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The temperature dependence of the stimulation of photosynthesis by elevated carbon dioxide in wheat and barley

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Abstract

The temperature dependencies of the solubility of carbon dioxide and oxygen in water and the temperature dependency of the kinetic characteristics of the ribulose-1,5 bisphosphate carboxylase/oxygenase (Rubisco) enzyme result in the short-term stimulation of photosynthesis with a doubling of carbon dioxide from 350 to 700 μmol mol^{-1} usually decreasing from about 90% at 30 °C to about 25% at 10 °C at high photon flux. In field-grown wheat and barley, the expected values at 30 °C were observed, but also values as high as 60% at 10 °C. The much larger than expected stimulation at cool temperatures in these species also occurred in plants grown at 15 °C, but not at 23 °C in controlled environment chambers. Gas exchange analysis indicated that an unusually high diffusive limitation was not an explanation for the large response. Assessment of the apparent *in vivo* specificity of Rubisco by determining the carbon dioxide concentration at which carboxylation equalled carbon dioxide release from oxygenation, indicated that growth at low temperatures altered the apparent enzyme specificity in these species compared to these species grown at the warmer temperature. Inserting the observed specificities into a biochemical model of photosynthesis indicated that altered Rubisco specificity was consistent with the observed rates of assimilation. Whether altered apparent Rubisco specificity is caused by altered stoichiometry of photorespiration or an actual change in enzyme specificity, the results indicate that the temperature dependence of the stimulation of photosynthesis by elevated carbon dioxide may vary greatly with species and with prior exposure to low temperatures.

Key words: Barley, carbon dioxide, photosynthesis, temperature, wheat.

Introduction

In predicting the responses of photosynthesis of C_3 plants to the increasing concentration of carbon dioxide in the atmosphere, it has been recognized that the stimulation of photosynthesis by increased carbon dioxide usually increases strongly with increasing temperature. This temperature dependence has a firm theoretical basis (Long, 1991; Kirschbaum, 1994) related to the temperature dependencies of the aqueous solubilities of oxygen and carbon dioxide and of kinetic characteristics of the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) enzyme. Some of the kinetic characteristics of Rubisco are conveniently summarized as the 'specificity factor' (Jordan and Ogren, 1984). While much work has been done comparing the specificity factor of Rubisco among species (Delgado *et al.*, 1995), the temperature dependence of the specificity factor has been determined for only a few species (cf. Brooks and Farquhar, 1985). Nevertheless, much experimental data on the temperature dependence of the stimulation of photosynthesis by elevated carbon dioxide (Sage and Sharkey, 1987; Sage *et al.*, 1995) agrees with the predictions from a biochemical model of C_3 photosynthesis based primarily on the kinetic characteristics of Rubisco from spinach.

The strong temperature dependence of the stimulation of C_3 photosynthesis by elevated carbon dioxide has potentially important implications for the response of global vegetation to the increasing concentration of carbon dioxide in the atmosphere. Only a minor stimulation of photosynthesis in cool climates and a much larger

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stimulation in warm climates would be expected. However, there are some reports indicating much larger than expected photosynthetic stimulation by elevated carbon dioxide at cool temperatures (Bowler and Press, 1996; Greer *et al*., 1995; Tesky, 1997). It is reported here that wheat and barley can have an unexpectedly large photosynthetic stimulation by elevated carbon dioxide at low temperatures, depending on the growth temperature. The apparent temperature dependence of Rubisco specificity determined *in vivo* was examined, and the possibility of a high diffusive limitation as an explanation for this large photosynthetic stimulation by elevated carbon dioxide at low temperatures was evaluated.

Materials and methods

Winter wheat (*Triticum aestivum* L.) cv. Coker, and winter barley (*Hordeum vulgare* L.) cv. Wyson were grown in field plots and in controlled environment chambers. In the field plots, seeds were sown in early October of 1994 and 1995 in Beltsville, Maryland, and measurements were made the following springs. Planting densities and fertilization followed normal agronomic practice in the region and weeds were removed by hand. In the controlled environment chambers, plants were grown in 15 cm diameter pots filled with vermiculite and flushed daily with a complete nutrient solution. Plants were grown at both 15 ° C and 23 °C constant day/night temperatures. Carbon dioxide was injected when the concentration fell below 350 μmol mol^{-1}, as measured by an absolute infrared analyser which monitored the chamber air continuously. Air scrubbed of carbon dioxide was added as needed to maintain the concentration below 380 μmol mol^{-1} at night. There were 14 h d^{-1} of light from metal halide and high pressure sodium lamps at a photosynthetic photon flux density (*PPFD*) of 1.0 μmol m^{-2} s^{-1}. For plants grown in the chamber at the warmer temperature, six plants per species were removed from the chamber and exposed to a single 24 h period with maximum/minimum temperatures of 15/8 °C by placing them outdoors early in the morning of a clear day. The plants were returned to the chamber the following morning, and the response of gas exchange to increased carbon dioxide (see later) was measured several hours after being returned to the chamber.

