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Effect of Cold Hardening on the Components of Respiratory Decarboxylation in the Light and in the Dark in Leaves of Winter Rye[1]

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In the dark, all decarboxylation reactions are associated with the oxidase reactions of mitochondrial electron transport. In the light, photorespiration is also active in photosynthetic cells. In winter rye (*Secale cereale* L.), cold hardening resulted in a 2-fold increase in the rate of dark respiratory CO_2 release from leaves compared with nonhardened (NH) controls. However, in the light, NH and cold-hardened (CH) leaves had comparable rates of oxidase decarboxylation and total intracellular decarboxylation. Furthermore, whereas CH leaves showed similar rates of total oxidase decarboxylation in the dark and light, NH leaves showed a 2-fold increase in total oxidase activity in the light compared with the dark. Light suppressed oxidase decarboxylation of end products of photosynthesis 2-fold in NH leaves and 3-fold in CH leaves in air. However, in high-CO_2, light did not suppress the oxidase decarboxylation of end products. Thus, the decrease in oxidase decarboxylation of end products observed in the light and in air reflected glycolate-cycle-related inhibition of tricarboxylic acid cycle activity. We also showed that the glycolate cycle was involved in the decarboxylation of the end products of photosynthesis in both NH and CH leaves, suggesting a flow of fixed carbon out of the starch pool in the light.

Plants from arctic and alpine regions typically exhibit higher rates of leaf dark respiration (Mooney, 1963; Klikoff, 1968; Billings et al., 1971) and photorespiration (Mächler and Nösberger, 1978) at a given measurement temperature than their temperate or lowland relatives. Similarly, leaf dark respiration is higher at a given measurement temperature in cold-tolerant compared with cold-sensitive herbaceous plants following growth at low temperatures (Steffen and Palta, 1989; Hurry et al., 1992). This increase in the capacity of dark respiration in leaves appears to be associated with increased amounts of alternative oxidase protein (Vanlerberghe and McIntosh, 1992) and increases in oxidative enzymes (Krasnuk et al., 1976; Stitt et al., 1990).

These increases in leaf respiratory activity are generally attributed to the accumulation of soluble carbohydrates in the cytosol (Wager, 1941; Caspar et al., 1985; Stitt et al., 1990). In the short term (hours to days), soluble carbohydrates may accumulate in leaves shifted to low temperatures because of reduced export capacity from source cells and/or reduced demand for carbon skeletons by synthetic processes within these cells. Such short-term accumulation often leads to the down-regulation of photosynthetic enzymes (Krapp et al., 1991, 1993). However, over the long term (weeks to months), the accumulation of soluble carbohydrates in leaves of cold-tolerant over-wintering annuals is associated with increases in the activity of photosynthetic enzymes (Guy et al., 1992; Holaday et al., 1992; Hurry et al., 1994, 1995a). Therefore, we have suggested that the increase in activity of these enzymes represents an acclimation to the low growth temperature (Hurry et al., 1994, 1995a), facilitating the accumulation of sugars with possible cryoprotective functions (Anchordoguy et al., 1987) and supporting basal metabolism during over-wintering.

It remains unclear, however, whether the concomitant increase in dark respiration following growth at low temperature occurs because of increased synthetic reactions (i.e. an increased demand for respiratory energy) or whether the high substrate pools (soluble carbohydrates) are driving the higher rates of dark respiration (Azcón-Bieto and Osmond, 1983). Furthermore, it is unclear whether the increase in dark respiration reported upon cold hardening also represents an increase in oxidase activity in the light, as the increase in oxidative enzymes in sink-limited plants would suggest (Stitt et al., 1990).

