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Minireview

Photosynthesis, photoinhibition and low temperature acclimation in cold tolerant plants

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Abstract

Cold acclimation requires adjustment to a combination of light and low temperature, conditions which are potentially photoinhibitory. The photosynthetic response of plants to low temperature is dependent upon time of exposure and the developmental history of the leaves. Exposure of fully expanded leaves of winter cereals to short-term, low temperature shifts *inhibits* whereas low temperature growth *stimulates* electron transport capacity and carbon assimilation. However, the photosynthetic response to low temperature is clearly species and cultivar dependent. Winter annuals and algae which actively grow and develop at low temperature and moderate irradiance acquire a resistance to irradiance 5- to 6-fold higher than their growth irradiance. Resistance to short-term photoinhibition (hours) in winter cereals is a reflection of the increased capacity to keep Q_A oxidized under high light conditions and low temperature. This is due to an increased capacity for photosynthesis. These characteristics reflect photosynthetic acclimation to low growth temperature and can be used to predict the freezing tolerance of cereals. It is proposed that the enhanced photosynthetic capacity reflects an increased flux of fixed carbon through to sucrose in source tissue as a consequence of the combined effects of increased storage of carbohydrate as fructans in the vacuole of leaf mesophyll cells and an enhanced export to the crown due to its increased sink activity. Long-term exposure (months) of cereals to low temperature photoinhibition indicates that this reduction of photochemical efficiency of PS II represents a stable, long-term down regulation of PS II to match the energy requirements for CO_2 fixation. Thus, photoinhibition in vivo should be viewed as the capacity of plants to adjust photosynthetically to the prevailing environmental conditions rather than a process which necessarily results in damage or injury to plants. Not all cold tolerant, herbaceous annuals use the same mechanism to acquire resistance to photoinhibition. In contrast to annuals and algae, overwintering evergreens become dormant during the cold hardening period and generally remain susceptible to photoinhibition. It is concluded that the photosynthetic response to low temperatures and susceptibility to photoinhibition are consequences of the overwintering strategy of the plant species.

Abbreviations: Asc – ascorbate; BQ – benzoquinone; CAP – chloramphenicol; DAD – diaminodurene; DCPIP – dichlorophenolindophenol; DHA – dehydroascorbate; DHQ – dihydroduroquinone; DPH – diphenylhexatriene; EPR – electron spin resonance; FBP – fructose bisphosphate; FBPase – fructose-1,6-bisphosphatase; F-2,6-BP – fructose-2,6-bisphosphate; F_O – minimum fluorescence with all PS II traps open in dark adapted leaves; F_M – maximum fluorescence with all PS II traps closed in dark adapted leaves; F_V –

variable fluorescence ($F_V = F_M - F_O$); F'_O – minimum fluorescence when all traps are open in light adapted leaves; F'_M – maximum fluorescence when all traps are closed in light adapted leaves; F'_V – variable fluorescence in light adapted leaves ($F'_V = F'_M - F'_O$); H – cold hardened state; HL – high-light (1200 μmol m^{-2} s^{-1}) grown plants; LD – plants grown with a 16 h photoperiod; LHC IIb – oligomeric form of the light harvesting complex of PS II; LT_{50} – freezing temperature at which 50% of the plants die; MDA – monodehydroascorbate; MV – methylviologen; NH – nonhardened state; PCR – photosynthetic carbon reduction cycle; PGA – phosphoglycerate; PQ – plastoquinone; PSmax – maximum, light saturated rates of photosynthesis; q_P – photochemical quenching parameter; q_N – nonphotochemical quenching parameter; $\phi_{PS\ II}$ – yield of PS II electron transport; $(Q_A)_{ox}$ – oxidized form of the primary, stable electron acceptor for PS II; $(Q_A)_{red}$ – reduced form of the primary, stable electron acceptor for PS II; RuBP – ribulose-1,5-bisphosphate; SD – plants grown with an 8 h photoperiod; SOD – superoxide dismutase; SPS – sucrose phosphate synthase; SUC-P – sucrose phosphate; TMPD – tetramethylphenylenediamine; TP – triose phosphate.

I. Introduction

Photosynthetic organisms are constantly confronted with reconciling excessive energy supply with the demands of the photosynthetic carbon reduction cycle (PCR) for the products of electron transport, ATP and NADPH. Photosynthetic control is the mechanism by which plants maintain a balance between energy conversion through electron transport and energy consumption by carbon fixation (Foyer et al. 1990). This is required in order to protect PS II photochemistry from excessive excitation and potential photodamage and at the same time ensure that the rate of ATP and NADPH synthesis is sufficient for the regeneration of PCR intermediates necessary to maintain sufficient rates of CO_2 fixation and triose phosphate (TP) export for sucrose synthesis. Exposure of plants to a combination of light and either sudden, short-term shifts from high to low temperature or long-term growth and development at low temperature will perturbate the balance between energy conversion and energy consumption. The photosynthetic responses of plants to such potential stresses and the mechanisms by which plants adjust to such extreme conditions are summarized in this review. The response of organisms to changes in the external environment generally occur over one of three time scales (Prosser 1986). *Adaptation* is a response to long-term changes which result in inheritable genetic alterations. These alterations are stable and will remain in the population over generations. *Acclimation*, on the other hand, is a response induced by an environmental change which causes a phenotypic alteration over a single generation time without any compositional change in the genetic complement. However, acclimative responses can be differentiated further into: (i) transient physiological and biochemical adjustments induced by abrupt or short-term changes in the environment, that is a *stress response* which subsequently may lead to senescence and (ii) stable, long-term adjustments which may reflect a *developmental response* to a new environmental condition. The extent to which a plant can respond to these different time courses is ultimately under genetic control and the degree of plant plasticity will, in turn, be dependent upon the regulation and expression of many genes some of which may be tissue specific. Therefore, it is important to define the time frame of the observed morphological, physiological and biochemical adjustments to environmental change so that transient stress responses can be separated from the more stable, acclimative responses.

In this review, we focus on recent adaptive and acclimative responses of photosynthesis to low temperature in cold tolerant plants only. In turn, we relate the capacity for photosynthetic adjustment to the plant's response to photoinhibition of photosynthesis at low temperature. For more extensive reviews of photosynthetic responses to temperature, the reader is referred to Berry and Björkman (1980), Graham and Patterson (1982), Öquist and Martin (1986) as well as a monograph edited by Long and Woodward (1988). For general reviews of cold acclimation and freezing tolerance we refer the reader to Levitt (1980), Steponkus (1984) and Guy (1990). The photosynthetic responses of chilling sensitive plants to low temperature will not be examined here but have been reviewed recently (Baker et al. 1988, Baker 1991).

II. Photosynthetic response to low temperature shifts

II.A. CO_2 uptake and O_2 evolution

There is general consensus that the optimum temperature for photosynthesis exhibited by a plant species reflects the environmental temperature range to which the species has adapted with desert species exhibiting higher temperature optima than cool, temperate species (Berry and Björkman 1980, Grace 1988). However, plants exhibit a high degree of plasticity with respect to the temperature response of photosynthesis. Photosynthetic acclimation to temperature shifts has been characterized generally by an altered temperature optimum which is biased towards the new low temperature regime (Berry and Björkman 1980, Graham and Patterson 1982, Öquist and Martin 1986, Mawson et al. 1986).

Recently, Holaday et al. (1992) reported that spinach exposed to 10 °C for 10 d exhibited higher rates of photosynthesis than spinach plants maintained at 24 °C. In contrast, Boese and Huner (1990) reported that the light saturated rate of CO_2 uptake ($PSmax_{CO_2}$) under ambient CO_2 concentrations and the light saturated rate of O_2 evolution ($PSmax_{O_2}$) in CO_2 saturated air were inhibited by 30% to 50% when spinach plants grown at 16 °C were abruptly transferred to 5 °C for 12 d with all other conditions held constant. In addition, exposure to such a temperature shift also resulted in a 20% depression in the apparent quantum yield for CO_2 uptake ($\phi_{app}CO_2$) and O_2 evolution ($\phi_{app}O_2$). Similar inhibition of photosynthesis was observed for spinach plants grown at 5 °C and subsequently shifted to 16 °C. This was observed regardless of the measuring temperature and occurred with no changes in internal CO_2 concentrations (Boese and Huner 1990). They concluded that fully expanded spinach leaves can not acclimate photosynthetically but in fact are stressed when exposed to an abrupt change in temperature. Similar trends have been reported by Somersalo and Krause (1989) for spinach and Maciejewska et al. (1984, 1987) for winter rape subjected to low temperature shifts. Employing additional controls, Boese and Huner (1990) showed that aging during the shift period can account for most of the observed reduction in the rate of photosynthesis.