For plants grown in the field, leaf gas exchange measurements were made using a CIRAS–1 portable photosynthesis system (PP Systems, Haverhill MA) with automatic carbon dioxide control. Measurements were made in full midday sunlight ($PPFD > 1.5$ μmol m^{-2} s^{-1}) and at the ambient conditions of air temperature and water vapour pressure. The leaf cuvette has a window with an infrared filter, and ventillated internal and external heat exchange surfaces. Leaf temperatures were less than 3 °C above that of the ouside air. Leaves were first exposed in the cuvette to air at 350 μmol mol^{-1}, and steady-state rates of photosynthesis and leaf conductance to water vapour were determined. The carbon dioxide concentration was then increased to 700 μmol mol^{-1}, and steady-state rates of gas exchange again determined. It usually took no more than 5 min for gas exchange rates to stabilize after increasing the carbon dioxide concentration. Whether or not stomatal conductance changed with the change in carbon dioxide concentration depended on the leaf-to-air water vapour pressure difference (Bunce, 1998), but in either case, stomatal conductance was stable when gas exchange rates were recorded. Such gas exchange measurements were made on six fully illuminated, mature upper canopy leaves per species every 1–2 weeks from the end of March to the end of May in both years.

For plants grown in the controlled environment chamber, similar measurements of responses of photosynthesis to change in external carbon dioxide concentration from 350 to 700 μmol mol^{-1} were made with the CIRAS-1 system placed inside a growth chamber, with leaves exposed to the growth chamber conditions of light and humidity and leaf temperatures of either 17 or 25 °C. More complete leaf gas exchange measurements on plants grown in the chamber were made with a laboratory-based open gas exchange system with control of light, temperature, humidity and carbon dioxide concentration. Carbon dioxide and water vapour exchange rates were determined using a Li-Cor 6262 infrared CO_2/H_2O analyser (Li-Cor Inc., Lincoln NB) in differential mode. Absolute concentrations of CO_2 and H_2O in the reference air stream were measured with an absolute infrared CO_2 analyser, and an optical condensation dew point hygrometer, respectively. The response of photosynthesis to carbon dioxide concentrations from about 30 to 1000 μmol mol^{-1} was determined at leaf temperatures of 17 °C and 25 °C at both 1.0 and 0.15 μmol m^{-2} s^{-1} *PPFD*s. At each temperature, the intersection of the initial parts of the carbon dioxide response curves measured at the two *PPFD*s was used to determine the CO_2 compensation point in the absence of dark respiration, Γ^*, which is the carbon dioxide concentration at which CO_2 fixation by carboxylation equals CO_2 release by oxygenation (Brooks and Farquhar, 1985). Γ^* is inversely proportional to the Rubisco specificity factor (i.e. $\Gamma^* = 0.5\ [O_2]/S$, where S is the 'specificity factor', Brooks and Farquhar, 1985). These meaurements were routinely made on three leaves per treatment, because more extensive measurements indicated that leaf-to-leaf variation was small (see later). The carbon dioxide response measurements at the higher *PPFD* were also used to determine the stimulation in photosynthesis from 350 to 700 μmol mol^{-1} external concentrations at 17 °C and 25 °C, for comparison with measurements made with the CIRAS-1 system on the same leaves.

A biochemical model of C_3 photosynthesis was used to aid in the interpretation of the experimental results. The model used is based on that of Farquhar *et al*. (1980). The temperature dependency functions for the solubilities of oxygen and carbon dioxide and the kinetic characteristics of Rubisco used by Long (1991) were incorporated, with the exception that J_{max}, the light-saturated potential rate of electron transport, and V_{cmax}, the maximum ribulose bisphosphate saturated rate of carboxylation, were varied as input parameters.

