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# Low Growth Temperature Effects a Differential Inhibition of Photosynthesis in Spring and Winter Wheat[1]

**Vaughan M. Hurry and Norman P. A. Huner***

*Department of Plant Sciences, University of Western Ontario, London, Ontario N6A 5B7, Canada*

## ABSTRACT

***In vivo* room temperature chlorophyll *a* fluorescence coupled with $CO_2$ and $O_2$ exchange was measured to determine photosynthetic limitation(s) for spring and winter wheat (*Triticum aestivum* L.) grown at cold-hardening temperatures (5°C/5°C, day/night). Plants of comparable physiological stage, but grown at nonhardening temperatures (20°C/16°C, day/night) were used in comparison. Winter wheat cultivars grown at 5°C had light-saturated rates of $CO_2$ exchange and apparent photon yields for $CO_2$ exchange and $O_2$ evolution that were equal to or greater than those of winter cultivars grown at 20°C. In contrast, spring wheat cultivars grown at 5°C showed 35% lower apparent photon yields for $CO_2$ exchange and 25% lower light-saturated rates of $CO_2$ exchange compared to 20°C grown controls. The lower $CO_2$ exchange capacity is not associated with a lower efficiency of photosystem II activity measured as either the apparent photon yield for $O_2$ evolution, the ratio of variable to maximal fluorescence, or the level of reduced primary quinone electron acceptor maintained at steady-state photosynthesis, and is most likely associated with carbon metabolism. The lower $CO_2$ exchange capacity of the spring cultivars developed following long-term exposure to low temperature and did not occur following overnight exposure of nonhardened plants to 5°C.**

Attainment of maximum freezing resistance by cold-tolerant cereals such as winter rye and winter wheat (*Triticum aestivum* L.) is dependent upon growth and development during prolonged exposure of seedlings to low, nonfreezing temperatures (0–5°C) prior to onset of freezing conditions. Growth and development at low temperature are therefore prerequisites for the expression of freezing resistance in these cold-tolerant cereals (8, 14, 17). Because photosynthesis provides the energy for this growth and development, we are interested in understanding the mechanisms by which overwintering cereals maintain optimal photosynthetic capacity at low, cold-hardening growth temperatures.

The most extensive work on the effects of prolonged exposure to low, nonfreezing (0–5°C) as well as freezing temperatures on photosynthesis in cold-tolerant plants has been carried out in conifers (21, 22, 24). However, conifers, unlike cereals such as wheat and rye, do not undergo significant growth during exposure to cold-hardening conditions (21).

Winter rye plants grown at 5°C develop a resistance to photoinhibition at low temperatures (23) similar to that observed for spinach (32), and are able to maintain light- and $CO_2$-saturated rates of photosynthesis equivalent to or higher than plants grown at 20°C (12). To exhibit these characteristics, winter rye must develop at low temperatures. Mature leaves developed at 20°C and subsequently exposed to low temperatures for up to 25 d do not exhibit these photosynthetic traits (23). Similarly, *Lolium temulentum* (27) and dicotyledonous winter annuals (4, 29) have equivalent rates of $CO_2$ exchange under cold-hardening and nonhardening conditions. In contrast, when pines are exposed to cold-hardening conditions they become photosynthetically inhibited (21, 24).

Much of our present knowledge of cold acclimation and freezing tolerance has been gained through comparative studies of spring and winter cereal cultivars grown at cold-hardening and nonhardening temperatures (8, 9, 17, 19). There are published data concerning the effects of measurement temperature (10, 11) and water stress (10, 13, 20) on carbon assimilation in spring and winter wheat, but there is little published information on the effects of development temperature on photosynthesis in cold-tolerant cereals.

Light and $CO_2$ are required during exposure of winter cereals and other herbaceous crops to low temperature in order to attain maximum cold-hardening (1, 7, 16). An important question, therefore, is whether cold-tolerant winter cereals remain photosynthetically more competent during prolonged exposure to low temperature than the less tolerant spring cultivars. In this study, we use measurements of *in vivo* room temperature Chl *a* fluorescence coupled with $CO_2$ and $O_2$ gas exchange to determine the photosynthetic limitation(s) imposed on spring and winter wheat during growth and development at cold-hardening temperatures.

## MATERIALS AND METHODS

### Plant Material

Three cultivars of winter (cv Monopol, cv Kharkov, cv Augusta) and three of spring (cv Glenlea, cv Katepwa, cv Marquis) wheat (*Triticum aestivum* L.) were grown in coarse vermiculite in 7-cm plastic pots at a density of five plants per pot. Water and nutrients were supplied as required in the form of a modified Hoagland solution as described previously (14). Seeds were germinated under controlled environment conditions with a day/night temperature regimen of 20°C/16°C, at a PPFD of 250 $\mu$mol $m^{-2}$ $s^{-1}$ and a 16-h photoperiod. After 7 d, both winter and spring seedlings were cold-hardened by transfer to a temperature regime of 5°C/5°C with photo-

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period and light intensity the same as controls. Control plants remained in the 20°C/16°C regimen.

### Comparative Growth Kinetics

Aerial portions of seedlings from two pots exposed to nonhardening conditions were harvested every 3 d; those exposed to cold-hardening conditions were harvested every 7 d. Leaves were counted, then shoot tissues were dried at 105°C to constant weight. Growth coefficients were calculated from the slope of ln of total shoot dry weight and leaf number *versus* time (19).