To understand the acclimation process further, we have used radiogasometric analysis to discover whether the increase in dark respiration observed upon cold hardening of winter rye (*Secale cereale* L.) reflects an increase in respiration in the light, and whether cold hardening has affected the substrates for the decarboxylation reactions in the light. We have compared both NH and CH leaves of winter rye measured at a warm temperature rather than at their respective growth temperatures. The same measurement temperature was used so that we could establish whether changes to respiratory metabolism had occurred in the light and so we could separate adaptive changes from the direct effects of low temperature. In photosynthetic cells,

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Abbreviations: CH, cold-hardened; NH, nonhardened; P_g, gross photosynthesis; R_i, intrafoliar reassimilation; RuBP, ribulose-1,5-bisphosphate; TCA, tricarboxylic acid.

two different mechanisms account for most of the decarboxylation reactions in the light. First, photorespiratory metabolism related to the oxygenation reaction of Rubisco leads to mitochondrial decarboxylation of Gly. Second, the decarboxylation reactions connected to the TCA cycle are associated with oxidase reactions of the mitochondrial electron transport chain (Cyt oxidase and alternative oxidase). For the purposes of this study, we have defined four different types of respiratory metabolism in the light: (a) oxygenase decarboxylation of primary products of photosynthesis; (b) oxygenase decarboxylation of end products of photosynthesis; (c) oxidase decarboxylation of primary products of photosynthesis; and (d) oxidase decarboxylation of end products of photosynthesis. Photorespiration typically includes both types a and b. Type c represents the direct flow of triose phosphates into the TCA cycle in the light. Type d represents the catabolism of end products such as starch or Suc via the TCA cycle and is the only form of respiration that occurs in the dark.

MATERIALS AND METHODS

Plant Material

Seedlings of winter rye (*Secale cereale* L. cv Musketeer, obtained from Mrs. E. Wilson [Crane Valley, Saskatchewan, Canada]) were grown five seedlings/13-cm pot in coarse vermiculite with water and nutrients supplied as required. Seeds were germinated under controlled-environment conditions: 250 μmol m^{-2} s^{-1} PPFD; day/night temperature regime 24/16°C; photoperiod 17 h. After 7 d, when the primary leaf had expanded, seedlings were CH under similar light conditions but with a day/night temperature regime of 5/5°C. All measurements were made on third or fourth leaves of 21- to 25-d-old NH and 70- to 75-d-old CH plants. At these ages, the leaves were fully expanded and the seedlings were considered to be at similar physiological stages of development as judged by leaf number and leaf dry weight (data not shown).

Measurement of CO_2 Exchange

Rates of CO_2 and $^{14}CO_2$ fluxes were measured at 24°C with a quick-operating, multi-channel, gas-exchange system (Special Design Office, Estonian Academy of Sciences, Tallinn, Estonia). The leaf chamber contained six fully expanded third or fourth leaves (total leaf area 14 cm^2) attached to six different plants. The leaves were illuminated using a xenon lamp (model DKsEI 1000-5, Riga Lamps Factory, Riga, Latvia) at an irradiance that saturated photosynthesis in both NH and CH leaves (1000 μmol m^{-2} s^{-1} PPFD). Gas mixtures containing $^{14}CO_2$ (specific activity 260 Bq $nmol^{-1}$ CO_2), CO_2, and O_2 were used as indicated. The components of CO_2 exchange, including net photosynthesis, dark respiration, and CO_2 efflux into CO_2-free air, were measured by IR gas analysis (model Infralyt 4, Junkalor, DDR, Dessau, Germany) using the open system attached to the chamber. Exposure of leaves to $^{14}CO_2$ and measurement of CO_2 and $^{14}CO_2$ efflux were carried out in a closed gas system (Pärnik and Keerberg, 1995).

Determination of the Rate of Decarboxylation of Primary Products of Photosynthesis

Primary products of photosynthesis, defined as compounds with a half-time for turnover measured in minutes, are saturated with ^{14}C during exposure to $^{14}CO_2$ of less than 10 min (Pärnik et al., 1972). These compounds include intermediates of the reductive pentose phosphate cycle, phosphorylated intermediates of starch and Suc synthesis, and possible substrates of decarboxylation reactions, such as Gly and pyruvate. To find the rate of $^{14}CO_2$ evolution from the primary products, leaves were preilluminated in either 300 or 2300 μL L^{-1} CO_2 until a constant rate of photosynthesis was established. Leaves were then exposed to either 300 or 2300 μL L^{-1} $^{14}CO_2$ for 10 min to saturate the primary products with ^{14}C, and the leaf chamber was cleaned for 4 s with either 300 or 2300 μL L^{-1} CO_2. Efflux of $^{14}CO_2$ in the light was recorded in either 300 or 2300 μL L^{-1} CO_2 to find the steady-state rate of $^{14}CO_2$ evolution from primary products. Evolution of $^{14}CO_2$ in 30 mL L^{-1} CO_2 was measured to find the rates of intracellular decarboxylation and of R_r (Pärnik and Keerberg, 1995).