The discrepancy in the published results may, in part, reflect a difference in the physiological ages of the leaves being compared. The capacity to acclimate photosynthetically to low temperature appears to be dependent upon the physiological age of the tissue and the developmental history of the leaf (Krol and Huner 1985). Similar conclusions have been reached by Rütten and Santarius (1992). However, fully expanded leaves of the perennial grass, *Lolium temulentum*, exhibited a 24% increase in $PSmax_{O_2}$ after a temperature shift from 20 °C to 5 °C for 28 d (Pollock et al. 1983). Thus, the natural life cycle of the species (annual versus perennial) may also play an important role in a plant's photosynthetic response to changes in temperature.

It has been reported that abrupt shifts of C_3 plants from high (20–30 °C) to low measuring temperatures (2–10 °C) results in O_2-insensitivity of CO_2 fixation (Joliffe and Tregunna 1973, Cornic and Louason 1980, Mächler et al. 1984, Leegood 1985, Schnyder et al. 1986, Labate and Leegood 1988, Labate et al. 1990, Paul et al. 1990, Paul et al. 1992). This lack of stimulation of photosynthesis by 2% O_2 results in characteristic oscillations in CO_2 fixation and Chl fluorescence. Similar effects have been reported after plants have been water stressed (Sharkey 1985a,b). The O_2 insensitivity as well as the oscillations in CO_2 fixation and Chl fluorescence can be alleviated by Pi feeding or exacerbated by mannose feeding through the transpiration stream (Leegood and Furbank 1986, Labate and Leegood 1988, Bailey and Walker 1992).

The effects of measuring temperature on the light response curves for photosynthesis have been investigated extensively (Berry and Björkman 1980, Stitt and Grosse 1988). Measuring temperature has minimum effects on the light limited rates of photosynthesis but reduces light saturated rates as the temperature is decreased between 30 and 10 °C. In addition, the shape of the light response curve for photosynthesis is sensitive to measuring temperature. Decreasing temperature led to a decrease in the irradiance required to saturate photosynthesis (Berry and Björkman 1980, Stitt and Grosse 1988, Falk et al. 1992). This has been interpreted to reflect the accumulation of photosynthetic metabolites at low temperature as a result of the restriction of sucrose synthesis at low temperatures and light saturating irradiance (Stitt and Grosse 1988).

II.B. Carbon metabolism

Evidence indicates that the low measuring temperature-induced O_2 insensitivity of CO_2 fixation reflects Pi limitation of photosynthesis (Leegood 1985, Sharkey 1985a,b, Sharkey et al. 1986, Stitt et al. 1987). This may, in part, be due to the fact that optimal rates of photosynthesis at low measuring temperature require higher Pi concentrations than photosynthesis at moderate to high temperatures (Leegood 1985). In addition, phosphorylated metabolites such as hexose phosphates tend to accumulate to higher levels after exposure to low measuring temperatures compared to high measuring temperatures reducing the available Pi in the stroma for photosynthesis at low temperatures (Mächler et al. 1984, Labate and Leegood 1989, Labate et al. 1990). For example, the ratio of PGA/TP, which reflects the redox as well as the phosphorylation potential of the chloroplast, rises as leaf measuring temperature decreases in spinach and wheat under ambient CO_2 concentrations (Fig.1) (Kobza and Edwards 1987, Stitt and Grosse 1988, Labate and Leegood 1990). This should favour starch accumulation within the chloroplast over sucrose synthesis in the cytoplasm (Stitt et al. 1987). The rise in PGA/TP may occur because of the low temperature restriction of electron transport and thus, a decreased capacity to generate the necessary ATP and NADPH. Such a decrease in phosphorylation and reducing potential would cause a significant limitation on CO_2 fixation. However, in contrast to spinach and wheat, the ratio of PGA/TP in barley stays constant or decreases as leaf temperature is lowered. Clearly, there are species differences with respect to the response of carbon metabolism to abrupt changes in temperature.

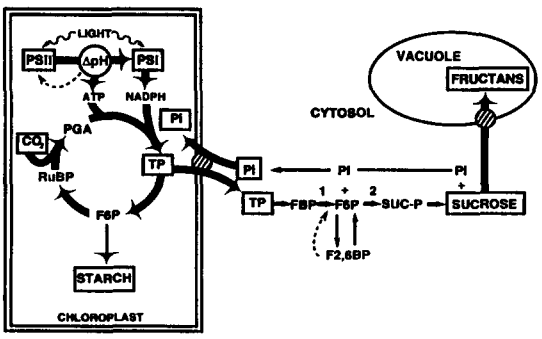

Fig. 1. Summary of chloroplastic and cytosolic carbon metabolism leading to the synthesis of sucrose. 1, FBPase; 2, Sucrose-P synthase.

Sucrose synthesis in certain plant species is inhibited at low temperature (Stitt et al. 1987). This appears to be the consequence of the increased sensitivity of the cytosolic FBPase to the important regulatory metabolite, F-2,6-BP and AMP (Fig.1) (Stitt et al. 1987). This would lead to an increase in phosphorylated intermediates and a decrease in cytosolic Pi (Stitt et al. 1987). However, further work is required to determine the actual levels of F-2,6-BP and AMP accumulated during leaf exposure to measuring temperatures in the range of 0 to 5 °C. Furthermore, it has been suggested that maximal rates of photosynthesis are ultimately limited by the capacity for sucrose synthesis through restriction of the rate at which cytosolic Pi can be exchanged for stromal TP by the chloroplastic phosphate translocator (Stitt 1986). Thus, it is possible that low temperature limitation of photosynthesis occurs at the level of the Pi translocator rather than sucrose synthesis *per se*. This would eventually limit the consumption of photosynthetically-derived ATP and NADPH and reduce PS II photochemical efficiency and rates of electron transport through photosynthetic control.

Short-term (14 d) low temperature exposure of warm grown wheat seedlings stimulates the amount of sucrose synthase present (Crespi et al. 1991, Newsted et al. 1991). This increase was reversed when plants were shifted back to warm temperatures. Northern blot analyses confirmed a 5- to 6-fold induction of sucrose synthase expression during the low temperature shift. Guy et al. (1992) have shown that warm grown spinach exposed to a 14 d shift to low temperature exhibit increased sucrose synthase activity. However, no concomitant photosynthetic measurements were made on these tissue. It is interesting to note that Guy et al. (1992) interpreted these results with respect to the ability of sucrose to act as a cryoprotectant rather than in terms of regulation of sucrose/starch interconversion. Species differences in the level of sucrose/starch accumulation in response to low temperature shifts may reflect genetic differences with respect to photosynthetic end product accumulation (Goldschmidt and Huber 1992).

II.C. PS II electron transport

In vivo Chl *a* fluorescence has provided evidence

that measuring temperature has a significant effect on the relation between electron transport and carbon metabolism. Stitt and Grosse (1988) showed that reducing leaf temperature from 30 °C to 15 °C more than doubled the level of reduced Q_A, estimated by the photochemical quenching parameter, q_P, under light saturated but not light limiting conditions. This was coupled to a concomitant increase in the level of energy-dependent quenching (q_E) of Chl *a* fluorescence and a decrease in the yield of PS II electron transport ($\phi_{PS\ II}$) in barley and spinach as leaf temperature decreased (Labate et al. 1990, Holaday et al. 1992). These results have been interpreted to reflect the low temperature limitation of carbon metabolism which causes energization of the thylakoid due to a reduced ratio of ATP/ADP as a consequence of Pi limitation of photosynthesis. This causes the build up of q_E and energy is dissipated nonphotochemically. However, as the temperature is decreased to lower and lower temperatures the rate at which Q_A is reduced under saturating light overtakes the rate of nonphotochemical dissipation of energy resulting in the accumulation of reduced Q_A. We have observed also that merely shifting mature 20 °C-grown rye plants to 5 °C in the light results in a marked decrease in $\phi_{PS\ II}$ which is the result of a build up of reduced Q_A (Huner, unpublished). Somersalo and Krause (1990) also reported that both light saturated rates of PS II electron transport ($H_2O \rightarrow BQ$) and $\phi_{PS\ II}$ were significantly lower in thylakoids isolated from warm grown spinach shifted to low temperatures for 10 d than warm grown controls. Thermoluminescence studies indicate that spinach exposed to low temperature for 10 d exhibit fewer active PS II reaction centers than controls maintained at warm temperatures (Briantais et al. 1992). Subsequent exposure to high light at 4 °C affected the shifted plants to a lesser extent than the controls. These authors conclude that photoinhibition in spinach gives rise to non-functional PS II reaction centers that do not give rise to either thermoluminescence or variable chlorophyll fluorescence (Briantais et al. 1992). Thus, shifting plants to low temperature generally has a negative impact on the function of PS II.

