ± 0.6	35.6 ± 0.9	45.1 ± 1.0
	F_V/F_M	0.74 ± 0.01	0.75 ± 0.01	0.74 ± 0.02	0.71 ± 0.01

[a] μmol O_2 m^{-2} s^{-1}.

vars. In addition, the Rubisco carbamylation ratio increased 1.5-fold (Tables III and IV). However, as a result of growth at 20/800, spring wheat did not change Rubisco parameters relative to the 20/250 plants (Table IV). In contrast, winter wheat exhibited a 20% decrease in total amount and total activity of Rubisco and a 50% decrease in the Rubisco carbamylation ratio (Table III). Upon growth at 5/250, both wheat cultivars demonstrated a 15% increase in total Rubisco activity, a 30 to 40% increase in total amount of Rubisco protein, and a 20 to 50% increase in carbamylation ratio of Rubisco when compared with the same plants grown at 20/250 (Tables III and IV).

Winter and spring cultivars grown at 20°C under increasing irradiance from 50 to 800 μmol m^{-2} s^{-1} resulted in an increase of stromal and cytosolic FBPase and SPS activities for both cultivars. Growth of both Monopol and Katepwa at 5/250 resulted in stromal and cytosolic FBPase activities that increased 30 to 40 and 30 to 70%, respectively, in comparison to the same plants grown at 20/250. However, growth at 5/250 resulted in a 3-fold increase in SPS activity in the winter cv Monopol but did not affect the SPS activity in the spring cv Katepwa (Tables III and IV).

Plants grown under similar PSII excitation pressures (20/800, 5/250) were also compared. Katepwa spring wheat grown at 5/250 exhibited in a 20 to 30% decrease in cytosolic FBPase and SPS activity with no change observed in stromal FBPase activity in comparison to plants grown at 20/800 (Table IV). In contrast, Monopol winter wheat grown at 5/250 resulted in a 2.5-fold increase in SPS activity with no changes in stromal or cytosolic FBPase activity in comparison to plants grown at 20/800 (Table III). Thus, winter wheat appears to exhibit a specific, differential stimulation of SPS activity in response to growth at 5/250.

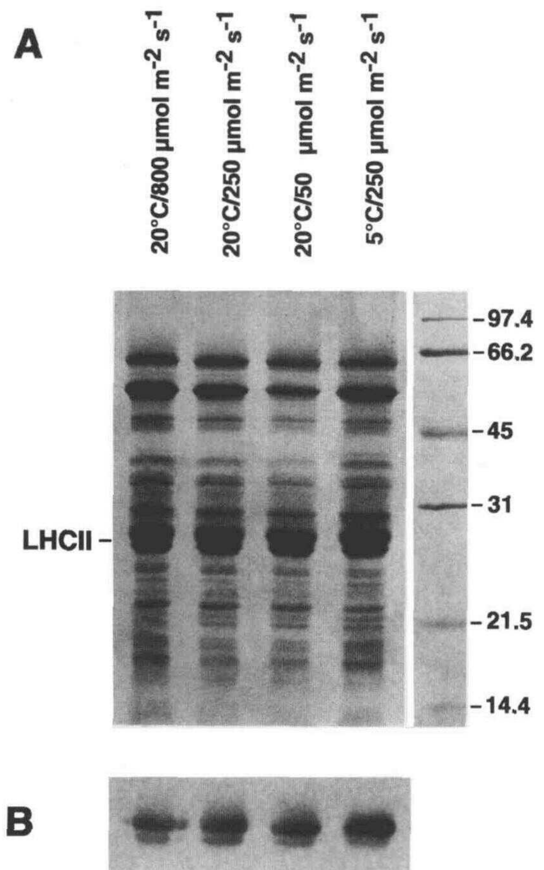

Figure 1. A, Coomassie blue-stained SDS-PAGE gel of thylakoid membrane proteins of winter rye (*S. cereale* L. cv Musketeer) grown under the indicated temperature/irradiance regimes. Lanes were loaded on an equal Chl basis (3 μg). LHCII polypeptides are indicated to the left. Molecular mass markers (kD) are indicated to the right. B, Immunoblot analysis of the 26-kD LHCII polypeptide from a duplicate gel of the above (see "Materials and Methods").