Determination of the Rate of Decarboxylation of End Products of Photosynthesis

Decarboxylation of end products was determined after measurement of the decarboxylation of primary products using the same set of leaves. We define end products of photosynthesis as compounds with a half-time for turnover measured in hours; these include compounds such as starch, Suc, and malate. Long-term exposure (3 to 4 h) of leaves to $^{14}CO_2$ is required to achieve any measurable level of the specific radioactivity of carbon in these compounds. Leaves were exposed to 300 μL L^{-1} $^{14}CO_2$ for 4 h and were then exposed to 300 μL L^{-1} CO_2 for 30 min to eliminate the ^{14}C from the primary product pools. The efflux of $^{14}CO_2$ from the leaves at either 300 or 2300 μL L^{-1} CO_2 in the light was then measured to find the steady-state rate of $^{14}CO_2$ evolution from end products. Efflux of $^{14}CO_2$ and CO_2 was also measured in CO_2-free air to find the specific radioactivity of CO_2 evolved from the leaves. From the kinetics of $^{14}CO_2$ and CO_2 evolution, the rates of the different components of intracellular decarboxylation were calculated as described in detail previously (Pärnik and Keerberg, 1995).

RESULTS

Photosynthesis and Carboxylation/Oxygenation Characteristics of Rubisco

The rate of gross photosynthetic CO_2 fixation (P_t = net photosynthesis + total respiration in the light) at 24°C and in 300 μL L^{-1} CO_2 was similar for NH and CH leaves at either 210 or 15 mL L^{-1} O_2. However, at high CO_2, P_t was 40% higher in CH rye, whatever the partial pressure of oxygen. Similarly, CH rye leaves showed elevated rates of carboxylation and regeneration of RuBP under high CO_2 (Table I). These data agree with previous findings that the elevated rates of photosynthesis and RuBP regeneration reported for CH cereals only become evident either at high

Table I. *The rate of P_t (net photosynthesis plus total respiration in the light), RuBP carboxylation (P_c = P_t plus R_r), RuBP oxygenation (P_o), RuBP regeneration (P_r), internal CO_2 concentration (C_w), and Rubisco specificity in leaves of NH and CH winter rye*

CO_2 exchange was measured using a cuvette containing six attached leaves on six separate plants (combined leaf area 14 cm^2). These leaves were illuminated at 1000 μmol m^{-2} s^{-1} PPFD at 24°C and 300 or 2300 μL L^{-1} CO_2 under either 210 or 15 mL^{-1} L O_2 until a stable rate of photosynthesis was established. Data represent the rate (±SE) calculated from the regression function used to fit the $^{14}CO_2$ evolution curves.

Treatment	P_t	$P_c = P_t + R_r$	P_o	$P_r = P_c + P_o$	P_c/P_o	C_w	Rubisco Specificity[a]
	μmol CO_2 m^{-2} s^{-1}		*μmol RuBP m^{-2} s^{-1}*			*μmol CO_2 L^{-1}*	
300 μL L^{-1} CO_2							
NH-210 mL L^{-1} O_2	10.85 ± 0.22	12.72 ± 0.23	5.28 ± 0.28	18.00 ± 0.36	2.41 ± 0.17	7.94 ± 0.14	72.8 ± 6.5
CH-210 mL L^{-1} O_2	10.59 ± 0.23	13.06 ± 0.24	6.72 ± 0.28	19.78 ± 0.37	1.94 ± 0.12	6.55 ± 0.07	71.2 ± 5.0
NH-15 mL L^{-1} O_2	13.87 ± 0.66	14.55 ± 0.66	0.36 ± 0.06	14.91 ± 0.66	–[b]	–	–
CH-15 mL L^{-1} O_2	13.05 ± 0.62	13.78 ± 0.63	0.50 ± 0.06	14.28 ± 0.63	–	–	–
2300 μL L^{-1} CO_2							
NH-210 mL L^{-1} O_2	17.51 ± 0.60	18.33 ± 0.6	1.10 ± 0.22	19.43 ± 0.64	16.66 ± 3.88	61.1 ± 2.6	65.4 ± 18.0
CH-210 mL L^{-1} O_2	24.06 ± 0.71	25.93 ± 0.71	2.12 ± 0.82	28.05 ± 1.08	12.23 ± 5.07	41.5 ± 1.7	70.7 ± 32.2
NH-15 mL L^{-1} O_2	17.43 ± 0.67	17.99 ± 0.67	0.08 ± 0.04	18.07 ± 0.67	–	–	–
CH-15 mL L^{-1} O_2	25.39 ± 1.40	26.56 ± 1.41	0.14 ± 0.14	26.70 ± 1.42	–	–	–