### Chl Content

Fresh leaf tissue was extracted in 80% (v/v) acetone. The Chl content was determined according to Arnon (2).

### Measurement of $CO_2$ Gas Exchange

$CO_2$ exchange rates were measured on attached, fully expanded, third and fourth leaves of 25-d-old nonhardened and 75-d-old cold-hardened plants in a closed system using an LI-COR 6200 (Lincoln, NE) IR $CO_2$ analyzer. The fourth leaf of Monopol at 20°C was about 60%, but others were fully expanded (Fig. 1), and plants were considered to be at similar physiological stages of development.

Measurements were made at 20 and 5°C air temperature in a controlled-environment growth chamber such that the temperature of the measured leaf and the remainder of the plant were comparable. Relative humidity of the air stream was approximately 50%. Ambient $CO_2$ ranged from 350 to 450 $\mu L\ L^{-1}$. Plants grown at 20°C to be measured at 5°C and those grown at 5°C to be measured at 20°C were equilibrated at the measurement temperature during the regular 8-h dark period prior to measurement of $CO_2$ exchange to ensure that the entire plant, not just the leaf being measured, was equilibrated to the measurement temperature. Gas exchange measurements were made 2 h after the beginning of the photoperiod. Temperature shifted plants were therefore equilibrated at the measurement temperature for a total of 10 h prior to measurement of $CO_2$ exchange. Stomatal conductance and calculated internal $CO_2$ concentration were measured with the LI-COR 6200 and were unaffected by this temperature shift.

$\Phi_{app}$[2] for $CO_2$ exccange (mol $CO_2$ exchanged/mol incident photons) was calculated from the slope of rate *versus* PPFD in the light-limited (4–125 $\mu$mol $m^{-2}\ s^{-1}$ PPFD) range. Plants were allowed to equilibrate for 1 h to changes in PPFD, which was monitored at leaf height with a quantum sensor (LI-COR model LI-190S-1). $CO_2$ exchange rates were measured at PPFDs from 0 to 1500 $\mu$mol $m^{-2}\ s^{-1}$ to determine PPFD saturation.

[2] $\Phi_{app}$, apparent photon yield; $F_o$, minimum fluorescence with all photosystem II reaction centers open; $(F_v)_m$, maximal variable fluorescence; $F_v$, variable fluorescence; $(F_v)_s$, saturation level of variable fluorescence; $F_v/F_m$, ratio of variable to maximal fluorescence; $q_N$, non-photochemical quenching; $q_P$, photochemical quenching; $Q_A$, primary quinone electron acceptor for PSII.

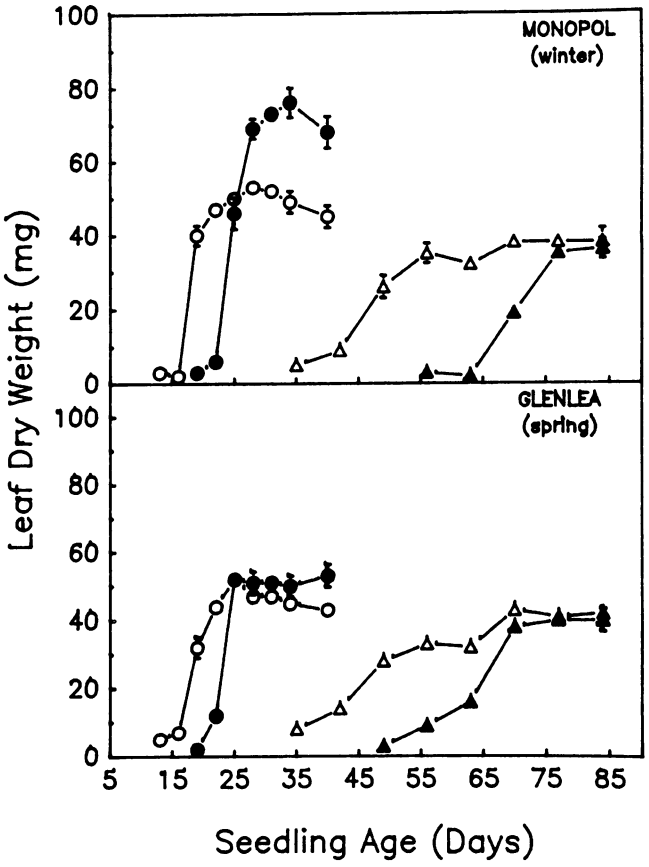


**Figure 1.** Leaf dry weight accumulation for winter (Monopol) and spring (Glenlea) wheat during growth under cold-hardening and nonhardening conditions. Leaf 3 (○) and leaf 4 (●) at 20°C; leaf 3 (△) and leaf 4 (▲) at 5°C. Each point is the mean of 10 plants from two different pots. Bars represent SE.

### Measurements of Fluorescence

Fluorescence induction kinetics were measured at room temperature using a PAM fluorometer (Heinz Walz, Effeltrich, FRG) (31). Measurements were made on detached, fully expanded, third and fourth leaves of both 75-d-old cold-hardened and 25-d-old nonhardened winter (Monopol) and spring (Glenlea) wheat.