PSII Excitation Pressure and Tolerance to Photoinhibition

The results in Table V indicate that increasing growth irradiance of Musketeer rye from 50 to 800 μmol m^{-2} s^{-1}

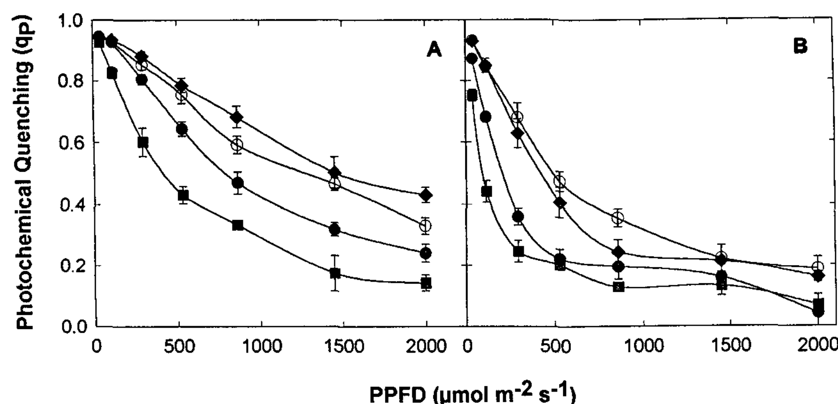

Figure 2. Effect of growth regime in winter rye (*S. cereale* L. cv Musketeer) on q_P as a function of irradiance measured at 20°C (A) and 5°C (B). Growth regimes were 20°C (closed symbols) and 5°C (open symbols) at 50 (■), 250 (●, ○), and 800 μmol m^{-2} s^{-1} (◆). Results are means ± SE; n = 3. When not present, error bars are smaller than symbol size.

had minimal effects on the content of β-carotene, lutein, and neoxanthin but resulted in a 2.7-fold increase in the xanthophyll pool size. However, no major changes in the CVS or EPS were observed. Regardless of growth regime, exposure to photoinhibition at 5°C resulted in minimal changes in all photosynthetic pigments but stimulated the conversion of violaxanthin to zeaxanthin, which resulted in a significant reduction in the EPS from approximately 0.9 to 0.3 and increased the CVS from approximately 0.1 to 0.8 (Table V). The same trends with respect to the effects of growth regime and photoinhibition were observed for winter and spring wheat (data not shown), which is consistent with a previous report (Hurry et al., 1992).

Regardless of the growth irradiance, the F_V/F_M of winter wheat (Fig. 3A), spring wheat (Fig. 3B), and winter rye leaves (Fig. 3C) developed at 20°C decreased with time during exposure to a photoinhibitory irradiance of 1600 μmol m^{-2} s^{-1} at 5°C. However, increasing the growth irradiance from 50 to 800 μmol m^{-2} s^{-1} at 20°C increased the tolerance of all cultivars to photoinhibition at 5°C. Furthermore, winter wheat, spring wheat, and winter rye grown at 5/250 exhibited a similar tolerance to photoinhibition as plants grown at 20/800 (Fig. 3). Thus, it appears that the increased tolerance to photoinhibition observed in plants grown at 5/250 reflects photosynthetic adjustment to growth under high PSII excitation pressure rather than photosynthetic adjustment to growth temperature.