II.D. PS I electron transport

In contrast to PS II, shifting mature 20 °C-grown rye to 5 °C in the light or dark results in a 50%–80% stimulation of light saturated PS I activity of isolated thylakoids measured as electron transport from reduced DCPIP to MV. The observed stimulation occurs independent of leaf number and leaf age. However, this stimulation is transient with a maximum occurring after about 36 h at 5 °C and subsequently decaying to below the initial activity after 96 to 120 h (Huner, unpublished). The basis for this transient, low temperature stimulation of PS I and its physiological significance are presently unknown. However, in vivo heat stimulation of PS I activity has been reported also (Thomas et al. 1986, Havaux et al. 1991).

III. Photosynthetic response to growth and development at low temperature

It has been documented that light and CO_2 are required during exposure to low temperature in order to attain *maximum* cold tolerance in a variety of plant species (Dexter 1933, Vasil'yev 1956, Steponkus and Lanphear 1968, Lawrence et al. 1973, Levitt 1980). Furthermore, the accumulation of excess photosynthetically fixed carbon in the form of sucrose during cold acclimation and its positive correlation with cold tolerance has been known for some time (Andersson 1944, Vasil'yev 1956, Levitt 1980). Thus, photosynthesis provides the energy required for the induction and long-term maintenance of the maximum cold hardened state (H). For herbaceous plants, this is generally acquired by growth and development at low temperature (Fowler et al. 1979).

Much of our present understanding of cold acclimation has been gained through comparative studies of the cold tolerant winter cereals and the less tolerant spring cereals grown at low, cold hardening or high, nonhardening temperatures (Macdowall 1974, Levitt 1980, Steponkus 1984). Due to intensive breeding programmes, winter cereals such as wheat and rye are not only the most cold tolerant herbaceous plants but also exhibit the greatest range in cold tolerance (Macdowall 1974, Fowler et al. 1977).

III.A. CO_2 uptake and O_2 evolution

In order to characterize the photosynthetic response of plants grown at 5 and 20 °C, the growth kinetics

of the species or cultivar must be assessed at both temperatures to ensure comparison of leaf tissue at a similar physiological age (Krol et al. 1984, Hurry and Huner 1991). Regardless of the parameter used, exponential growth rate at 5 °C is about 1/3 of that observed at 20 °C for spinach and cereals (Boese and Huner 1990, Hurry and Huner 1991). Concomitantly, winter cultivars typically exhibit a 3-fold increase in the ratios of leaf dry weight:leaf area and dry weight:fresh weight (Krol et al. 1984, Cadieux et al. 1988, Hurry and Huner 1991). Leaves of winter cultivars developed at low temperatures are anatomically, morphologically and physiologically distinct from leaves developed at warm temperatures (Table 1). This underscores the importance of comparing leaf tissue of equivalent *physiological age* and not the same *chronological age* in the study of plant acclimation to an altered environment in order to separate stress or plant aging effects from acclimation (Krol et al. 1984, Krol and Huner 1985, Boese and Huner 1990, Hurry and Huner 1991, Boese and Huner 1992).

Acclimation of temperate species to lowered growth temperatures is accompanied by an increased capacity for carbon metabolism (Berry and Björkman 1980). After exposure to growth and development at 5 °C (H), winter wheat and rye exhibit $PSmax_{CO_2}$ or $PSmax_{O_2}$ that are greater than the same cultivars grown at 20 °C (NH) regardless of measuring temperature between 5 °C and 25 °C (Huner et al. 1986, Hurry and Huner 1991, Öquist and Huner 1992, Öquist et al. 1993). Similar trends have been reported for 30 °C and 13 °C-grown *Brassica napus* (Paul et al. 1990). For example, PSmax(H) / PSmax(NH) varies from a maximum value of about 3 to a minimum value of 1 for winter rye (Musketeer) and winter wheat (Kharkov, Augusta and Monopol) respectively (Table 2). This is accompanied by a significantly greater V_{max} for purified Rubisco regardless of measuring temperature (Huner and Macdowall 1979). However, growth temperature does not affect the quantum yield for photosynthesis measured as either $\phi_{app}CO_2$ or $\phi_{app}O_2$ (Huner et al. 1986, Hurry and Huner 1991, Öquist and Huner 1992). Thus, winter wheat and rye are able to modulate photosynthetic rates during growth at low temperatures such that photosynthetic capacity is increased [PSmax(H) / PSmax(NH) > 1] with no change in photosynthetic efficiency. This is in sharp contrast to the inhibition of photosynthesis observed when cereals are shifted from warm to cold temperatures. The extent of this low growth temperature stimulation of PSmax is cultivar dependent.

Table 1. Summary of the relative effects of growth temperature on leaf morphology, anatomy and physiology

Leaf characteristic	Rye	Spinach
Chl/area	1.4 × increase	No change
Dry weight/area	3 × increase	3 × increase
Thickness	1.5 × increase	1.5 × increase
Palisade layers	No change	2 × increase
Mesophyll cell size	1.5 × increase	1.5 × increase
Osmotic potential	2 × increase	2 × increase
LT_{50}	Increase from −4 °C to −29 °C	Increase from −3 °C to −9 °C

In contrast to winter cereals, cold grown spinach did not exhibit any significant change in $PSmax_{CO_2}$, $\phi_{app}CO_2$, $PSmax_{O_2}$ or $\phi_{app}O_2$ compared to physiologically equivalent warm grown spinach (Boese and Huner 1990). Thus, for spinach, PSmax(H)/PSmax(NH) = 1.0. Furthermore, $PSmax_{CO_2}$ and $PSmax_{O_2}$ for the less cold tolerant spring wheats (Katepwa, Glenlea and Marquis) (Table 2) are 25 to 30% lower after growth at 5 °C than 20 °C (Hurry and Huner 1991, Öquist and Huner 1992). Thus, spring wheats exposed for long periods to low, cold hardening temperatures and moderate growth irradiance (250 μmol $m^{-2}s^{-1}$) are photosynthetically *less* competent than when grown at nonhardening temperatures. This results in values of PSmax(H)/PSmax(NH) < 1.0 (Table 2). This inhibition is reversible by a 10 h exposure of cold hardened spring wheat to 20 °C in the dark. This differential ability to modulate photosynthetic capacity is not due to stomatal effects since neither stomatal conductance nor internal CO_2 concentration are affected significantly by growth temperature in any of the cultivars tested (Huner et al. 1986, Hurry and Huner 1991). The response of photosynthetic capacity to low growth temperature is clearly species dependent.

When PSmax(H)/PSmax(NH) is plotted as a function of maximum LT_{50} of the cultivar a positive, linear correlation is observed ($r^2 = 0.933$) (Öquist et al. 1993). We note that it is the capacity to *adjust* or *change* light saturated rates of photosynthesis rather

Table 2. Capacity of cold tolerant plants to modulate photosynthetic capacity (PSmax) and the redox state of Q_A as a function of cold hardening

Species	Cultivar	PSmax(H) / PSmax(NH)	Q_A(H) / Q_A(NH)	LT_{50}
Secale cereale L.	Musketeer	2.30	2.20	–32
Triticum aestivum L.	Kharkov	1.31	1.85	–21
	Augusta	1.23	nd	–13
	Monopol	1.03	1.50	–12
	Katepwa	0.77	nd	–9
	Glenlea	0.55	1.10	–8
	Marquis	0.46	nd	–7

than the absolute rates of photosynthesis that are correlated with freezing tolerance.

III.B. Carbon metabolism

Paul et al. (1990) reported that growth of *Brassica napus* at 30 °C resulted in a higher degree of O_2 insensitivity than 13 °C-grown plants at all measurement temperatures between 25 and 5 °C. This may be interpreted to indicate that the 13 °C-grown plants are less limited by TP utilization, and thus less Pi limited, than the 30 °C-grown plants (Fig.1). As expected, analyses of phosphorylated intermediates indicate that the 13 °C-grown plants exhibit 2- to 3-fold higher levels of hexose-P, FBP and TP than the 30 °C-grown plants. However, PGA/TP is 4-fold lower in the 13 °C than the 30 °C-grown plants which, in turn, is coupled to 4-fold higher levels of sucrose in the former than the latter. The ratio of sucrose:starch was 2.5 for the 13 °C plants compared to 1.1 for the 30 °C plants (Paul et al. 1990). Thus, it appears that temperate plants such as *Brassica napus* grown at cool temperatures are able to overcome, to some extent, the limitations imposed by low temperature upon photosynthesis and carbon metabolism. This is not observed when plants are shifted abruptly from high to low temperature.

Pollock and Lloyd (1987) have shown that starch synthesis is more sensitive to low temperature than sucrose and fructan synthesis in 8 cold tolerant species. They propose that the use of sucrose and fructans as storage carbohydrates (Fig.1) may counteract the predisposition for Pi limitation at low temperatures. This should result in a more effective utilization of absorbed light at low temperatures. In addition, conversion of sucrose to fructans in the vacuole partially overcomes the possible negative osmotic effects of high sucrose accumulation in the cytosol.

