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THE RELATIONSHIP BETWEEN CHILLING TOLERANCE
AND SUSCEPTIBILITY TO PHOTOINHIBITION IN *Zea mays* (L.)

by

LINDA JEAN LANGILLE, B.Sc.

A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfilment of
the requirements for the degree of
Master of Science

Department of Biology
Carleton University
Ottawa, Ontario
January 15, 1990

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TOLERANCE AND SUSCEPTIBILITY TO
PHOTOINHIBITION IN *Zea mays* (L.)

submitted by Linda Jean Langille, B.Sc.
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the degree of Master of Science

Thesis Supervisor

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January 31, 1990

ABSTRACT

An apparatus was constructed in which individual, attached second leaves of 10 to 15 day old corn (*Zea mays* L.) seedlings could be treated to a microclimate of specific photon flux density (PFD), temperature, and humidity. The apparatus was used to investigate the relationship between the chilling tolerance of a corn genotype and its ability to resist photoinhibitory damage at chilling temperatures. Three corn lines differing in chilling tolerance, judged by the ability of seedlings to grow and survive 30 days at about 10°C, were employed. Barley (*Hordeum vulgare* L. cv. Birka), not ranked against the corn lines, was included in one photoinhibition experiment.

Although some trends were found, it was not possible to conclude that increased whole-plant chilling tolerance lends increased resistance to photoinhibition at 5°C. In part, this was attributed to differences between the short term chill-lability of the photosynthetic apparatus and longer-term whole-plant chilling tolerance.

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GLOSSARY OF TERMS

cyt b_6/f	cytochrome b_6/f complex
D1	The 32 kDa Qb apoprotein having herbicide-binding properties. It and the 34 kDa D2 protein are thought to bind the photosystem II reaction centre.
Fd	soluble ferridoxin
Fp/FMR	ferredoxin-NADP oxidoreductase
F_o	initial fluorescence level
F_m	maximal fluorescence level
F_v	variable fluorescence ($F_v = F_m - F_o$)
Gaspé	Gaspé Flint. The most chilling-tolerant corn line employed in the experiments described in this thesis, based upon the ability of seedlings to grow and survive at chilling temperatures.
HIWF	Highland Intermediate White Flint. The corn line least able to grow under the above chilling conditions.
LG3	A corn hybrid intermediate in chilling sensitivity under the above conditions.

K _D	rate constant for non-radiative dissipation of excitation energy by antennae pigments
K _P	rate constant for photochemistry of photosystem II
LHC II	the chlorophyll a/b light-harvesting complex of photosystem II
OEC	the oxygen evolving complex of PS II
P	peak fluorescence level
PAR	photosynthetically active radiation
PC	plastocyanin
P/F ₀	ratio of peak over initial fluorescence
PFD	photon flux density
Pheo	pheophytin
PS II	photosystem II
PS I	photosystem I
PQ	plastoquinone
P680	the photochemical reaction centre of photosystem II
P700	the photochemical reaction centre of photosystem I
ΔpH	transthylakoid proton gradient
Q _a	the primary quinone electron acceptor of photosystem II
Q _b	the secondary quinone electron acceptor of photosystem II

qQ	photochemical fluorescence quenching
qNP	non-photochemical fluorescence quenching (includes qE, qI, and qT)
qE	energy-dependent fluorescence quenching
qI	photoinhibitory fluorescence quenching
qT	ATP-dependent fluorescence quenching
SCFH	standard cubic feet per hour
S, M	components of the room temperature fluorescence induction transient thought to reflect the induction of carbon metabolism
T	terminal or steady state fluorescence level
VPD	water vapour pressure deficit
Z	the secondary electron donor to photosystem II

CHAPTER 1

INTRODUCTION

A few years ago, Powles *et al.* (1983) observed that photosynthesis was photoinhibited to a greater extent in some plant species (*Lycopersicon esculentum*, *Gossipium hirsutum*, and *Atriplex triangularis*), than in others (*Nerium oleander*, *Atriplex expansa*, and *Atriplex rosea*) when leaves were exposed to a high photon flux density (PFD) at low, non-freezing (chilling) temperatures. Although the relative chilling tolerances of the six species were not known, the differences were attributed to either a greater chilling tolerance of plants in the latter group, or the requirement for a longer treatment period to show the same extent of photoinhibition in all species (Powles *et al.*, 1983). More recently, Greer *et al.* (1986) proposed that the extent of photoinhibition was more severe in the first group of species, as low temperatures may have inhibited protein synthesis-mediated repair to a greater extent than in the second group. Again, this implied that species in the former group were more chilling-sensitive than those in the latter.

This prompted the question: are plants that are relatively chilling-intolerant more susceptible to photoinhibition at low, non-freezing temperatures than plants with greater chilling resistance? Short-term photoinhibition experiments at 5°C with two rice (*Oryza sativa*) cultivars and barley (*Hordeum vulgare*) (Moll and Steinback, 1986) seemed to support this

theory, but the authors did not state how the chilling tolerances of the genotypes were determined. Because of this, it was decided that the relationship between chilling tolerance and susceptibility to photoinhibition at chilling temperatures should be reinvestigated. In the experiments described in this thesis, three lines of corn (*Zea mays* L.), differing greatly in the ability to grow and survive at 10°C (Dr. Robert Hamilton, personal communication) were employed for this purpose. Barley (cv. Birka) was included for comparison, but was not ranked against the corn lines with respect to tolerance of chilling temperatures.

Corn is a thermophilic plant of subtropical origin (Miedema, 1982), but the great genetic diversity of the species permits its cultivation in many climatic regions (Vallejos, 1979), including cool temperate zones found in southern Canada. In cool temperate areas, corn seedlings commonly encounter a combination of chilling temperatures and high PFDs in the first weeks after planting; overnight temperatures in May and early June commonly dip below 10°C, followed by cool, sunny mornings (Long *et al.*, 1983). Such early morning conditions are known to cause photoinhibition of photosynthesis in the field, and may slow the rate of seedling growth, decreasing the time available for the crop to mature (Farage and Long, 1987). Therefore, it would be useful to find corn genotypes with some resistance to photoinhibition at chilling temperatures.

In the first part of this chapter, a description of the structure and function of the photosynthetic apparatus is presented, paying particular attention to PS II, and the phenomenon of fluorescence. This is followed by a literature review of photoinhibition. The basis of chilling sensitivity is

discussed, as are the inhibitory effects of chilling temperatures on the photosynthetic apparatus. Finally the objectives of the research presented in this thesis are given.

1.1. Photosynthesis

Photosynthesis is the process by which higher plants, algae, cyanobacteria, and purple and green bacteria capture energy from the sun, making it available to all life on earth. In simple terms, it is the conversion of light energy, absorbed by chlorophyll molecules, to chemical energy in the form of ATP (adenosine triphosphate) and reducing power NADPH (the reduced form of nicotinamide adenine dinucleotide phosphate), and the use of these compounds to fix carbon dioxide into carbohydrates. Photosynthesis may be summarised by the following equation:

What follows is a discussion of photosynthesis as it pertains to higher plants and green algae, both chlorophyll b-containing organisms.

1.1.1 The chloroplast

In the cells of higher plants and algae, photosynthesis is carried out within double-membrane bound organelles known as chloroplasts. Approximately 5 to 10 μm in diameter (Glazer and Melis, 1987), chloroplasts contain highly-ordered thylakoid membranes and amorphous stroma. The thylakoid membrane is the location of photosynthetic electron transport. In

mature chloroplasts, thylakoids have the appearance of flattened sacs, which are piled into stacks called grana, or appear as non-appressed lamellar regions (Fig. 1) (Stryer, 1981). These membranes are composed of polar (approximately 25%) and electroneutral (the remainder) lipids, which form a bilayer structure that is interspersed with protein molecules (Barber, 1987). The proteins are positioned within the membrane, or at its surface, depending on their relative hydrophilic or hydrophobic properties. Thylakoids possess five major protein complexes (Fig. 2) (Barber, 1987), discussed below. The stroma, an aqueous proteinaceous matrix surrounding the thylakoids (Fig. 1), contains enzymes for the fixation of carbon dioxide, and synthesis of carbohydrates (Arnon, 1987).

Leaves of *Zea mays* and other C₄ plants possess a characteristic morphology known as Kranz anatomy. Veins are encompassed by a layer of bundle-sheath cells, densely-packed with chloroplasts. Mesophyll cells surround those of the bundle-sheath (Bidwell, 1979). Bundle sheath chloroplasts show little or no granaal stacking (Bidwell, 1979). The two chloroplast types play two distinctive roles in CO₂ fixation (see section 1.1.3).

1.1.2 Photosynthetic Electron Transport

In the reactions of non-cyclic electron transport, electrons and protons are transferred from water to NADP⁺ in a series of redox reactions powered by light energy absorbed by two separate, but interacting photosystems, two (PS II) and one (PS I) (Figs. 2 and 3). The three major components, PS II, the cytochrome b₆/f complex, and PS I are held on three of the five major polypeptide complexes (Fig. 2) (Barber, 1987).

Fig. 1. The structure of a chloroplast, with a view through the double membrane (detail from Bidwell, 1979).

Fig. 2. Schematic diagram of the thylakoid membrane (from Barber, 1987), showing the lipid bilayer and locations of the five major protein complexes: the chlorophyll a/b light-harvesting complex of PS II (LHC II), photosystem II (PS II), cytochrome b_6/f (cyt b_6/f), photosystem I (PS I), and the ATP synthase. Between PS II and cyt b_6/f is the plastoquinone pool (PQ). Plastocyanin (PC) mediates electron transport between cyt b_6/f and PS I. Other components are: Fd, soluble ferridoxin; Fp, $NADP^+$ -ferridoxin oxidoreductase or FNR. Refer to text for details.

Fig. 3. A schematic diagram of the pathways for non-cyclic and cyclic electron transport in higher plants, showing midpoint redox potentials at pH 7.0 (from Barber, 1987). Refer to text for details.

1.1.2.1 *Photosystem II*

Electron transport within PS II

The PS II complex oxidises water to molecular oxygen, releasing electrons and hydrogen ions which reduce molecules of the plastoquinone (PQ) pool (Figs. 2 and 3). The primary reaction occurs with the absorption of a photon of light by P680, the photochemical reaction centre of PS II, which is thought to be a special chlorophyll a molecule or dimer (Glazer and Melis, 1987). This excites P680 to the singlet state P680* (Fig. 3), resulting in a charge separation between the reaction centre pigment and pheophytin (Pheo), the primary electron acceptor of PS II (equation 2). As a result, an electron is passed to the primary and secondary bound quinone acceptors, Q_a and Q_b:

(Krause, 1988). The electron received by Z^+ , the secondary electron donor, is donated by water. Z is thought to be a tyrosine residue on the D1 PS II reaction centre polypeptide (Barber, 1988).

Q_b operates by a two-electron gate mechanism. The semiquinone anion (Q_b^-) accepts a second electron and two protons from the stroma, forming plastoquinol (Q_bH_2) (Fig. 2) (Barber, 1987). This reduced state detaches from the PS II complex and is replaced with oxidised Q_b (Barber, 1987). Upon excitation of P680, the reduction of Q_a occurs within about 1 μ s; reduction of Q_b occurs within 1 ms (Barber, 1987).

Structure of PS II core complex

There are at least 8 major proteins in the photosystem II complex. Three are extrinsic hydrophilic membrane proteins included in the oxygen evolving complex (OEC) (discussed below). The remaining five types are chloroplast-encoded hydrophobic intrinsic membrane polypeptides with molecular weights of approximately 47, 43, 34, 32 kDa, and a low molecular weight b-type cytochrome dimer with units of 4 and 9 kDa (cytochrome b₅₅₉) (Barber, 1987).

The exact arrangement of the PS II complex is not clear. The model currently gaining favour places the PS II reaction centre on the 32 (D1) and 34 (D2) kDa polypeptides (Fig. 4) (Barber, 1987; Nanbah and Satoh, 1987; Satoh, 1988; Andréasson and Vänngård, 1988), based on amino acid sequence homology with the L and M polypeptides at the photosynthetic reaction centre of procaryotic purple bacteria (Deisenhofer *et al.*, 1985 a,b).

According to this model, the 43 and 47 kDa polypeptides act as core

Fig. 4. Proposed structure of the PS II complex (from Barber, 1987).

antenna complexes, binding approximately 50 chlorophyll a and 10 carotenoid molecules per reaction centre (Ghanotakis and Yocum, 1985). Cyt b₅₅₉ might act as a charge balancer (Sato, 1988).

The complex is located in appressed regions of the thylakoid membrane (Fig. 2) (Barber, 1987).

The Chlorophyll a/b light-harvesting complex

Associated with PS II is the chlorophyll a/b light-harvesting complex (LHC II). This antenna complex is composed of nuclear-encoded polypeptides of molecular weight 25 to 29 kDa, to which are bound chlorophyll a and b, little or no β -carotene, and some xanthophylls (Barber, 1987).

LHC II exists in two forms, one tightly associated to the PS II core antenna (LHC II-intrinsic), the second a mobile pool in the thylakoid membrane (LHC II-peripheral). The former contains 80 ± 20 chlorophyll (a and b) molecules; the latter possesses about 120 additional chlorophyll (a and b) molecules (Glazer and Melis, 1987). In higher plants, the mobile complexes are thought to undergo reversible phosphorylation by a membrane-bound kinase, whose activity is controlled by the redox state of the plastoquinone pool (Barber, 1987). When excitation of PS II is greater than that of PS I, the plastoquinone pool becomes highly reduced, and mobile complexes are phosphorylated. The mobile complexes may then migrate to PS I, where they may serve as antennae for PS I reaction centres, favouring the excitation of PS I with respect to PS II. The mobile complexes are dephosphorylated by a membrane-bound phosphatase in dim light or light favouring the excitation of PS I (Barber, 1987). In this way, the distribution of excitation energy

between PS II and PS I is regulated (Bennett *et al.*, 1980) so that the quantum efficiency of photosynthetic electron transport is maximised, particularly when light is limited (Barber, 1987).

The oxygen-evolving complex

The oxygen evolution complex (OEC), situated at the luminal surface of the thylakoid membrane (Fig. 2) (Govindjee *et al.*, 1985; Murata and Miyao, 1985), is thought to be composed of at least three nuclear-encoded proteins with molecular weights of approximately 33, 23, and 16 kDa (Fig. 4) (Barber, 1987). The 33 kDa polypeptide, required for the evolution of oxygen, binds Mn (probably 4 atoms) at an unknown water-splitting catalytic site (Govindjee *et al.*, 1985; Murata and Miyao, 1985).

The PS II reaction centre must turn over four times to produce one molecule of oxygen from two water molecules (Joliot and Kok, 1975). Four hydrogen ions are released into the thylakoid lumen (Fig. 2) (Govindjee *et al.*, 1985), establishing a transthylakoid proton gradient, while the electrons are accepted by Z^+ .

1.1.2.2 Cytochrome *b₆/f*

The cytochrome *b₆/f* complex accepts electrons from reduced plastoquinone, passing them to PS I via plastocyanin (PC), and also mediates cyclic electron transport (Figs. 2 and 3) (Barber, 1987). The complex pumps protons from reduced plastoquinone into the lumen (Fig. 2), adding to the transthylakoid proton gradient. To maintain electrical neutrality, the transfer of a proton is accompanied by the passage of a Mg^{2+} ion in the opposite

direction (one Mg^{2+} per two protons), or a Cl^- in the same direction as the proton (Stryer, 1981).

The complex is composed of a 31 and 34 kDa doublet (cyt f), a 22 kDa molecule (cyt b_{563} or b_6), a Rieske FeS protein of 20 kDa (ReFeS), and an unknown 17 kDa protein. The ferredoxin-NADP oxidoreductase (Fp or FMR) is thought to associate with the complex during cyclic electron transport via PS I (Fig. 3) (Barber, 1987).

1.1.2.3 *The photosystem I complex and the synthesis of NADPH*

From plastocyanin, the PS I complex transports electrons to soluble ferridoxin (Fd), which passes them to the ferridoxin-NADP oxidoreductase for the synthesis of NADPH (Figs. 2 and 3), the final product of non-cyclic electron transport.

Electron transport through PS I requires the absorption of a photon of light, causing a charge separation between the PS I reaction centre P700 (a chlorophyll a dimer (Glazer and Melis, 1987)) and the primary electron acceptor A_0 . From A_0 , electrons pass via A_1 to the membrane-bound iron-sulphur centres A_2 , FeSB, and FeSA (Andréasson and Vänngård, 1988). A_0 may be a chlorophyll a molecule (Andréasson and Vänngård, 1988), while A_1 has the spectroscopic properties of a quinone (Thurnauer and Gast, 1985).

The reaction centre core of PS I may be a tetramer of chloroplast-encoded polypeptides with approximate molecular weights of 70 kDa (Lundell *et al.*, 1985), binding P700 and a total of about 130 chlorophyll a and 16 carotenoid molecules (Glazer and Melis, 1987). PS I possesses an accessory

light-harvesting complex of nuclear-coded proteins with molecular weights 20 to 30 kDa (Andréasson and Vänngård, 1988). These bind an additional 60 to 80 chlorophyll a and b molecules (Glazer and Melis, 1987). Thus, the total antenna size of the PS I unit is about 200 chlorophyll (a and b) molecules.

Upon reduction of soluble ferredoxin by the membrane-bound ferredoxins Fe-SA and Fe-Sb of PS I, reduced soluble ferredoxin transfers electrons to NADP⁺. The reaction is catalysed by the ferredoxin-NADP oxidoreductase, a water-soluble, iron-sulphur protein (Barber, 1987) with FAD (oxidised flavin adenine dinucleotide) as its prosthetic group (Stryer, 1981). Since the reduction of NADP⁺ to NADPH requires two electrons, and ferredoxin is a one-electron carrier, the electron transfer process is repeated twice (Stryer, 1981):

Both PS I and the ATP synthase complex (discussed below) tend to be situated in non-appressed regions of the thylakoid membrane (Fig. 2) (Barber, 1987).

1.1.2.4 Photophosphorylation or ATP synthesis

ATP required for the manufacture of carbohydrates is synthesised by the multi-peptide ATP synthase (CF₀-CF₁) complex. This apparatus, which spans the thylakoid membrane (Fig. 2), is composed of two main units: CF₀, a set of intrinsic membrane proteins forming a trans-thylakoid proton channel, and

CF₁, a group of extrinsic polypeptides fixed to CF₀ at the stromal surface. The knoblike CF₁ contains the catalytic sites for ATP synthesis from substrates ADP (adenosine diphosphate) and inorganic phosphate (P_i) (Barber, 1987).

Operation of the ATP synthase requires light-induced electron transport, as a transthylakoid proton gradient of at least two pH units is needed to drive ATP synthesis (Stryer, 1981). ATP synthesis occurs during both cyclic and non-cyclic electron transport.

1.1.2.5 *Electron transport in C₄ species*

Although mesophyll chloroplasts can carry out non-cyclic electron transport (synthesis of both NADPH and ATP), the bundle sheath chloroplasts of *Zea mays* lack PS II (Stamp, 1984) and LHC II associated with appressed thylakoid regions (Barber, 1987). As a result, bundle-sheath chloroplasts can carry out only cyclic electron transport via PS I, forming only ATP (Bidwell, 1979).

1.1.3 The assimilation of CO₂

In the so-called "dark" or light-independent reactions of photosynthesis, ATP and NADPH are used to reduce atmospheric carbon dioxide to carbohydrates. This sequence of reactions, catalysed by enzymes in the chloroplast stroma, is the C₃, or Calvin cycle. The net reaction for the Calvin cycle is:

(Stryer, 1981).