DISCUSSION

Somersalo and Krause (1989, 1990) were the first to report that spinach grown at low, cold-hardening temperatures (5°C) but moderate irradiance (250 μmol m^{-2} s^{-1}) exhibited a tolerance to light that was 5- to 6-fold higher than the growth irradiance. Subsequently, this phenomenon was confirmed in rye and wheat in which tolerance to photoinhibition was shown to be related to an increased capacity to keep Q_A oxidized through increased PS_{max}, regardless of measuring temperature or measuring irradiance (Huner et al., 1993; Öquist and Huner, 1993; Öquist et al., 1993b). In addition, it was suggested that growth at low temperature was an absolute requirement for this phenomenon (Boese and Huner, 1990, 1992; Gray et al., 1994). In all of the experiments previously reported regarding low-temperature-induced tolerance to photoinhibition, plants grown at 5/250 were compared with control plants grown at 20/250. Since the only difference in growth condition was the growth temperature, the assumption was that any difference in photosynthetic response must represent a growth temperature response. Thus, the development of low-temperature-induced tolerance to photoinhibition has been interpreted as a low-temperature-growth response (Huner et al., 1993). In the present report, we show that this interpretation is incorrect. We suggest that the development of increased tolerance to photoinhibition upon

Table III. *Temperature and irradiance responses of photosynthetic enzymes in winter wheat (T. aestivum L. cv Monopol)*

All assays were performed at 25°C. SPS activity was determined using the V_{max} assay (see "Materials and Methods"). All values represent means ± SE; n = 3 to 6.

Enzyme	Characteristic	Growth Regime (°C μmol^{-1} m^{-2} s^{-1})			
		20/50	20/250	20/800	5/250
Rubisco					
	Total activity (μmol m^{-2} s^{-1})	28.0 ± 1.3	65.8 ± 4.8	52.5 ± 2.2	74.7 ± 7.2
	Total amount (μmol m^{-2})	1.5 ± 0.1	3.2 ± 0.3	2.6 ± 0.2	4.3 ± 0.2
	Carbamylation ratio (%)	40	61	32	75
FBPase					
Stromal	Activity (μmol m^{-2} s^{-1})	3.6 ± 0.4	9.1 ± 0.8	10.4 ± 0.3	11.6 ± 0.6
Cytosolic	Activity (μmol m^{-2} s^{-1})	1.2 ± 0.1	1.5 ± 0.1	2.2 ± 0.1	1.9 ± 0.1
SPS					
	Activity (μmol m^{-2} s^{-1})	0.9 ± 0.1	1.6 ± 0.1	2.0 ± 0.2	5.0 ± 1.0

Table IV. *Temperature and irradiance responses of photosynthetic enzymes in spring wheat (T. aestivum L. cv Katepwa)*

All assays were performed at 25°C. SPS activity was determined using the V_{max} assay (see "Materials and Methods"). All values represent means ± SE; n = 3 to 6.

Enzyme	Characteristic	Growth Regime (°C μmol^{-1} m^{-2} s^{-1})			
		20/50	20/250	20/800	5/250
Rubisco					
	Total activity (μmol m^{-2} s^{-1})	19.2 ± 3.7	62.8 ± 3.7	60.7 ± 4.1	72.5 ± 4.4
	Total amount (μmol m^{-2})	0.8 ± 0.2	3.2 ± 0.3	2.8 ± 0.3	4.6 ± 0.5
	Carbamylation ratio (%)	37	55	51	83
FBPase					
Stromal	Activity (μmol m^{-2} s^{-1})	4.2 ± 0.4	9.3 ± 0.9	11.4 ± 0.7	12.8 ± 0.7
Cytosolic	Activity (μmol m^{-2} s^{-1})	0.8 ± 0.1	1.3 ± 0.2	3.0 ± 0.3	2.2 ± 0.2
SPS					
	Activity (μmol m^{-2} s^{-1})	0.9 ± 0.1	1.6 ± 0.1	2.0 ± 0.1	1.6 ± 0.1

growth of cold-tolerant herbaceous plants at low temperature can be explained on the basis of growth under high PSII excitation pressure.