The model was used to calculate predicted values of photosynthesis at 700 μmol mol^{-1} external carbon dioxide concentration from gas exchange characteristics measured at 350 μmol mol^{-1} and the measured substomatal carbon dioxide concentrations at 350 and 700 μmol mol^{-1}, for plants grown in the controlled environment chamber. This was done by using a high, non-limiting value of J_{max}, and determining what value of V_{cmax} fit the observed combination of assimilation rate and substomatal carbon dioxide at the lower measurement concentration at the measurement temperature. This value of V_{cmax} and the observed value of substomatal carbon dioxide concentration at 700 μmol mol^{-1} were used to predict the photosynthetic rate at 700 μmol mol^{-1}. Fitted values of V_{cmax} were obtained separately for both measurement temperatures.

When the value of Γ^* for a given treatment differed substantially from the value expected for the measurement temperature (see Results), the process of obtaining V_{cmax} and predicting the rate of photosynthesis at 700 μmol mol^{-1} was also accomplished by running the model with a value of the

Michaelis constant of Rubisco for carbon dioxide consistent with the measured value of Γ^*. The Michaelis constant of Rubisco for carbon dioxide is the temperature-dependent parameter determining the Rubisco specificity factor and Γ^* (Long, 1991).

Results

Field measurements of the stimulation of photosynthesis by increasing the measurement carbon dioxide from 350 to 700 μmol mol^{-1} were made on days where the midday temperatures ranged from less than 10 °C to over 30 °C. In both species, the relative stimulation tended to increase with leaf temperature (Fig. 1). The stimulation observed at the higher temperatures approximated that predicted from the photosynthesis model in both species, or else was less than predicted. In contrast, the stimulation at temperatures below about 20 °C generally exceeded that predicted by the model in both species (Fig. 1). The increase in substomatal carbon dioxide concentration with the change in external concentration decreased slightly with increasing temperature in both species (Fig. 2a), because of an increase with temperature in the value at the lower external concentration (Fig. 2b). Using the substomatal concentrations of carbon dioxide at 10 °C and 35 °C obtained from the data in Fig. 2a did not substantially affect the stimulation of photosynthesis predicted by the model at low temperature (Fig. 1), but slightly reduced the predicted stimulation at high temperature (Fig. 1).

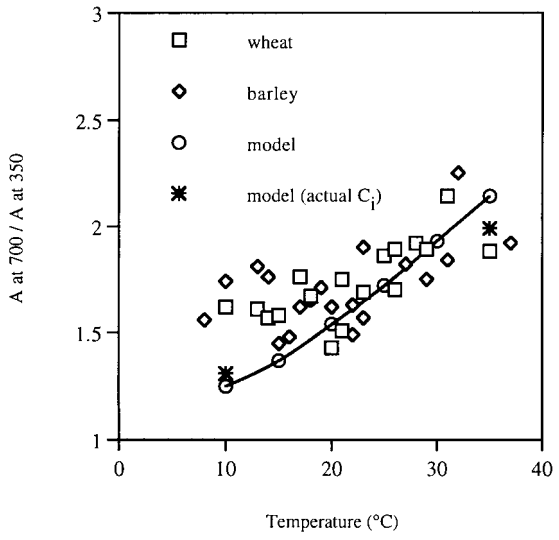

Fig. 1. The ratio of photosynthetic CO_2 assimilation (A) measured at an external CO_2 concentration (C_a) of 700 μmol mol^{-1} to that at 350 μmol mol^{-1} for wheat and barley plants as a function of measurement temperature. Each point represents a mean value for six leaves measured on one day. Measurements were made near midday ($PPFD > 1.5$ mol m^{-2} s^{-1}) in the field under ambient conditions of temperature. The curve is the relationship predicted by the photosynthesis model (see text), with internal CO_2 concentrations (C_i) of 225, and 450 at C_as of 350 and 700 μmol mol^{-1}, respectively. * Indicates the ratio predicted by the model, using the average C_is obtained from Fig. 2a.