[a] Rubisco specificity = $P_c[O_2]_w/P_o[CO_2]_w$. [b] –, Not done.

CO_2 concentrations (Huner et al., 1986) or at low temperature (Hurry et al., 1994).

The calculated specificity factor of Rubisco was the same in CH and NH rye at both concentrations of CO_2 tested (Table I). The small increase in oxygenase activity in CH rye at 300 μL L^{-1} CO_2 coincided with lower internal CO_2 concentrations in these leaves, a result of slight stomatal closure in CH rye at the higher temperature (Table I). Thus, cold hardening did not change the O_2/CO_2 discrimination properties of Rubisco of winter rye.

Oxidase Decarboxylation of End Products in the Light and in the Dark

Of the four respiration components, oxidase decarboxylation of end products of photosynthesis is the only one that is operative in the dark. In the dark at 24°C, 300 μL L^{-1} CO_2, and 210 mL L^{-1} O_2, CH leaves showed a 2-fold increase in oxidase decarboxylation of end products compared with NH leaves (Fig. 1). Under these same gas concentrations, light suppressed oxidase decarboxylation of end products 2-fold in NH and 3-fold in CH rye leaves (Fig. 1). Thus, although CH leaves showed a 2-fold-higher dark rate of oxidase decarboxylation of end products, the rate in the light was comparable to the rate for NH leaves. Light did not completely suppress the oxidase decarboxylation of end products at 300 μL L^{-1} CO_2, and it represented 6 and 8% of total intracellular decarboxylation in the light for NH and CH leaves, respectively (Figs. 1 and 2). It is interesting that under high CO_2, no light inhibition of oxidase decarboxylation of end products occurred. Additionally, the concentration of CO_2 did not affect the rate of oxidase decarboxylation of end products in either NH or CH rye in the dark (Fig. 1).

Total Respiratory Decarboxylation in the Light

In contrast to dark decarboxylation of end products of photosynthesis, total intracellular decarboxylation in the light in 300 μL L^{-1} CO_2 and 210 mL L^{-1} O_2 was similar in both NH and CH leaves (Fig. 2A). Not surprisingly, under these gas concentrations oxygenase decarboxylation was the prevailing mechanism. Nevertheless, the oxidase component did contribute 30 and 25% of the intracellular decarboxylation in the light in NH and CH rye, respectively (Fig. 2A). It is interesting that when the rates of total oxidase activity in the light (Fig. 2A) were compared with the rates in the dark (Fig. 1), we found that CH leaves had similar rates of total oxidase activity in the light and the dark, but NH leaves showed a 2-fold increase in the light compared with the dark.

When oxygenase decarboxylation was suppressed at 2300 μL L^{-1} CO_2, no change in oxidase decarboxylation was found in NH leaves, but oxidase decarboxylation increased 1.5-fold in CH leaves (Fig. 2B).