III.C. PS II electron transport

Photochemical quenching (q_P) analyses performed according to Genty et al. (1989) and measured as a function of irradiance (50 – 2000 μmol m^{-2} s^{-1}) and temperature (5–25 °C) indicated that 5 °C-grown rye maintained a higher level of oxidized Q_A regardless of irradiance and measuring temperature (Hurry et al. 1992, Öquist and Huner 1992). Furthermore, the difference between 5 and 20 °C-grown rye in the capacity to keep Q_A oxidized was greater under CO_2 saturated conditions than under ambient CO_2 levels. Three-fold higher light levels were required to reduce the ratio of $(Q_A)_{ox}$ / $(Q_A)_{red}$ to 0.5 in 5 °C than 20 °C-grown rye. $\phi_{PS\,II}$ as estimated by Genty et al. (1989) is the product of F_V'/F_M' and q_P. Rye and wheat grown at 5 °C exhibit higher $\phi_{PS\,II}$ than 20 °C-grown plants regardless of irradiance and measuring temperature. This is primarily due to low growth temperature effects on q_P rather than F_V'/F_M'(Hurry et al. 1992, Öquist and Huner 1992). Plots of $\phi_{PS\,II}$ / q_P versus irradiance and $\phi_{PS\,II}$ / q_P versus q_N according to Weis and Berry (1987) indicate that the response of open PS II reaction centers to irradiance and to regulation through non-photochemical quenching are identical for leaves developed at 5 or 20 °C. Thus, growth at low temperatures appears to alter the proportion of open reaction centers and not the nature of the PS II reaction centers themselves.

In a survey of winter rye, spring and winter wheats

the capacity to modulate the redox state of Q_A in response to growth temperature [q_P(H)/q_P(NH)] was shown to be positively correlated to the maximum LT_{50} of the cultivar (Öquist et al. 1993). Those cultivars with the greatest capacity to modulate the redox state of Q_A exhibit the greatest freezing tolerance (Table 2).

III.D. PS I electron transport

The increased $\phi_{PS\,II}$ is associated with a doubling in the PQ pool size (Griffith et al. 1984) with no detectable change in thylakoid membrane viscosity (Huner et al. 1987). Growth at low temperature also results in 50% to 100% higher rates of in vitro, light saturated PS I electron transport with no effect on light limited rates using MV as the terminal electron acceptor (Huner 1985, Huner and Reynolds 1989, Reynolds and Huner 1990). The higher PS I activity was also observed in rye chloroplast biogenesis at 5 °C (Krol et al. 1988). Low temperature stimulation of light saturated PS I activity was also observed in periwinkle leaves during natural overwintering conditions (Huner et al. 1988). This stimulation was observed using H_2O or reduced DCPIP as electron donors but not with the more lipophilic electron carriers DAD, TMPD or DHQ (Huner and Reynolds 1989). Thus, growth temperature appears to affect the site at which DCPIP donates electrons for PS I electron transport but not the more lipophilic reductants. This may explain why Cadieux et al. (1988) failed to observe stimulation of PS I activity in isolated thylakoids of 5 °C-grown wheat. The concentration of DCPIP required to produce 50% of the maximal rate of PS I electron transport was not significantly different for thylakoids from 5 and 20 °C-grown plants. Thus, it was concluded that the higher PS I activity in 5 °C plants could not be due to a differential accessibility of DCPIP to the PS I site of donation (Huner and Reynolds 1989). However, the higher PS I activity in thylakoids from 5 °C-grown plants could be completely eliminated by isolation of the thylakoids in the absence of Na^+ and Mg^{2+} (Huner and Reynolds 1989). This resulted in identical light response curves for PS I activity in isolated thylakoids from 5 and 20 °C-grown plants. Readdition of Ca^{2+}, Mn^{2+} or Mg^{2+} in vitro to final concentrations of 10 mM re-established the higher rates of PS I activity in thylakoids from 5 °C-grown leaves. Monovalent cations could also restore this activity but 10-fold higher concentrations were required (Chapman and Huner, in preparation). Thus, in contrast to the transient stimulation of PS I activity observed upon shifting plants from 20 to 5 °C, growth at 5 °C results in stable, higher light saturated PS I activity. Clearly, cations are required to stabilize this higher activity.

In summary, short-term, low temperature shifts of fully expanded leaves of winter cereals generally *inhibits* photosynthetic electron transport and carbon assimilation. In contrast, long-term exposures to low growth temperature generally *stimulates* PS II and PS I as well as overall photosynthetic capacity. The extent of these responses are species and cultivar dependent. If exposure to low temperature predisposes plants to Pi limitation of photosynthesis (Leegood 1985, Sharkey 1985a,b, Sharkey et al. 1986, Stitt et al. 1987), cold tolerant plants which grow at low temperature and exhibit increased rates of photosynthesis must overcome this limitation in some way. Presently, there is a paucity of available literature on carbon metabolism at low temperature. More important, further information is required on the effects of both short-term as well as long-term low temperature on carbon metabolism so that stress responses can be clearly separated from low temperature acclimation responses. Only then will we be able to construct a comprehensive model of the regulatory interactions of photosynthetic electron transport and carbon metabolism at low temperature.

IV. Susceptibility to low temperature photoinhibition

Herbaceous, overwintering annuals such as wheat, rye and spinach must photosynthesize for prolonged periods of time (weeks to months) under suboptimal conditions of low temperature and moderate to high light during the autumn in order to attain maximum cold tolerance. Hence, these plants are faced with a conundrum: although growth under conditions of light and low temperature are required during cold acclimation, these same conditions expose the plants to potential photoinhibition of photosynthesis. Low temperature-induced photoinhibition has been postulated to result from: (1) the disproportionate suppression of the rates the thermochemical reactions compared to the photochemical reactions of photosynthesis by low temperature which results in the

over-excitation and over-reduction of PS II, (2) the low temperature dependent suppression of the rates of de novo protein synthesis required for the repair of photodamage to PS II and (3) the inhibition or impairment of alternative pathways for the dissipation of excess light energy (Greer et al. 1986, Öquist et al. 1987, Krause 1988, Greer et al. 1991).

IV.A. Short-term photoinhibition

Somersalo and Krause (1989, 1990) were the first to report that cold acclimated plants exhibit a unique capacity to increase tolerance to photoinhibition. They showed that cold acclimated spinach show a greater resistance to short-term, low temperature-induced photoinhibition than control, nonacclimated spinach when measured as a decrease in F_V/F_M of dark adapted leaves pre-exposed for several hours to high light at 4 °C. This has been confirmed for cereals as well as spinach (Boese and Huner 1990, Öquist and Huner 1991, Hurry and Huner 1992). Recently, it has been reported that 5 °C-grown winter wheat cultivars can be distinguished from 5 °C-grown spring cultivars with the former being less susceptible to short-term, low temperature photoinhibition than the latter when measured as a decrease in F_V/F_M or a decrease in the $\phi_{app}O_2$ (Hurry and Huner 1992). A unique feature of resistance to photoinhibition is that growth at 5 °C and *low to moderate* irradiance (250 μmol m^{-2} s^{-1}) results in a significant decrease in susceptibility to *high* irradiance (1500 μmol $m^{-2}s^{-1}$), that is, 6 times the growth irradiance. Usually resistance to photoinhibition is associated with preexposure to high light. For example, plants exposed to full sunlight are typically less sensitive to photoinhibition than the same plants exposed to a shade environment (Powles 1984, Ögren and Rosenqvist 1992).

Öquist and Huner (1991) showed that development of winter rye at low temperature is an absolute requirement for the acquisition of resistance to photoinhibition. Fully expanded leaves of rye grown at 20 °C and subsequently shifted to 5 °C for up to 3 weeks do not acquire any significant level of resistance to low temperature photoinhibition. Rye plants exposed to such a temperature shift for 18 days exhibit a depression of F_V/F_M from 0.80 ± 0.02 to 0.44 ± 0.03 (Boese and Huner 1990). A detailed study of this developmental requirement has been reported for spinach (Boese and Huner 1990, 1992) and Huner 1992) and confirm that only those leaves which expand at low temperature acquire an increased resistance to low temperature photoinhibition. This occurs independently of photoperiod (Gray and Huner, unpublished results) but is dependent upon the photoinhibitory irradiance, time of exposure at the photoinhibitory irradiance and leaf age with the oldest, fully expanded leaves exhibiting the greatest susceptibility. The resistance to short-term photoinhibition acquired during growth at low temperature is completely lost after exposure to a 12 day shift to 16 °C (Boese and Huner 1992). Growth of *Chlamydomonas reinhardtii* at 12 °C and moderate light also results in increased resistance to photoinhibition compared to cells grown at 27 °C (Falk 1991, Falk et al. 1990, Kirilovsky et al. 1990). Thus, low temperature growth induced resistance to photoinhibition may be a general phenomenon in cold tolerant organisms.