For plants grown in the controlled environment chambers at 15 and 23 °C, the stimulation in photosynthesis from 350 to 700 mmol mol^{-1} at 25 °C was between 1.6 and 1.9 for both species (Table 1). At the measurement temperature of 17 °C, the stimulation was about 1.4 for plants grown at 23 °C, but about 1.7 for plants grown at 15 °C (Table 1). Measurements with both gas exchange systems produced similar results (Fig. 3). For plants of both species grown at 23 °C, exposing them to one cool 24 h period lowered the absolute values of photosynthesis, but increased the relative stimulation of photosynthesis caused by doubling the carbon dioxide concentration (Table 1). In wheat, but not in barley, the cool 24 h period lowered the substomatal carbon dioxide concentration for both external concentrations (Table 1).

The values of Γ^* for both barley and wheat grown at 23 °C were quite consistent with previously reported values for spinach and other species at both the 17 °C and 25 °C measurement temperatures (Fig. 4). Growth at 15 °C increased the values of Γ^* at both measurement temperatures in barley, and at the lower measurement temperature in wheat (Fig. 5). Exposure to the single cool 24 h period increased Γ^* in barley (Fig. 5), but not in wheat (not shown).

For leaves in which Γ^* was close to the expected value for the temperature, the model predicted the relative stimulation of photosynthesis with doubling of carbon dioxide quite accurately (Fig. 6). For leaves in which Γ^* was higher than expected for the measurement temperature, i.e. for both species grown at 15 °C and for barley leaves exposed to a cool 24 h period, the relative stimulation of photosynthesis by carbon dioxide doubling was larger than predicted by the photosynthesis model (Fig. 6). For such leaves, altering the Michaelis constant of Rubisco for carbon dioxide such that the value of Γ^* matched the observed value, produced accurate predictions of the stimulation of photosynthesis with carbon dioxide doubling (Fig. 6).

Discussion

In this study, the measurement temperatures in the field were close to the daytime maximum temperatures. The overnight minimum temperatures averaged about 12 °C lower, so the plants had been exposed to quite low temperatures at the times when the relative stimulation was larger than expected. The data for wheat and barley grown at cool temperatures from this study, which indicate an unexpectedly large relative stimulation in photosynthesis at cool measurement temperatures, agree with a few other reports for species grown under cool conditions. For example, Tesky (1997) found, in field measurements on loblolly pine in winter, that the relative