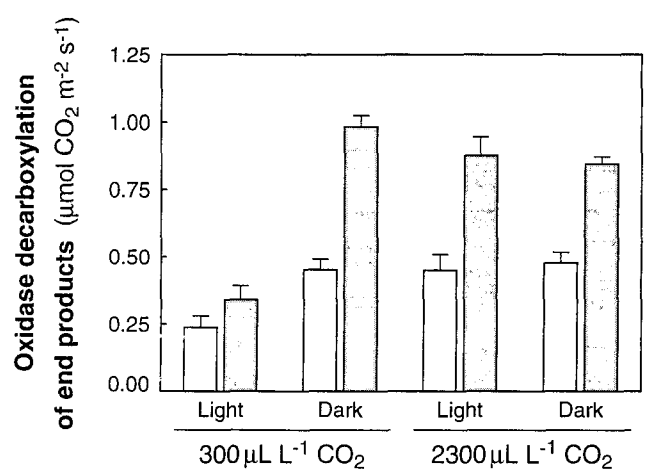

Figure 1. The rate of oxidase decarboxylation of end products of photosynthesis in the light and in the dark for NH (open bars) and CH (shaded bars) leaves of winter rye. Six attached leaves from six separate plants (combined leaf area 14 cm^2) were illuminated at 1000 μmol m^{-2} s^{-1} PPFD at 24°C in 210 mL L^{-1} O_2 and 300 or 2300 μL L^{-1} CO_2. Respiration in the dark was measured 10 min after switching off the light. Data represent the rate (±SE) calculated from the regression function used to fit the $^{14}CO_2$ evolution curves.

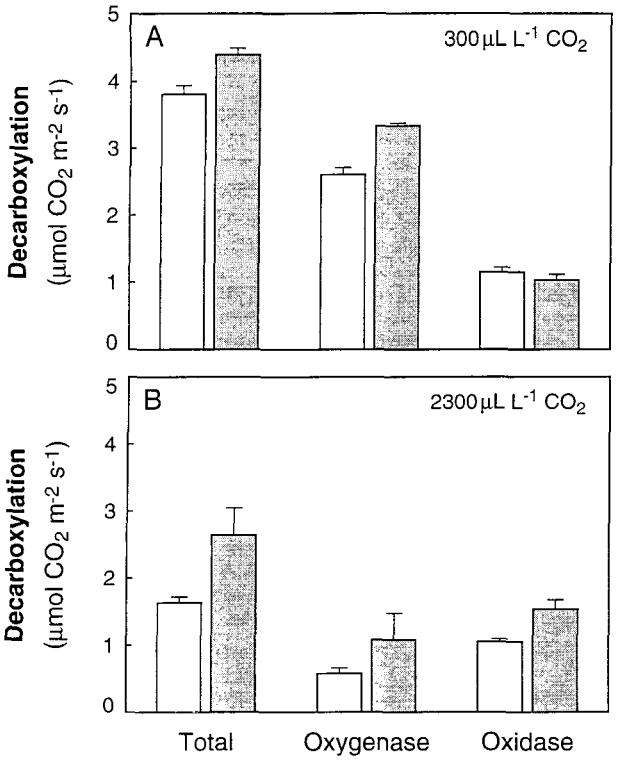

Figure 2. Total rates of intracellular decarboxylation in the light in leaves of NH (open bars) and CH (shaded bars) winter rye and the relative contributions of oxygenase and oxidase activity. Six attached leaves from six separate plants (combined leaf area 14 cm^2) were illuminated at 1000 μmol m^{-2} s^{-1} PPFD at 24°C in 210 mL L^{-1} O_2 and 300 (A) or 2300 (B) μL L^{-1} CO_2. Data represent the rate (±SE) calculated from the regression function used to fit the $^{14}CO_2$ evolution curves.

Primary and End-Product Usage by Oxygenase and Oxidase Decarboxylation in the Light

In the light at 210 mL L^{-1} O_2 and 300 μL L^{-1} CO_2, primary products were the main substrates for oxygenase decarboxylation, i.e. the oxygenation of RuBP regenerated from newly formed triose phosphates. However, 18 and 27% of the substrate for oxygenase decarboxylation came from the end products of photosynthesis in NH and CH leaves, respectively (Fig. 3A). These data support the hypothesis that the glycolate cycle is involved in the decarboxylation of compounds derived from the degradation of end products of photosynthesis (Pärnik and Keerberg, 1995).