Using the NADPH and ATP formed during photosynthetic electron transport, C_4 species have the ability to store CO_2 inside mesophyll cells as the β -carboxyl group of a 4-carbon chain acid. In *Zea mays*, this C_4 acid is malate (Stamp, 1984). In this form CO_2 is transported to the bundle sheath cells, where the C_4 acid is decarboxylated (Fig. 5) and CO_2 is released for participation in the Calvin cycle inside the bundle sheath chloroplasts. NADPH is synthesised upon the decarboxylation of malate to pyruvate (Fig. 5). Since non-cyclic electron transport does not occur in the bundle sheath chloroplasts of NADP-malic enzyme species such as corn, the decarboxylation step may meet the demand for the NADPH required in the Calvin cycle (Bidwell, 1979).

1.1.4 Fluorescence

1.1.4.1 The phenomenon of fluorescence

Light energy falling upon a plant is absorbed by chlorophyll and carotenoid antennae pigments, travelling by intermolecular transfer to the specialised chlorophyll a molecule(s) at the photosynthetic reaction centres. Each reaction centre possesses approximately 300 antenna chlorophyll

Fig. 5. Reactions of the C₄ cycle in *Zea mays*, a NADP-malic enzyme species (from Stamp, 1984). Enzymes are: 1, phosphoenolpyruvate (PEP) carboxylase; 2, NADP- malate dehydrogenase; 3, NADP-malic enzyme; 4, Pyruvate Pi dikinase; 5, ribulose bisphosphate (RUBP) carboxylase

molecules (Lavorel and Etienne, 1977).

Chlorophylls and carotenoids are very effective energy collectors and relayers due to their extensive network of conjugated double bonds. Absorption of light results in the excitation of electrons from ground-state bonding π orbitals to empty π^* orbitals, while n orbitals, associated with lone-pair electrons of nitrogen or oxygen, may participate in $n \rightarrow \pi^*$ transitions (Hipkins and Baker, 1986).

Upon absorption of a photon, and excitation of an electron from the ground state to an excited state, electrons initially reside in a higher rotational or vibrational sub-level of the excited state. Because of a very large rate constant for radiationless decay to lower sub-states, an excited electron will fall to the lowest singlet state sub-level (Hipkins and Baker, 1986). At this point, decay to the ground state can occur in a number of ways: the molecule can emit the energy as a photon of light (fluorescence), the excess energy can be released as heat (non-radiative dissipation), energy can be transferred on to a neighbouring pigment molecule, or is used in photochemistry (Hipkins and Baker, 1986). Alternatively, a molecule in an excited singlet state can go to a lower-energy triplet excited state in a process known as intersystem crossing (Hipkins and Baker, 1986).

Because excited electrons always decay to the lowest (long wavelength) singlet sub-state before falling to the ground state, fluorescence is emitted in the red end of the spectrum, at a longer wavelength than the absorbed energy (Hipkins and Baker, 1986).

1.1.4.2 Room temperature fluorescence in intact leaf tissue

Fluorescence emitted at room temperature from higher plant chloroplasts shows a peak at 680-685 nm, with a smaller, broad shoulder at approximately 740 nm (Hipkins and Baker, 1986). This signal arises mostly from the chlorophyll a molecules of PS II and LHC II (Vredenberg and Slooten, 1967), while only a minimal amount is emitted at the longer wavelengths by those of PS I (Kyle *et al.*, 1983). The other pigments, chlorophyll b and carotenoids, absorb photons but do not themselves fluoresce, instead transferring their excitons to chlorophyll a (Lavorel and Etienne, 1977; Hipkins and Baker, 1986).

Upon illumination of dark-adapted leaves with a constant light intensity at an ambient temperature, a pattern of fluorescence emission with characteristic phases F_0 , I, D, P, S, M, and T is observed. Fig. 6 shows an example of such an induction transient, also known as the Kautsky effect.

Initial or constant fluorescence, F_0 , is thought to arise from excited antenna chlorophyll a molecules before excitation energy has reached the photosynthetic reaction centre; this occurs after dark adaptation when PS II reaction centres and Q_a are fully oxidised or "open" (Krause and Weis, 1984). The chlorophyll a of PS I antenna may also contribute to F_0 at the longer wavelengths mentioned above (Kyle *et al.*, 1983).

The remaining components of the Kautsky curve constitute variable fluorescence. Variable fluorescence is strictly a PS II phenomenon, depending largely, but not entirely, upon the redox state of Q_a (Krause and Weis, 1984). Thus, in C₄ plants such as *Zea mays*, variable fluorescence is emitted

Fig. 6. A typical fluorescence induction transient, measured from the untreated second leaf of a 13 day old Gaspé seedling. F_0 , I, and D could not be resolved with the apparatus used, although I and D appear as a "shoulder" on the rise to P. Refer to sections 1.1.4.2 and 1.1.4.3 for further details.

exclusively from the chloroplasts of the mesophyll cells (Furbank and Walker, 1985).

If Q_a is already reduced or "closed", absorption of a second photon and the subsequent charge separation at the reaction centre will be followed by a charge recombination back reaction:

(Krause and Weis, 1984). The excitation energy is returned to the PS II antennae pigments, where it is emitted as fluorescence (Klimov and Krasnovskii, 1981).

As Q_a becomes reduced, the fluorescence level rises to I, followed by a slight dip at D, caused by oxidation of Q_a as electrons move toward PS I. The I to D transition is not always clearly visible, often appearing as a "shoulder" in the transient during the rise to peak fluorescence, P (Hipkins and Baker, 1986) (Fig. 6). At P, Q_a and the plastoquinone pool are highly reduced (Hipkins and Baker, 1986). This may be due to a temporary blockage of electron flow through PS I, caused by a lag in the initiation of CO_2 assimilation and oxidation of NADPH (Krause and Weis, 1984). Although the height of P depends on the intensity of the exciting light (Krause and Weis, 1984), fluorescence emitted at P is not maximal; Q_a is not totally reduced, since electrons continue to flow from Q_a to PS I. Complete closure seldom occurs, even when light intensities are photosynthetically-saturating (Hipkins and

Baker, 1986). The F_0 to P transition usually occurs within about two seconds (Hipkins and Baker, 1986).

From P the fluorescence emission dips to S, rises to a second peak M, then gradually reaches a steady-state level, T (Fig. 6). This part of the induction transient occurs over several minutes, and is related to the induction of carbon metabolism (Ireland *et al.*, 1984).

1.1.4.3 Quenching mechanisms during fluorescence induction

The P to T portion of the induction transient is thought to be shaped by the simultaneous influence of two main fluorescence quenching mechanisms. These are photochemical quenching, qQ, controlled by the redox level of Q_a , and energy-dependent quenching, qE, dependent on the magnitude of the transthylakoid pH gradient (ΔpH) (Krause and Weis, 1984; Hipkins and Baker, 1986). Energy-dependent quenching is the major non-photochemical quenching (qNP) mechanism in unstressed, intact photosynthetic systems (Krause *et al.*, 1982; Krause and Laasch, 1987).

Upon induction of carbon metabolism, the rate of non-cyclic electron transport increases, excitation energy is directed toward $NADP^+$, and fluorescence is quenched (qQ). Energy-dependent quenching (qE) is thought to occur upon cation movement through the thylakoid membrane during electron transport and ATP synthesis, causing ultrastructural changes which may result in the dissipation of excitation energy as heat (Krause *et al.*, 1982, 1983). As non-photochemical quenching predominates when incident light levels are above those saturating for photosynthesis (Horton and Hague, 1988), it has been suggested that qE may play some role in protecting PS II

against photoinhibition (Somersalo and Krause, 1989).

Photochemical quenching qQ is favoured when Calvin cycle activity is high, with fast reoxidation of NADPH and turnover of ATP (Krause and Weis, 1984). However, if the rate of photosynthetic electron transport becomes limited, due to reduced availability of NADP^+ , qQ is released. Slowed Calvin cycle activity and the reduction in the use of ATP will inhibit the dissipation of the transthylakoid proton gradient, promoting qE (Krause and Weis, 1984). In this way, oscillations in the fluorescence transient from P to T may be caused by oscillations in the rate of carbon metabolism prior to reaching a steady state of operation (Krause and Weis, 1984).

A second non-photochemical factor that probably plays only a small role *in vivo* is ATP-dependent quenching, qT , thought to occur upon phosphorylation of LHC II (Horton and Hague, 1988). qT may account for only a minor component of the P to S dip (Krause and Weis, 1984), as its participation seems to be significant only at low light levels (Horton and Hague, 1988). At higher light intensities, decreases in qT have been correlated with a rise in qE (Horton and Hague, 1988).

Leaves subjected to high light stress show an additional component of non-photochemical quenching, known as photoinhibitory quenching (qI) (refer to section 1.2).

It is evident that the degree of quenching, and ultimately, the shape of the induction transient from a leaf is dependent upon its physiological state. This can make interpretation of room temperature fluorescence signals, particularly the P to T region, rather difficult.

1.1.4.4 *A useful probe for assessing the effects of environmental stresses on the photosynthetic apparatus*

As variable fluorescence is a good indicator of perturbations in the thylakoid membrane (Lavorel and Etienne, 1977), it is a convenient method for examining the effects of photoinhibition (Fork *et al.*, 1981; Powles and Björkman, 1982; Baker *et al.*, 1983; Ögren and Öquist, 1984b; Greer *et al.*, 1986), and chilling temperatures (Smillie, 1979; Hetherington *et al.*, 1983; Havaux and Lannoye, 1984) on intact leaf tissue. In particular, the ratio of peak over initial fluorescence (P/F_0) at room temperature is a useful monitor of the photochemical activity of PS II.

1.2 Photoinhibition

Photoinhibition is the light-dependent inhibition of photosynthesis that occurs when plants encounter light levels in excess of what can be dissipated through photosynthesis. Photoinhibition commonly occurs at ambient temperatures when incident light levels are higher than the growth PFD (Powles, 1984; Greer *et al.*, 1986; Demmig and Björkman, 1987), or even under moderate light levels, when accompanied by environmental stresses that limit the rate of photosynthesis, such as chilling temperatures (Ort and Martin, 1983; Powles, 1984; Hodgson and Raison, 1989).

The extent of photoinhibition generally depends upon external conditions such as the PFD and the time of light exposure (Krause, 1988), but may also depend upon the ongoing rate of repair of the damage (Greer *et al.*,

1986), or ability of the plant to protect itself against high light damage (Greer *et al.*, 1988; Krause, 1988).

1.2.1 The site(s) of photoinhibitory damage

It is well-established that the thylakoid membrane is the primary target of photoinhibition. Jones and Kok (1966) provided early evidence for damage to the light-dependent reactions, finding that the action spectrum of light-induced damage was correlated with the absorption spectrum of chlorophyll. Some authors have reported that PS I is a site of photoinhibitory damage (Satoh, 1970; Barényi and Krause, 1985), but this observation seems to be limited to studies where chloroplasts or thylakoids were exposed to high photon flux densities after isolation. Critchley (1981a, b) localised the photoinhibition site at or near the PS II complex. This was subsequently confirmed by other researchers (Powles and Björkman, 1982; Arntzen *et al.*, 1984; Kyle *et al.*, 1984) but there is still controversy as to the exact location and nature of damage within the PS II complex.

Many researchers have reported that initial damage is incurred at or close to the PS II reaction centre. Isolated spinach thylakoids exposed to high light stress at room temperature showed decreases in the amplitude of absorbance changes at 320 nm (Cleland and Critchley, 1985; Cleland *et al.*, 1986) and from 540 to 550 nm (Cleland *et al.*, 1986). This was indicative of inhibition of charge separation between P680 and Q_a. Cleland *et al.* (1986) postulated that charge separation may be prevented if damaged reaction centres, still coupled to the LHC II antenna, act as quenchers of excitation energy.

Damage could also be located on the oxidising side of the PS II reaction centre. Critchley (1981a, b) found that electron transport from diphenylcarbazide to methyl viologen, and from catechol to methyl viologen (both bypassing the OEC) was blocked in thylakoids isolated from photoinhibited cucumber leaves. This suggested that, rather than directly damaging the the OEC, electron flow to the reaction centre was inhibited, preventing the reduction of P680⁺.

Quenching of variable fluorescence upon exposure to high light stress (qI) has been taken as evidence for the inactivation of the PS II reaction centre (Fork *et al.*, 1981; Powles and Björkman, 1982; Ögren and Öquist, 1984b; Bradbury and Baker, 1986; Demmig and Björkman, 1987, Percival *et al.*, 1987; Somersalo and Krause, 1989). Decreases in variable fluorescence may be the result of modification of the LHC II and/or its disconnection from the PS II reaction centre (Hayden *et al.*, 1987; Percival *et al.*, 1987). Alternatively, qI may involve involve dissipation of the excitation energy as heat by antennae (Powles and Björkman, 1982; Ögren and Öquist, 1984b; Demmig and Björkman, 1987) or reaction centre (Ögren and Öquist, 1984b) chlorophylls. These mechanisms may be distinguished according to their effect on the initial fluorescence level, F₀ (Demmig and Björkman, 1987).

Others authors have identified the 32 kDa Q_b apoprotein (also known as the herbicide-binding protein) as the primary target of photoinhibitory damage (Arntzen *et al.*, 1984; Kyle *et al.*, 1984; Ohad *et al.*, 1984). After exposure of *Chlamydomonas reinhardtii* cells to high light stress at room temperature, Kyle *et al.* (1984) found a strong correlation between the loss in ¹⁴C-atrazine binding sites on thylakoids, and decreases in both *in vivo* room

temperature variable fluorescence and uncoupled electron transport through PS II. A similar loss of atrazine binding, variable fluorescence, and electron transport through PS II was observed when second leaves of corn seedlings were photoinhibited for 6 h at 5°C and $1500 \mu\text{E m}^{-2} \text{s}^{-1}$ (Percival *et al.*, 1987). In *Chlamydomonas*, recovery of variable fluorescence was correlated with *de novo* synthesis of the 32 kDa protein (Ohad *et al.*, 1984).

According to Kyle *et al.* (1984) and Artnzen *et al.* (1984), photoinhibition occurs when the PQ pool becomes fully reduced so that the hydroplastoquinone at the Q_b binding site cannot be replaced with an oxidised quinone. The reduced quinone, a strong reductant in the Q_b^{2-} form, may rebind to the Q_b site and directly alter the Q_b protein, disrupting electron transport. Alternatively, it may transfer its energy to an oxygen molecule, creating an active oxygen species which could then inactivate the quinone-binding site. The Q_a site might also be affected, causing variable fluorescence to be quenched.

Although photoinhibition causes an inhibition of the light-limited and light-saturated rates of oxygen evolution (Björkman, 1987), the OEC does not appear to be a primary target (Kyle *et al.*, 1984; Bradbury and Baker, 1986). It may, however, be inactivated upon prolonged exposure to high light stress (Kyle *et al.*, 1984; Bradbury and Baker, 1986).

In summary, the primary site of photoinhibition lies within the PS II complex, but its exact location has yet to be resolved (Krause, 1988). If the PS II reaction centre lies on the D1/D2 heterodimer as suggested by Nanbah and Satoh (1987), it is entirely possible that photoinhibition of the reaction centre could damage other components in the complex, such as the 32 kDa protein.

Alternatively, the operation of more than one photoinhibitory mechanism may indeed be responsible for the discrepancies in the literature (Bradbury and Baker, 1986; Krause, 1988).

Photoinhibition does not limit photosynthesis by stomatal closure, nor does it involve the photooxidation of leaf pigments (Powles, 1984).

1.2.2 Photoinhibition as a protective mechanism

Because they are thought to dissipate excitation energy as heat when light levels are above saturating for photosynthesis (Horton and Hague, 1988), qI and qE may work in tandem to protect the photosynthetic apparatus against severe damage (Krause, 1988; Somersalo and Krause, 1989). Photoinhibitory quenching, induced within minutes to hours after exposure to excess light, may take over when qE, occurring in the order of seconds to minutes, becomes light-saturated (Somersalo and Krause, 1989).

1.3 Chilling temperatures

1.3.1 The basis of chilling sensitivity

Chilling temperatures are generally considered to be those in the range from 0 to 15°C (Raison and Lyons, 1986). According to Raison and Lyons (1986) a plant is considered to be chilling-sensitive if it "suffers damage when exposed to chilling temperatures beyond a certain limited time". If maintained at these temperatures, the chilling-sensitive plant "develop(s) a variety of external symptoms and eventually die(s)". In contrast, chilling-

insensitive plants are those that can grow and survive under chilling conditions (Raison and Lyons, 1986).

Lyons (1973) postulated that the primary site of chilling injury may be the cellular membranes. At some critical temperature in the chilling range, membrane lipids are thought to undergo a phase transition from a fluid liquid-crystalline state to a solid gel. In corn, the phase transition of thylakoid lipids occurs from 19 to 4°C (Havaux and Lannoye, 1983). In chilling-tolerant plants, such as barley, these phase changes are thought to occur below 0°C (Havaux and Lannoye, 1983).

This primary event is followed by the secondary manifestations of chilling injury (Fig. 7) (Lyons, 1973). Changes in the physical state of membrane lipids could alter the conformation of the active site of membrane-bound enzymes, increasing their energies of activation. Solidification of the membrane could induce increased permeability and loss of membrane integrity. Both of these changes could in turn lead to disruption of cell metabolism (Fig. 7). If exposure is prolonged, cell injury and death occur (Lyons, 1973). However, it is still not known precisely how these membrane phase changes would be translated into cell injury (Yoshida *et al.*, 1989). The severity of the secondary chilling events may depend on the duration of exposure, the temperature, and the physical state of the plant during the chilling treatment (Stamp, 1984; Raison and Lyons, 1986).

Lyons (1973) attributed the decreased low temperature fluidity of chilling-sensitive plant membranes to the possession of a higher percentage of lipid species with saturated fatty acids. In recent years, this hypothesis has been questioned. There is no consistent evidence for the correlation of degree

Fig. 7. The sequence of events thought to lead to chilling injury in chilling-sensitive plants (from Lyons, 1973).

of bulk lipid unsaturation with adaptation of a plant to growth at chilling temperatures (Patterson *et al.*, 1978). This is particularly true for the bulk lipids of thylakoid membranes (Chapman *et al.*, 1983; Martin, 1986). As lipids possessing saturated fatty acids make up only a minor component of the thylakoid membrane (Martin, 1986), it has been suggested that only discrete areas of membrane lipids may undergo phase changes (Patterson *et al.*, 1978; Martin, 1986). Murata and colleagues (Murata, 1983; Murata and Yamaya, 1984; Roughan, 1985) have suggested that disaturated species of the phospholipid phosphatidylglycerol (which make up only about 10% of the total lipid content of thylakoids) may be the molecules responsible for phase transitions in the thylakoid membranes of chilling-sensitive plants.

1.3.2 Inhibitory effects of chilling temperatures on photosynthesis

In chilling-intolerant plants, the loss of thylakoid fluidity is thought to be a major cause of inhibition of photosynthesis at chilling temperatures. A strong correlation was found between the temperature at which inhibition of light-dependent oxygen evolution first occurred, and the temperature at which phase transitions of isolated polar thylakoid lipids took place, as detected by differential scanning calorimetry (Hodgson *et al.*, 1987). Low temperatures may hinder the diffusion of mobile plastoquinone between the PS II and cytochrome b_6/f complexes in chilling-sensitive genotypes, preventing the oxidation of Q_A^- (Havaux, 1987). Chilling carried out at moderate light levels may restrict electron transport on the reducing side of PS I (Hodgson and Raison, 1989), or inhibit thylakoid-associated functions, such as phenazine methosulphate-dependent cyclic phosphorylation, proton

uptake, osmotic properties of the thylakoids, and Ca^{2+} -dependent ATPase activity (Garber, 1977). Damage to the photochemical steps of photosynthesis, causing quenching of variable fluorescence, occurs only when chilling is carried out in the light (Oquist, 1983).

Chilling temperatures also limit photosynthesis at sites independent of the thylakoid membrane. Chilling reduces CO_2 assimilation rates (Long *et al.*, 1983; Ort and Martin, 1983; Öquist, 1983; Powles *et al.*, 1983) by limiting activities of stromal enzymes (Stamp, 1984; Taylor *et al.*, 1974) and inducing stomatal closure (Long *et al.*, 1983; Öquist, 1983; Ort and Martin, 1983).