PSII excitation pressure ($1 - q_P$) is a measure of the redox state of Q_A and, thus, reflects the balance between light energy absorbed through the temperature-independent photochemical reactions of PSII and the energy utilized through the temperature-dependent reactions of electron transport and CO_2 fixation at physiologically relevant temperatures. Therefore, PSII excitation pressure may be elevated either by increasing irradiance at constant temperature or by decreasing temperature at constant irradiance. This was demonstrated by the data presented in Table I. Growth of wheat and rye at 5/250 or 20/800 results in higher PSII excitation pressure in comparison to that of plants grown at 20/250. Thus, cold-tolerant plants grown at low temperature and moderate irradiance (5/250) should be photosynthetically comparable to the same plants grown at high temperature and high light (20/800). The results for winter wheat and winter rye are consistent with this hypothesis. First, Monopol and Musketeer grown at 5/250 or 20/800 exhibited increased PS_{max} O_2 (Table II). Second, rye grown at 5/250 was comparable to plants grown at 20/800 with respect to its capacity to keep Q_A oxidized as a function of irradiance when measured at either 20 or 5°C (Fig. 2). Third, winter wheat (Table III) and winter rye (Hurry et al., 1994, 1995) grown at low temperature and moderate irradiance exhibit enhanced SPS activity analogous to plants grown at high light. Last, Monopol, Katepwa, and Musketeer grown at 5/250 exhibited a tolerance to photoinhibition similar to that of plants grown at 20/800 (Fig. 3). Thus, we conclude that cold-tolerant, herbaceous plants grown at 5/250 exhibit a greater tolerance to photoinhibition than plants grown at 20/250 not because of low growth temperature per se but rather as a consequence of growth under high PSII excitation pressure.

It has been shown that low-temperature stress results in a decrease in CO_2 assimilation rates associated with an inhibition of Suc and starch biosynthesis (Pollock and Lloyd, 1987). However, cold acclimation may result in an adjustment of Suc biosynthesis and increased rates of carbon fixation due to an adjustment of enzyme capacity in the Suc biosynthetic pathway. In our experiments, we have shown that winter wheat grown at 5/250 exhibits a 3-fold higher SPS activity relative to that grown at 20/250 (Table III). Similar results have been reported for Musketeer rye grown at low and high temperatures at a constant irradiance of 300 μmol m^{-2} s^{-1} (Hurry et al., 1995). In contrast, spring wheat exhibited no adjustment in SPS activity in response to growth at 5/250 (Table IV). We suggest that the differential capacity of winter versus spring cultivars to adjust SPS activity in response to growth regime may, in part, account for the increased capacity for photosynthesis (Galtier et al., 1993) and increased Suc accumulation typi-

Table V. *Effect of growth regime and photoinhibitory treatment on photosynthetic pigment content of winter rye (S. cereale L. cv Musketeer)*

All data are expressed as mmol mol^{-1} Chl *a* + *b* and were obtained before (control) or after photoinhibition (+ PI). Photoinhibition occurred at 5°C with an irradiance of 1600 μmol m^{-2}s^{-1}. Pigments were separated and quantified by HPLC (see "Materials and Methods"). β-Car, β-Carotene; Lut, lutein; Neo, neoxanthin; A, antheraxanthin; V, violaxanthin; Z, zeaxanthin; V+A+Z, violaxanthin + antheraxanthin + zeaxanthin; nd, not detected. All values represent means ± SE; n = 3.