In the light at 210 mL L^{-1} O_2 and 300 μL L^{-1} CO_2, primary products were also the main substrates for oxidase decarboxylation (Fig. 3A). These data suggest a considerable flow of triose phosphates through glycolysis and the TCA cycle in the light that was comparable in NH and CH leaves (0.94 and 0.72 μmol CO_2 m^{-2} s^{-1} in NH and CH leaves, respectively). The contribution of end products to oxidase decarboxylation in the light was also comparable in NH and CH leaves (0.24 and 0.34 μmol CO_2 m^{-2} s^{-1} in NH and CH leaves, respectively) (Figs. 1 and 3A). Thus, in the light, total oxidase activity increased 2-fold in NH leaves due to the strong consumption of primary products. However, in CH leaves oxidase activity remained constant in the light and dark, but in the light end-product usage was strongly suppressed and was replaced by primary-product usage.

At high CO_2, rates of oxidase decarboxylation of end products increased from 0.24 to 0.45 μmol CO_2 m^{-2} s^{-1} in NH leaves and from 0.34 to 0.88 μmol CO_2 m^{-2} s^{-1} in CH leaves (Fig. 3, A and B), reflecting the relaxation of "light suppression" of oxidase decarboxylation of end products in these leaves (Fig. 1). However, the release of suppression in NH leaves did not lead to an increase in total oxidase activity in the light, but instead led to a concomitant drop in primary-product usage, from 0.94 to 0.60 μmol CO_2 m^{-2} s^{-1}. This was not so for CH leaves, in which the release of suppression was not followed by a drop in oxidase decarboxylation of primary products (0.72 to 0.69 μmol CO_2 m^{-2} s^{-1}), and as a result, total oxidase activity was elevated 1.5-fold under these conditions (Fig. 2B).

These data also show that whereas high concentrations of CO_2 almost completely suppressed the oxygenase decarboxylation of end products, total suppression of primary product usage requires both high CO_2 and low O_2 (Fig. 3; Table I).

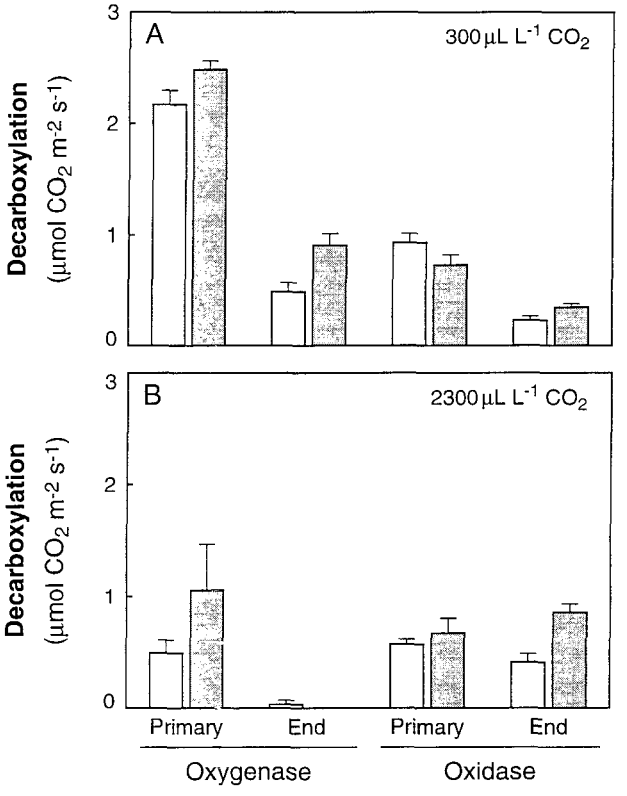

Figure 3. Relative contribution of pools of primary and end products of photosynthesis to oxygenase and oxidase decarboxylation in the light in leaves of NH (open bars) and CH (shaded bars) winter rye. Six attached leaves from six separate plants (combined leaf area 14 cm^2) were illuminated at 1000 μmol m^{-2} s^{-1} PPFD at 24°C in 210 mL L^{-1} O_2 and 300 (A) or 2300 (B) μL L^{-1} CO_2. Data represent the rate (±SE) calculated from the regression function used to fit the $^{14}CO_2$ evolution curves.