1.3.3 Photoinhibition at chilling temperatures

By slowing the rate of photosynthesis, and therefore dissipation of excess light energy from PS II, chilling temperatures predispose the photosynthetic apparatus to photoinhibition. In both chilling-sensitive and -tolerant plants, simultaneous exposure to chilling and high PFDs induces a greater degree of photoinhibitory damage, and loss of variable fluorescence, than when leaves are treated to the same PFD at room temperature (Baker *et al.*, 1983; Long *et al.*, 1983; Ögren *et al.*, 1984; Greer *et al.*, 1988). Chilling also predisposes leaves to photoinhibition under moderate or low light levels that are not damaging at room temperature (Ögren *et al.*, 1984; Smillie *et al.*, 1988).

1.4 Objectives

The two major objectives of this thesis were as follows:

I) To design and construct an apparatus with the capacity to chill single, intact, attached leaves of corn seedlings, in a microclimate of specific light, temperature, and humidity levels.

II) To determine if plants that are chilling-tolerant have increased resistance to photoinhibition when leaves are chilled to 5°C in the presence of high PFDs, than plants that are more sensitive to chilling temperatures.

Three corn lines, differing greatly in the ability to survive and grow at chilling temperatures, were employed for this purpose.

CHAPTER 2

MATERIALS AND METHODS

2.1 Preliminary experiments with isolated mesophyll cells

It was originally decided that photoinhibition experiments would be performed with intact mesophyll cells isolated from corn leaves, since intact cells have several advantages over intact leaf tissue. Firstly, the use of isolated cells would permit the simultaneous measurement of oxygen evolution and fluorescence with a modulated oxygen electrode device already in the laboratory. Secondly, a suspension of cells would allow a more uniform photoinhibitory treatment. There is often a gradient of photoinhibitory damage in leaves exposed to high light intensities, caused by shading of some cells by others (Critchley, 1988).

Mesophyll cells were isolated using a procedure based on the protocol of Chen *et al.* (1974). However, attempts to isolate whole cells were unsuccessful; cells that were released did not appear to be covered by a wall, but had the spherical appearance of protoplasts. As much more work than had previously been anticipated was necessary to perfect the isolation technique, it was decided that all subsequent experiments would be performed with intact leaf tissue.

2.2 Equipment used for the photoinhibition of leaf material

2.2.1 The leaf chamber

All photoinhibition and temperature experiments were carried out within a specially-constructed leaf chamber. This apparatus, modelled after the cuvette of Baker *et al.* (1983) and Long *et al.* (1983), allowed the exposure of a single, intact, attached leaf to a microclimate of specific PFD (photon flux density), temperature, and humidity conditions. A leaf chamber was employed, rather than chilling the entire plant, so that light and temperature treatment effects would not be confounded by the effects of whole-plant water stress and wilting. Rapidly chilling the entire plant and the soil surrounding the roots is known to inhibit water uptake and movement through the roots (Kramer, 1956), since the viscosity of water increases greatly at low temperatures.

Two versions were used. Preliminary experiments were conducted with a single-chambered prototype, which could hold only one leaf at a time. A double-chambered model, capable of holding an experimental and a control leaf simultaneously, was employed for the remaining experiments.

Both versions were constructed of opaque black perspex. Each leaf chamber, essentially a long, narrow slot with a clear colourless glass lid, was surrounded on its bottom and three sides by a water jacket, through which distilled water or a 30% (v/v) aqueous methanol solution was pumped by a Lauda K-2 circulator (Brinkmann Instruments, Rexdale, Ont.) to maintain the desired temperature at the chamber floor. The leaf chambers and their water jackets were encased in styrofoam (plate 1), and the Tygon tubing leading to

and from the circulating unit covered in Armaflex foam rubber insulation (plate 2).

During an experiment, the leaf (as shown schematically in Fig. 8) was held in thermal contact with the chamber floor by two soft foam pads attached to the glass lid (plate 3). The temperature of the floor beneath the leaf was monitored with one or two 5 mm diameter thermistors (Fenwal Electronics #KB21J1; Electrosonic Inc., Willowdale, Ont.) (Fig. 8) fixed to the floor with black electrical tape. The resistance of these previously-calibrated thermistors was displayed on a Hewlett-Packard 34702A digital multimeter (Hewlett-Packard, Loveland, CO) (Fig. 8, plate 2), then converted to temperature.

2.2.2 Photoinhibitory light source

Each leaf chamber was equipped with an incandescent reflector flood lamp (Philips R40 500W/120V) (plate 2) held in a retort stand and centred over the midpoint of the leaf (Fig. 8). The PFD emitted by each lamp was controlled with a variable autotransformer (plate 2).

The considerable heat given off by these light sources was minimised by the placement of a infrared-filtering device (plate 4) between each lamp and leaf chamber. Held together with 6 brass screws, each filter unit consisted of an approximately 1.3 cm-thick aluminum ring sandwiched between a 1.3 cm-thick circle of clear, colourless perspex below, and a 36 cm diameter pyrex petri dish bottom above (plate 4). A Fresnel lens (# 29; Thomas Electronic Security Ltd., Montréal, P.Q.) was fixed to the bottom of each unit to focus the desired PFD on a 3 cm long portion of leaf directly below the lamp. Inside each heat filter were two 5 x 5 cm infrared mirrors (Opticon Corporation Ltd., Waterloo,

Plate 1. Two leaf chambered model, showing styrofoam insulation and points of entry for humidified air stream (see arrows).

Plate 2. Overview of apparatus for dual leaf chamber with lampshade removed.

Fig. 8. Schematic diagram of the leaf chamber apparatus. showing position of an attached, intact second leaf during photoinhibition and chilling experiments.

Plate 3. Leaves were held against the chamber floor by soft foam pads attached to the chamber lid.

Plate 4. Detail of heat filter apparatus.

Ont.) held end to end on a perspex, or a perspex and aluminum frame.

Aluminum foil covered the rest of the filter floor interior. Cold tap water flowed constantly through the filters.

During all experiments a water filter and lamp apparatus was positioned directly over each leaf chamber slot, on top of the styrofoam jacket. To protect and maintain the rest of the plant at the room PFD and temperature during high light treatment of a leaf, an aluminum foil-covered "lampshade" surrounded each water filter and light source (plate 5).

2.2.3 Humidity apparatus

A device was constructed to provide air to the leaf chamber at a desired humidity level. All components, except for the compressed air supply and gas gauges, were immersed in distilled water inside an 8L styrofoam-insulated perspex aquarium (Fig. 9, plate 2), and maintained at the desired temperature by a second circulating unit (Lauda K-2/RD; Brinkmann Instruments Inc., Rexdale, Ont.).

Compressed air was carried through Tygon tubing and split into two streams (Fig. 9) with flow rates regulated by Brooks-Mite gauges (Brooks Instrument Division, Emerson Electric (Canada) Inc.). Each stream was passed through a separate copper coil (each 1.5 m total length with 4 mm internal diameter) immersed in the aquarium, to heat or cool the air to the desired temperature. One air stream was bubbled through a gas washing bottle containing 450 mL distilled water, then passed into a perspex cylinder, where it was mixed with the second unhumidified stream (Fig. 9). Air flow rates through the humidified and unhumidified lines could be adjusted so

Plate 5. A lampshade surrounded the incandescent lamps so that the rest of the plant could be maintained at the room light level.

Fig. 9. Schematic diagram of humidity apparatus. Refer to text (section 2.2.3) for further details.

that the desired absolute humidity was obtained. From the mixing cylinder, the combined air stream entered the leaf chamber at the end opposite the leaf insertion point (Fig. 9, plate 1). Relative humidity of the airstream was monitored with an Atkins thermistor psychrometer, model 3Z02B (Atkins Technical Inc., Gainesville, Florida),

2.3 Selection of corn lines varying in long term chilling tolerance

Seeds of corn (*Zea mays* L.) cultivars Gaspé Flint, Highland Intermediate White Flint (HIWF), and the hybrid LG3 were obtained from Dr. Robert Hamilton at Agriculture Canada, Ottawa. These three lines, tested against 33 others, were selected on the basis of their growth performance in chilling trials carried out at Agriculture Canada (described below).

Seeds were germinated on moist filter paper in the dark at 26°C for 3 to 4 days, until the coleoptile was at least 1 cm in length. The seedlings were pinned to a styrofoam float placed in Hoagland's solution, and moved inside a Conviron cabinet to a temperature of 14 to 17°C during the day and 10°C at night, a PFD of 500 to 600 $\mu\text{E m}^{-2} \text{s}^{-1}$, and a 16h photoperiod. After 28 to 30 days, various growth characters (Table I) were assessed.

The parameters root rating, vigour and colour (Table I) were subjectively ranked on a scale of 1 to 5. Root rating, essentially relative root volume and bulk, is highly correlated with root mass, with 1 signifying relatively little root growth and 5 the greatest relative amount of root material. Vigour is a term encompassing dry weight biomass, and the general

Table I

Results of the long-term chilling tolerance survey conducted at Agriculture Canada (Dr. Robert Hamilton, personal communication) for 36 corn lines including Gaspé, LG3, and HIWF. Presented are various growth characters for 30 day-old Gaspé, LG3, and HIWF seedlings, following 30 days at approximately 10°C, 500-600 $\mu\text{E m}^{-2} \text{s}^{-1}$, and 16 h photoperiod. Means judged not significantly different by Duncan's Multiple Range Test (comparisonwise error rate $\alpha = 0.05$) within each growth character are indicated by the same letter. Refer to section 2.3 for further details.

growth character	sample size (n)	line	rank out of 36 lines	mean value
root rating (1-5)	10	Gaspé	2	4.6000 A
	10	LG3	18	3.2000 B
	7	HIWF	35	1.8571 C
root length (cm)	10	Gaspé	3	12.550 A
	10	LG3	22	8.700 B
	7	HIWF	32	7.357 B
vigour (1-5)	40	Gaspé	2	4.4750 A
	40	LG3	7	4.1500 A
	28	HIWF	36	0 (died)
colour (1-5)	40	Gaspé	11	3.7000 A
	40	LG3	1	4.4500 B
	28	HIWF	35	1.5000 C

Table I (continued)

growth character	sample size (n)	line	rank out of 36 lines	mean value
leaf number	40	Gaspé	1	3.6125 A
	40	LG3	12	2.9625 B
	28	HIWF	34	2.0821 C
coleoptile mass (g) "	15	Gaspé	3	0.53557 A
	16	LG3	9	0.41995 A
	7	HIWF	35	0.05790 B
	10	Gaspé	4	0.54106 A
	11	LG3	8	0.41288 A
	7	HIWF	35	0.05790 B
seed mass (g) "	15	Gaspé	1	not given A
	16	LG3	22	0.11877 B
	7	HIWF	17	0.11080 B
	10	Gaspé	1	0.04132 A
	11	LG3	23	0.11765 B
	7	HIWF	20	0.11080 B
radicle mass (g) "	15	Gaspé	7	0.16493 A
	16	LG3	19	0.08719 B
	7	HIWF	35	0.02299 C
	10	Gaspé	5	0.18486 A
	11	LG3	18	0.09025 B
	7	HIWF	35	0.02299 C

overall visual appearance of the whole plant, where 1 is the least vigorous and 5 the most. Leaf colour was rated as 1 if chlorophyll production had ceased, to 5, if the leaves remained healthy and green. All dead plants received a 0 designation (Dr. Robert Hamilton, personal communication).

On the basis of the Table I data, Gaspé (an inbred line, homozygous for chilling-resistance) was judged most cold-tolerant, HIWF the least cold-tolerant, and LG3 intermediate in chilling-tolerance.

2.4 Growth conditions for plants used in preliminary, photoinhibition, and short-term chilling experiments

Seeds were surface-sterilised prior to planting. This was done by soaking them for 30 seconds in a 6% sodium hypochlorite solution, followed by a thorough rinse in tap water. Seeds were sown into 6 x 6 cm plastic pots filled with vermiculite (1 seed per pot).

Seedlings were grown in a temperature-controlled room beneath a bank of 4 fluorescent (96" 215W Vitalite ultra high output; Duro Test Electric, Ltd., Rexdale, Ont.) and 24 incandescent (40 W; GTE Sylvania Ltd., Drummondville, P.Q.) lamps. To minimise PFD drop-off near the perimeter of the growing area, the plants were placed within a wooden enclosure (width 40 cm, length 185 cm, height 39 cm) with a reflective white-painted interior.

The lamps were positioned so that plants received a PFD of either 300 or 150 $\mu\text{E m}^{-2} \text{s}^{-1}$ PAR (400-700 nm) at the level of the second leaf, as measured with a LI-170 quantum sensor (Lambda Instruments). Plants were grown

under a 16 h photoperiod, at a temperature of approximately 23°C day/ 20°C night, and uncontrolled humidity, and were watered daily with modified Hoagland's solution equilibrated to the growth temperature. This solution contained 5 mM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 5 mM KNO_3 , 2 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1 mM KH_2PO_4 , 18 μM $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 10 μM H_3BO_3 , 1.5 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.6 μM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.032 μM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, and 178 μM Fe_3O_4 obtained from Sequestrene (Ciba Geigy) (Lana Reid, personal communication).

2.5 Experimental procedure

All experiments were carried out with intact, attached second leaves of healthy, green 12 to 15 day-old seedlings at the 4 to 4 1/2 leaf stage.

2.5.1 Preliminary experiments

2.5.1.1 *Determination of a suitable dark adaptation period*

HIWF seedlings grown at 150 $\mu\text{E m}^{-2} \text{s}^{-1}$ were dark-adapted for 0, 15, 30, 60, or 90 minutes, and room temperature fluorescence transients measured (as described in section 2.6). Experiments were performed 8 to 11 h after the start of the photoperiod.

2.5.1.2 Confirmation of diurnal changes in variable fluorescence

Fluorescence transients were recorded from second leaves of untreated 300 $\mu\text{E m}^{-2} \text{s}^{-1}$ -grown HIWF seedlings (as described in section 2.6). Trials were carried out 3 to 4.5 h and 12 to 14 h after photoperiod initiation.

2.5.2 Photoinhibition and short-term chilling experiments

During all experiments described below, the air stream supplied to each leaf chamber was maintained at a relatively constant absolute humidity of saturation to 0.2 kPa below saturation, and was delivered at a flow rate of 4 standard cubic feet per hour (SCFH). The humidity of the air stream was kept high to avoid water stress effects, since the extent of inhibition of photosynthesis at high PFDs has been found to increase with the water vapour pressure deficit (VPD) between the leaf and surrounding air (East *et al.*, 1981; Long *et al.*, 1983).

While the second leaf was treated to the specific PFD and temperature conditions (described below) in the leaf chamber, the rest of the plant was maintained under room PFD (approximately 10 $\mu\text{E m}^{-2} \text{s}^{-1}$), and at room temperature. Inside each chamber, the PFD supplied by the floodlamp was not found to vary more than $\pm 10\%$ over the treated area of the leaf. Preliminary photoinhibition experiments were carried out with seedlings grown at 300 $\mu\text{E m}^{-2} \text{s}^{-1}$. Subsequent photoinhibition and short-term chilling experiments (experiments 1 to 3) employed 150 $\mu\text{E m}^{-2} \text{s}^{-1}$ -grown seedlings.

2.5.2.1 Preliminary photoinhibition experiments

1100 $\mu\text{E m}^{-2} \text{s}^{-1}$ 1 h trials

Second leaves of Gaspé and HIWF seedlings were inserted in the leaf chamber so that a 3 cm-long area at the midpoint of the leaf was exposed to a PFD of 1100 $\mu\text{E m}^{-2} \text{s}^{-1}$ for 1 hour at a chamber floor temperature of 5°C. Control leaves received the same temperature treatment, except that the lamp was switched off. Trials were repeated with a leaf chamber temperature of 20°C. Following treatment, a 2.5 cm-long leaf segment was cut from the centre of the leaf, and dark-adapted for 10 minutes prior to measurement of room temperature fluorescence. Leaves were not examined during set time blocks.

1100 $\mu\text{E m}^{-2} \text{s}^{-1}$ 3 h trials

Second leaves of Gaspé and HIWF seedlings were treated for 3 h at 5°C, and PFD of 1100 $\mu\text{E m}^{-2} \text{s}^{-1}$ as described above, but fluorescence transients were recorded (as outlined in section 2.6) 9.5 to 13 h after the start of the photoperiod. Control leaves were maintained 3 h at 5°C with the lamp off, or at the growth PFD (300 $\mu\text{E m}^{-2} \text{s}^{-1}$).

2.5.2.2 Experiment 1

The second leaf of a Gaspé, LG3, or HIWF seedling was positioned in the leaf chamber so that the midpoint was exposed to 3000 $\mu\text{E m}^{-2} \text{s}^{-1}$ for 6 h at a floor temperature of 5°C (experiment 1a). Control leaves were maintained at the growth PFD (150 $\mu\text{E m}^{-2} \text{s}^{-1}$) and 5°C for 6 h.

To examine the effects of a short-term chill at the growth PFD on photosynthesis (experiment 1b), the 6 h 5°C 150 $\mu\text{E m}^{-2} \text{s}^{-1}$ -treated controls of experiment 1a were compared to untreated leaves maintained under normal growing conditions.

Experiments were performed during two consecutive 6 h periods over the experimental day; leaves were examined 10 to 12 h and 16 to 18 h after the start of the photoperiod.

2.5.2.3 Experiment 2

To avoid the significant loss of bulk chlorophyll observed during experiment 1a, 6 h trials at 5°C were repeated with a PFD of 1500 $\mu\text{E m}^{-2} \text{s}^{-1}$ (experiment 2b). Control leaves were exposed for 6 h to a chamber floor temperature of 5°C at the growth PFD (150 $\mu\text{E m}^{-2} \text{s}^{-1}$).

Again, to observe the effect of a 6 h 5°C chill on photosynthetic parameters (experiment 2c), the controls used in experiment 2b were compared to untreated leaves.

For both experiments 2b and 2c, the three corn lines were tested against spring barley (*Hordeum vulgare* L. cv. Birka), a grass of temperate origin (Smillie *et al.*, 1988). The young barley plants were grown to the same developmental stage as the corn seedlings, and under identical conditions.

Trials with LG3 were repeated at 20°C, so that the extent of photoinhibition incurred at 5°C and at 20°C could be compared (experiment 2a).

For all trials, room temperature fluorescence and O₂ evolution rates were examined 10.5 to 12.5 h after photoperiod initiation.

2.5.2.4 *Experiment 3*

To simulate an overnight chill in the field, Gaspé and HIWF seedlings were removed from the growth room at the end of a normal photoperiod, and placed in a dark incubator (Percival, Boone, Iowa), where they received an overnight chill in the dark at 10°C and 100% relative humidity. This overnight dark chilling period (referred to as the pre-chill) was 12 or 15 h in duration.

At the end of the pre-chilling period, the second leaf was inserted into the leaf chamber, where it was exposed to a 3 h photoinhibitory treatment at 1500 $\mu\text{E m}^{-2} \text{s}^{-1}$ and a chamber floor temperature of 5°C (experiment 3a). Controls were leaves that had received an overnight pre-chill, then were subjected to 3 h at the growth PFD (150 $\mu\text{E m}^{-2} \text{s}^{-1}$) and 5°C.

The effect on pre-chilled leaves of the 3 h 5°C chilling period at the growth PFD was examined (experiment 3b). The controls of experiment 3a were compared to "pre-chilled untreated" leaves (leaves that had received an overnight chill in the dark followed by 3 h under normal growing conditions at 23°C).

The effects of the pre-chill treatments on photosynthetic parameters were determined (experiment 3c) by comparing the "pre-chilled untreated" leaves to untreated leaves.

Second leaves from plants in the 12 h pre-chill group were examined 6.5 to 8.5 h after the start of the usual photoperiod. Second leaves of the 15 h pre-chilled plants were examined 9.5 to 11.5 h after normal photoperiod initiation.