Growth Regime (°C μmol^{-1} m^{-2} s^{-1})	Pigment Concentration								
	β-Car	Lut	Neo	A	V	Z	V+A+Z	CVS	EPS
20/50 (control)	87 ± 0	121 ± 0	14 ± 2	nd	31 ± 4	2 ± 1	33 ± 4	0.07 ± 0.03	0.93 ± 0.03
(+ PI)	75 ± 0	130 ± 3	15 ± 3	nd	10 ± 1	24 ± 3	35 ± 2	0.70 ± 0.04	0.31 ± 0.04
20/250 (control)	95 ± 1	130 ± 6	16 ± 2	nd	45 ± 6	4 ± 1	48 ± 7	0.07 ± 0.01	0.93 ± 0.01
(+ PI)	88 ± 2	146 ± 8	15 ± 1	4 ± 3	10 ± 0	38 ± 3	52 ± 7	0.80 ± 0.02	0.23 ± 0.01
20/800 (control)	102 ± 4	152 ± 4	19 ± 1	2 ± 2	83 ± 1	6 ± 1	91 ± 4	0.09 ± 0.03	0.92 ± 0.02
(+ PI)	108 ± 4	160 ± 4	19 ± 2	20 ± 3	14 ± 1	67 ± 2	101 ± 3	0.86 ± 0.01	0.24 ± 0.02
5/250 (control)	88 ± 2	152 ± 8	19 ± 1	1 ± 1	68 ± 3	7 ± 2	76 ± 2	0.11 ± 0.02	0.90 ± 0.02
(+ PI)	91 ± 2	158 ± 9	22 ± 3	21 ± 5	17 ± 1	45 ± 2	84 ± 3	0.79 ± 0.01	0.33 ± 0.02

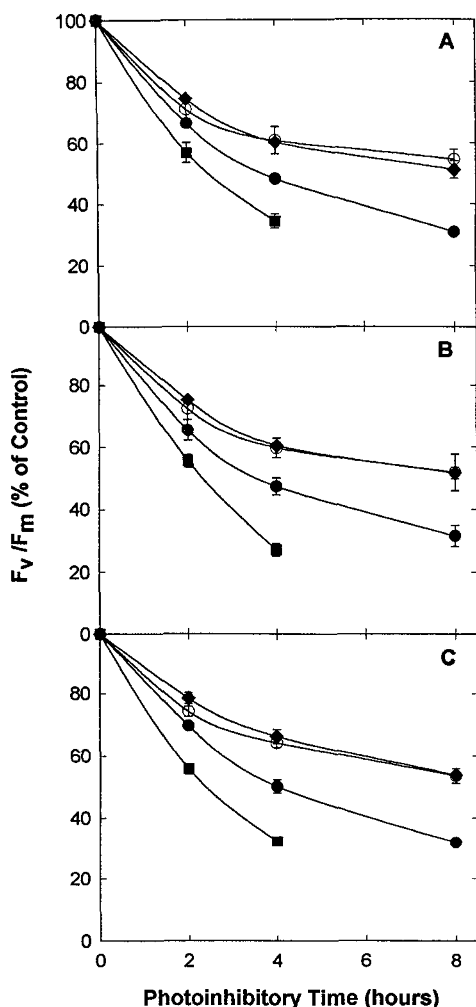

Figure 3. Effect of growth regime on tolerance to photoinhibition measured as F_V/F_M as a function of photoinhibitory time in winter wheat (*T. aestivum* L. cv Monopol) (A), spring wheat (*T. aestivum* L. cv Katepwa) (B), and winter rye (*S. cereale* L. cv Musketeer) (C). Photoinhibition occurred at 5°C with a PPFD of 1600 μmol m^{-2} s^{-1}. Growth regimes were 20°C (closed symbols) and 5°C (open symbols) at 50 (■), 250 (●, ○), and 800 μmol m^{-2} s^{-1} (◆). Results are means ± SE; n = 3. When not present, error bars are smaller than symbol size.

cally associated with exposure to low temperature (Guy et al., 1992; Holaday et al., 1992; Huner et al., 1993; Öquist et al., 1993a; Hurry et al., 1995). Previously, we reported that the ability of cereals to increase PS_{max} as a consequence of low growth temperature was correlated with their freezing tolerance, with spring cereals being less able to adjust PS_{max} than winter cereals (Öquist et al., 1993a). The results presented in Table II (20/250, 5/250) are consistent with this report. However, the data in Table II, as well as Tables III and IV, indicate that PS_{max} and SPS activity are sensitive to growth irradiance as well as growth temperature. These data coupled with a recent, detailed analysis of CO_2 gas exchange, enzyme activities, and metabolite pool sizes (L.V. Savitch, G.R. Gray, and N.P.A. Huner, unpublished results) indicate that spring and winter wheat exhibit a differential capacity to adjust photosynthetic carbon metabolism to PSII excitation pressure rather than to growth temperature as previously assumed.