IV.B. Mechanism of resistance to short-term photoinhibition

IV.B.i. Photosynthetic capacity. Leaf anatomy significantly attenuates light quality and irradiance within the leaf (Vogelman et al. 1989). Growth of annuals at low temperature results in significant alterations in epidermal cell wall structure as well as an increase in leaf thickness (Table 1). Lapointe and Huner (1992) reported that a suspension of intact, photosynthetically active mesophyll cells isolated from 5 or 20 °C grown rye exhibited similar differential resistance to photoinhibition as intact leaves. However, isolated thylakoids from cold hardened and non-hardened rye exhibited similar sensitivities to photoinhibition (Lapointe et al. 1991). Thus, leaf anatomy can not account for the differential sensitivity to photoinhibition observed between 5 and 20 °C-grown rye. Moreover, resistance to photoinhibition must reflect the interaction between electron transport and carbon fixation. However, the results with isolated thylakoids are equivocal since the thylakoids were acceptor-limited during photoinhibition.

Öquist and Huner (1992) reported that the differential resistance to photoinhibition in 5 and 20 °C-grown rye reflects a differential capacity to keep Q_A oxidized under high light and low temperature. When the redox state of Q_A was artificially equalized for 5 and 20 °C-grown rye prior to exposure to low

temperature photoinhibition the differential resistance observed between these plants was eliminated. These results indicate that the redox state of Q_A can account totally for the differential sensitivity to photoinhibition in rye. How do rye plants keep Q_A oxidized under conditions of high light and low temperature? Any process which can accept electrons directly or indirectly from Q_A could influence the proportion of $(Q_A)_{ox}$ / $(Q_A)_{red}$. In addition to photosynthesis and photosynthetic electron transport, other processes such as photorespiration, Mehler reaction and Cyt b_{559}-mediated cyclic electron transport around PS II are possibilities. Alternatively, increased capacity for non-radiative dissipation of excitation energy through the antenna of PS II could also prevent over-reduction of Q_A (Krause and Weis 1991).

As discussed above, cold tolerant cereals modulate photosynthetic capacity [PSmax(H) / PSmax(NH)] at low growth temperature such that $PSmax_{CO_2}$ or $PSmax_{O_2}$ are double that observed for warm grown plants regardless of temperature (Huner et al. 1986, Hurry and Huner 1991, Öquist and Huner 1992, Öquist et al. 1993). The finding that the ϕ_{O_2} for open reaction centres (ϕ_{O_2}/q_P), based on absorbed photons, is similar for 5 °C and 20 °C-grown rye supports the notion that the increased proportion of $(Q_A)_{ox}/(Q_A)_{red}$ is controlled by the higher capacity for photosynthesis in 5 °C leaves. This increased resistance to photoinhibition and capacity for photosynthesis in leaves developed at 5 °C is also correlated to an increased availability of Pi (Hurry et al. 1993). Furthermore, it appears that photorespiration does not contribute significantly since the difference in the capacity to keep Q_A oxidized is greater at 5% CO_2 than ambient CO_2 conditions (Öquist and Huner 1992).

However, spinach does not modulate either its photosynthetic capacity or the proportion of Q_A(ox)/ Q_A(red) significantly as a function of growth temperature (Boese and Huner 1990, Gray and Huner, unpublished). Thus, resistance to photoinhibition in spinach must be due to a different mechanism than for winter cereals. Schöner and Krause (1990) report 30% to 80% increases in total SOD, ascorbate peroxidase and MDA reductase activity but decreases in catalase and DHA reductase activities in spinach exposed to low temperature which may provide partial protection against photoinhibition of photosynthesis. However, these activities do not appear to have a significant impact on susceptibility to photoinhibition of PS II at low temperature (van Wijk and Krause 1991, van Wijk 1992). Furthermore, all assays were performed at 20 °C whereas photoinhibition was performed at 5 °C. It will be important to determine the specific activities of these enzymes at low temperature. O_2 effects may be exacerbated at low temperature since the solubility of O_2 increases at low temperature as the rates of these enzyme catalyzed reactions decrease.

IV.B.ii. Xanthophyll cycle pigments. Increased levels of zeaxanthin in the antenna have been shown to protect PS II upon exposure to high light by dissipating excess absorbed light energy as heat in higher plants (Demmig-Adams 1990) and algae (Franklin et al. 1992). Zeaxanthin was not detectable in 20 °C-grown spring and winter wheat exposed to 250 μmol $m^{-2}s^{-1}$, whereas 5 °C-grown plants exhibited 18 to 24 nmol zeaxanthin gm^{-1} fresh weight (Hurry et al. 1992). However, a plot of ϕ_{O_2}/q_P versus q_N indicated that non-photochemical quenching regulates the yield of open reaction centers similarly in leaves of 5 and 20 °C-grown plants (Öquist and Huner 1992). Furthermore, growth at 5 °C stimulated zeaxanthin production to the same extent in winter and spring cultivars even though they exhibited significantly different susceptibilities to photoinhibition. Thus, dissipation of excess energy through xanthophyll cycle pigments can not account for the differential susceptibility to photoinhibition in cereals.

Schöner and Krause (1990) suggest that increased levels of the xanthophylls in spinach may have a protective role against low temperature photoinhibition in this plant species. However, we have not observed any significant changes in xanthophyll cycle pigments or q_N during either low temperature growth or low temperature shift experiments with spinach of comparable physiological age (Boese and Huner 1990, Gray and Huner, unpublished). The discrepancy in the results for xanthophyll accumulation may reflect comparison of spinach leaf tissue of different physiological ages.

IV.B.iii. PS II repair cycle. Guenther and Melis (1990a,b) propose that photoinhibition and recovery are linked in a PS II repair cycle. Upon photoinhibition, PS IIα-centers are replaced by PS IIβ-

centers (Neale and Melis 1990, Tyystjärvi and Aro 1990) and the amount of PS II Q_B-reducing centers decrease while Q_B-nonreducing centers increase (Falk et al. 1992). This repair cycle (Fig. 2A) is dependent upon low light, lateral diffusion of damaged PS IIα centers from the granal to the stromal lamellae for replacement of damaged D1 protein by de novo protein synthesis and subsequent migration of PS II units back to the grana during recovery (Kyle 1987, Mattoo et al. 1989). The efficiency of this repair cycle does influence susceptibility to photoinhibition as indicated by the increased reduction in F_V/F_M when chloroplast protein synthesis is inhibited during exposure to high light regardless of the photoinhibitory temperature (Hurry and Huner 1992). However, the extent of photoinhibition is significantly less at high temperature than at low temperature. This may be due to a combination of a more efficient repair of damage as well as higher rates of photosynthesis at 25 than 5 °C.

Low temperature-induced photoinhibition will impose two important limitations: reduced rate of diffusion of PS II units between the grana and the stroma lamellae due to an increased membrane viscosity and a reduction in the rate of protease activity involved in removing photodamaged D1 protein and subsequent de novo biosynthesis of D1 protein (Aro et al. 1990). Thus, it has been postulated that resistance to low temperature photoinhibition may be the result of an increased capacity to repair damage to PS II at low temperature (Öquist et al. 1987, Greer et al. 1991, Andersson and Styring 1991, Barber and Andersson 1992).