2.6 Measurement of Room Temperature Fluorescence

Prior to the measurement of room temperature fluorescence transients, the whole plant was dark-adapted for 30 minutes (unless stated otherwise) at 23°C. Following the dark adaptation period, a 2.5 cm-long segment was cut from the midpoint of the second leaf, and was immediately transferred to the probe cup of a Plant Productivity Fluorometer (model SF-20, Richard Branker Research, Ottawa, Ont.). This procedure was performed under dim green light supplied by a 25W incandescent bulb. An excitatory PFD (670 nm) of 16 to 18 $\mu\text{E m}^{-2} \text{s}^{-1}$ was employed for fluorescence measurements; chlorophyll fluorescence was detected at wavelengths longer than 710 nm. Fluorescence transients were recorded on a Kipp and Zonen BD41 (Delft, Holland) chart recorder.

Rather than presenting peak fluorescence (P) as an absolute value, it was normalised on the initial fluorescence level (F_0). This minimised the variation caused by differences in chlorophyll content between individual leaves (Miranda *et al.*, 1981).

2.7 Measurement of the Rate of Oxygen Evolution

The rate of CO₂-saturated, light-dependent oxygen evolution from the cut leaf segment was recorded immediately following measurement of room temperature fluorescence (within 25 minutes of removing the segment from the leaf).

Using scissors, the leaf segment was cut across the midrib into 3 mm-wide strips. These were placed into a side arm Erlenmeyer with approximately 20 mL of an aqueous solution containing 50 mM Tricine (pH 7.0) (Sigma) and 0.5% NaHCO_3 . The strips were vacuum-infiltrated for 4 minutes with gentle suction, then were quickly transferred to the sample chamber of an oxygen electrode device (model 53, Yellow Springs Instrument Co., Yellow Springs, Ohio) containing 4.0 mL of the above solution. The rate of oxygen evolution was followed at 25°C, using a non-saturating light intensity of $5.5 \times 10^4 \text{ ergs cm}^{-2} \text{ s}^{-1}$ provided by a 300W incandescent floodlamp (125-130V, Westinghouse). Rates were expressed as the absolute rate of O_2 evolution ($\mu\text{mole O}_2 \text{ evolved} \cdot \text{mg chlorophyll}^{-1} \cdot \text{h}^{-1}$) while the leaf strips were illuminated.

2.8 Determination of Leaf Bulk Chlorophyll Content

Following fluorescence and/or oxygen evolution measurements, leaf segments or strips were placed in a mortar and ground for approximately 15 seconds with about 10 mL 80% acetone. The brei was thoroughly rinsed from the mortar with an additional 5 mL acetone, and centrifuged at 412 G for 5 minutes. Absorbance of the supernatant was measured at 649 and 665 nm with a Spectronic 70 spectrophotometer (Bausch and Lomb, Rochester, NY) and the chlorophyll concentration determined as:

$$\frac{\mu\text{g chlorophyll}}{\text{mL}} = 17.72 (A_{649}) + 6.45 (A_{665})$$

(Vernon, 1960). Chlorophyll contents were expressed as μg chlorophyll per g leaf tissue, calculated as:

$$\frac{\text{chlorophyll solution concentration } (\mu\text{g/mL})}{\text{mass leaf segment (g), measured immediately after measurement of room temperature fluorescence .}} \times \frac{\text{volume of chlorophyll solution (mL)}}{1}$$

2.9 Data Analysis

Data for all experiments (excluding preliminary 1 and 3 h $1100 \mu\text{E m}^{-2} \text{s}^{-1}$ photoinhibition trials) were analysed by analysis of variance, using the SPSS-X computer package on CP-6. Details of analyses of variance, and relevant p values are given in Appendices II to V. For all comparisons, the experimentwise error rate was held at $\alpha = 0.05$. Pairwise comparisons of corn lines within a treatment (when the total number of lines tested was at least three) were made with the Student-Newman-Keuls test (experimentwise error rate $\alpha = 0.05$).

CHAPTER 3

RESULTS

3.1 Preliminary experiments

3.1.1 Preliminary photoinhibition experiments

Data from initial 1 h photoinhibition trials at $1100 \mu\text{E m}^{-2} \text{s}^{-1}$, and 5 or 20°C (Appendix Ia) were difficult to interpret. Leaves were not fully or consistently dark adapted prior to measurement of fluorescence, and P/F_0 decreased appreciably over the experimental day. This prompted the determination of a more suitable dark adaptation period, and the use of longer treatment periods during discreet time blocks.

Increasing the time of high light treatment to 3h (Appendix Ib) decreased P/F_0 values, indicative of some photoinhibition. However, there was still little distinction between the cultivars. In an attempt to increase the amount of photoinhibition, plants for subsequent experiments were grown at a lower PFD ($150 \mu\text{E m}^{-2} \text{s}^{-1}$), since low light-grown plants are more susceptible to high light stress than are plants cultivated under higher PFDs (Lichtenthaler *et al.*, 1983; Demmig and Björkman, 1987; Greer and Laing, 1988). This may be due to diminished rates of light-saturated photosynthesis in low-light grown plants (Greer and Laing, 1988; Demmig and Björkman, 1987), combined with an enhanced light-harvesting capacity per PS II reaction centre (Anderson and Osmond, 1987). In addition, lower light -grown plants

may possess reduced rates of repair of photoinhibitory damage (Demmig and Björkman, 1987) and/or a diminished ability to dissipate the excess excitation energy as heat (Greer and Laing, 1988; Horton and Hague, 1988).

3.1.2 Dark adaptation period

The general shape of room temperature fluorescence transients from second leaves of 'untreated $150 \mu\text{E m}^{-2} \text{s}^{-1}$ -grown HIWF seedlings suggested that dark adaptation required 30 minutes of whole-plant dark exposure. Although maximal P/F_0 values were reached within 15 minutes of dark adaptation (Fig. 10), all aspects of the fluorescence transient (particularly M and S) were not fully visible until 30 minutes of dark exposure. Subsequently, all plants were dark-adapted for 30 minutes prior to measurement of room temperature fluorescence.

Details of analyses of variance are given in Appendix II.

3.1.3 Diurnal changes in variable fluorescence

Significant diurnal variation in the magnitude of variable fluorescence, first noted during the preliminary photoinhibition experiments, was confirmed (Fig. 11). P/F_0 showed a significant 12% drop over about 9 hours under normal growing conditions (Fig. 11a). This was due to a decrease in P , rather than any significant change in F_0 (Fig. 11b). The fluorescence transient was flattened over the course of the experimental day, with elimination of the S to M rise.

Details of analyses of variance are given in Appendix II.

Fig. 10. The ratio of peak to initial fluorescence with respect to the length of the whole-plant dark adaptation period. Room temperature fluorescence transients were measured from second leaves of $150 \mu\text{E m}^{-2} \text{s}^{-1}$ -grown HIWF seedlings. Values are presented as mean $\pm$ standard deviation, with $n = 7$ plants for each dark adaptation period. Means were very significantly different (ANOVA, $p = 0.0000$), with means of plants dark-adapted for 15, 30, and 90 minutes not significantly different (Student-Newman-Keuls test, $\alpha = 0.05$).

Fig. 11. Preliminary experiments with $300 \mu\text{E m}^{-2} \text{s}^{-1}$ -grown HIWF seedlings, showing **a)** a significant decrease in the ratio of peak to initial fluorescence (P/F_0) over the course of the day (ANOVA, $p = 0.0004$). **b)** This was due to a significant drop in P (ANOVA, $p = 0.001$), rather than a change in F_0 (ANOVA, $p = 0.408$). Room temperature fluorescence transients were measured from the second leaf, with $n = 6$ plants per time period. Values presented are mean $\pm$ standard deviation.

3.2 Experiment 1

Details of analyses of variance for experiments 1a and 1b are found in Appendices IIIA and IIIB, respectively.

3.2.1 Effect of time on the data

In both the 6 h photoinhibition trials (experiment 1a) and the 6 h chilling trials (experiment 1b) P/F_O was the only photosynthetic parameter to decrease significantly over the experimental day (ANOVA, main time effect in each experiment $p < 0.001$). As a result, means for F_O , oxygen evolution rate, and steady state fluorescence (Tables II and IV) were not calculated for the two individual time periods, as was done for P/F_O (Figs 12 a and b), but for all data collected.

In the photoinhibition trials (experiment 1a), the very significant diurnal decrease in P/F_O (Figs. 12a and b) occurred for control leaves chilled at the growth PFD, rather than for high light-treated leaves. Taken over all corn lines, there was no change in P/F_O measured from $3000 \mu\text{E m}^{-2} \text{s}^{-1}$ -treated leaves (ANOVA, $p = 0.998$), whereas P/F_O from chilled control leaves decreased very significantly by 6 to 14% over the day (ANOVA, $p < 0.001$). This accounted for a very significant interaction between time and treatment (ANOVA, $p < 0.001$).

In the chilling trials (experiment 1b), P/F_O values decreased over the day to a similar extent, both for leaves chilled at 5°C and $150 \mu\text{E m}^{-2} \text{s}^{-1}$ (the controls described above) and untreated leaves (ANOVA, $p < 0.001$). The diurnal decrease in P/F_O was significant within all corn lines (Gaspé $p < 0.001$,

Fig. 12. P/F_0 ratios from second leaves of Gaspé, LG3, and HIWF corn following 6 h exposure to $3000 \mu\text{E m}^{-2} \text{s}^{-1}$ at 5°C (experiment 1a), and 6 h chilling treatment at 5°C and $150 \mu\text{E m}^{-2} \text{s}^{-1}$ (experiment 1b). Values are presented as mean $\pm$ standard deviation of the data ($n=8$ per treatment group per line within each time period). Time period 1 data (a) were measured 10 to 12 h after the start of the photoperiod; time period 2 data (b) were collected 16 to 18 h after photoperiod initiation. For $150 \mu\text{E m}^{-2} \text{s}^{-1}$ 5°C -treated, and untreated leaves, the magnitude of P/F_0 decreased significantly over the experimental day. Refer to text for further details.

a) time period 1

b) time period 2

LG3 $p < 0.001$, HIWF $p = 0.003$, collapsed over both temperature treatments). Untreated leaves showed the largest decrease in P/F_0 over the day, decreasing by up to 19%.

3.2.2 Experiment 1a: 6 h photoinhibition trials at $3000 \mu\text{E m}^{-2} \text{s}^{-1}$ and 5°C

In all lines, exposure to $3000 \mu\text{E m}^{-2} \text{s}^{-1}$ flattened the fluorescence transient (shown for LG3 in Fig. 13), causing a very significant drop in P/F_0 (Figs. 12a and b). Ratios for high light-treated leaves were very significantly lower than those for $150 \mu\text{E m}^{-2} \text{s}^{-1}$ 5°C controls ($p < 0.001$ within each line, when collapsed over the two time periods; $p < 0.001$ within each time period, when collapsed over all corn lines).

For P/F_0 , there was a significant interaction between corn line and PFD treatment (ANOVA, $p = 0.001$); this suggested that the corn lines were affected differently by 6 h exposure to $3000 \mu\text{E m}^{-2} \text{s}^{-1}$ at 5°C . In reality, all lines were photoinhibited to the same end point (Figs. 12a and b). When $3000 \mu\text{E m}^{-2} \text{s}^{-1}$ data for each time period were combined (due to the absence of a significant time by cultivar interaction), there was little difference between P/F_0 values for the three corn lines (ANOVA, $p = 0.846$). Instead, genotypic differences arose within the $150 \mu\text{E m}^{-2} \text{s}^{-1}$ 5°C control leaf P/F_0 data (Figs. 12a and b); collapsed over both 6 h time periods, all lines were significantly different from one another (ANOVA, $p < 0.001$, Student-Newman-Keuls test, $\alpha = 0.05$).

When high light P/F_0 data for each corn line are presented as a percentage of the chilled controls (Table III), HIWF does appear to show a

Fig. 13. Representative room temperature fluorescence transients measured from second leaves of LG3 seedlings during experiments 1a and 1b. Treatments were as follows: **A**, 6h, 3000 $\mu\text{E m}^{-2} \text{s}^{-1}$, 5°C; **B**, 6h, 150 $\mu\text{E m}^{-2} \text{s}^{-1}$, 5°C; **C**, untreated. The transients were recorded 10 to 12 hours after the start of the photoperiod.

FLUORESCENCE (relative units)

69

60s
TIME

greater photoinhibitory loss of P/F_0 than do Gaspé or LG3, at least during the earlier time period.

F_0 was not significantly affected by the photoinhibitory treatment within any line (Table II).

Oxygen evolution rates and steady state fluorescence levels were significantly lowered by 6 h exposure to $3000 \mu\text{E m}^{-2} \text{s}^{-1}$ at 5°C (Table II). Although the high light treatment led to a very significant reduction of oxygen evolution rates in all three lines (ANOVA, $p < 0.001$ in each line), Gaspé and HIWF were not affected significantly differently by the treatment (Table II). Similarly, steady state fluorescence was lowered in all lines (ANOVA, Gaspé $p = 0.002$, LG3 $p = 0.006$, HIWF $p < 0.001$), but there was no significant interaction between treatment and corn line ($p = 0.099$).

Unfortunately, the photoinhibitory treatment caused a significant (ANOVA, $p < 0.001$) photooxidative loss of bulk chlorophyll in HIWF. Although some photobleaching took place in leaves of Gaspé and LG3 (Table II), the loss was not significant in these two lines ($p = 0.146$, and $p = 0.062$, respectively). Leaves of all corn lines showed a slight "silvering" of the upper leaf surface in the areas exposed to the high PFD (plate 6). Control leaves chilled at the growth PFD showed no visible surface photobleaching.

3.2.3 Temperature effects: 6 h $150 \mu\text{E m}^{-2} \text{s}^{-1}$ 5°C versus untreated leaves

When the controls of experiment 1a (leaves chilled to 5°C at the growth PFD of $150 \mu\text{E m}^{-2} \text{s}^{-1}$) were compared to untreated leaves, it was evident that P/F_0 was lowered by 6 h of chilling (Figs. 12a and b). There was a significant

Plate 6. Visible photobleaching of the upper surface of a Gaspé leaf exposed to the conditions of experiment 1a (left). Arrows indicate unbleached bands (areas of the leaf covered by the foam pads of the chamber lid during treatment). An untreated leaf, or "growth room control" is shown for comparison (right).

three-way interaction between time, treatment, and line terms (ANOVA, $p = 0.004$). This suggested that the corn genotypes were affected differently by chilling, and that the chilling effect on each line varied with the time of day. Compared to untreated leaf values, P/F_0 was significantly decreased for chilled leaves of Gaspé during both time periods, whereas LG3 showed significant chilling effects only in time period 1, and HIWF only during time period 2 (Student-Newman-Keuls, $\alpha = 0.05$).

Chilling affected the three corn lines differently with respect to P/F_0 (ANOVA, $p = 0.043$). Overall, there were distinct genotypic differences for chilled leaves (ANOVA, $p < 0.001$), while untreated leaves of all lines had similar P/F_0 values (ANOVA, $p = 0.302$). Within the first 6 h time period, the chilling effects seemed to be **inversely** related to the long-term chilling tolerance of each line; only Gaspé and LG3 showed a significant drop in P/F_0 (Student-Newman-Keuls, $\alpha = 0.05$) upon treatment at 5°C, as compared to untreated leaves (Fig. 12a). Similarly, only Gaspé steady state fluorescence was significantly decreased (Table IV), although there was no statistically significant interaction between line and temperature treatment terms ($p = 0.171$).

Within the later experimental period (Fig. 12b), the inverse relationship between long-term and short-term chilling tolerance was not apparent. While chilled leaves of both HIWF and Gaspé showed significant decreases in P/F_0 as compared to untreated leaves (Student-Newman-Keuls, $\alpha = 0.05$), those of chilled LG3 leaves did not (Fig. 12b). No significant differences between corn lines were apparent within each temperature treatment.

Chilling at the growth PFD did not significantly affect the rate of oxygen evolution, F_0 , or bulk chlorophyll contents in any of the corn lines (Table IV).

3.3 Experiment 2

Details of analyses of variance for experiments 2a, 2b and 2c are found in Appendices IVA, B and C, respectively.

3.3.1 Experiment 2a: The effect of temperature on the degree of photoinhibition in LG3 leaves

With respect to controls chilled at the growth PFD, P/F_0 decreased very significantly upon 6 h exposure to $1500 \mu\text{E m}^{-2} \text{s}^{-1}$, both at 5°C and 20°C (Table Va). Leaves photoinhibited at chilling temperatures showed a significantly greater loss of P/F_0 than was observed at 20°C . High light treatment at either temperature had little effect on F_0 or steady state fluorescence. There was no photooxidation of bulk leaf pigments at 5°C , nor at 20°C (Table Va).

Because there was little difference in P/F_0 , F_0 , and steady state fluorescence between leaves treated in the leaf chamber at 20°C and $150 \mu\text{E m}^{-2} \text{s}^{-1}$ (Table Va), and untreated leaves maintained under normal growing conditions (Table Vb), this justified the use of untreated leaves as temperature controls in chilling experiments.

3.3.2 Experiment 2b: 6 h photoinhibition trials at 1500 $\mu\text{E m}^{-2} \text{s}^{-1}$ and 5°C

Decreasing the photoinhibitory PFD by half to 1500 $\mu\text{E m}^{-2} \text{s}^{-1}$ prevented the photooxidation of bulk leaf pigments (Table VI), but did not prevent a slight bleaching of the upper surface of high light-treated leaves.

As in experiment 1a, 6 h exposure to 1500 $\mu\text{E m}^{-2} \text{s}^{-1}$ and 5°C caused very significant decreases in both P/F_0 and the rate of oxygen evolution in all corn lines and in Birka barley (Table VI). Steady state fluorescence was not significantly affected for any line (Table VI). F_0 was increased slightly in all corn lines and in barley, but the genotypes were not affected significantly differently by the high light treatment (ANOVA, $p = 0.235$). Again, P/F_0 was the only parameter for which lines were affected significantly differently by the high light treatment ($p = 0.005$). However, as in experiment 1a, differences between the genotypes did not lie with the high light-treated leaves (ANOVA, $p = 0.940$, Table VI), but with control leaves chilled at the growth PFD (ANOVA, $p < 0.001$).

The trend to a greater extent of photoinhibition with increasing chilling tolerance of a corn line was again suggested (Table III).

3.3.3 Experiment 2c. Temperature Effects: 6 h 150 $\mu\text{E m}^{-2} \text{s}^{-1}$ 5°C-treated versus untreated leaves

In contrast to experiment 1b, barley and Gaspé and HIWF corn lines were little affected by the 6 h chilling at the growth PFD with respect to P/F_0 (Table VII). There was little genotypic difference between values for the chilled leaves (ANOVA, $p = 0.038$) (Table VII). Rather, significant genotypic

differences were due to differences for untreated leaves (ANOVA, $p = 0.001$). However, untreated P/F_0 levels for the least and most chilling sensitive corn lines (Gaspé and HIWF, respectively), and Gaspé and barley were not significantly different (Table VII, Student-Newman-Keuls, $\alpha = 0.05$).

F_0 and steady state fluorescence did drop in response to chilling (ANOVA, $p = 0.006$ and 0.002 for F_0 and steady state fluorescence levels, respectively), but only for the most chilling-sensitive line HIWF. Interactions between line and temperature treatment were not significant (ANOVA, $p > 0.225$) for these parameters.

Oxygen evolution rates were not significantly affected by the chilling treatment (Table VII).

3.4 Experiment 3: Pre-chill experiments

Details of analyses of variance for experiment 3a are found in Appendix VA, those for experiment 3b in Appendices VB, C, and D.