Prior to fluorescence measurements, all leaves were dark-adapted for 30 min at room temperature. $F_o$ was determined by illuminating dark-adapted leaves with a low intensity measuring beam (<1 $\mu$mol $m^{-2}\ s^{-1}$ PPFD) from a light-emitting diode. $(F_v)_m$ was determined by application of a second beam of saturating (3000 $\mu$mol $m^{-2}\ s^{-1}$ PPFD) actinic white light applied in four short (400 ms) pulses at 10 s intervals. The difference between the mean height of the four peaks and the $F_o$ level was taken to be $(F_v)_m$. Following determination of $F_o$ and $(F_v)_m$, $F_v$ and $(F_v)_s$ were monitored during induction. This was accomplished by simultaneous illumination of the sample with a red actinic light source of low intensity (60 $\mu$mol $m^{-2}\ s^{-1}$ PPFD) to stimulate fluorescence induction, and a saturating white light (3000 $\mu$mol $m^{-2}\ s^{-1}$ PPFD) source applied repetitively in 400 ms pulses at 10 s intervals. $q_N$ and $q_P$

**Table I.** *Estimated Growth Coefficients for Winter and Spring Wheat*

Ten seedlings from two pots grown at nonhardening and cold-hardening temperatures were harvested every 3 and 7 d, respectively. Growth coefficients ($k_1'$) were calculated from the plot of ln of total shoot dry weight and leaf number *versus* time.

| Cultivar | Growth Parameter | $k_1'$ ($day^{-1}$) | | H:NH |
|---|---|---|---|---|
| | | Hardened (H) | Nonhardened (NH) | |
| Monopol (winter) | Dry wt | 0.060 | 0.184 | 0.33 |
| | Leaf No. | 0.039 | 0.109 | 0.36 |
| Glenlea (spring) | Dry wt | 0.058 | 0.182 | 0.32 |
| | Leaf No. | 0.044 | 0.121 | 0.36 |

coefficients were calculated using the equations of Schreiber *et al.* (31).

$$q_N = \frac{(F_v)_m - (F_v)_s}{(F_v)_m} \quad (1)$$

and

$$q_P = \frac{(F_v)_s - F_v}{(F_v)_s} \quad (2)$$

### Measurement of $O_2$ Evolution

Oxygen evolution was measured with a leaf-disc electrode (HansaTech, model LD2, Kings Lynn, Norfolk, UK) at 20°C with an initial gas mixture 5% $CO_2$:5% $O_2$:90% $N_2$. Light was provided by a set of photodiodes (HansaTech; $\lambda_{max}$ 660 nm). Measurements were made on leaf discs punched from the mid region of fully expanded third and fourth leaves of both 75-d-old cold-hardened and 25-d-old nonhardened winter (Monopol) and spring (Glenlea) wheat. $\Phi_{app}$ for $O_2$ evolution was calculated from the plot of $O_2$ evolution rate *versus* PPFD in the light-limited (1–60 $\mu$mol $m^{-2}$ $s^{-1}$ PPFD) range.

## RESULTS

### Comparative Growth Kinetics

It is important to consider both chronological age and the relative stage of development, since growth temperature has a pronounced effect on the rate of both growth and development. Accordingly, the growth characteristics of all six cultivars were compared at cold-hardening (5°C) and nonhardening (20°C) temperatures to determine at which chronological ages the different growth temperatures yielded experimental material at a similar physiological stage of development. Results are shown only for the winter wheat Monopol and the spring wheat Glenlea because the results of all winter and spring wheat cultivars were comparable within their respective groups.

Growth coefficients for winter and spring cultivars were similar for dry weight accumulation and leaf initiation, but coefficients achieved at 5°C were about one-third those at 20°C (Table I). These results are consistent with previous reports for growth of cereals at cold-hardening temperatures (9, 14, 19). Thus, for all subsequent measurements of $CO_2$ exchange, $O_2$ evolution, and fluorescence characteristics, the third and fourth leaves of 25-d-old nonhardened plants were compared with those of 75-d-old cold-hardened plants.

### Effect of Growth and Measuring Temperature on Apparent Photon Yield for $CO_2$ Exchange

The winter wheat cultivars Monopol, Kharkov, and Augusta exhibited similar $\Phi_{app}$ for $CO_2$ exchange whether grown at 5 or 20°C (Table II). Furthermore, measurement temperature had no effect on $\Phi_{app}$ for $CO_2$ exchange for either cold-hardened or nonhardened Monopol, Kharkov, or Augusta winter wheat (Table II). In contrast, the $\Phi_{app}$ for $CO_2$ exchange of Glenlea, Marquis, and Katepwa spring wheat following growth at low, cold-hardening temperatures was 35 to 45% lower than the $\Phi_{app}$ for $CO_2$ exchange of the same cultivars grown at 20°C (Table II). The $\Phi_{app}$ for $CO_2$ exchange for both hardened and nonhardened Katepwa plants was not affected by measurement temperature (Table II). However, cold-hard-

**Table II.** *Effect of Growth and Measurement Temperature on Apparent Photon Yield for $CO_2$ Exchange of Winter and Spring Wheat Cultivars*

Light-response curves for $CO_2$ exchange were measured at 5 and 20°C on attached, fully expanded third and fourth leaves of 25-d-old nonhardened and 75-d-old cold-hardened plants. Data represent the mean and (SE), $n = 4$.