Recent data indicate that resistance to in vivo photoinhibition in winter annuals can not be accounted for by a differential capacity for repair of PS II photochemistry. First, rye, wheat and spinach grown at either 5 or 20 °C exhibit similar capacities to recover between 20–25 °C at low light after photoinhibition at 5 °C (Öquist and Huner 1991, Boese and Huner 1992, Hurry and Huner 1992). Second, after application of CAP, which was shown to inhibit ^{35}S-methionine incorporation into the PS II reaction center D1 polypeptide, 5 °C- grown wheat was still significantly more resistant to short-term low temperature photoinhibition than 20 °C-grown wheat (Hurry and Huner 1992). Third, recovery after low temperature photoinhibition exhibits a rapid initial phase which is independent of protein

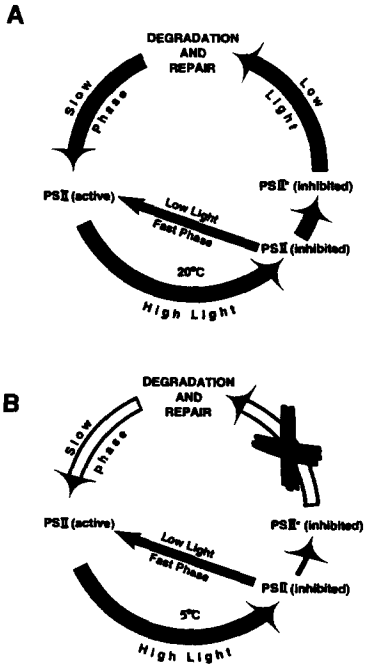

Fig. 2. Photoinhibition and recovery of PS II reaction centers in cold tolerant cereals and algae. We assume two forms of inhibited PS II reaction centers: PS II (inhibited) are inactive forms of PS II which act as quenchers and can be converted to the active, non-quenching form by subtle conformational changes. This conversion is not dependent upon de novo protein synthesis. This conversion reflects the fast phase of recovery. PS II* (inhibited) are inactive forms of PS II which can not act as quenchers but are tagged (*) to enter the repair cycle. Conversion of PS II*(inhibited) to active PS II centers requires de novo protein synthesis and reflects the slow phase of recovery. Formation of the PS II*(inhibited) form from the PS II (inhibited) form is irreversible. (A) Photoinhibition of cold tolerant cereals and algae at 20 °C is minimal due, in part, to a combination of an efficient repair cycle which converts PS II* (inhibited) centers to active PS II centers repair through de novo protein synthesis (slow phase) and the rapid conversion of PS II(inhibited) to active PS II centers which occurs independent of protein synthesis (fast phase). (B) Photoinhibition at 5 °C is exacerbated by the limitations imposed upon the repair cycle by low temperature. Thus, repair through de novo synthesis at low temperature is severely inhibited and this is reflected in the absence of the slow phase during recovery at low temperature and low light. However, the presence of the fast phase and indicates the capacity to convert PS II (inhibited) centers to PS II (active) centers independent of temperature and de novo protein synthesis.

synthesis followed by a slower phase recovery which is dependent upon protein synthesis (Hurry and Huner 1992, van Wijk 1992). Similar results have

been reported for green algae grown at 27 °C (Falk and Samuelsson 1992). This is consistent with published data indicating that there is no correlation between degradation of D1 protein and the extent of photoinhibition as measured by F_V/F_M (Chow et al. 1989, Gong and Nilsen 1989, Ottander at al 1992). Furthermore, this rapid initial phase of recovery is also independent of temperature (Hurry and Huner 1992, van Wijk 1992). However, we note that results utilizing CAP as an inhibitor of protein synthesis are equivocal since CAP has been shown to have other effects (Okada et al. 1991).

We postulate that after photoinhibition at low temperature, a population of inhibited PS II reaction centers can revert rapidly to photochemically active PS II centers possibly through subtle but, as yet unknown, conformational changes (Fig. 2 B). Green algae exhibit this capacity when photoinhibited at either high or low temperature (Falk and Samuelsson 1992). We suggest that this capacity to circumvent irreversible damage to PS II reaction centers, which requires de novo protein synthesis for repair, may be imperative for successful acclimation to life at low temperatures in cold tolerant plants and algae. This characteristic may separate chilling sensitive plants from cold tolerant plant species.

Although resistance to low temperature photoinhibition of photosynthesis in winter annuals can not be accounted for by a more efficient PS II repair process, complete recovery from photoinhibition does require de novo protein synthesis (Hurry and Huner 1992, Falk et al. 1990). The dependence on de novo protein synthesis for the recovery from photoinhibition in vivo may change as a function of the conditions under which it is imposed. For example, in the absence of protein synthesis, photoinhibition of wheat at 20 °C results in a significant increase in F_0 and a decrease in F_V whereas at 5 °C in the absence of protein synthesis, photoinhibition is due solely to a decrease in F_V (Hurry and Huner 1992). This supports the suggestion that PS II inhibited centers may be converted to quenching centers upon photoinhibition. Clearly, further experimentation is required to delineate the role of protein synthesis in photoinhibition and recovery.

IV.C. Long-term photoinhibition

IV.C.i. Growth and photosynthetic response. Long-term, repetitive, daily exposures of wheat to photoinhibition at 5 °C and 1200 μmol $m^{-2}s^{-1}$ (HL) results in daily reductions in F_V/F_M of up to 40% of nonphotoinhibited controls exposed to 5 °C and 250 μmol m^{-2} s^{-1} (Fig. 3) and a concomitant reduction in ϕ_{O_2} evolution based on absorbed photons (Hurry et al. 1992). The HL plants also exhibited a 20% increase in Chl *a/b* and a decrease in leaf absorptance from about 0.88 to 0.82 (Hurry et al. 1992). However, over the period of 56 days at 5 °C, HL-treated Kharkov and Katepwa *increased* their growth rate on a dry weight basis by 25% and 10% respectively and altered their pattern of carbon allocation to favour the shoot by 10% to 30% (Hurry 1991, Hurry et al. 1992). This occurred with no change in the winter or spring growth habit.

Although $\phi_{PS\ II}$ was lower for HL plants than the 5 °C-grown controls under light limiting conditions both exhibited equal $\phi_{PS\ II}$ under light saturating conditions. The HL plants were grown at an irradiance that was 5-fold higher than control plants. The calculated flux of electrons through PS II ($\phi_{PS\ II}$ × I) was about double in the HL plants during exposure to the photoinhibitory light compared to controls. Thus, the saturating light levels available during photoinhibition of these cold tolerant plants was sufficient to maintain a significant capacity for carbon assimilation even though the efficiency of light utilization was reduced. Cold tolerant cereals can take advantage of the available light to increase net carbon gain during exposure to low temperature photoinhibition. In contrast, it has been estimated that about 10% of the net carbon gain of peripheral willow shoots can be lost due to daily photoinhibition (Ögren and Sjöström 1990). However, this discrepancy may be due to the fact that loss of daily carbon gain is exacerbated by the fluctuating irradiance typical for field conditions in contrast to the constant HL conditions in the controlled experiments of Hurry et al. (1992). Clearly, photoinhibition does not necessarily limit plant productivity measured as net carbon gain. The long-term studies of Hurry et al. (1992) strengthen the view that photoinhibition in vivo represents a state of acclimation through which electron transport is adjusted to match the energy requirements for carbon assimilation under the prevailing light conditions.

IV.C.ii. Zeaxanthin accumulation. Growth at 5 °C and HL stimulated the levels of zeaxanthin by an

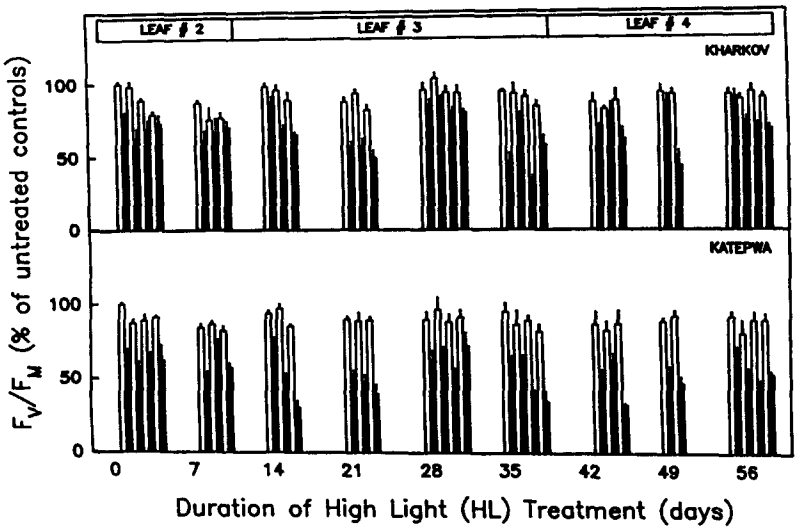

Fig. 3. Effect of long term exposure to low temperature photoinhibition on F_V/F_M of leaves from Kharkov and Katepwa wheat. Cold hardened plants were exposed on a daily basis to a 16 h photoperiod and an irradiance of 250 μmol m^{-2} s^{-1} for 2h, subsequently shifted to an irradiance of 1200 μmol m^{-2} s^{-1} and then returned to an irradiance of 250 μmol m^{-2} s^{-1} for an additional 2h. All growth occurred at a constant temperature of 5 °C. F_V/F_M was measured each day for dark adapted leaves before (open bars) and after exposure (closed bars) to 1200 μmol m^{-2} s^{-1}. All values are the average 6 replicate measurements SD.

additional 3- to 4-fold on a fresh weight basis at the expense of violaxanthin relative to plants grown under standard low temperature conditions. However, the increased levels of zeaxanthin did not alter the susceptibilities of these cultivars to low temperature photoinhibition (Hurry et al. 1992) measured as either reductions in F_V/F_M or ϕ_{O_2} evolution based on absorbed photons. Furthermore, q_N under light saturating conditions (1000–2000 μmol m^{-2} s^{-1}) was not significantly altered in HL-treated plants compared to controls. These results are consistent with previous results for cereals and indicate that dissipation of light energy by non-photochemical mechanisms can not account for resistance to short-term or long-term photoinhibition in cereals.