3.4.1 Experiment 3a: 3 h photoinhibition trials at $1500 \mu\text{E m}^{-2} \text{s}^{-1}$ and 5°C with plants receiving an overnight 12 or 15 h pre-chill

3.4.1.1 12 h pre-chill

The oxygen evolution rate, P/F_0 , and steady state fluorescence were significantly diminished when leaves received a 3 h exposure to $1500 \mu\text{E m}^{-2} \text{s}^{-1}$ at 5°C following a 12 h overnight pre-chill (Table VIII). For oxygen

evolution and P/F_0 , Gaspé and HIWF were similarly affected (Table VIII); cultivar-treatment interaction terms were $p = 0.170$ and $p = 0.632$ for P/F_0 and the rate of oxygen evolution, respectively. However, HIWF showed a more significant loss of steady state fluorescence than did Gaspé (ANOVA, $p = 0.016$). HIWF levels fell to 83% ($p = 0.016$) of the control, while for Gaspé, steady state fluorescence levels were little affected ($p = 0.293$).

F_0 was increased by the high light-chilling treatment, but only for Gaspé (cultivar-treatment interaction term, $p = 0.014$), which showed a significant (ANOVA, $p = 0.011$) 18% increase with respect to control leaves. Leaf bulk chlorophyll content was unchanged by the photoinhibitory treatment (Table VIII).

3.4.1.2 15 h pre-chill

When the same photoinhibitory treatment followed a 15 h pre-chill, it again caused significant reductions in P/F_0 and the rate of oxygen evolution (ANOVA, $p = 0.002$ and $p < 0.001$ for P/F_0 and oxygen evolution rate, respectively). Again, there was little significant difference between Gaspé and HIWF in their response to the high light-chilling treatment, with respect to P/F_0 (ANOVA, $p = 0.063$), or the oxygen evolution rate (ANOVA, $p = 0.231$). However, trends for a greater reduction of the oxygen evolution rate and P/F_0 in HIWF than in Gaspé were apparent (Table VIII).

F_0 , steady state fluorescence, and bulk chlorophyll content were not affected by high light treatment in either cultivar (Table VIII).

3.4.2 Experiment 3b. Temperature effects

3.4.2.1 *Effect of the 3 h chill at 5°C and 150 $\mu\text{E m}^{-2} \text{s}^{-1}$ on pre-chilled leaves*

P/F_0 was significantly lowered in leaves receiving 3 h 5°C chilling treatments at the growth PFD preceded by 12 or 15 hour dark pre-chills (the controls for experiment 3a) with respect to leaves receiving the pre-chill treatment only (Table IX). Gaspé and HIWF were not affected significantly differently (ANOVA, $p = 0.679$ for leaves pre-chilled 12 h, $p = 0.719$ for leaves pre-chilled 15 h). The 3 h chilling treatment did not affect other photosynthetic parameters of pre-chilled leaves, nor did it cause a loss of leaf bulk chlorophyll (Table IX).

3.4.2.2 *Effect of the dark pre-chill on photosynthetic parameters*

The overnight pre-chill, followed by 3 h under normal growing conditions, had little effect on any parameter except P/F_0 measured from leaves pre-chilled for 15 h (Table IX). The 15 h pre-chill caused a general increase in P/F_0 with respect to untreated leaves, particularly for Gaspé (Table IX), although there was no significant interaction between cultivar and chilling treatment (ANOVA, $p = 0.639$).

As observed in preliminary experiments and experiment 1, untreated leaves showed a 13 to 16% drop in P/F_0 between the two test periods (Table IX). No other parameter displayed such a diurnal change (Table IX). In this analysis, the decrease in P/F_0 was significant only for Gaspé (ANOVA, $p = 0.014$), but HIWF also showed a trend (ANOVA, $p = 0.028$) to loss of variable fluorescence (Table IX).

Table II Experiment 1a

The effects of a 6 h photoinhibitory treatment at 3000 $\mu\text{E m}^{-2} \text{s}^{-1}$ and 5°C on room temperature initial and steady-state fluorescence levels, rate of oxygen evolution, and bulk chlorophyll content for three corn lines. Control leaves were subjected to the growth PFD (150 $\mu\text{E m}^{-2} \text{s}^{-1}$) at 5°C. Refer to Materials and Methods for further experimental detail.

Values presented are the mean $\pm$ standard deviation, using data for both daily 6 h time periods (described in Fig. 12). N = 16 per treatment group per corn line, except for the oxygen evolution rate, where n = 8.

Means within a treatment group judged not significantly different by the Student-Newman-Keuls test ($\alpha = 0.05$) are joined by lines.

Asterisks denote high light treatment means judged significantly different from their corresponding control means at $p \leq 0.017$ (experimentwise error rate $\alpha = 0.05$ / 3 corn line comparisons) by analysis of variance.

PFD at 5°C [$\mu\text{E m}^{-2} \text{s}^{-1}$]	line	O ₂ evolution rate [$\mu\text{mole O}_2$ mg chl·h]	initial fluorescence level (F ₀) [relative fluorescence units]	steady state fluorescence level (T) [relative fluorescence units]	bulk chlorophyll content [$\mu\text{g chlorophyll}$ g leaf tissue]
3000	Gaspé LG3 HIWF	***0.183 ± 0.144	158 ± 6	** 152 ± 8	1877 ± 240
		***0.273 ± 0.144	150 ± 12	** 146 ± 11	1801 ± 212
		***0.340 ± 0.124	140 ± 6	***135 ± 6	***1962 ± 246
150	Gaspé LG3 HIWF	1.28 ± 0.11	155 ± 10	164 ± 13	2005 ± 197
		1.72 ± 0.27	146 ± 9	156 ± 10	1965 ± 295
		1.41 ± 0.24	139 ± 10	156 ± 12	2251 ± 239

no asterisk $p > 0.017$ * $0.010 < p \leq 0.017$ ** $0.001 < p \leq 0.010$ *** $p \leq 0.001$

Table III

P/F₀ values from photoinhibition experiments 1a (Figs. 12a and b) and 2b (Table VI), with data presented as the mean of the high light (HL) values divided by the mean of the low light (LL) 150 $\mu\text{E m}^{-2} \text{s}^{-1}$, 5°C controls. Error (σ) was calculated as:

$$\sigma = \frac{\overline{\text{HL}}}{\overline{\text{LL}}} \sqrt{\left(\frac{\text{sd HL}}{\overline{\text{HL}}}\right)^2 + \left(\frac{\text{sd LL}}{\overline{\text{LL}}}\right)^2}.$$

experiment	line	$\left[\frac{\overline{\text{HL}}}{\overline{\text{LL}}} \pm \sigma \right] \times 100\%$
3000 $\mu\text{E m}^{-2} \text{s}^{-1}$ 6h 5°C		
	Gaspé	80.2 $\pm$ 5.8
	LG3	78.0 $\pm$ 9.6
	HIWF	68.9 $\pm$ 5.2
time period 2	Gaspé	88.6 $\pm$ 6.5
	LG3	82.4 $\pm$ 8.6
	HIWF	80.3 $\pm$ 8.9
1500 $\mu\text{E m}^{-2} \text{s}^{-1}$ 6h 5°C		
	Gaspé	76.1 $\pm$ 8.8
	LG3	74.7 $\pm$ 8.2
	HIWF	67.1 $\pm$ 6.7
	Birka barley	77.9 $\pm$ 3.6

Table IV Experiment 1b

The effects of a 6 h chill at 5°C on initial and steady-state fluorescence, and rate of oxygen evolution measured at room temperature from second leaves of corn seedlings. Intact, attached leaves were chilled at the growth PFD (150 $\mu\text{E m}^{-2} \text{s}^{-1}$), and are compared to untreated leaves maintained under normal growing conditions. See Materials and Methods for further experimental detail.

Data for chilled and untreated leaves from the two consecutive 6 h experimental periods are presented and analysed as outlined for experiment 1a, Table II. For each treatment group $\bar{x}$, $n = 16$, except for the oxygen evolution rate data, where $n = 8$.

2

OF/DE

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MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS
STANDARD REFERENCE MATERIAL 1010a
(ANSI and ISO TEST CHART No. 2)

treatment temperature	line	O ₂ evolution rate [$\mu\text{mole O}_2$ mg chl·h]	initial fluorescence level (F ₀) [relative fluorescence units]	steady state fluorescence level (T) [relative fluorescence units]	bulk chlorophyll content [$\mu\text{g chlorophyll}$ g leaf tissue]
[°C]					
5	Gaspé LG3 HIWF	1.28 ± 0.11 1.72 ± 0.27 1.41 ± 0.24	155 ± 10 146 ± 9 139 ± 10	** 164 ± 13 156 ± 10 156 ± 12	2005 ± 197 1965 ± 295 2251 ± 239
23 (untreated leaves)	Gaspé LG3 HIWF	1.52 ± 0.36 1.80 ± 0.41 1.59 ± 0.37	163 ± 12 151 ± 7 146 ± 10	176 ± 10 164 ± 10 158 ± 11	2054 ± 309 2035 ± 271 2104 ± 181

no asterisk p > 0.017 * 0.010 < p ≤ 0.017 ** 0.001 < p ≤ 0.010 *** p ≤ 0.001

Table V Experiment 2a

Room temperature fluorescence parameters and bulk chlorophyll contents from second leaves of LG3 seedlings.
a) Effects of temperature on the extent of photoinhibition. Intact, attached leaves were exposed to $1500 \mu\text{E m}^{-2} \text{s}^{-1}$ for 6 h at either 5 or 20°C . Controls were maintained at the growth PFD ($150 \mu\text{E m}^{-2} \text{s}^{-1}$). b) The same fluorescence parameters, obtained from untreated leaves taken directly from the growth chamber.

All fluorescence transients were measured 10.5 to 12.5 h after the start of the photoperiod, with $n = 8$ for each treatment group. Values presented are the mean $\pm$ standard deviation.

Lines join means within each light level treatment group judged not significantly different by analysis of variance at $p \leq 0.025$ (experimentwise error $\alpha = 0.05/2$ light treatment groups).

Asterisks indicate means significantly different from the corresponding control, as determined by analysis of variance. Means for $1500 \mu\text{E m}^{-2} \text{s}^{-1}$ -treated leaves are compared to their respective $150 \mu\text{E m}^{-2} \text{s}^{-1}$ -treated controls, while $150 \mu\text{E m}^{-2} \text{s}^{-1}$ -treated leaves (a) are contrasted with untreated leaves (b).

PFD [$\mu\text{E m}^{-2} \text{s}^{-1}$]	temperature during treatment [°C]	ratio of peak to initial fluorescence (P/F ₀)	initial fluorescence level (F ₀) [relative fluorescence units]	steady state fluorescence level (T) [relative fluorescence units]	bulk chlorophyll content [$\mu\text{g chlorophyll}$ g leaf tissue]
a)					
1500	5	***1.06 ± 0.03	111 ± 5	109 ± 6	1976 ± 133
	20	***1.28 ± 0.08	113 ± 7	112 ± 6	1880 ± 207
150	5	***1.42 ± 0.13	104 ± 11	111 ± 8	1906 ± 296
	20	1.71 ± 0.16	110 ± 8	117 ± 9	2055 ± 224
b)					
150	23 ¹	1.81 ± 0.13	107 ± 6	116 ± 7	1936 ± 261
(untreated leaves.)	23 ²	1.72 ± 0.13	102 ± 5	113 ± 7	1949 ± 176

no asterisk $p > 0.025$ * $0.010 < p \leq 0.025$ ** $0.001 < p \leq 0.010$ *** $p \leq 0.001$

1 untreated leaves examined during same period as those treated at 5°C

2 untreated leaves examined during same period as those treated at 20°C

Table V' Experiment 2b

The effects of a 6 h photoinhibitory treatment at 1500 $\mu\text{E m}^{-2} \text{s}^{-1}$ and 5°C on room temperature fluorescence, rate of oxygen evolution, and bulk chlorophyll content of second leaves of Gaspé, LG3, and HIWF corn and Birka barley seedlings. Controls were maintained at 5°C and the growth PFD (150 $\mu\text{E m}^{-2} \text{s}^{-1}$). See Materials and Methods for further experimental detail.

Data are presented as mean $\pm$ standard deviation, with $n = 8$ plants per treatment group per genotype. Data were recorded 10.5 to 12.5 h after the start of the photoperiod.

Lines denote means within a treatment group judged not significantly different by the Student-Newman-Keuls test ($\alpha = 0.05$). For oxygen evolution rates only, lines connect means within a treatment group that are not significantly different, as determined by analysis of variance at $p \leq 0.025$ (experimentwise error rate $\alpha = 0.05 / 2$ light level treatment groups).

Asterisks identify high light treatment means significantly different from their corresponding controls at $p \leq 0.013$ (0.05/4 line comparisons), as determined by analysis of variance.

PFD at 5°C [μE/ m ² ·s]	line	O ₂ evolution rate [μmole O ₂ mg chl·h]	ratio of peak to initial fluorescence level (P/F ₀)	initial fluorescence level (F ₀) [relative fluorescence units]	steady state fluorescence level (T) [relative fluorescence units]	bulk leaf chlorophyll content [μg chlorophyll g leaf tissue]
1500	Gaspé	***0.732 ± 0.205	***1.08 ± 0.05	112 ± 9	109 ± 8	2104 ± 544
	LG3	--	***1.06 ± 0.03	111 ± 5	109 ± 6	1976 ± 133
	HIWF	***0.428 ± 0.218	***1.08 ± 0.04	**108 ± 8	105 ± 6	1920 ± 331
	Birka	--	***1.09 ± 0.02	127 ± 8	124 ± 7	1560 ± 79
150	Gaspé	1.48 ± 0.51	1.42 ± 0.15	110 ± 9	117 ± 9	2122 ± 604
	LG3	--	1.42 ± 0.13	104 ± 11	111 ± 8	1906 ± 296
	HIWF	1.14 ± 0.40	1.61 ± 0.5	94 ± 10	105 ± 8	1907 ± 522
	Birka	--	1.40 ± 0.06	123 ± 9	127 ± 8	1601 ± 100

no asterisk p > 0.013 * 0.010 < p ≤ 0.013 ** 0.001 < p ≤ 0.010 *** p ≤ 0.001

Table VII Experiment 2c

Comparison of the effects of a 6 h chilling treatment at 5°C on room temperature fluorescence and oxygen evolution rate of second leaves of Gaspé, LG3, and HIWF corn and Birka barley seedlings. Leaves were maintained at the growth PFD ($150 \mu\text{E m}^{-2} \text{s}^{-1}$) throughout the chilling treatment. Chilled leaves are compared to untreated leaves kept under normal growing conditions. See Materials and Methods for further experimental detail.

All measurements were recorded 10.5 to 12.5 h after the start of the photoperiod. Data are presented as the mean $\pm$ standard deviation, with $n = 8$ plants per treatment group per line.

Chilled and untreated leaf data were analysed, compared, and presented as outlined for Experiment 2b, Table VI.

treatment temperature [°C]	line	O ₂ evolution rate [$\mu\text{mole O}_2$ mg chl·h]	ratio of peak to initial fluorescence level (P/F ₀)	initial fluorescence level (F ₀) [relative fluorescence units]	steady state fluorescence level (T) [relative fluorescence units]	bulk leaf chlorophyll content [$\mu\text{g chlorophyll}$ g leaf tissue]
5	Gaspé	1.48 ± 0.51	1.42 ± 0.15	110 ± 9	117 ± 9	2122 ± 604
	LG3	--	***1.42 ± 0.13	104 ± 11	111 ± 8	1906 ± 296
	HIWF	1.14 ± 0.40	1.61 ± 0.15	**94 ± 10	**105 ± 8	1907 ± 522
	Birka	--	1.40 ± 0.06	123 ± 9	127 ± 8	1601 ± 100
23 (untreated leaves)	Gaspé	1.66 ± 0.53	1.59 ± 0.24	114 ± 10	127 ± 8	2140 ± 518
	LG3	--	1.81 ± 0.13	107 ± 6	116 ± 7	1936 ± 261
	HIWF	1.22 ± 0.29	1.73 ± 0.26	106 ± 7	117 ± 7	2082 ± 531
	Birka	--	1.48 ± 0.09	124 ± 4	129 ± 4	1686 ± 113

no asterisk p > 0.013 * 0.010 < p ≤ 0.013 ** 0.001 < p ≤ 0.010 *** p ≤ 0.001

Table VIII Experiment 3a

The effects of a 3 h photoinhibitory treatment at $1500 \mu\text{E m}^{-2} \text{s}^{-1}$ and 5°C on on room temperature fluorescence parameters, rate of oxygen evolution, and bulk chlorophyll contents for second leaves of Gaspé and HIWF. Control leaves were exposed to the growth PFD ($150 \mu\text{E m}^{-2} \text{s}^{-1}$) at 5°C . Both high light-treated and control seedlings were subjected to an overnight chill at 5°C in the dark (12 or 15 h in duration) prior to treatment in the leaf chamber. Refer to Materials and Methods for further experimental details.

Data are presented as the mean $\pm$ standard deviation, with $n = 8$ plants per treatment group per cultivar. Leaves from plants exposed to a 12 h pre-chill were examined 6.5 to 8.5 h after the start of the usual photoperiod. Leaves from 15 h-pre-chilled seedlings were examined 9.5 to 11.5 h after usual photoperiod initiation.

Lines join cultivar means within a treatment group judged not significantly different by analysis of variance at $p \leq 0.025$ (experimentwise error rate $\alpha = 0.05 / 2$ light level treatment groups).

Asterisks identify high light treatment means significantly different from their controls at $p \leq 0.025$ ($0.05/2$ cultivars), as determined by analysis of variance.

PFD at 5°C [μE/ m ² ·s]	cultivar	O ₂ evolution rate [μmole O ₂ mg chl·h]	ratio of peak to initial fluorescence levels (P/F ₀)	initial fluorescence level (F ₀) [relative fluorescence units]	steady state fluorescence level (T) [relative fluorescence units]	bulk chlorophyll content [μg chlorophyll g leaf tissue]
12 h pre-chill						
1500	Gaspé HIW/F	***0.660 ± 0.104 ***0.843 ± 0.212	1.10 ± 0.02 ** 1.11 ± 0.03	*129 ± 24 105 ± 8	131 ± 24 *106 ± 7	2263 ± 280 2004 ± 184
150	Gaspé HIW/F	1.21 ± 0.35 1.50 ± 0.45	1.16 ± 0.07 1.27 ± 0.18	109 ± 9 112 ± 10	123 ± 15 127 ± 14	2283 ± 500 1927 ± 185
15 h pre-chill						
1500	Gaspé HIW/F	*0.839 ± 0.206 ***0.789 ± 0.407	1.09 ± 0.03 ***1.13 ± 0.04	118 ± 8 112 ± 10	119 ± 7 117 ± 14	2519 ± 248 2147 ± 193
150	Gaspé HIW/F	1.30 ± 0.22 1.56 ± 0.51	1.14 ± 0.02 1.32 ± 0.19	116 ± 4 113 ± 11	127 ± 5 125 ± 11	2698 ± 314 2238 ± 387

no asterisk p > 0.025 * 0.010 < p ≤ 0.025 ** 0.001 < p ≤ 0.010 *** p ≤ 0.001

Table IX Experiment 3b

The effects of a 3 h chill at 5°C and the growth PFD ($150 \mu\text{E m}^{-2} \text{s}^{-1}$) on fluorescence parameters and the rate of oxygen evolution measured at room temperature from second leaves of Gaspé and HIWF. As for experiment 3a, whole seedlings were chilled in the dark at 10°C for 12 or 15 h prior to the 3 h chilling treatment. Leaves identified as "23°C pre-chilled" were maintained in the growth room under normal growing conditions (23°C , $150 \mu\text{E m}^{-2} \text{s}^{-1}$) for 3 h following the pre-chill treatment. Data for non pre-chilled (untreated) leaves are also presented.

Data are given as the mean $\pm$ standard deviation, with $n = 8$ per treatment group per cultivar.

Data were analysed as for Table VIII, experiment 3a. Asterisks identify 5°C-treated pre-chilled leaf means significantly different from their-same cultivar 23°C pre-chilled control means at $p \leq 0.025$ (experimentwise error rate $\alpha = 0.05 / 2$ cultivars), as determined by analysis of variance. In a similar manner, 23°C pre-chilled leaf data are compared to data for untreated leaves.