Recently, we reported that the acquisition of increased tolerance to photoinhibition in *C. vulgaris* can be rationalized on the basis of PSII excitation pressure (Maxwell et al., 1994, 1995a). Here we extend the concept of PSII excitation pressure and tolerance to photoinhibition and show that it is equally applicable to higher plants. However, the mechanisms of photosynthetic adjustment to high PSII excitation pressure in green algae and cereals are significantly different. *C. vulgaris* responds to growth at high PSII excitation pressure by decreasing its capacity to absorb incident radiation through a reduction in LHCII and Chl $cell^{-1}$ as well as increased capacity for nonradiative dissipation of excess energy through the xanthophyll zeaxanthin (Maxwell et al., 1995a). In contrast, growth of winter cereals at high PSII excitation pressure results in an increase in Chl content (Table II), minimal changes in LHCII content (Fig. 1), and no significant changes in CVS or EPS (Table V). Furthermore, *C. vulgaris* is unable to adjust at the level of the Suc biosynthetic pathway (L.V. Savitch, D.P. Maxwell, and N.P.A. Huner, unpublished results), whereas winter cereals appear to exhibit an enhanced PS_{max}, in part, as a consequence of increased SPS activity (Table III; Hurry et al., 1995). A detailed discussion and comparison of these two mechanisms are the subject of a forthcoming report from this laboratory.

In this report we have shown that at 20°C an increase in growth irradiance from 50 to 800 μmol m^{-2} s^{-1} resulted in an increased PS_{max} O_2 and an associated increase in the capacity of Rubisco, stromal and cytosolic FBPase, and SPS. However, highest PS_{max} O_2 values were observed in plants grown under conditions of high PSII excitation pressure (20/800), when a decline in total Rubisco activity, total amount of Rubisco protein, and the carbamylation ratio of Rubisco was observed. We suggest that the changes in Rubisco parameters in 20/800-grown plants are related to feedback effects that occur when the rate of photosynthesis reaches the maximum capacity for starch and Suc synthesis. It has been shown previously that, when photosynthesis becomes feedback limited, a decline in Rubisco activity and the carbamylation ratio of Rubisco is expected (Schnyder et al., 1986; Sharkey et al., 1986). However, it appears that during growth at 20/800 photosynthesis is not limited by Rubisco activity. Similarly, during photoinhibition, CO_2 assimilation is not limited by Rubisco but rather by the regeneration of the substrate, RuBP, related to a restriction on the supply of reducing equivalents (Dujardyn and Foyer, 1989). Growth at 5/250 resulted in a similar increase in both cultivars of total Rubisco activity, total amount of Rubisco protein, and Rubisco carbamylation ratio in both winter and spring wheat, suggesting no limitation of photosynthesis at the level of Rubisco (Table III and IV). In addition to the adjusted Rubisco activity, growth at 5/250 in both winter and spring cultivars resulted in an increase of stromal and cytosolic FBPase activity in comparison to plants grown at 20/250. However, we have shown that PS_{max} O_2 in Monopol winter wheat grown at 5/250 was higher than PS_{max} O_2 observed in 20/250 grown material and

was comparable to that of plants grown at 20/800. Alternatively, there was no increase in PS_{max} O_2 in Katepwa spring wheat upon growth at 5/250. This difference in PS_{max} O_2 reflects a differential stimulation of SPS activity in the winter and spring cultivars at low temperature.

In summary, we conclude that tolerance to photoinhibition in higher plants and algae reflects a response to PSII excitation pressure. However, whereas algae adjust their capacity for light absorption, cereals modulate their ability to utilize light through the increased capacity of carbon metabolism at the level of Suc biosynthesis.

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