| Cultivars | Measurement Temperature | | | |
|---|---|---|---|---|
| | Nonhardened | | Cold-hardened | |
| | 20°C | 5°C | 20°C | 5°C |
| | *mol $CO_2$ $mol^{-1}$ photons* | | | |
| Winter | | | | |
| Monopol | 0.041 (0.002) | 0.044 (0.001) | 0.042 (0.002) | 0.042 (0.002) |
| Kharkov | 0.040 (0.001) | 0.040 (0.002) | 0.045 (0.002) | 0.045 (0.002) |
| Augusta | 0.042 (0.002) | 0.045 (0.001) | 0.044 (0.001) | 0.040 (0.002) |
| Spring | | | | |
| Glenlea | 0.043 (0.001) | 0.045 (0.001) | 0.034 (0.001) | 0.024 (0.001) |
| Katepwa | 0.048 (0.002) | 0.047 (0.003) | 0.027 (0.001) | 0.026 (0.001) |
| Marquis | 0.046 (0.001) | 0.045 (0.001) | 0.035 (0.002) | 0.029 (0.002) |

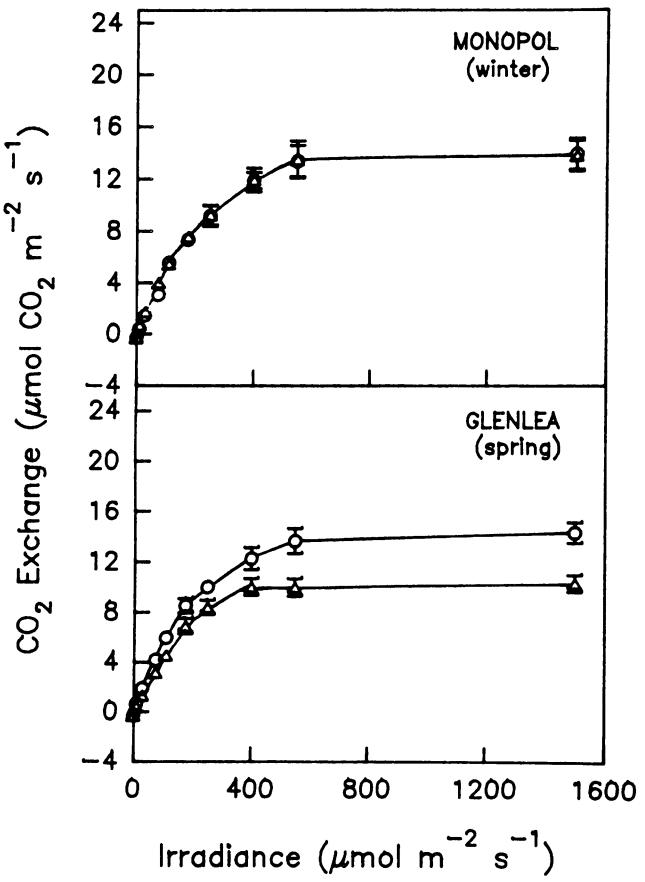


**Figure 2.** Irradiance response curves for $CO_2$ exchange in winter (Monopol) and spring (Glenlea) wheat measured at 5°C. (○) Plants grown at 20°C; (▲) plants grown at 5°C. Each point is the mean of 12 leaves from four different pots. Bars represent SE.

ened Glenlea and Marquis spring wheat exhibited only a 15 to 24% lower $\Phi_{app}$ for $CO_2$ exchange, relative to nonhardened leaves, after being equilibrated at 20°C for 10 h (8 h darkness + 2 h at 250 $\mu$mol $m^{-2}$ $s^{-1}$ PPFD). This indicates that partial recovery of $\Phi_{app}$ for $CO_2$ exchange is possible provided the measurement temperature is increased above the 5°C growth temperature (Table II).

### Effect of Growth and Measuring Temperature on Light-Saturated Rates of $CO_2$ Exchange

For simplicity, only the data for one winter and one spring cultivar are shown in Figure 2 because the three winter cultivars tested responded similarly, as did the three spring cultivars. The light-saturated rates of $CO_2$ exchange of winter wheat cultivars were similar when the cultivars were grown at either 5 or 20°C and measured at 5°C (Fig. 2). In contrast, the three spring wheat cultivars had 25 to 35% lower light-saturated rates of $CO_2$ exchange when grown at 5°C compared with nonhardened plants (Fig. 2). Furthermore, cold-hardened seedlings of spring wheat exhibited lower light-saturated rates of $CO_2$ exchange, relative to cold-hardened seedlings of winter wheat (Fig. 2). All cultivars exhibited 20 to 25% higher light-saturated rates of $CO_2$ exchange when measured at 20°C than when measured at 5°C.

### Effect of Growth Temperature on Apparent Photon Yield for $O_2$ Evolution and $F_v/F_m$

Measurements of $CO_2$ gas exchange give an indication of overall photosynthetic competence. The 35 to 45% decrease in $\Phi_{app}$ for $CO_2$ exchange points to a significant loss in photosynthetic efficiency, but does not provide any information with respect to a possible site for the perturbation. Butler and Kitijima (5) first showed that $\Phi_{app}$ for $O_2$ evolution is directly related to $F_v/F_m$. This has been confirmed experimentally with a number of plant species (6) including wheat (Fig. 3). Both these parameters can be used as estimates of the efficiency of PSII photochemistry.