DISCUSSION

Long-term cold hardening of winter rye at 5°C resulted in a 2-fold increase in oxidase decarboxylation of photosynthetic end products in the dark when compared with NH leaves at the same temperature and gas concentration. Although not directly comparable, this finding is similar to the increases in dark respiration reported previously for cold-acclimated annuals (Steffen and Palta, 1989; Hurry et al., 1992) and alpine ecotypes (Mooney, 1963; Klikoff, 1968; Billings et al., 1971) measured in the dark as increased O_2 consumption. However, our results also show that the increase in oxidase decarboxylation of the end products in the dark was not associated with an increase in total decarboxylation in the light. In the light at 210 mL L^{-1} oxygen and 300 μL L^{-1} CO_2, oxygenase decarboxylation accounted for 70 to 75% of the total decarboxylation reactions in both NH and CH leaves, with the remainder being made up by oxidase decarboxylation. Thus, despite the increase in oxidase decarboxylation of end products measured in CH leaves in the dark, cold hardening did not enhance total rates of decarboxylation in the light, nor did it change the relative contribution of the oxidase component in the light.

Earlier studies of altitudinal ecotypes of *Trifolium repens* have suggested that plants from alpine habitats and plants from either alpine or lowland habitats grown at low temperatures show increased rates of photorespiration. This was estimated either as increased ^{14}C activity in Gly and Ser (Mächler et al., 1977) or as increased sensitivity of photosynthesis to oxygen (Mächler and Nösberger, 1978). In the current experiments with winter rye, some increased oxygenase activity was seen in CH leaves (Table I; Fig. 2A). However, this was also associated with a slight decrease in the internal partial pressure in CO_2 (Table I). This suggested that the increase in oxygenase activity in CH winter rye was a consequence of increased stomatal resistance after the CH leaves were moved to the warmer measurement temperature, not of increased photorespiration. The change in oxygen sensitivity of photosynthesis shown for alpine ecotypes or low-temperature-grown plants is a better indicator of the phosphate status of the plant (Leegood and Furbank, 1986) than of photorespiratory activity. In accordance with these earlier findings (Mächler and Nösberger, 1978), low growth temperatures have been shown to increase the pools of phosphorylated intermediates (Hurry et al., 1994, 1995a). Further, we found no change in the O_2/CO_2 specificity of Rubisco following cold hardening of winter rye (Table I). Thus, we conclude that cold hardening did not increase photorespiration in winter rye.

The oxygenase decarboxylation reaction used mainly primary products, as might be expected given that this reaction uses RuBP as a substrate. However, the supply of some end products to oxygenase decarboxylation shows a back-flow of fixed carbon from the end-product pools in both NH and CH leaves. We suggest that this is probably a consequence of mobilization of part of the starch pool in the light. It is possible that some end-product substrates for the oxygenase reaction may have been from other end-product pools such as Suc. However, such pools are partitioned outside the chloroplast and their decarboxylation via photorespiration would require that these products were imported back into the chloroplast in the light. It seems much more likely that these end products originated in the starch pool. Net starch mobilization in the light has been shown to occur in spinach (Stitt and Heldt, 1981; Beck and Ziegler, 1989). Furthermore, the increased decarboxylation of end products by CH leaves via oxygenase activity (Fig. 3A) may reflect an increase in starch mobilization in CH leaves. However, whether this reflects a true increase in flux out of the starch pool or simply an increase due to a larger substrate pool in CH leaves is unclear from these data.

Oxidase decarboxylation in the light is required for the provision of carbon skeletons used in synthetic reactions, such as amino acid synthesis and nitrate assimilation (Graham, 1980). Oxidase activity may also provide substrates for oxidative phosphorylation to produce part of the ATP necessary for cytosolic reactions such as Suc synthesis (Hanson, 1992; Krömer et al., 1993). However, ATP production can be associated with Gly oxidation, which is linked to the glycolate cycle (Gardeström and Lernmark, 1995; Krömer, 1995). Consequently, oxidative phosphorylation could, under photorespiratory conditions, be partly disconnected from oxidase decarboxylation (Gardeström and Lernmark, 1995). The rate of oxidase decarboxylation of primary products in the light shows that 7 and 9% of the triose phosphates produced by photosynthesis are diverted to the TCA cycle in CH and NH leaves, respectively (Table I; Fig. 3). The rates of oxidase decarboxylation of end products were also comparable in the light in CH and NH leaves (Figs. 1 and 3A). These data suggest that there is probably not a greater demand for carbon skeletons in the light in winter rye leaves following cold hardening.