Significant diurnal changes in photosynthetic parameters for untreated leaves are indicated with a dagger † (ANOVA, $p < 0.025 = 0.05 / 2$ cultivar comparisons).

treatment	cultivar	O ₂ evolution rate [$\mu\text{mole O}_2$ mg chl·h]	ratio of peak to initial fluorescence levels (P/F ₀)	initial fluorescence level (F ₀) [relative fluorescence units]	steady state fluorescence level (T) [relative fluorescence units]	bulk chlorophyll content [$\mu\text{g chlorophyll}$ g leaf tissue]
12 h pre-chill, measured 6.5 to 8.5 h after start of the normal photoperiod						
5°C (prechilled)	Gaspé HIWF	1.21 ± 0.35 1.49 ± 0.45	***1.16 ± 0.07 ***1.27 ± 0.18	109 ± 9 112 ± 10	123 ± 15 127 ± 14	2284 ± 501 1927 ± 185
23°C (prechilled)	Gaspé HIWF	1.15 ± 0.23 1.53 ± 0.46	1.90 ± 0.08 1.98 ± 0.08	116 ± 8 114 ± 13	133 ± 7 124 ± 13	2466 ± 354 2160 ± 401
untreated	Gaspé HIWF	1.26 ± 0.40 1.19 ± 0.27	† 1.82 ± 0.18 2.07 ± 0.10	115 ± 6 106 ± 11	125 ± 4 117 ± 9	2391 ± 461 2418 ± 260
15 h pre-chill, measured 9.5 to 11.5 h after start of the normal photoperiod						
5°C (prechilled)	Gaspé HIWF	1.30 ± 0.22 1.56 ± 0.51	***1.14 ± 0.02 ***1.32 ± 0.19	116 ± 4 113 ± 11	127 ± 5 125 ± 11	2698 ± 314 2238 ± 387
23°C (prechilled)	Gaspé HIWF	1.31 ± 0.12 1.43 ± 0.40	**1.84 ± 0.18 2.05 ± 0.09	118 ± 9 107 ± 7	137 ± 15 117 ± 8	2342 ± 427 2252 ± 266
untreated	Gaspé HIWF	1.13 ± 0.21 1.21 ± 0.24	1.53 ± 0.31 1.81 ± 0.24	114 ± 13 116 ± 17	132 ± 18 128 ± 18	2325 ± 316 2539 ± 411

no asterisk p > 0.025 * 0.01 < p ≤ 0.025 ** 0.001 < p ≤ 0.01 *** p ≤ 0.001

CHAPTER 4

DISCUSSION

4.1 Diurnal changes in fluorescence emission

P/F_0 values for untreated leaves (experiments 1b and 3b), and leaves chilled at the growth PFD (experiment 1) dropped significantly over the experimental day. Visible as a gradual flattening of the fluorescence transient, this quenching was due to a large reduction in peak fluorescence, P , rather than any significant change in initial fluorescence, F_0 . In contrast, there was little evidence for diurnal change in the oxygen evolution rate, or steady state fluorescence, T , in any of the corn lines. This decrease in P/F_0 was quite similar to fluorescence quenching reported for plants cultivated under artificial illumination (Greer and Laing, 1988), and natural sunlight (Björkman and Powles, 1984; Ben *et al.*, 1987). Leaf tissue from kiwifruit (*Actinidia deliciosa*) grown at 25°C and 1300 $\mu\text{E m}^{-2} \text{s}^{-1}$ (65% of full sunlight) showed significant diurnal decreases in variable fluorescence measured at 77°K (Greer and Laing, 1988). Between "dawn" and up to 6.7 hours after photoperiod initiation, variable fluorescence emissions dropped by almost 33%. Leaves grown at half of this PFD, showed a slight, although non-significant, quenching of variable fluorescence, comparable in magnitude to some of the changes observed for P/F_0 from 150 $\mu\text{E m}^{-2} \text{s}^{-1}$ -grown corn.

Leaves of C₃ species *Nerium oleander* (Björkman and Powles, 1984), *Helianthus annuus* and *Xanthium strumarium* (Ben et al., 1987), grown under full natural sunlight, have shown decreased levels of variable and maximal fluorescence (F_m) measured at 77°K, but these changes closely followed variations in the sunlight's intensity over the day.

Fluorescence quenching observed under normal growing conditions has been referred to as "incipient" (Björkman and Powles, 1984) or "chronic" (Greer and Laing, 1988) photoinhibition. Since such quenching appears to be readily reversible upon exposure to lower light levels (Björkman and Powles, 1984), it may reflect a redistribution of excitation energy within PS II (Greer and Laing, 1988), rather than extensive physical damage to the photosynthetic apparatus. According to the model for total fluorescence yield presented by Björkman (1987) (Appendix VI), damage to PS II reaction centres would be expected to cause a decrease in K_p , the rate constant for photochemistry of PS II. A drop in K_p could cause an increase in F_o (Appendix VI), but no significant changes in this parameter were detected in experiments 1 and 3b (Tables II, IV, and IX), or by Greer and Laing (1988). Alternatively, the quenching could be due to an increase in the rate constant for non-radiative dissipation of excitation energy, K_D (Greer and Laing, 1988) over the day. An increase in K_D would be expected to cause decreased emissions of both F_o and F_m (Appendix VI), and might be due to an inability to dissipate excitation energy by photosynthetic electron transport as the day progresses. The gradual quenching of variable fluorescence in the three corn lines seemed to parallel diurnal decreases in the rate of light-saturated, uncoupled whole-chain electron transport reported by Lonergan (1984).

4.2 Photoinhibition experiments

It was expected that high light treatment at 5°C would cause a general quenching of variable and steady state fluorescence and rate of oxygen evolution with respect to measurements from control leaves chilled at the growth PFD. Also, it was anticipated that the extent of chilling-induced photoinhibition observed for a corn line would be directly related to its growth performance at 10°C, determined in the study at Agriculture Canada. However, a clear relationship between the long term chilling sensitivity of the corn lines and extent of photoinhibitory damage incurred at 5°C was not found. Some trends, discussed below, suggested that HIWF might be somewhat more susceptible to high light stress at 5°C than Gaspé or LG3, but these trends were not consistent.

4.2.1 Variable and initial fluorescence

As expected, exposure to light levels higher than the growth PFD caused a very significant photoinhibitory quenching (qI) of the P/F_0 ratio. This was indicative of damage in the vicinity of the PS II reaction centre (Krause, 1988). Other authors (East *et al.*, 1981; Baker *et al.*, 1983; Richards and Hall 1986; Somersalo and Krause, 1989) have reported a similar loss of *in vivo* room temperature variable fluorescence when leaves were exposed to high PFDs at chilling temperatures.

The extent of photoinhibition is known to increase with the PFD to which leaves are exposed (Powles and Björkman, 1982; Long *et al.*, 1983; Powles *et al.*, 1983; Ögren *et al.*, 1984; Ögren and Öquist, 1984a; Greer and Laing, 1988). However, for leaves receiving a 6 h treatment at $3000 \mu\text{E m}^{-2} \text{s}^{-1}$ or $1500 \mu\text{E m}^{-2} \text{s}^{-1}$, there was little difference in the extent of photoinhibition for treatments carried out during the same time period (Table III). It is likely that a 6 h exposure to $1500 \mu\text{E m}^{-2} \text{s}^{-1}$ was sufficient to almost completely inhibit PS II, and that the additional high light stress at $3000 \mu\text{E m}^{-2} \text{s}^{-1}$ only led to the photooxidation of HIWF bulk leaf pigments (Table II).

The greater extent of photoinhibitory damage when high light treatment was carried out at 5°C than at 20°C (experiment 2a) agrees with previous findings (East *et al.*, 1981; Powles and Björkman, 1982; Baker *et al.*, 1983; Long *et al.*, 1983; Powles *et al.*, 1983; Greer *et al.*, 1988; Greer and Laing, 1988). This may be due to inhibition of photosynthetic rates and/or repair processes at chilling temperatures (discussed below).

The corn lines were affected significantly differently by high light treatment, but only with respect to their chilled controls (Table III). This was because P/F_0 values for the corn and barley lines differed for the control leaves rather than for the high light treated leaves. As a result, it is too early to conclude if the apparent significant differences between corn lines, or between corn and barley, are due to genotypic differences in the extent of photoinhibition, or are reflective of variation in the range of P/F_0 for each line.

In contrast to P/F_0 , F_0 levels were not significantly altered by high light treatment at 5°C . F_0 increased significantly in some instances (experiments 2b

and 3a), but these changes were not always consistent with HIWF showing the greatest extent of photoinhibition. Similar increases in F_0 have been observed in both chilling-tolerant *Lemna gibba* (Ögren and Öquist, 1984b) and spinach (Somersalo and Krause, 1989) and chilling-intolerant *Nerium oleander* (Powles and Björkman, 1982), corn (Baker *et al.*, 1983), and kiwifruit (Greer *et al.*, 1988) after several hours' exposure to light levels above the growth PFD at chilling temperatures. Measurements from corn (Baker *et al.*, 1983) leaves were recorded at room temperature, while those from the other species were emissions from PS II antennae, measured at 77°K. Both methods of measurement were used for spinach (Somersalo and Krause, 1989).

4.2.2 Steady state fluorescence and bulk chlorophyll contents

With the exception of the experiment 2a data, high light treatment at 5°C caused a general quenching of steady state fluorescence with respect to control leaves, as expected. At first glance, it appeared that HIWF may have suffered the most photoinhibition, but results were too inconsistent to make definite conclusions. For corn exposed to $3000 \mu\text{E m}^{-2} \text{s}^{-1}$ for 6 h (experiment 1a), the lines were not significantly different in degree of quenching of T; the slightly greater loss of steady state fluorescence in HIWF may have been due to the photooxidative loss of bulk chlorophyll (Table II). Three hours at $1500 \mu\text{E m}^{-2} \text{s}^{-1}$ following a 12 h overnight dark chill (experiment 3a) induced a greater photoinhibitory loss of steady state fluorescence in HIWF than in Gaspé, but there was no significant difference between the two cultivars when the experiment was repeated 3 h later with plants that had received a 15h overnight chill (Table VIII). However, as HIWF was the only line to suffer a

significant photooxidative loss of bulk chlorophyll (Table II), this might alone suggest that HIWF was the most susceptible to photoinhibition.

Photobleaching of leaf pigments is thought to occur only after severe photoinhibitory damage has taken place (Powles, 1984).

4.2.3 Possible reasons for changes in fluorescence levels during photoinhibition

Changes in the levels of F_0 and variable fluorescence may be a result of two separate processes (Demmig and Björkman, 1987). First, according to the model of total fluorescence yield (Björkman, 1987) (Appendix VI), an increase in the level of F_0 emitted from antennae chlorophyll a molecules upon photoinhibitory damage to PS II reaction centres could be caused by a drop in K_P (Ögren and Öquist, 1984b; Demmig and Björkman, 1987). Second, a rise in F_0 could be due to a decrease in the rate constant for transfer of excitation energy from antennae pigments to the PS II reaction centre (Ögren and Öquist 1984b). Again, this could be the result of direct damage to the PS II reaction centre (Percival *et al.*, 1987), leading to decreased trapping efficiency (Somersalo and Krause, 1989), or could be due to modification of the protein complement of the light harvesting chlorophyll apparatus of PS II (LHC II) (Hayden *et al.*, 1987) and/or its detachment from the PS II reaction centre (Percival *et al.*, 1987). It is obvious that any increase in excitation energy emitted as F_0 would lead to a decrease in the emission of variable fluorescence.

Photoinhibitory quenching of variable fluorescence may also be due to an increase in K_D , the rate constant for non-radiative dissipation of excitation

energy by antennae pigments (Powles and Björkman, 1982; Ögren and Öquist, 1984b, Demmig and Björkman, 1987). This may be associated with a modification of the pigment bed (Bradbury and Baker, 1986) and may have been responsible for the general quenching of P, M and T in high light-treated leaves (Fig. 13) (Bradbury and Baker, 1986). A quenching of F_0 would also be expected from Björkman's model, but this was not observed in any of the experiments reported here.

The consistency of F_0 levels may be because the magnitude of K_D was small compared to that of K_P ; in this situation an increase in K_D might cause a significant quenching of F_m , with a small, possibly undetectable, drop in F_0 (Demmig and Björkman, 1987). Quenching of variable fluorescence, but not F_0 , may also be attributed to an increase in emission of excitation energy as heat at PS II reaction centres, rather than at antenna chlorophyll (Ögren and Öquist, 1984b). Alternatively, since absolute fluorescence values (such as F_0 and steady state fluorescence) measured from leaves at room temperature may vary with the optical properties or chlorophyll contents of individual leaves (Hipkins and Baker, 1986), any real differences between photoinhibited and control leaves may have been obscured.

4.2.4 Oxygen evolution rates

In all experiments, high light treatment caused a general drop in the rate of light-limited oxygen evolution from leaf tissue, consistent with damage in the vicinity of PS II. Other authors have reported similar decreases in oxygen evolution rates measured from leaf tissue subjected to high PFDs at 5°C (Baker *et al.*, 1983), or at room temperature (Greer *et al.*, 1986; Greer and

Laing, 1988). As for the fluorescence parameters, oxygen evolution rates did not detect clear significant genotypic differences in the extent of photoinhibition.

4.3 Short term chilling experiments

In general, short term chilling treatments at 5°C and the growth PFD caused decreases in P/F_0 for all corn lines and Birka barley, but the effect on other parameters was less consistent.

4.3.1 Effects of short term chilling treatments on variable fluorescence and rate of oxygen evolution

With respect to P/F_0 , the lack of a significant difference between corn lines (particularly Gaspé and HIWF) and Birka barley for response to 6 h chilling treatments was contrary to what was expected. Although Birka was not tested against corn for its ability to grow under long-term chilling conditions, the temperate origins of barley (Smillie *et al.*, 1988) suggest that it should be more tolerant of chilling conditions than corn, a grass of tropical origin (Smillie *et al.*, 1988).

Quite unexpectedly, short term chilling effects for the corn lines seemed to be inversely related to the long term chilling sensitivity of each line. This was most apparent for P/F_0 recorded from leaves chilled 6 h at the growth PFD, examined 10 to 12.5 hours after photoperiod initiation (time period 1). In this earlier time period of experiment 1b (Fig. 12a), P/F_0 measured from

Gaspé and LG3 dropped significantly to 79 and 78%, respectively, of untreated leaf values, while HIWF was not significantly affected by the 6 h chill.

Repetition of the experiment (experiment 2c) revealed a similar pattern: although LG3 was the only line with a significant loss of P/F_0 in this analysis, Gaspé still showed a slightly greater (ANOVA, $p = 0.018$) decrease than did HIWF (ANOVA, $p = 0.156$) (Table VII).

This relationship was not apparent for leaves chilled later in the day during experiment 1b (Fig. 12b): with respect to untreated leaves, P/F_0 from Gaspé and HIWF were equally lowered by the 6 hour chill, whereas P/F_0 did not change significantly for LG3. The relationship may have been obscured by a significant diurnal decrease in P/F_0 for untreated leaves of Gaspé and LG3, but not HIWF, making the chilling-induced drop in P/F_0 apparently less significant for Gaspé and LG3, than for HIWF.

The significant decreases in P/F_0 upon chilling at the growth PFD were probably largely due to photoinhibition (Öquist, 1983), induced by the depression of photosynthetic rates at low temperatures. This is supported by similar short term chilling experiments with the aquatic plant *Lemna gibba* (Ögren *et al.*, 1984) and 15 other species (Smillie *et al.*, 1988). Light was required for the inhibition of PS II photochemistry, even at PFDs as low as $150 \mu\text{E m}^{-2} \text{s}^{-1}$ (slightly above the growth PFD of *Lemna gibba*); chilling of leaf tissue in the dark for the same length of time had no effect on variable fluorescence levels measured at 77°K.

In short-term chilling trials of several hours' duration carried out at low to moderate light levels, the chill-labile site(s) limiting photosynthesis may lie on the reducing side of PS I, at the ferredoxin-FeS complex or NADP-

ferredoxin oxidoreductase (Hodgson and Raison, 1989) and/or at the level of the Calvin cycle or C₄ enzymes (Taylor *et al.*, 1974; Stamp, 1984; Hodgson and Raison, 1989). Six hour chilling of 25°C-grown corn (Hybrid Wisc. 575) seedlings at 10°C at the growth PFD (approximately 800 $\mu\text{E m}^{-2} \text{s}^{-1}$) caused significant inhibition of NADP-malate dehydrogenase and pyruvate Pi dikinase activity when the enzymes were isolated following chilling treatment and assayed at 25°C (Taylor *et al.*, 1974). Leaf catalase activity was also decreased by this treatment (Taylor *et al.*, 1974), suggesting that chilling could increase the likelihood of photoinhibitory damage at PS II caused by activated oxygen species (Graham and Patterson, 1982).

As expected, the greatest chilling-induced drop in P/F_0 was observed when 3 h treatments at 5°C and 150 $\mu\text{E m}^{-2} \text{s}^{-1}$, were preceded by an overnight dark pre-chill (experiment 3b). However, there was still no correlation between the long term and short term chilling susceptibilities of the corn lines: Gaspé and HIWF were equally affected by this chill in the light, with a 36 to 39% loss of P/F_0 with respect to leaves maintained 3 h under normal growing conditions following the pre-chill. Again, this quenching was probably mostly due to photoinhibition, to which plants were predisposed by the slowing of photosynthetic rates during the pre-chill. A dark overnight chill (16 h at 1°C, and 100% relative humidity), similar to that received by the corn seedlings, caused significant inhibition of CO₂- and light-saturated rates of CO₂ uptake (Ort and Martin, 1983) in leaves of young tomato plants. Less than one-third of this inhibition was attributed to stomatal closure, the rest to a direct effect at the chloroplast level. It was suggested that the Calvin cycle enzymes were affected by chilling

temperatures, as the dark overnight chill had no significant effect on electron transport activity through PS II, or on the light-dependent rate of CO₂ uptake (Ort and Martin, 1983).

Lesions occurring after Q_a would prevent the oxidation of Q_a⁻, increasing the level of variable fluorescence emitted. This may account for the increase in P/F₀ measured from leaves receiving only a 15 hour overnight dark chill, with respect to untreated leaves (Table IX). This chill-induced increase was greater for Gaspé, than for HIWF, indicating again the lack of correspondence between short- and long-term chill tolerance. Curiously, this relationship was not apparent for the 12 h pre-chill data, as a longer dark chilling period may have been required to observe a difference between cultivars.

Havaux and Lannoye (1984) suggested that a loss of P/F₀ in corn seedlings chilled at the growth PFD was due to inactivation of the oxygen evolving complex (OEC), caused by a decrease in fluidity of the thylakoid membranes at chilling temperatures. However, light-dependent oxygen evolution rates were not significantly affected in any of the corn lines tested in experiments 1b, 2c or 3b. The OEC has been identified as the site of chilling stress primarily in experiments carried out with detached leaves of chilling-sensitive plants stored in the dark for several days (Kaniuga *et al.*, 1978; Smillie 1979; Smillie and Nott, 1979), clearly non-physiological conditions.

4.3.2 Effects of short-term chilling treatments on initial and steady state fluorescence levels.

In contrast to P/F_0 , the 6 h chilling treatments had rather inconsistent effects on steady state and initial fluorescence levels for the three corn lines. Steady state fluorescence data for experiment 1b (Table IV) did suggest a trend to the inverse chilling relationship observed with P/F_0 , but the reverse was suggested by the experiment 2c data (Table VII). Closer examination of p values for experiment 2c revealed that Gaspé was almost as significantly affected by the short term chilling treatment (ANOVA, $p = 0.018$) as was HIWF (ANOVA, $p = 0.002$). F_0 was also little affected by chilling treatment during experiment 1b (Table IV), and only for HIWF in experiment 2c (Table VII). However, line by treatment interaction terms were not significant for F_0 and T in either experiment 1b or 2c.