The results summarized in Table III indicate that both Glenlea spring and Monopol winter wheat grown at 20°C exhibit similar $\Phi_{app}$ for $O_2$ evolution. However, in contrast to the lower $\Phi_{app}$ for $CO_2$ exchange (Table II), growth at cold-hardening temperatures had no significant effect on $\Phi_{app}$ for $O_2$ evolution for spring or winter wheat (Table III). Growth at cold-hardening temperatures resulted in a 6% decrease in $F_v/F_m$ in both spring and winter wheat (Table III); however, cold-hardening did not cause a differential decrease in this fluorescence response. Winter wheat grown at 5°C showed an increased fluorescence signal as indicated by the increase in both $F_o$ and $F_v$, which can be explained by the 55% increase in Chl/unit leaf area (Table III). Thus, the fluorescence data are consistent with the data for $\Phi_{app}$ for $O_2$ evolution (Table

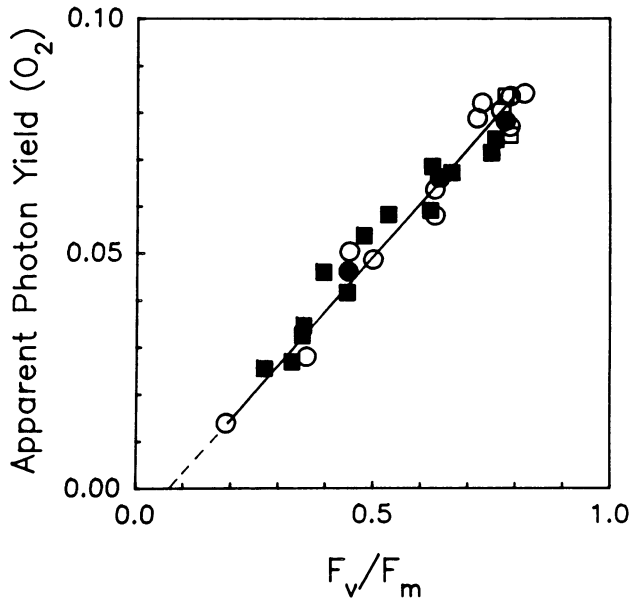


**Figure 3.** Relation between apparent photon yield for $O_2$ evolution (mol $O_2$ evolved/mol incident photons) and the ratio of variable to maximal fluorescence ($F_v/F_m$) measured at room temperature. Leaves 3 and 4 of nonhardened (open symbols, ○, □) and cold-hardened (closed symbols, ●, ■) Glenlea (squares, □, ■) spring and Monopol (circles, ○, ●) winter wheat were photoinhibited for varying periods of time at 5°C and 1200 $\mu$mol $m^{-2}$ $s^{-1}$ PPFD ($r$ = 0.98).

**Table III.** *Fluorescence Characteristics, Apparent Photon Yield of $O_2$ Evolution ($\Phi_{app}$), and Chl Concentration of Winter and Spring Wheat Leaves Grown at Two Temperatures*

Measurements were made on detached, fully expanded third and fourth leaves from 25-d-old nonhardened and 75-d-old cold-hardened plants. Data represent the mean and (SE), $n = 4$.

| Cultivar | Growth Temperature | $F_o$ | $F_v$ | $F_m$ | $F_v/F_m$ | $\Phi_{app}$ for $O_2$ evolution[a] | Chl |
|---|---|---|---|---|---|---|---|
| | °C | *relative fluorescence units* | | | | *mol $O_2$ mol$^{-1}$ photons* | *mg m$^{-2}$* |
| Monopol (winter) | 20/16 | 1.4 | 6.4 | 7.8 | 0.82 | 0.090 | 320 |
| | | (0.1) | (0.1) | (0.1) | (0.01) | (0.010) | (32) |
| | 5/5 | 2.0 | 7.2 | 9.2 | 0.78 | 0.084 | 510 |
| | | (0.2) | (0.4) | (0.4) | (0.01) | (0.010) | (47) |
| Glenlea (spring) | 20/16 | 1.5 | 6.2 | 7.7 | 0.80 | 0.086 | 320 |
| | | (0.1) | (0.2) | (0.2) | (0.01) | (0.010) | (27) |
| | 5/5 | 1.8 | 5.8 | 7.6 | 0.76 | 0.070 | 340 |
| | | (0.1) | (0.4) | (0.3) | (0.01) | (0.005) | (37) |

[a] Measured at 20°C.

III) which indicate that the photosynthetic efficiency of PSII was unaffected by low growth temperature in both the spring and winter cultivars.

### Effect of Growth Temperature on Room Temperature Fluorescence Induction

The slow fluorescence transients typically observed by *in vivo* room temperature Chl *a* fluorescence induction are thought to reflect processes associated with photosynthetic carbon reduction (26). Because growth at low temperature appeared to inhibit $\Phi_{app}$ for $CO_2$ exchange of spring but not winter wheat, we compared the slow fluorescence transients of cold-hardened and nonhardened winter wheat (Fig. 4). Nonhardened spring and winter wheat exhibited similar patterns for quenching maximum fluorescence, which was characterized by the presence of a prominent M-peak prior to the attainment of a minimum steady-state fluorescence yield. This M-peak, although somewhat reduced, was still present in spring cultivar after growth at low temperature. In contrast, the M-peak was not resolved in winter cultivar after cold-hardening (Fig. 4).