The increase in oxidase decarboxylation of end products in CH leaves in the dark or in the light at elevated CO_2 suggests that cold hardening has increased the capacity for oxidase activity in these leaves. It is possible that this increase in capacity reflects increases in total enzyme activity, perhaps analogous to that previously described for sink-limited plants (Stitt et al., 1990). However, it seems more likely that the high dark rates in CH leaves reflect acclimation during cold hardening, increasing enzymatic levels to compensate for lower activities at the low growth temperature. This "increase" in capacity may be observed only at the elevated measuring temperature in the dark or in the light when some regulatory mechanism is suppressed by elevated CO_2. Whether this is so, and what the consequences of that acclimation might be to the rates of and substrates for decarboxylation in the light at low temperatures, is currently under investigation.

The absence of light suppression of oxidase decarboxylation of end products at high CO_2 concentrations, where oxygenase activity is suppressed, supports the hypothesis for glycolate cycle suppression of TCA-cycle activity. A likely target enzyme for regulation is the pyruvate dehydrogenase complex (Budde et al., 1988; Schuller and Randall, 1989; Krömer, 1995). Steady-state pyruvate dehydrogenase complex activity is thought to decrease in the light due to increased activity of a protein kinase that inactivates pyruvate dehydrogenase. The activity of the protein kinase

is stimulated by NH_4^+ released during Gly oxidation (Schuller and Randall, 1989; Budde and Randall, 1990; Gemel and Randall, 1992) and by ATP (Budde et al., 1988), which can increase in photorespiratory conditions (Gardeström and Wigge, 1988). Cold hardening has also been shown to alter the adenylate status of the cell in winter rye (Hurry et al., 1995b). However, at this point we do not know whether the greater suppression of oxidase decarboxylation of end products in the light in CH leaves is due to altered pyruvate dehydrogenase complex activity in response to cold hardening, or whether it is due to the slight stomatal-induced increase in oxygenase activity or some other mechanism. Furthermore, it is notable that although we show evidence of light suppression of oxidase decarboxylation of end products, the decarboxylation of primary products by oxidase reactions leads to no net inhibition of total oxidase decarboxylation in the light in either NH or CH leaves.

The activities of active oxygen-scavenging enzymes have also been shown to increase during cold acclimation in a wide range of plant species (Esterbauer and Grill, 1978; Guy and Carter, 1984; Jahnke et al., 1991; Kuroda et al., 1991). Following chilling, regulation of the activity of the Cyt and alternative respiratory pathways appears to involve an adjustment to the accumulation of H_2O_2 and an increase in the levels of active oxygen-scavenging enzymes (Purvis and Shewfelt, 1993; Prasad et al., 1994a, 1994b; Wagner and Krab, 1995). These data suggest a role for coordinated increases in the capacity for removal of reduced species of molecular oxygen in the acclimation response of respiratory metabolism and possibly photosynthetic and cytosolic metabolism. Similarly, cold acclimation also leads to substantial changes in the lipid composition of plant membranes (Steponkus, 1984), which have been shown to reduce the permeability of plant membranes to solutes (Hincha and Schmitt, 1988). What effect this might have on interorganelle transport, e.g. of intermediates of the glycolate cycle, is unknown. Furthermore, we also have no information on what effect cold hardening has on the function or concentration of the various molecular transporters identified as important in interorganelle transport (Flügge and Heldt, 1991; Martinoia and Rentsch, 1994). These factors will need to be considered if an integrated picture of metabolic acclimation to low temperature is to be developed.

These experiments have shown that although cold hardening led to a 2-fold increase in oxidase decarboxylation of end products of photosynthesis in the dark, this was not associated with increases either in total decarboxylation or in oxidase decarboxylation in the light. Whether the increase in dark-respiratory CO_2 release was due to increased demands from synthetic or maintenance reactions during the dark period, or whether it was a consequence of increased amounts of enzymes or of larger substrate pools, is unclear. However, the increase in respiratory activity in the dark in CH leaves was clearly not carried over into the light period. Finally, the data showed that the glycolate cycle (oxygenase activity) was involved in the decarboxylation of compounds derived from the end products of photosynthesis, and that this may have been more pronounced in CH leaves.

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