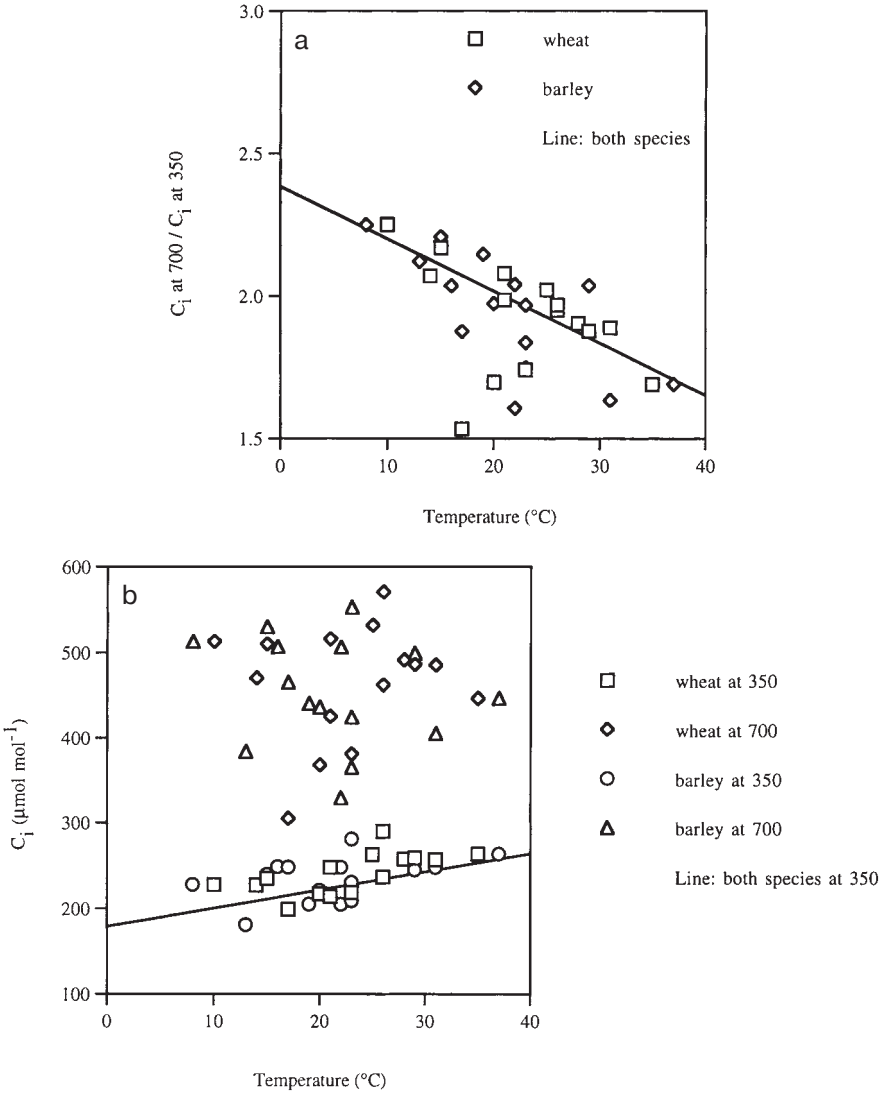

Fig. 2. The ratio of C_i at C_a of 700 to that at C_a of 350 μmol mol^{-1} (a), and values of C_i for C_as of 350 and 700 μmol mol^{-1} (b) as a function of temperature for wheat and barley leaves. Other conditions are as in Fig. 1. The line is the linear regression for the data combined for both species.

stimulation of photosynthesis from ambient to ambient +330 μmol mol^{-1} was a factor of 2.2 at temperature of 13 °C. The relative stimulation did not vary substantially with time of year, despite the large range of measurement temperatures (13–28 °C). He concluded that the unexpectedly large relative stimulation at cool temperatures was not caused by unusual internal carbon dioxide concentrations. Greer *et al.* (1995) found a mean relative stimulation between 350 and 700 μmol mol^{-1} external carbon dioxide concentrations of 1.58 at 12 °C in several cool season pasture species grown at 12/7 °C. The ratio exceeded 1.7 in nearly half of the species. The relative stimulation measured at the daytime growth temperature did not vary between 12 °C and 28 °C. Bowler and Press (1996) found a relative stimulation of 2 at 20 °C in a species of *Agrostis* grown at 20/15 °C, over the internal carbon dioxide interval of 220 to 440 μmol mol^{-1}.

Simple predictions of the increase in assimilation rate with an increase in carbon dioxide concentration are based on the specificity of Rubisco, an assumption that ribulose bisphosphate regeneration capacity is non-limiting, and the assumption of a constant ratio of C_i to C_a (Long, 1991; Fig. 1). There are several reasons why the relative stimulation of photosynthesis from 350 to 700 μmol mol^{-1} external concentrations at high photon flux might be less than that expected from the specificity of Rubisco. These include a smaller than expected change in substomatal carbon dioxide concentration, assimilation at high carbon dioxide becoming limited by the regeneration of ribulose bisphosphate, or limitation of assimilation rate by inorganic phosphate. The last two are possible explanations of the smaller than expected stimulation sometimes seen in the field data (i.e. points below the curve in Fig. 1). However, only lower than expected

Table 1. *Effect of growth and measurement temperature on the ratio of photosynthetic CO_2 assimilation rate at 700 to that at 350 μmol mol^{-1} external carbon dioxide concentration, and on the substomatal concentrations of carbon dioxide in barley and wheat*

A@350 is the photosynthetic CO_2 assimilation rate at 350 μmol mol^{-1} external CO_2 concentration. '15/8' indicates plants grown at 23 °C, but exposed to one 24 h period with maximum/minimum temperatures of 15/8 °C. Values in ( ) are standard deviations for $n=3$.

Species	Temperature (°C)			A@350 (μmol m^{-2} s^{-1})	Substomatal CO_2 at external concentration of:	
	Growth	Measurement	Ratio		350 μmol mol^{-1}	700 μmol mol^{-1}
Barley	15	25	1.85 (0.08)	26 (3)	212	354
	15	17	1.74 (0.07)	21 (2)	209	457
	23	25	1.60 (0.07)	22 (2)	215	427
	23	17	1.38 (0.05)	16 (1)	250	550
	15/8	25	2.00 (0.10)	13 (2)	214	405
	15/8	17	1.72 (0.07)	9 (2)	296	601
Wheat	15	25	1.73 (0.06)	26 (2)	170	310
	15	17	1.72 (0.07)	20 (2)	202	375
	23	25	1.64 (0.06)	24 (3)	185	388
	23	17	1.38 (0.04)	22 (2)	283	580
	15/8	25	2.27 (0.11)	15 (3)	153	343
	15/8	17	1.97 (0.10)	11 (2)	158	375