We have observed similar trends in room temperature fluorescence induction curves of cold-hardened and nonhardened spinach (SR Boese, NPA Huner, unpublished results) and rye (NPA Huner, unpublished results). Thus, it appears that the cold-hardened winter cultivars can quench maximum room temperature Chl *a* fluorescence more rapidly than cold-hardened spring cereals.

Fluorescence quenching kinetics of cold-hardened spring and winter wheats were examined during the induction period in more detail by the pulsed-modulated system recently developed by Schreiber and co-workers (31). This provides a means of separating fluorescence quenching into its constituent $q_P$ and $q_N$ components. $q_P$ reflects the redox state of $Q_A$ (31). Several different processes are thought to be associated with $q_N$. However, there appears to be a consensus that it reflects, primarily, the capacity to establish a *trans*-thylakoid pH gradient (15).

Nonhardened winter (Fig. 5A) and spring wheat (Fig. 5C) exhibited similar kinetics for the establishment of $q_P$ and $q_N$

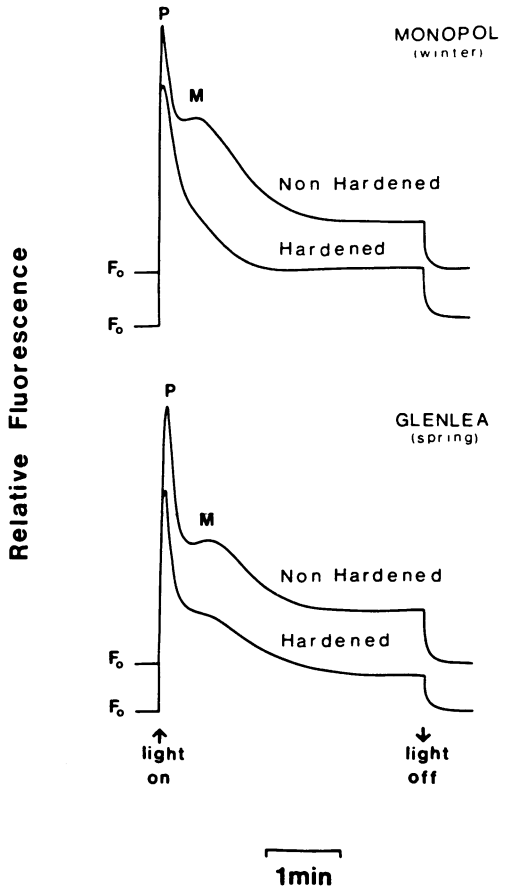


**Figure 4.** Representative traces of the slow fluorescence transients for cold-hardened and nonhardened winter (Monopol) and spring (Glenlea) wheat. Original traces generated by illuminating leaves with a low intensity (60 $\mu$mol m$^{-2}$ s$^{-1}$ PPFD) red actinic light to stimulate fluorescence induction.

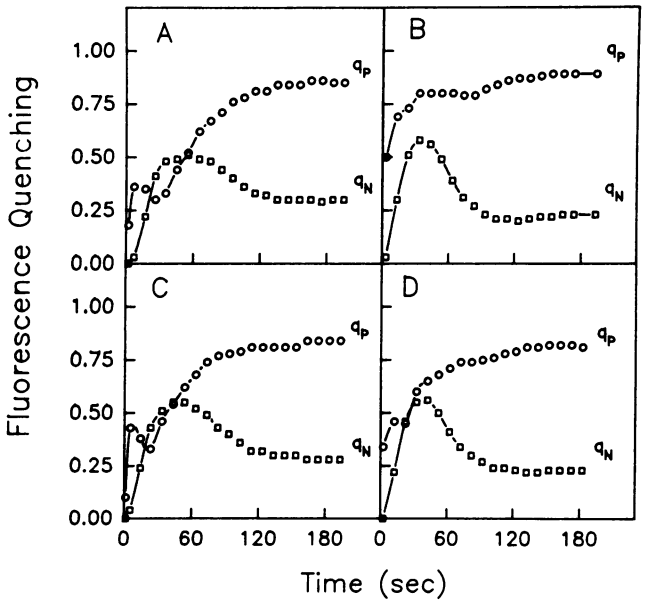


**Figure 5.** Comparison of the time courses of $q_P$ (○) and $q_N$ (□) during fluorescence induction in nonhardened (A) and cold-hardened (B) Monopol winter wheat, and in nonhardened (C) and cold-hardened (D) Glenlea spring wheat. Curves were calculated from the original induction curves that were generated by simultaneously illuminating leaves with a low intensity (60 $\mu$mol $m^{-2}$ $s^{-1}$ PPFD) red actinic light to stimulate fluorescence induction, and saturating white light (3000 $\mu$mol $m^{-2}$ $s^{-1}$ PPFD) applied in 400 ms pulses at 10 s intervals to fully reduce $Q_A$.

during the induction period. In both cases, cold-hardening appeared to increase significantly the relaxation of $q_N$ (Fig. 5B and D). In addition, both the spring and winter wheats exhibited a more rapid rise to maximum $q_P$ after growth at low temperature (Fig. 5B and D) than did plants grown at 20°C (Fig. 5A and C). However, cold-hardened winter wheat appeared to develop maximum $q_P$ more rapidly than cold-hardened spring wheat (Fig. 5B and D).