V. Cold tolerant evergreens

V.A. Photosynthetic response

Conifers become extremely cold and freezing tolerant when exposed to a combination of short days (SD) and low temperature (Levitt 1980), conditions normally experienced during autumn and early winter. This induces dormancy in conifers which is essential for maximum cold tolerance and winter survival. In contrast to cold tolerant herbaceous annuals and algae, exposure of Scots pine to 5 °C and SD under controlled environment conditions causes a significant depression in $PSmax_{CO_2}$ and electron transport (Öquist et al. 1980). Natural overwintering of Scots pine results in the inhibition of PS II activity and CO_2 assimilation (Martin et al. 1978, Strand and Öquist 1985, 1988). PSmax, $\phi_{app}O_2$ and F_V/F_M are generally reduced during exposure to natural overwintering of pine (Leverenz and Öquist 1987, Ottander and Öquist 1991), spruce (Bolhar-Nordenkampf and Lechner 1988), ivy (Oberhuber and Bauer 1991), holly (Groom et al. 1991) and periwinkle (Rezansoff and Huner, unpublished).

V.B. Response to photoinhibition

Several reports have indicated that photoinhibition of photosynthesis may occur at low temperatures under laboratory as well as field conditions in Scots pine (Öquist and Ögren 1985, Strand and Öquist 1988, Öquist and Malmberg 1989), Norway spruce

(Bolhar-Nordenkampf and Lechner 1988, Lundmark and Hällgren 1988), ivy (Oberhuber and Bauer 1991), holly (Groom et al. 1991) and wheat (Groom and Baker 1992). However, field grown pine exhibited full recovery from winter stress within several hours to days when overwintering branches were shifted to 20 °C and moderate light conditions in the laboratory (Ottander and Öquist 1991). Similar trends have been reported for photoinhibited holly (Groom et al. 1991) and ivy (Oberhuber and Bauer 1991). Light levels as low as 50 μmol m^{-2} s^{-1} are sufficient to induce significant reductions in the quantum yield of the upper, light exposed sides of needles during prolonged exposure to low temperature and SD under controlled conditions (Strand and Öquist 1988). Öquist and Huner (1991) reported that pine exposed to 5 °C and SD exhibit similar susceptibilities to low temperature photoinhibition as pine grown under summer conditions. They concluded that Scots pine is unable to respond to exposure to cold hardening conditions through an increased resistance to photoinhibition.

Exposure to severe and persistent freezing conditions in sunlight can lead to secondary photooxidative effects following photoinhibition (Senser and Beck 1977, Oberhuber and Bauer 1991). This may lead to permanent damage followed by leaf or needle senescence. Freezing in the absence of light can also result in a significant reduction on PSmax (Strand and Öquist 1985, DeLucia and Smith 1987, Hällgren et al. 1990). It appears that night frosts increase the susceptibility of Scots pine to photoinhibition (Hällgren et al. 1990). In a recent study of winter stress in Scots pine, Ottander and Öquist (1991) reported that the photochemical efficiency of PS II recovered more rapidly from winter stress than either $\phi_{app}O_2$ or $PSmax_{O_2}$. These data are consistent for both field and laboratory recovery experiments. They conclude that photoinhibition represents the mechanism by which winter stressed pine prevents photodamage to PS II when the consumption of ATP and NADPH by CO_2 fixation is largely inhibited (Ottander and Öquist 1991).

V.C. Pigments and antioxidants

Recently, we observed an increased resistance to photoinhibition as measured by F_V/F_M or $\phi_{app}O_2$ in primary needles of Jack pine (*Pinus banksiana* Lamb.) when four week old seedlings grown at 20 °C and 250 μmol m^{-2} s^{-1} with a 16 h photoperiod (LD) were shifted to 5 °C and 250 μmol m^{-2} s^{-1} with an 8 h photoperiod (SD) (Krol, Hurry, Gray, Öquist, Malek and Huner, in preparation). The increased resistance was dependent upon irradiance and photoperiod during the low temperature shift with SD plants more resistant than LD plants. This resistance to photoinhibition was a transitory phenomenon with maximum resistance occurring after a two month exposure. After four months, this effect could not be detected.

Although exposure to 5 °C, an irradiance of 250 μmol m^{-2} s^{-1} and SD stimulated the accumulation of zeaxanthin and the antioxidant, α-tocopherol, both LD and SD accumulated these compounds to the same extent and thus, could not account for the differential resistance to photoinhibition in Jack pine. However, resistance to photoinhibition appeared to be correlated to anthocyanin accumulation specifically in the epidermal cells of the upper side of the needles. LD, warm grown seedlings shifted to SD and low temperature for two months exhibited a 1.8-fold higher anthocyanin level (0.40 ± 0.08 μg/g fresh weight) than similar seedlings shifted to LD and low temperature (0.22 ± 0.03 μg/g fresh weight). Seedlings grown at 20 °C do not accumulate anthocyanin. Thus, the increased resistance to photoinhibition in Jack pine may be accounted for by a 'shading effect' caused by specific accumulation of anthocyanin in epidermal cells of the adaxial needle surface directly exposed to the light. However, further work is required to substantiate this hypothesis.

VI. Conclusions

Although evergreens such as pine exhibit a greater freezing tolerance but also greater susceptibility to low temperature photoinhibition than most herbaceous annuals, these trends must be considered in the context of the developmental strategy exhibited by these plants during the cold hardening process. Pine, unlike winter wheat and rye, must enter a photoperiod-dependent dormant growth state in the fall in order to attain maximum cold tolerance (Levitt 1980). Furthermore, evergreen leaves or needles that develop in the spring and early summer are subjected to yearly overwintering conditions. Thus, we conclude that in cold tolerant evergreens

there may be no selective advantage to modulate photosynthetic rates or development of long-term resistance to photoinhibition since growth, by and large, is terminated during the cold hardening period. However, it is incumbent upon the plant to protect the photosynthetic apparatus from excessive excitation during winter stress to prevent photooxidation and to ensure reasonable photosynthetic capacity when the winter stress conditions have been alleviated in early spring when new growth resumes. Due to thermodynamic constraints and the high cost of repair (Raven and Samuelsson 1986), dependence upon de novo protein synthesis and lateral diffusion of large membrane protein complexes appears unlikely as a primary mechanism at low temperature and is not supported by in vivo experiments with cold tolerant plant species. However, photoinhibition of photosynthesis would provide a mechanism for stable, long-term down regulation of PS II thereby providing a mechanism for safe dissipation of excessive light energy through nonphotochemical quenching. This would appear to be a reasonable mechanism to protect the photosynthetic apparatus from irreparable photodynamic damage (Fig. 4A). Thus, it is our contention that low temperature photoinhibition of photosynthesis in evergreens reflects a long-term regulation of PS II, that is a state of acclimation, which is of physiological and ecological significance for the successful establishment of evergreens in cold, temperate climates. However, a more extensive survey of different evergreen species as well as direct measurements of increased energy dissipation during cold acclimation of evergreens are required to confirm this hypothesis.

In contrast to evergreens, cold tolerant cereals and algae are not dormant during the cold hardening process but require growth and development at low temperature to exhibit maximum freezing tolerance. To support this low temperature growth, these plants must have a greater dependence on active photosynthesis for the supply of energy for the low temperature acclimation process than the dormant evergreens. The increased photosynthetic capacity maintains a greater proportion of open PS II reaction centers under conditions of high light and low temperature (Fig. 4B). As a consequence, winter cereals exhibit an increased resistance to photoinhibition. Thus, resistance to photoinhibition in cereals and algae is symptomatic of the photosynthetic response of the plant to development at low temperature. However, not all cold tolerant herbaceous plant species use the same mechanism as cereals. We suggest that the mechanism(s) for resistance to photoinhibition is/are species specific and may also change within the same species as a function of the prevailing environmental conditions and the carbon requirements of the plant.

What is the physiological role for the low temperature-induced increase in photosynthetic capacity in cold tolerant winter cereals? The demand for the photosynthetic end product, sucrose, remains high during growth at low temperature due to the energy requirements for the production and maintenance of the cold acclimated state which is a prerequisite for maximum freezing tolerance. This is associated with a change from the normal summer growth habit to a rosette pattern of short, thick leaves and minimal stem elongation. In contrast, spring varieties are unable to adjust either photosynthesis or plant morphology to growth and development at low temperature (Andersson 1944, Vasil'yev 1956, Levitt 1980, Krol et al. 1984, Hurry and Huner 1991). For successful overwintering, it is essential that the crown survive. Translocation of sucrose from leaves to the crown, a major sink during cold acclimation, is essential for the ultimate survival of overwintering cereals (Levitt 1980). Although there have been considerable data published on *measuring temperature* effects on carbon partitioning, information on the effects of low *growth temperature* on source-sink relationships is sparse (Farrar 1988). Clearly, much more experimentation on the effects of low growth temperature on source-sink relationships in both winter and spring cereals is required in order to understand the basis of the correlations between photosynthesis and freezing tolerance.