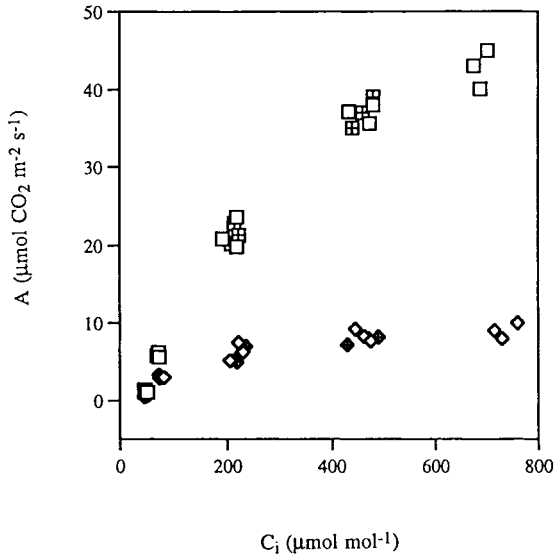

Fig. 3. Rates of photosynthetic CO_2 assimilation (A) as a function of internal CO_2 concentration (C_i) for barley plants grown at 15 °C, and measured at 17 °C at 1.0 (squares) or 0.15 (diamonds) μmol m^{-2} s^{-1} photosynthetic photon flux density, with either a CIRAS-1 portable system (closed symbols) or a laboratory system (open symbols).

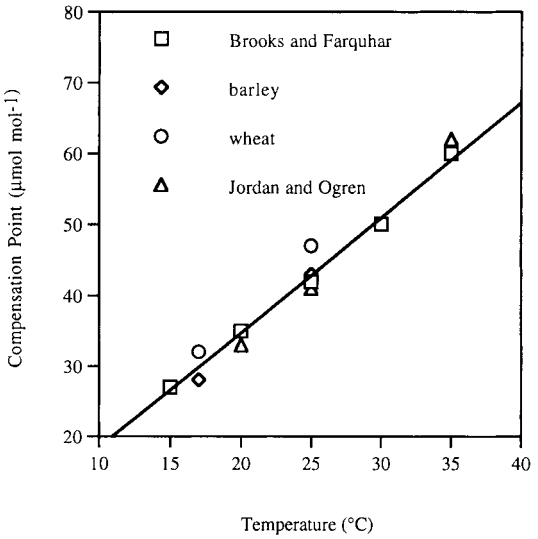

Fig. 4. Values of Γ^* for wheat and barley plants grown at 23 °C and measured at 17 °C and 25 °C, compared with values reported for spinach by Brooks and Farquhar (1985) and for soybean by Jordan and Ogren (1984). The line is a linear regression of the data of Brooks and Farquhar (1985).

values of C_i or larger than expected increase in C_i with increased CO_2 would be expected to give larger relative stimulation than predicted from the Rubisco specificity.

An example of the effect of low C_i in increasing the relative stimulation is the data for wheat after a cool night. The low night temperature reduced stomatal conductance and C_i, and resulted in a large relative stimulation, despite no change in Γ^*. Other cases of relative stimulation larger than expected were not due to low C_i, but all could be attributed to high Γ^*. Jacob and Lawlor (1993) found high Γ^* in sunflower plants deficient in inorganic phosphate. They suggested the possibility that this could be caused by altered stoichiometry of photorespiration rather than an actual change in Rubisco specificity, but did not measure either. The Γ^* value they reported deviated even further from the expected value than did the values reported here for wheat and barley exposed to cool temperatures. Estimates of Γ^* from gas exchange are based on the dubious assumption that the substomatal CO_2 concentration is the same as the CO_2 concentration at the site of carboxylation. However, recalculating Γ^* by assuming a constant mesophyll resistance to CO_2 diffusion equal to half of the maximum stomatal resistance (a high estimate, cf. Laisk and Loreto, 1996) did not affect the values of Γ^*. This indicates that