Although growth at low temperature affects the kinetics for the relaxation of $q_N$ and rise in $q_P$, steady-state levels of $q_N$ and $q_P$ were not affected by growth temperature. At steady-state, at least 85% of $Q_A$ was maintained in the oxidized state at the actinic light intensity employed, regardless of growth temperature. Thus, the inability to resolve the M-peak in cold-hardened winter wheat appears to be associated with an increased rate for the development of photochemical quenching.

## DISCUSSION

We have shown for the first time that spring wheat grown at 5°C and 250 $\mu$mol $m^{-2}$ $s^{-1}$ PPFD exhibits lower $\Phi_{app}$ (35–45%) and lower light-saturated rates (25–35%) of $CO_2$ exchange compared to 20°C grown controls. In contrast, $\Phi_{app}$ and light-saturated rates of $CO_2$ exchange of winter wheat are similar for plants grown at both 20 and 5°C. The $\Phi_{app}$ for $CO_2$ exchange and carboxylation efficiency of winter rye (*Secale cereale* L.) are also similar when grown under cold-hardening (5°C) and nonhardening temperatures (20°C) (12). Thus, spring wheat, exposed for long periods to low, cold-hardening temperatures and moderate light intensities, are less photosynthetically competent than when grown at 20°C. Winter cereals adjust to prolonged exposure to low temperatures without sacrificing photosynthetic competence.

Similar losses in both $\Phi_{app}$ and light-saturated rates of $CO_2$ exchange, related to photoinhibitory damage to PSII, occur in chilling-sensitive plants following short-term exposure to low temperatures and high light (3, 18, 28). However, in contrast with earlier work on chilling sensitive plants, the spring wheat Glenlea showed no significant reduction in photochemical efficiency of PSII after cold-hardening, measured as either a reduction in $F_v/F_m$ or as a reduction in $\Phi_{app}$ for $O_2$ evolution. Thus, the reduction in $\Phi_{app}$ and light-saturated rates of $CO_2$ exchange observed for spring wheat during cold-hardening does not appear to be due to an inhibition at the level of PSII. This is supported by the fact that maximum photochemical quenching, that is, the oxidation state of $Q_A$, is similar for spring and winter wheat regardless of growth temperature.

Ortiz-Lopez and co-workers (25) reported that exposure of chilling sensitive *Zea mays* to low temperatures and light intensities results in a reduction in $\Phi_{app}$ for $CO_2$ exchange without affecting either the photochemical efficiency of PSII or coupling factor activity. In the current study, spring wheat exhibited a capacity to generate and relax $q_N$ similar to that of winter wheat regardless of the growth temperature. Because the build-up of $q_N$ reflects, in part at least, the establishment of the *trans*-thylakoid pH gradient (15), the relaxation of $q_N$ indicates the collapse of that gradient through the synthesis of ATP by coupling factor 1. Therefore, the low temperature-induced depression of $\Phi_{app}$ for $CO_2$ exchange in spring wheat does not appear to be the result of a decreased capacity to establish or to utilize the *trans*-thylakoid pH gradient. The quenching data indicate that the inhibition of $\Phi_{app}$ for $CO_2$ exchange at low growth temperatures may be associated with the photosynthetic carbon reduction reactions of the stroma rather than the thylakoid membrane.

Chilling at high light is known to reduce the activity of key enzymes involved in carbon reduction (30), and this may be a contributing factor to the inhibition of $CO_2$ exchange observed in the spring wheat. However, all nonhardened spring cultivars were equilibrated at 5°C for 10 h (8 h darkness + 2 h at 250 $\mu$mol $m^{-2}$ $s^{-1}$ PPFD) prior to measurement of $\Phi_{app}$ and light-saturated rates of $CO_2$ exchange at 5°C (Table II) without any observed reduction in these parameters relative to the same plants measured at 20°C. Thus, if there is a reduction in the activity of key enzymes during cold-hardening of spring wheat cultivars, it must be dependent on low temperature-high light interactions or it must develop as a function of leaf growth and development at low temperature rather than as a consequence of the brief exposure of fully expanded nonhardened leaves to low temperature.

In conclusion, winter wheat cultivars can be distinguished from spring cultivars by their ability to maintain comparable photosynthetic efficiency and light-saturated rates of $CO_2$ exchange at cold-hardening and nonhardening temperatures. Stomatal conductance and internal $CO_2$ concentration are not affected by growth temperature in any of the six wheat

cultivars tested. The site of low temperature inhibition of $CO_2$ exchange in the spring cultivars is not associated with PSII or the thylakoid membrane but is likely associated with enzymes involved in photosynthetic carbon reduction. This inhibition is partially reversible by exposure of 5°C grown plants to 20°C for at least 10 h. Further work is required to pinpoint the specific site of inhibition.