4.4 The relationship between susceptibility to chilling-induced photoinhibition and chilling tolerance

Despite the ambiguous results of the photoinhibition experiments, recent comparisons within rice, and between rice and barley (Moll and Steinback, 1986; Smillie *et al.*, 1988) and a number of other species (Smillie *et al.*, 1988) do suggest that cultivars or species with a greater chilling sensitivity are more susceptible to photoinhibition at chilling temperatures than are plants known for some degree of chilling tolerance. The chilling tolerances of

these plants were presumably determined according to their ability to grow under low, non-freezing temperatures, but this was not clearly stated by the authors.

There does seem to be some genetic variability within *Zea mays* for susceptibility to high light stress at 5°C (Long *et al.*, 1987). Lines considered to be generally cold-tolerant showed a high resistance to the loss of light-dependent CO₂ uptake. However, correlation of the degree of photoinhibition with chilling sensitivity of the lines was not possible, as the relative chilling tolerances of all genotypes were not given.

Chilling-sensitive species or cultivars might be particularly prone to photoinhibition at suboptimal temperatures for several reasons.

1. Genotypic differences in chill-lability of the photosynthetic apparatus.

A plant's intolerance of chilling temperatures may imply that its photosynthetic apparatus is also chill-labile, leading to inhibition of photosynthetic rates at low temperatures. This would limit the dissipation of excitation energy through PS II (as discussed above), predisposing plants with a greater chill-sensitivity of photosynthesis to a greater extent of photoinhibition.

At the level of the thylakoid membrane, an increase in the viscosity of the thylakoid lipid matrix in more chilling-sensitive plants could hinder the diffusion of plastoquinone between PS II and cytochrome b₆/f complexes, blocking electron transport through PS II (Havaux, 1987). In fact, within both *Zea mays* (Havaux, 1987) and *Oryza sativa* (Moll and Steinback, 1986), chilled leaves of relatively chilling-intolerant genotypes showed greater levels of reduced Q_a than did lines considered to be generally more chilling-tolerant.

Moll and Steinback (1986) presented evidence that chilling temperatures inhibited phosphorylation of the chlorophyll a/b light-harvesting complex (LHC II) in rice but not in barley, both *in vivo* and *in vitro*. The authors proposed that spillover of excitation energy from PS II to PS I would be prevented in rice, causing light energy to arrive at PS II in excess of what would be dissipated by electron transport. This might explain the greater inhibition of variable fluorescence in rice than in barley, after 2 h treatment of intact leaves at $2000 \mu\text{E m}^{-2} \text{s}^{-1}$ and 5°C (Moll and Steinback, 1986). However, more recent work (Demmig *et al.*, 1987) suggests that phosphorylation of LHC II may not play a protective role against photoinhibitory damage, at least at room temperature. Under photoinhibitory conditions at 25°C causing significant irreversible quenching of variable fluorescence correlated with an increase in ΔpH , the LHC II was dephosphorylated (Demmig *et al.*, 1987).

As discussed above, dissipation of excitation energy away from PS II may be limited at chilling temperatures by the inhibition of photosynthesis on the reducing side of PS I (Taylor *et al.*, 1974; Ort and Martin, 1983; Stamp, 1984; Hodgson and Raison, 1989). Sugiyama and Boku (1976) found a strong correlation between the climatic origins of several corn genotypes and the *in vitro* activity of the C_4 enzyme pyruvate Pi dikinase, extracted from leaves of warm-grown seedlings cultivated under identical conditions. Lines adapted to northern cool temperate regions showed a higher enzyme activity at 0°C than did lines originating from more southern zones (Sugiyama and Boku, 1976). The activity of NADP-malate dehydrogenase may also limit the rate of CO_2 assimilation in chilling-intolerant corn genotypes subjected to short term chilling treatments at the growth PFD (Stamp, 1984).

2. Differences in rates of repair of photoinhibitory damage.

It is also possible that chilling temperatures may inhibit the rate of repair of photoinhibitory damage, thought to involve protein synthesis (Ohad *et al.*, 1984; Greer *et al.*, 1986), to a greater extent in chilling-intolerant plants than in those more resistant to chilling temperatures (Greer *et al.*, 1986).

The absence of a clear relationship between the long term chilling tolerances of the three corn lines and the severity of photoinhibitory damage when leaves were chilled to 5°C could be attributed to the apparent differences between their long term and short term chilling tolerances. In particular, the data for leaves chilled 6 h at the growth light level suggest that the photosynthetic apparatus of HIWF is less chill-labile in the short term, than that of Gaspé or LG3. MacRae *et al.* (1986) found a similar lack of correlation between long-term whole-plant chilling-tolerance and short-term chill-lability of the photosynthetic apparatus. *Passiflora* species, judged very sensitive to chilling by the extent of visible injury to leaves in growth chamber studies, showed little short term chill-lability of photosynthesis. Chilling sensitivity of the photosynthetic apparatus was judged according to the number of hours required to show a 50% decrease in the rate of rise of variable fluorescence for leaf tissue maintained in the dark at 0°C (Hetherington *et al.*, 1983). However, short term chill-lability of photosynthesis does not necessarily mean that a plant will be more susceptible to photoinhibition at chilling temperatures. Smillie *et al.* (1988) and Hetherington *et al.* (1989) found no linear relationship between chill

tolerance of the photosynthetic apparatus of 15 plant species (measured by the method of Hetherington *et al.*, 1983), and the extent of photoinhibitory damage after 20 h at 7°C and 300 $\mu\text{E m}^{-2} \text{s}^{-1}$. Therefore, contrary to the assumption discussed above, short term exposure to chilling temperatures may not be the factor predisposing some genotypes (such as HIWF) to photoinhibition.

Instead, Smillie *et al.* (1988) have suggested that chilling-intolerant plants may show more photoinhibitory damage because they could be more sensitive to light at both chilling and non-chilling temperatures. They found that species considered to be generally chilling-intolerant continued to show a more significant depression of the ratio of variable over maximal fluorescence (measured at 77°K) after 20 h treatment at 21°C and 900 $\mu\text{E m}^{-2} \text{s}^{-1}$, than did those species considered relatively chilling-tolerant. Genotypic differences in the ability to dissipate excess excitation energy as heat might account for these differences in susceptibility to photoinhibition (Greer *et al.*, 1988). If HIWF is more light-sensitive than the other lines, this may account for the significant photooxidative loss of chlorophyll after 6 h exposure to 3000 $\mu\text{E m}^{-2} \text{s}^{-1}$ at 5°C, and the strong photoinhibition of photosynthetic parameters at 5°C, even though HIWF seemed to be the least affected by short term chilling exposure. Photoinhibition experiments carried out at 20°C would be required to confirm if HIWF is indeed more light-sensitive at all temperatures.

A better correlation between the long term growth performances of the corn lines and extent of photoinhibition might have existed if the chill-lability of the photosynthetic apparatus was used as the indicator of relative

chilling tolerance. This could have been accomplished by the methods of experiments 1b, 2c or 3b, or Hetherington *et al.* (1983). However, this underlines the ambiguity of the terms "chilling-tolerant" or "chilling-sensitive". In the literature, the definition of chilling sensitivity is rather vague, particularly with respect to the nature and location of damage before it becomes visible as chlorosis or necrosis of leaf tissue. In the field, the chilling tolerance of a plant ultimately depends upon its ability to grow under, and survive exposure to suboptimal temperatures, which may effect many aspects of metabolism (e.g. respiration, uptake of nutrients from the soil, translocation) (Stamp, 1984), in addition to photosynthesis. Therefore, judging the chilling-tolerance of a plant on the basis of chilling effects on a single trait, as a number of authors have done (e.g. Smillie, 1979; Hetherington *et al.*, 1983), may be misleading (Stamp, 1984). Growth performance under controlled environmental conditions remains the best method for the estimation of general performance of a genotype at chilling temperatures (MacRae *et al.*, 1986).

In conclusion, it was not possible to positively confirm within *Zea mays* the relationship between long-term chilling tolerance and ability to resist photoinhibition at a chilling temperature. However, the literature suggests that the ability of a plant species or cultivar to grow and survive at chilling temperatures may not necessarily confer high resistance to photoinhibition in this temperature range, particularly when the stress conditions are only of several hours' duration.

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Appendix Ia

Results of preliminary 1 h photoinhibition experiments with $300 \mu\text{E m}^{-2} \text{s}^{-1}$ - grown Gaspé and HIWF seedlings. Intact, attached second leaves were exposed to $1100 \mu\text{E m}^{-2} \text{s}^{-1}$ for 1 h at 5 or 20°C. Control leaves were maintained at 5 or 20°C with the lamp off (approximately $10 \mu\text{E m}^{-2} \text{s}^{-1}$). Refer to section 2.5.2.1 for further experimental details.

Values presented (when $n \geq 3$) are mean $\pm$ standard deviation of the data, with sample sizes as indicated. Data were placed into two arbitrary time periods, according to the time when room temperature fluorescence was measured.

treatment temperature	PFD	cultivar	n	ratio of peak to initial fluorescence (P/F ₀)	bulk chlorophyll content
[°C]	[μE m ⁻² s ⁻¹]				[μg chlorophyll g leaf tissue]
i) examined 7 to 12 h after start of photoperiod					
5	1100	Gaspé	3	1.17 ± 0.05	---
		HIWF	3	1.16 ± 0.03	2561 ± 198
	10	Gaspé	2	1.39, 1.47	---
		HIWF	1	1.65	2795
20	1100	Gaspé	3	1.53 ± 0.14	2403 ± 350
		HIWF	3	1.60 ± 0.08	2640 ± 190
	10	Gaspé	3	1.45 ± 0.29	2556 ± 222
		HIWF	3	1.32 ± 0.19	2231 ± 124
untreated		Gaspé	8	1.81 ± 0.24	---
		HIWF	7	1.33 ± 0.27	2511 ± 256
ii) examined 12 to 17 h after start of photoperiod					
5	1100	Gaspé	4	1.17 ± 0.04	---
		HIWF	4	1.38 ± 0.02	2742 ± 181
	10	Gaspé	4	1.43 ± 0.14	---
		HIWF	4	1.22 ± 0.03	2861 ± 200
20	1100	Gaspé	1	1.37	3226
		HIWF	---	---	---
	10	Gaspé	1	1.60	2869
		HIWF	2	1.37, 1.23	2279, 2525
untreated		Gaspé	3	1.62 ± 0.18	---
		HIWF	6	1.24 ± 0.08	---

Appendix Ib

Results of preliminary 3h photoinhibition experiments with 300 $\mu\text{E m}^{-2} \text{s}^{-1}$ - grown Gaspé and HIWF seedlings. Intact, attached second leaves were exposed to 1100 $\mu\text{E m}^{-2} \text{s}^{-1}$ for 3h at 5°C, controls to approximately 10 (lamp off) or 300 $\mu\text{E m}^{-2} \text{s}^{-1}$ at 5°C. Fluorescence transients were measured 9.5 to 13h after photoperiod initiation. Refer to section 2.5.2.1 for further experimental details. Data are presented as mean $\pm$ standard deviation, with sample sizes as indicated.

PFD at 5°C [$\mu\text{E m}^{-2} \text{s}^{-1}$]	cultivar	n	ratio of peak to initial fluorescence (P/F ₀)	bulk chlorophyll content [$\mu\text{g chlorophyll}$ g leaf tissue]
1100	Gaspé	4	1.10 $\pm$ 0.05	2296 $\pm$ 370
	HIWF	4	1.15 $\pm$ 0.06	2612 $\pm$ 402
10	Gaspé	3	1.22 $\pm$ 0.04	2326 $\pm$ 187
	HIWF	3	1.35 $\pm$ 0.07	2469 $\pm$ 459
300	Gaspé HIWF	--- 5	--- 1.37 $\pm$ 0.07	--- 2221 $\pm$ 266

Appendix II

Analyses of Variance for Preliminary Experiments

1. Dark adaptation of $150 \mu\text{E m}^{-2} \text{s}^{-1}$ -grown HIWF seedlings. Comparison of P/F_0 values measured from the second leaf after 0, 15, 30, 60, or 90 minutes of whole-plant dark exposure at room temperature.

ANOVA TABLE

Source	d. f.	Mean Square	F ratio	p
between groups	4	0.5416	68.7303	0.0000
within groups	30	0.0079		

2. Diurnal change in P/F_0 from second leaves of $300 \mu\text{E m}^{-2} \text{s}^{-1}$ -grown HIWF seedlings, examined 3 to 4.5 hours and 12 to 14 hours after photoperiod initiation.

ANOVA TABLE

Source	d. f.	Mean Square	F ratio	p
between groups	1	0.1945	27.3653	0.0004
within groups	10	0.0071		

3. Diurnal changes in F_0 and P from second leaves of $300 \mu\text{E m}^{-2} \text{s}^{-1}$ -grown HIWF seedlings, measured 3 to 4.5 hours and 12 to 14 hours after start of the photoperiod.

ANOVA TABLE

Source	d. f.	Mean Square	F ratio	p
between groups				
fluorescence parameter	1	9165.04167	27.92305	0.000
time period	1	3876.04167	11.80910	0.003
interaction	1	1650.04167	5.02717	0.036
within groups	20	328.22500		

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
between groups				
time within F_0	1	234.08333	0.71318	0.408
time within P	1	5292.00000	16.12309	0.001
within groups	20	328.22500		

Appendix III

Analyses of Variance for Experiments 1a and b

A) Experiment 1a: Effects of 6h photoinhibitory treatment at $3000 \mu\text{E m}^{-2} \text{s}^{-1}$ and 5°C on photosynthetic parameters measured from second leaves of 3 corn lines. Control leaves were treated for 6h at $150 \mu\text{E m}^{-2} \text{s}^{-1}$ and 5°C .

Treatments were carried out during two daily time periods so that data were collected 10 to 12h (time period 1) and 16 to 18h (time period 2) after the start of the photoperiod.

1. O_2 evolution rate

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
time period	1	0.07341	2.22106	0.145
PFD	1	17.41172	526.80363	< 0.001
line	2	0.28507	8.62512	0.001
time by PFD by line	2	0.00764	0.23128	0.795
time by PFD	1	0.00052	0.01584	0.901
PFD by line	2	0.18154	5.49271	0.008
time by line	2	0.05626	1.70221	0.197
within groups	36	0.03305		

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
PFD within Gaspé	1	4.79663	145.12523	< 0.001
PFD within LG3	1	8.41099	254.48016	< 0.001
PFD within HIWF	1	4.56720	138.18366	< 0.001
line within 3000 $\mu\text{E m}^{-2} \text{s}^{-1}$	2	0.04907	1.48453	0.240
line within 150 $\mu\text{E m}^{-2} \text{s}^{-1}$	2	0.41755	12.63330	< 0.001

2. P/F_0

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
time period	1	0.10228	14.27363	< 0.001
PFD	1	1.66240	231.99642	< 0.001
line	2	0.06667	9.30346	< 0.001
time by PFD by line	2	0.00972	1.35643	0.263
time by PFD	1	0.10212	14.25177	< 0.001
PFD by line	2	0.05702	7.95783	0.001
time by line	2	0.00790	1.10284	0.337
within groups	84	0.00717		

relevant simple main effects				
Source	d. f.	Mean Square	F ratio	p
time within 3000 $\mu\text{E m}^{-2} \text{ s}^{-1}$	1	0.00000	0.00000	0.998
time within 150 $\mu\text{E m}^{-2} \text{ s}^{-1}$	1	0.20440	28.52540	< 0.001
PFD within time period 1	1	1.29429	180.62506	< 0.001
PFD within time period 2	1	0.47023	65.62314	< 0.001
PFD within Gaspé	1	0.27662	38.60368	< 0.001
PFD within LG3	1	0.50115	69.93824	< 0.001
PFD within HIWF	1	0.99867	139.37015	< 0.001
line within 3000 $\mu\text{E m}^{-2} \text{ s}^{-1}$	2	0.00120	0.16731	0.846
line within 150 $\mu\text{E m}^{-2} \text{ s}^{-1}$	2	0.12249	17.09398	< 0.001

3. Initial fluorescence level, F_0 **ANOVA TABLE: MAIN EFFECTS**

Source	d. f.	Mean Square	F ratio	p
between groups				
time period	1	8.76042	0.10491	0.747
PFD	1	168.01042	2.01199	0.160
line	2	2452.34375	29.36782	< 0.001
time by PFD by line	2	128.13542	1.53447	0.222
time by PFD	1	8.76042	0.10491	0.747
PFD by line	2	20.13542	0.24113	0.786
time by line	2	58.88542	0.70518	0.497
within groups	84	83.50446		

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
PFD within Gaspé	1	78.12500	0.93558	0.336
PFD within LG3	1	124.03125	1.48532	0.226
PFD within HIWF	1	6.12500	0.07335	0.787

4. Steady state fluorescence level, T

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
time period	1	234.37500	2.20730	0.141
PFD	1	4845.04167	45.62979	< 0.001
line	2	1135.01042	10.68934	< 0.001
time by PFD by line	2	7.69792	0.07250	0.930
time by PFD	1	322.66667	3.03882	0.085
PFD by line	2	252.32292	2.37633	0.099
time by line	2	36.21875	0.34110	0.712
within groups	84	106.18155		

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
PFD within Gaspé	1	1116.28125	10.51295	0.002
PFD within LG3	1	830.28125	7.81945	0.006
PFD within HIWF	1	3403.12500	32.05006	< 0.001

5. Leaf bulk chlorophyll content

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
time period	1	490.51042	0.00813	0.928
PFD	1	898420.51042	14.89559	< 0.001
line	2	428728.53125	7.10822	0.001
time by PFD by line	2	23982.38542	0.39762	0.673
time by PFD	1	32671.26042	0.54168	0.464
PFD by line	2	56884.07292	0.94312	0.393
time by line	2	19745.51042	0.32738	0.722
within groups	84	60314.51042		

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
PFD within Gaspé	1	130177.53125	2.15831	0.146
PFD within LG3	1	216153.125000	3.58377	0.062
PFD within HIWF	1	665858.00000	11.03976	0.001

B) Experiment 1b: Effects of 6h chilling treatment at 5°C and 150 $\mu\text{E m}^{-2} \text{s}^{-1}$ on photosynthetic parameters measured from second leaves of 3 corn lines. Control leaves were maintained concurrently under normal growing conditions (150 $\mu\text{E m}^{-2} \text{s}^{-1}$, 23°C). Treatments were carried out during the same two daily time periods described in Experiment 1a.