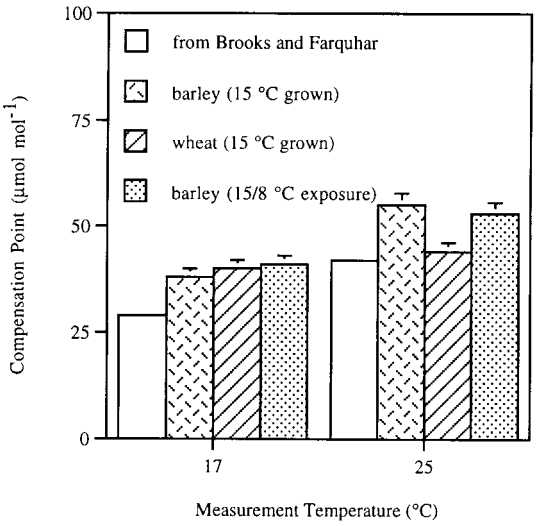

Fig. 5. Values of Γ^* for wheat and barley plants grown at 15 °C and measured at 17 °C and 25 °C, and for barley plants grown at 25 °C and then exposed to a single cool day (max/min of 15/8 °C), compared with values reported for spinach by Brooks and Farquhar (1985). Bars represent standard errors, for $n=3$.

any possible changes in mesophyll resistance are unlikely to have affected the estimated values of Γ^*.

Actual variation in the temperature dependence of the specificity of Rubisco also can not be ruled out by the data shown here and is supported by some earlier work. For example, Hall and Keys (1983) found that the ratio of oxygenation to carboxylation of Rubisco from wheat varied little with temperature from 5–25 °C and then increased with temperature. Brooks and Farquhar (1985) used these data to calculate the temperature dependence of the specificity factor in wheat, and found a much flatter response between 5 °C and 25 °C than occurred in spinach. These results are consistent with our measurements indicating that Γ^* in this species was nearly the same at 17 °C and 25 °C measurement temperatures, when plants were grown at the cool temperature.

Whether altered stoichiometry of photorespiration or an actual change in enzyme specificity caused the altered apparent Rubisco specificity and the unexpectedly large stimulation in photosynthesis at cool temperatures observed here in wheat and barley, and by others in different species, these data indicate that the temperature dependence of the stimulation of photosynthesis by elevated carbon dioxide may vary greatly with species and with prior exposure to low temperatures.

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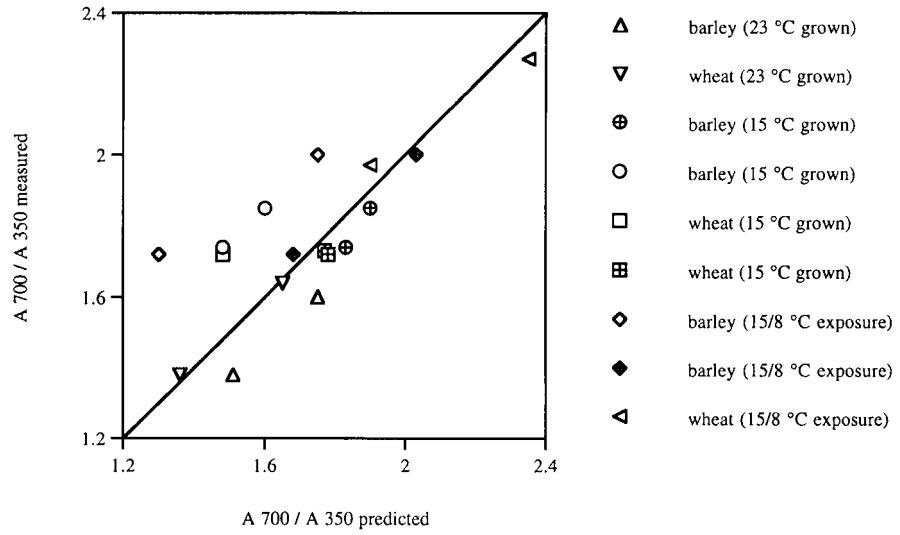

Fig. 6. Measured and predicted values of the ratio of photosynthetic CO_2 assimilation (A) at 700 to that at 350 μmol mol^{-1} external CO_2 concentrations. Predicted values were obtained using the photosynthesis model with the observed values of C_i and either the expected value of Γ^* (open symbols), or actual values of Γ^* (filled symbols), when the two were significantly different. The line is the 1 : 1 line.

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