## LITERATURE CITED


1. **Andrews CJ, Pomeroy MK, de la Roche IA** (1974) The influence of light and diurnal freezing temperature on the cold hardiness of winter wheat. Can J Bot **52:** 2539–2546
2. **Arnon DL** (1949) Copper enzymes in isolated chloroplasts: polyphenoloxidase in *Beta vulgaris*. Plant Physiol **24:** 1–15
3. **Baker NR, Bradbury M, Farage PK, Ireland CR, Long SP** (1989) Measurements of the quantum yield of carbon assimilation and chlorophyll fluorescence for assessment of photosynthetic performance of crops in the field. Philos Trans R Soc Lond B Biol Sci **323:** 295–308
4. **Boese SR, Huner NPA** (1990) Effect of growth temperature and temperature shifts on spinach leaf morphology and photosynthesis. Plant Physiol **94:** 1830–1836
5. **Butler WL, Kitijima M** (1975) Fluorescence quenching in photosystem II of chloroplasts. Biochim Biophys Acta **376:** 116–125
6. **Bjorkman O, Demmig B** (1987) Photon yield of $O_2$ evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. Planta **170:** 489–504
7. **Dexter ST** (1933) Effect of several environmental factors on the hardening of plants. Plant Physiol **8:** 122–139
8. **Fowler DB, Gusta LV** (1977) Influence of fall growth and development on cold tolerance of rye and wheat. Can J Plant Sci **57:** 751–755
9. **Fowler DB, Carles RJ** (1979) Growth, development and cold tolerance of fall acclimated cereal grains. Crop Sci **19:** 915–922
10. **Frank AB, Power JF, Willis WO** (1973) Effect of temperature and plant water stress on photosynthesis, diffusion resistance and leaf water potential in spring wheat. Agron J **65:** 777–780
11. **Friend DJC** (1969) Net assimilation rate of wheat as affected by light intensity and temperature. Can J Bot **47:** 1781–1787
12. **Huner NPA, Migus W, Tollenaar M** (1986) Leaf $CO_2$ exchange rates in winter rye grown at cold-hardening and non-hardening temperatures. Can J Plant Sci **66:** 443–452
13. **Johnson RR, Moss DN** (1976) Effect of water stress on $^{14}CO_2$ fixation and translocation in wheat during grain filling. Crop Sci **16:** 697–701
14. **Krol M, Griffith M, Huner, NPA** (1984) An appropriate physiological control for environmental temperature studies: comparative growth kinetics of winter rye. Can J Bot **62:** 1062–1068
15. **Krause GH, Weis E** (1984) Chlorophyll fluorescence as a tool in plant physiology. II. Interpretation of fluorescence signals. Photosynth Res **5:** 139–157
16. **Lawrence T, Cooper JP, Breese EL** (1973) Cold tolerance and winter hardiness in *Lolium perenne*. II. Influence of light and temperature during growth and hardening. J Agric Sci **80:** 341–348
17. **Levitt J** (1980) Chilling, freezing, and high temperature stresses. *In* Responses of Plants to Environmental Stresses, Ed 2 Vol 1. Academic Press, New York, p 497
18. **Long SP, East TM, Baker NR** (1983) Chilling damage to photosynthesis in young *Zea mays*. J Exp Bot **34:** 177–188
19. **Macdowall FDH** (1974) Growth kinetics of Marquis wheat. VI. Genetic dependence and winter hardening. Can J Bot **52:** 151–157
20. **Morgan JA, Willis WO** (1983) Gas exchange and water relations of "Olaf" spring wheat. Crop Sci **23:** 541–546
21. **Oquist G, Martin B** (1986) Cold climates. *In* NR Baker, SP Long, eds, Photosynthesis in Contrasting Environments, Vol 7. Elsevier, New York, pp 237–293
22. **Oquist G, Greer DH, Ogren E** (1987) Light stress at low temperature. *In* DJ Kyle, CB Osmond, CJ Arntzen, eds, Photoinhibition, Vol 9. Elsevier, New York, pp 67–88
23. **Oquist G, Huner NPA** (1989) Effects of cold acclimation on the susceptibility of photosynthesis to photoinhibition. *In* M Baltscheffsky, ed, Current Research in Photosynthesis, Vol II. Kluwer Academic Publishers, Netherlands, pp 471–474
24. **Oquist G, Malmberg G** (1989) Light and temperature dependent inhibition of photosynthesis in frost-hardened and unhardened seedlings of pine. Photosynth Res **20:** 261–277
25. **Ortiz-Lopez A, Nie GY, Ort DR, Baker NR** (1990) The involvment of the photoinhibition of photosystem II and impaired membrane energisation in the reduced quantum yield of carbon assimilation in chilled maize. Planta **181:** 78–84
26. **Papageorgio G** (1975) Chlorophyll fluorescence: an intrinsic probe of photosynthesis. *In* Govindjee ed, Bioenergetics of Photosynthesis. Academic Press, New York, pp 319–371
27. **Pollock CJ, Lloyd EJ, Thomas H, Stoddart JL** (1984) Changes in photosynthetic capacity during prolonged growth of *Lolium temulentum* at low temperature. Photosynthetica **18:** 478–481
28. **Powles SB, Berry JA, Bjorkman O** (1983) Interaction between light and chilling temperature on the inhibition of photosynthesis in chilling-sensitive plants. Plant Cell Environ **6:** 117–123
29. **Regehr DL, Bazzaz FA** (1976) Low temperature photosynthesis in successional winter annuals. Ecology **57:** 1297–1303
30. **Sassenrath GF, Ort DR, Portis AR Jr** (1987) Effect of chilling on the activity of enzymes of the photosynthetic carbon reduction cycle. *In* J Biggins, ed, Progress in Photosynthesis Research, Vol IV. Martinus Nijhoff, Dordrecht, pp 103–106
31. **Schreiber U, Schwliwa U, Bilger W** (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth Res **10:** 51–62
32. **Sommersalo S, Krause GH** (1989) Photoinhibition at chilling temperature. Fluorescence characteristics of unhardened and cold-acclimated spinach leaves. Planta **177:** 409–416