In addition, storage of carbohydrate as fructans in the vacuole of source tissue may also help to maintain high rates of carbon flow into sucrose at low temperature. Sucrose accumulation at low temperature results in enhanced levels of fructans in the vacuole (Fig. 5). This provides a much larger storage compartment than starch in the chloroplast (Pollock and Chatterton 1988). It appears that the metabolic conversion of sucrose to fructans is much less temperature sensitive than starch synthesis (Pollock and Lloyd 1987, Pollock and Chatterton 1988). Furthermore, herbaceous plants (McNulty

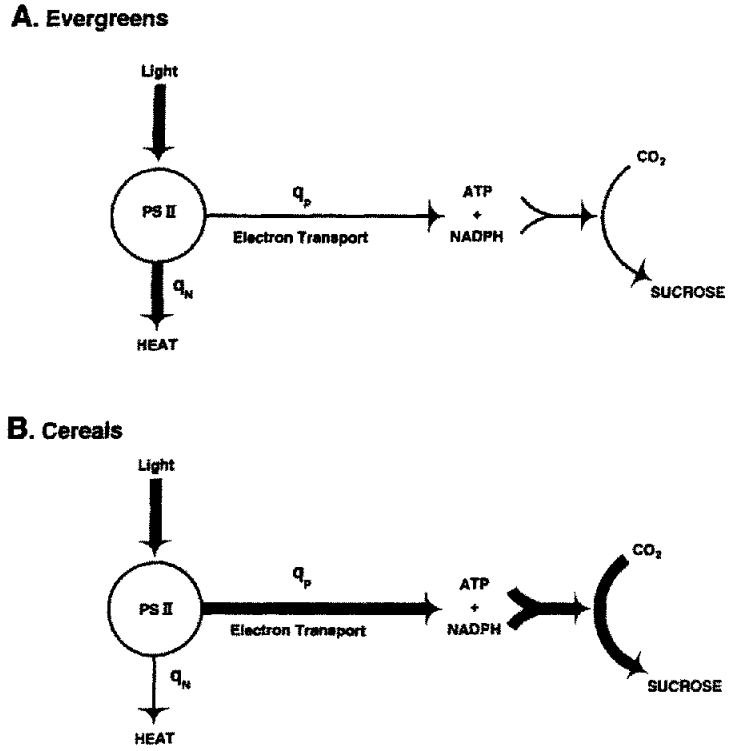

Fig. 4. Mechanisms for the protection of PS II from low temperature photoinhibition. (A) Evergreens through nonphotochemical quenching (q_N) mechanisms and (B) cereals through photochemical quenching (q_p).

and Cummins 1987, Collier and Cummins 1990, Hurry et al. 1992) and algae (Maxwell, Falk and Huner, unpublished) typically exhibit enhanced rates of respiration upon growth at low temperature. Enhanced demands of the crown for photosynthate, accumulation of fructans in the vacuole and the enhanced rates of respiration may operate in concert to prevent significant feedback inhibition of photosynthesis by sucrose. In addition, alterations in the regulatory properties of key enzymes of carbon metabolism such as cytosolic FBPase and sucrose-P synthase (Fig. 5) as a consequence of high sucrose accumulation at low growth temperature may also be important in maintaining increased rates of photosynthesis. For example, the sensitivity of cytosolic FBPase to F-2,6-BP may be altered as a consequence of growth at low temperature. Recently, Hurry et al. (1993) showed that *growth* at low temperature does indeed increase Pi availability in cold tolerant cereals. This is contrary to expectations based on our current knowledge of the effects of low measuring temperature or low temperature shifts on photosynthesis and carbon metabolism. Thus, we believe that cold tolerant plants *grown* at low temperature represent excellent experimental systems to investigate the mechanism(s) by which plants possibly circumvent or escape potential Pi limitation of photosynthesis thought to be imposed by low temperature upon carbon metabolism (Sharkey 1985b, Sharkey et al. 1986, Stitt et al. 1987). The use of antisense mutations to block various steps of carbon metabolism and sink activities as well as over-expression of these genes (Fig. 5) in cold tolerant plants may prove to be a fruitful approach to analyse the contributions of various metabolic pathways to the photosynthetic response of plants during low temperature acclimation and ultimately to the acquisition of freezing tolerance.

On the basis of our model (Fig. 5), we propose that cold tolerant winter cereals are be able to maintain a higher flux of carbon through the Calvin cycle and through to sucrose synthesis than spring cereals due, in part, to higher sink activity as well as increased capacity to store carbohydrate as fructans.

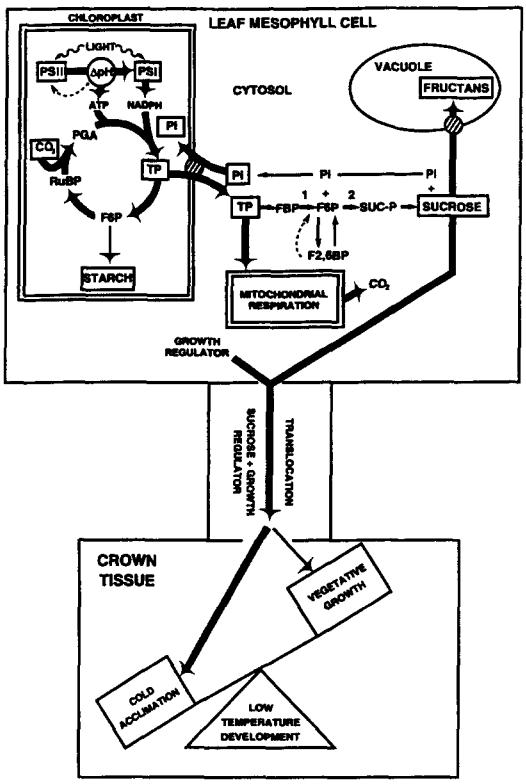

Fig. 5. 'Balance model' relating leaf photosynthesis, sucrose synthesis and cold acclimation of the crown in cold hardened winter annuals. In the less hardy spring annuals, 'the balance' will be tipped in favour of the maintenance of vegetative growth. 1, FBPase; 2, Sucrose-P synthase.

This is reflected at the level of PS II through feedback regulation. As a consequence, winter varieties exhibit a higher capacity to adjust the proportion of oxidized Q_A [Q_A(H)/Q_A(NH)] and a higher maximum rate of photosynthesis [PSmax(H)/ PSmax(NH)] as a function of growth temperature than spring varieties. This may be the reason that there is such a strong positive correlation between photosynthesis, sucrose accumulation and freezing tolerance. We believe that the accumulation of sucrose and other carbohydrates during growth at low temperature reflect specific adjustments in carbon metabolism which enhance the capacity of the plant to cold acclimate. Historically, the relationship between carbohydrate accumulation during cold acclimation and freezing tolerance has been interpreted principally on the basis of cryoprotection (Levitt 1980, Guy 1990). However, emphasis should be shifted to the fact that the accumulation of these carbohydrates reflects important regulatory adjustments in photosynthetic carbon metabolism. A greater understanding of the capacity to adjust overall metabolism during low temperature growth should lead to new insights into the process of cold acclimation and its relationship to freezing tolerance. Frost tolerance of cereals has not increased significantly since the turn of this century by conventional breeding programmes, in part, due to the lack of physiological markers for frost tolerance (Blum 1985). We believe that these photosynthetic characteristics are the first physiological markers that could be considered useful in breeding for freezing tolerance in cereals.

During growth at low temperatures, winter cultivars not only modulate photosynthetic capacity but also alter their growth form from the elongated summer habit at high temperature to the characteristic rosette pattern at low temperature. This reflects reallocation of energy away from vegetative growth to the development of a cold acclimated crown (Fig. 5). Thus, spring varieties not only suffer from depressed rates of photosynthesis at low temperature but also are unable to shift the energy balance from vegetative growth in favour of cold acclimation of the crown. This must be closely coupled to the known regulation of low temperature development of the crown by growth plant regulators (Chen and Li 1982). Thus, we suggest that cold acclimation of cereals includes, first, the accumulation of sufficient energy stores at low temperature through the modulation of photosynthetic capacity and carbon metabolism. Second, this is coupled to the preferential allocation of photosynthate away from vegetative growth and to cold acclimation of the crown for maximum winter survival and subsequent early spring growth.

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