1. O₂ evolution rate

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
time period	1	0.22948	2.49177	0.123
temperature	1	0.32863	3.56839	0.067
line	2	0.54902	5.96145	0.006
time by temp. by line	2	0.10451	1.13481	0.333
time by temperature	1	0.05335	0.57932	0.452
temperature by line	2	0.03033	0.32935	0.722
time by line	2	0.11742	1.27498	0.292
within groups	36	0.09210		

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
temp. within Gaspé	1	0.24111	2.61799	0.114
temp. within LG3	1	0.02117	0.22987	0.635
temp. within HIWF	1	0.12702	1.37923	0.248

2. P/F_0 **ANOVA TABLE: MAIN EFFECTS**

Source	d. f.	Mean Square	F ratio	p
between groups				
time period	1	0.73330	50.19215	< 0.001
temperature	1	1.03130	70.58878	< 0.001
line	2	0.09265	6.34127	0.003
time by temp. by line	2	0.08711	5.96230	0.004
time by temperature	1	0.04707	3.22170	0.076
temperature by line	2	0.04757	3.25574	0.043
time by line	2	0.01240	0.84888	0.432
within groups	84	0.01461		

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
time within Gaspé	1	0.30949	21.18340	< 0.001
time within LG3	1	0.31478	21.54573	< 0.001
time within HIWF	1	0.13384	9.16079	0.003
time within 5°C	1	0.20440	13.99064	< 0.001
time within untreated	1	0.57597	39.42321	< 0.001
line within 5°C	2	0.12249	8.38395	< 0.001
line within untreated	2	0.01772	1.21305	0.302

3. Initial fluorescence level, F_0 **ANOVA TABLE: MAIN EFFECTS**

Source	d. f.	Mean Square	F ratio	p
between groups				
time period	1	102.09375	1.09900	0.297
temperature	1	1046.76042	11.26793	0.001
line	2	2207.88542	23.76694	< 0.001
time by temp. by line	2	79.96875	0.86083	0.427
time by temperature	1	102.09375	1.09900	0.297
temperature by line	2	20.69792	0.22280	0.801
time by line	2	99.78125	1.07410	0.346
within groups	84	92.89732		

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
temp. within Gaspé	1	465.12500	5.00687	0.028
temp. within LG3	1	180.50000	1.94301	0.167
temp. within HIWF	1	442.53125	4.76366	0.032

4. Steady state fluorescence level, T

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
time period	1	121.50000	0.95539	0.331
temperature	1	1190.04167	9.35769	0.003
line	2	1416.69792	11.13996	< 0.001
time by temp. by line	2	89.13542	0.70090	0.499
time by temperature	1	495.04167	3.89267	0.052
temperature by line	2	229.38542	1.80373	0.171
time period by line	2	2.90625	0.02285	0.977
within groups	84	127.17262		

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
temp. within Gaspé	1	1188.28125	9.34385	0.003
temp. within LG3	1	442.53125	3.47977	0.066
temp. within HIWF	1	18.00000	0.14154	0.708

5. Leaf bulk chlorophyll content

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
time period	1	43947.04167	0.65603	0.420
temperature	1	2016.66667	0.03010	0.863
line	2	288701.65625	4.30963	0.017
time by temp. by line	2	7311.19792	0.10914	0.897
time by temperature	1	2604.16667	0.03887	0.844
temperature by line	2	113556.69792	1.69513	0.190
time period by line	2	42801.94792	0.63893	0.530
within groups	84	66989.81845		

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
temp. within Gaspé	1	18963.78125	0.28308	0.596
temp. within LG3	1	39200.00000	0.58516	0.446
temp. within HIWF	1	170966.28125	2.55212	0.114

Appendix IV

Analyses of Variance for Experiments 2a, 2b, and 2c

A) Experiment 2a

i) Comparison of extent of photoinhibition at 5°C versus 20°C for second leaves of LG3 seedlings exposed to 1500 $\mu\text{E m}^{-2} \text{s}^{-1}$ for 6h. The high light - treated leaves are compared to their corresponding same-temperature controls maintained for 6h at 150 $\mu\text{E m}^{-2} \text{s}^{-1}$.

1. P/F₀

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	0.53323	43.61063	< 0.001
IPFD during treatment	1	1.23135	100.70612	< 0.001
interaction	1	0.00901	0.73701	0.398
within groups	28	0.01223		

2. Initial fluorescence level, F_0

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	116.28125	1.89034	0.180
PFD during treatment	1	225.78125	3.67044	0.066
interaction	1	30.03125	0.48821	0.490
within groups	28	61.51339		

3. Steady state fluorescence level, T

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	175.78125	3.22878	0.083
PFD during treatment	1	124.03125	2.27823	0.142
interaction	1	22.78125	0.41845	0.523
within groups	28	54.44196		

4. Leaf bulk chlorophyll content

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	5805.03125	0.11685	0.735
PFD during treatment	1	21997.53125	0.44278	0.511
interaction	1	120909.03125	2.43375	0.130
within groups	28	49680.20982		

ii) Comparison of photosynthetic parameters for second leaves of LG3 seedlings treated in the leaf chamber at $150 \mu\text{E m}^{-2} \text{s}^{-1}$ and either 5 or 20°C , versus those for untreated leaves maintained concurrently under normal growing conditions ($150 \mu\text{E m}^{-2} \text{s}^{-1}$ and 23°C).

1. P/F_0

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	0.07719	4.18769	0.050
treatment location	1	0.32076	17.40287	< 0.001
interaction	1	0.29958	16.25355	< 0.001
within groups	28	0.01843		

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
5°C vs untreated leaves examined concurrently	1	0.62016	33.64660	< 0.001
20°C vs untreated leaves examined concurrently	1	0.00018	0.00981	0.922

2. Initial fluorescence level, F_0

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	1.12500	0.01927	0.891
treatment location	1	45.12500	0.77302	0.387
interaction	1	231.12500	3.95931	0.056
within groups	28	58.37500		

3. Steady state fluorescence level, T

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	19.53125	0.33343	0.568
treatment location	1	2.53125	0.04321	0.837
interaction	1	185.28125	3.16310	0.086
within groups	28	58.57589		

4. Leaf bulk chlorophyll content

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	52731.28125	0.88952	0.354
treatment location	1	11514.03125	0.19423	0.663
interaction	1	37743.78125	0.63670	0.432
within groups	28	59280.74554		

B) Experiment 2b: Effects of 6h high light treatment at $1500 \mu\text{E m}^{-2} \text{s}^{-1}$ and 5°C on photosynthetic parameters. High light-exposed leaves are compared to controls treated for 6h at $150 \mu\text{E m}^{-2} \text{s}^{-1}$ and 5°C .

1. O_2 evolution rate

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
PFD	1	4.26393	33.27074	< 0.001
line	1	0.82272	6.41958	0.017
interaction	1	0.00216	0.01687	0.898
within groups	28	0.12816		

2. P/F_0

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
PFD	1	2.39093	276.27020	< 0.001
line	3	0.04010	4.63373	0.006
interaction	3	0.04115	4.75500	0.005
within groups	56	0.00865		

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
PFD within Gaspé	1	0.46731	53.99726	< 0.001
PFD within LG3	1	0.51484	59.48968	< 0.001
PFD within HIWF	1	1.14774	132.62033	< 0.001
PFD within Birka	1	0.38449	44.42793	< 0.001

3. Initial fluorescence level, F_0 **ANOVA TABLE: MAIN EFFECTS**

Source	d. f.	Mean Square	F ratio	p
between groups				
PFD	1	742.56250	9.61079	0.003
line	3	1658.60417	21.46688	< 0.001
interaction	3	112.93750	1.46172	0.235
within groups	56	77.26339		

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
PFD within Gaspé	1	10.56250	0.13671	0.713
PFD within LG3	1	210.25000	2.72121	0.105
PFD within HIWF	1	784.00000	10.14711	0.002
PFD within Birka	1	76.56250	0.99093	0.324

4. Steady state fluorescence level, T

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
PFD	1	178.89063	3.03952	0.087
line	3	1216.68229	20.67257	< 0.001
interaction	3	52.14063	0.88592	0.454
within groups	56	58.85491		

5. Leaf bulk chlorophyll content

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
PFD	1	582.01563	0.00400	0.950
line	3	792566.55729	5.44164	0.002
interaction	3	9360.34896	0.06427	0.979
within groups	56	145648.35491		

C) Experiment 2c: Effects of 6h chilling treatment at 5°C and 150 $\mu\text{E m}^{-2} \text{s}^{-1}$ on photosynthetic parameters. Values for chilled leaves are compared to those for untreated leaves maintained under normal growing conditions (150 $\mu\text{E m}^{-2} \text{s}^{-1}$ and 23°C).

1. O₂ evolution rate

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	0.14298	0.72127	0.403
line	1	1.21251	6.11664	0.020
interaction	1	0.02179	0.10991	0.743
within groups	28	0.19823		

2. P/F₀

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	0.57549	21.62682	< 0.001
line	3	0.17622	6.62247	0.001
interaction	3	0.07882	2.96214	0.040
within groups	56	0.02661		

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
temperature within Gaspé	1	0.11007	4.13657	0.047
temperature within LG3	1	0.62016	23.30525	< 0.001
temperature within HIWF	1	0.05507	2.06960	0.156
temperature within Birka	1	0.02666	1.00183	0.321

3. Initial fluorescence level, F_0

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	415.14063	5.67108	0.021
line	3	1606.80729	21.94998	< 0.001
interaction	3	97.39063	1.33042	0.274
within groups	56	73.20312		

4. Steady state fluorescence level, T

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	798.06250	14.35410	< 0.001
line	3	969.56250	17.43873	< 0.001
interaction	3	83.06250	1.49398	0.226
within groups	56	55.59821		

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
temperature within Gaspé	1	333.06250	5.99053	0.018
temperature within LG3	1	115.56250	2.07853	0.155
temperature within HIWF	1	588.06250	10.57700	0.002
temperature within Birka	1	10.56250	0.18998	0.665

5. Leaf bulk chlorophyll content

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	95095.14063	0.55673	0.459
line	3	673825.43229	3.94489	0.013
interaction	3	20458.43229	0.11977	0.948
within groups	56	170809.59152		

Appendix V

Analyses of Variance for Experiments 3a and b

A) Experiment 3a: Effects of 3h high light treatment at $1500 \mu\text{E m}^{-2} \text{s}^{-1}$ and 5°C on photosynthetic parameters measured from second leaves of the two corn lines Gaspé and HIWF. High light-exposed leaves were compared to controls maintained 3h at $150 \mu\text{E m}^{-2} \text{s}^{-1}$ and 5°C . All leaves received an overnight chill in the dark, 12 or 15h in duration, prior to treatment in the leaf chamber.

i) 12 hour prechill

1. O_2 evolution rate

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
PFD	1	2.86771	30.01052	< 0.001
line	1	0.44517	4.65866	0.040
interaction	1	0.02243	0.23478	0.632
within groups	28	0.09556		

2. P/F₀**ANOVA TABLE: MAIN EFFECTS**

Source	d. f.	Mean Square	F ratio	p
between groups				
PFD	1	0.10393	10.99216	0.003
line	1	0.03083	3.26090	0.082
interaction	1	0.01877	1.98560	0.170
within groups	28	0.00946		

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
PFD within Gaspé	1	0.01718	1.81705	0.188
PFD within HIWF	1	0.10553	11.16072	0.002

3. Initial fluorescence level, F₀**ANOVA TABLE: MAIN EFFECTS**

Source	d. f.	Mean Square	F ratio	p
between groups				
PFD	1	306.28125	1.51802	0.228
line	1	979.03125	4.85237	0.036
interaction	1	1391.28125	6.89561	0.014
within groups	28	201.76339		

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
PFD within Gaspé	1	1501.56250	7.44219	0.011
PFD within HIWF	1	196.00000	0.97143	0.333

4. Steady state fluorescence level, T

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
PFD	1	300.12500	1.12425	0.298
line	1	924.50000	3.46313	0.073
interaction	1	1770.12500	6.63079	0.016
within groups	28	266.95536		

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
PFD within Gaspé	1	306.25000	1.14720	0.293
PFD within HIWF	1	1764.00000	6.60785	0.016

5. Leaf bulk chlorophyll content

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
PFD	1	6132.78125	0.06173	0.806
line	1	756142.53125	7.61069	0.010
interaction	1	19061.28125	0.19185	0.665
within groups	28	99352.66518		

ii) 15 hour prechill

1. O₂ evolution rate**ANOVA TABLE: MAIN EFFECTS**

Source	d. f.	Mean Square	F ratio	p
between groups				
PFD	1	3.02795	23.58114	< 0.001
line	1	0.08869	0.69073	0.413
interaction	1	0.19268	1.50056	0.231

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
PFD within Gaspé	1	0.84649	6.59232	0.016
PFD within HIWF	1	2.37414	18.48938	< 0.001

2. P/F₀

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
PFD	1	0.11732	12.11426	0.002
line	1	0.08770	9.05528	0.005
interaction	1	0.03627	3.74561	0.063
within groups	28	0.00968		

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
PFD within Gaspé	1	0.01156	1.19382	0.284
PFD within HIWF	1	0.14203	14.66605	0.001

3. Initial fluorescence level, F_0

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
PFD	1	2.00000	0.02649	0.872
line	1	171.125000	2.26682	0.143
interaction	1	8.00000	0.10597	0.747
within groups	28	75.49107		

4. Steady state fluorescence level, T

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
PFD	1	520.03125	5.43494	0.027
line	1	22.78125	0.23809	0.629
interaction	1	0.78125	0.00816	0.929
within groups	28	95.68304		

5. Leaf bulk chlorophyll content

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
PFD	1	146205.28125	1.68585	0.205
line	1	1383200.28125	15.94924	< 0.001
interaction	1	15444.03125	0.17808	0.676
within groups	28	86725.17411		

B) Experiment 3b: Effects of the 3h chilling treatment at 5°C and 150 $\mu\text{E m}^{-2} \text{s}^{-1}$ on photosynthetic parameters measured from second leaves of Gaspé and HIWF. Leaves subjected to the 3h chill were compared to controls (23°C, prechilled leaves) maintained 3h in the growth cabinet under normal growing conditions (150 $\mu\text{E m}^{-2} \text{s}^{-1}$, 23°C). All leaves received an overnight chill in the dark (12 or 15h) prior to the 3h treatment.

i) 12 hour prechill

1. O₂ evolution rate

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	0.00063	0.00420	0.949
line	1	0.87665	5.88665	0.022
interaction	1	0.01423	0.09558	0.759
within groups	28	0.14892		

2. P/F_0 **ANOVA TABLE: MAIN EFFECTS**

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	4.11623	334.76852	< 0.001
line	1	0.07090	5.76659	0.023
interaction	1	0.00215	0.17459	0.679
within groups	28	0.01230		

3. Initial fluorescence level, F_0 **ANOVA TABLE: MAIN EFFECTS**

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	180.50000	1.71105	0.201
line	1	0.12500	0.00118	0.973
interaction	1	40.50000	0.38392	0.541
within groups	28	105.49107		

4. Steady state fluorescence level, T

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	120.12500	0.73862	0.397
line	1	40.50000	0.24903	0.622
interaction	1	325.12500	1.99912	0.168
within groups	28	162.63393		

5. Leaf bulk chlorophyll content

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	343827.78125	2.40748	0.132
line	1	876819.03125	6.13947	0.020
interaction	1	5075.28125	0.03554	0.852
within groups	28	142816.71875		

ii) 15 hour prechill

1. O₂ evolution rate

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	0.02982	0.24875	0.622
line	1	0.29051	2.42365	0.131
interaction	1	0.03912	0.32633	0.572
within groups	28	0.11987		

2. P/F₀

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	4.05093	212.83579	< 0.001
line	1	0.28814	15.13864	0.001
interaction	1	0.00252	0.13233	0.719
within groups	28	0.01903		

3. Initial fluorescence level, F_0

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	42.78125	0.64719	0.428
line	1	442.53125	6.69460	0.015
interaction	1	116.28125	1.75910	0.195
within groups	28	66.10268		

4. Steady state fluorescence level, T

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	8.00000	0.07528	0.786
line	1	882.00000	8.29908	0.008
interaction	1	666.12500	6.26783	0.018
within groups	28	106.27679		

5. Leaf bulk chlorophyll content

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
temperature	1	233415.28125	1.86220	0.183
line	1	603076.53125	4.81138	0.037
interaction	1	274355.28125	2.18882	0.150
within groups	28	125343.81696		

C) Experiment 3b: Effects of an overnight chill in the dark (12 or 15 hours in duration) on photosynthetic parameters measured from second leaves of Gaspé and HIWF. Leaves chilled overnight (then kept under normal growing conditions for 3h) were compared to untreated leaves.

i) 12 hour prechill

1. O₂ evolution rate

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
treatment	1	0.11040	0.88732	0.354
line	1	0.17925	1.44066	0.240
interaction	1	0.39971	3.21249	0.084
within groups	28	0.12442		

2. P/F_0

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
treatment	1	0.00066	0.04634	0.831
line	1	0.21166	14.86493	0.001
interaction	1	0.05766	4.04923	0.054
within groups	28	0.01424		

3. Initial fluorescence level, F_0

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
treatment	1	166.53125	1.68009	0.205
line	1	247.53125	2.49728	0.125
interaction	1	81.28125	0.82002	0.373
within groups	28	99.12054		

4. Steady state fluorescence level, T

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
treatment	1	413.28125	4.99191	0.034
line	1	552.78125	6.67689	0.015
interaction	1	0.78125	0.00944	0.923
within groups	28	82.79018		

5. Leaf bulk chlorophyll content

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
treatment	1	67528.125000	0.47738	0.495
line	1	155403.12500	1.09860	0.304
interaction	1	221778.00000	1.56783	0.221
within groups	28	141455.31250		

ii) 15 hour prechill

1. O₂ evolution rate

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	P
between groups				
treatment	1	0.30652	4.40097	0.045
line	1	0.08061	1.15739	0.291
interaction	1	0.00328	0.04713	0.830
within groups	28	0.06965		

2. P/F₀

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	P
between groups				
treatment	1	0.58864	12.10741	0.002
line	1	0.47822	9.83619	0.004
interaction	1	0.01093	0.22489	0.639
within groups	28	0.04862		

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
treatment within Gaspé	1	0.38001	7.81623	0.009
treatment within HIWF	1	0.21956	4.51606	0.043

3. Initial fluorescence level, F_0

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
treatment	1	63.28125	0.44426	0.511
line	1	166.53125	1.16912	0.289
interaction	1	357.78125	2.51177	0.124
within groups	28	142.44196		

4. Steady state fluorescence level, T

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
treatment	1	75.03125	0.31595	0.579
line	1	1069.53125	4.50371	0.043
interaction	1	520.03125	2.18981	0.150
within groups	28	237.47768		

5. Leaf bulk chlorophyll content

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
treatment	1	146205.28125	1.11934	0.299
line	1	31187.53125	0.23877	0.629
interaction	1	184376.28125	1.41157	0.245
within groups	28	130617.43304		

D) Experiment 3b: Diurnal changes in photosynthetic parameters from second leaves of untreated Gaspé and HIWF seedlings. The leaves were examined during two time periods: 6.5 to 8.5 h, and 9.5 to 11.5 h after initiation of the normal photoperiod.

1. O₂ evolution rate

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
line	1	0.00008	0.00095	0.976
time period	1	0.02106	0.25309	0.619
interaction	1	0.04741	0.56977	0.457
within groups	28	0.08321		

2. P/F_0

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
line	1	0.55971	11.24888	0.002
time period	1	0.60850	12.22943	0.002
interaction	1	0.00230	0.04623	0.831
within groups	28	0.04976		

relevant simple main effects

Source	d. f.	Mean Square	F ratio	p
time within Gaspé	1	0.34281	6.88972	0.014
time within HiWF	1	0.26799	5.38595	0.028

3. Initial fluorescence level, F_0

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
line	1	87.78125	0.58321	0.451
time period	1	148.78125	0.98849	0.329
interaction	1	236.53125	1.57150	0.220
within groups	28	150.51339		

4. Steady state fluorescence level, T

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
line	1	264.50000	1.38930	0.248
time period	1	595.12500	3.12592	0.088
interaction	1	40.50000	0.21273	0.648
within groups	28	190.38393		

5. Leaf bulk chlorophyll content

ANOVA TABLE: MAIN EFFECTS

Source	d. f.	Mean Square	F ratio	p
between groups				
line	1	116523.78125	0.84965	0.365
time period	1	6077.53125	0.04432	0.835
interaction	1	70031.53125	0.51065	0.481
within groups	28	137142.75446		

Appendix VI

Model for total fluorescence yield ΦF (Björkman, 1987)

The total fluorescence yield, ΦF , may be expressed as:

$$\Phi F = \frac{F}{J} = \frac{K_F \cdot A}{K_F + K_D + K_T + K_P} + \frac{K_F (1-A)}{K_F + K_D + K_T} \quad (1)$$

where F is the fluorescence emitted, J the PFD of the light absorbed by photosynthetic pigments, K_F the rate constant for fluorescence, K_D the rate constant for non-radiative dissipation of excitation energy by antennae pigments, K_T the rate constant for transfer of excitation energy from PS II to PS I, K_P the rate constant for photochemistry of PS II, and A the fraction of open reaction centres (Björkman, 1987).

When all reaction centres are open at F_0 , $A=1$ and

$$\Phi F_0 = \frac{K_F}{K_F + K_D + K_T + K_P} \quad (2).$$

At F_m , the maximal level of fluorescence that can be emitted when all reaction centres are closed, $A=0$ and

$$\Phi F_m = \frac{K_F}{K_F + K_D + K_T} \quad (3).$$

END

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