Temperature Dependence of Photosynthesis in Cotton

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ABSTRACT

Cotton plants (*Gossypium hirsutum* L., var. Deltapine Smooth Leaf) were grown under controlled environmental conditions over a range of day/night temperatures from 20/15 to 40/35 C. Their photosynthetic characteristics were then measured over a comparable temperature range. Net photosynthesis tended stongly to be greatest, and intracellular resistance to CO_2 transport to be lowest, when the measurement temperature corresponded to the daytime growth temperature, suggesting pronounced acclimation of the plants to the growth temperature. The preferred growth temperature was close to the 25/20 C regime, since net photosynthesis of these plants, regardless of measurement temperature, was higher and intracellular resistance lower, than in plants from any other regime.

Ribulose diphosphate carboxylase activity per unit leaf area was highest in plants grown at 25/20 C, but did not show pronounced changes with growth temperature. Glycolate oxidase activity decreased and NADH-malate dehydrogenase activity tended to increase with increasing growth temperature. In contrast, changes in carbonic anhydrase activity with growth temperature showed a general similarity to changes in photosynthetic rate. This may suggest that the "chemical resistance" component of the intracellular resistance bears a relationship to the amount of carbonic anhydrase in the leaf.

Among the major ecological variables, temperature is always prominent in that it can limit the distribution of plants and also the diversity of plant forms that colonize or develop in a particular region. Temperature changes may act directly by modifying existing physiological processes, and indirectly by inducing an altered pattern of development subsequent to the imposition of the temperature change.

In studying photosynthetic acclimation to temperature, it is important to distinguish between the effects of different growth temperature regimes, which can result in marked differences in the anatomical and morphological characteristics of plants, as well as in different leaf photosynthetic characteristics and capacities and the effects of temperature of measurement on plants grown under a particular growth regime, which can affect stomatal diffusive resistance, as well as biochemical reaction rates. It is also desirable to examine, not only photosynthetic rates at normal CO_2 concentrations, but also aspects of photosynthetic capacity and efficiency, such as the maximum rate of photosynthesis under nonlimiting conditions, and the intracellular resistances to CO_2 transfer, respectively. The relationships between these over-all parameters and the activities of some of the primary photosynthetic enzymes should also be explored.

A number of studies of photosynthetic acclimation have been reported (3, 11, 13, 14, 16, 19, 20) but, so far as is known, there has been no study of all of the factors referred to above. The present paper describes experiments on the responses of photosynthetic processes, measured at various temperatures in plants grown under different temperature regimes. Changes in amounts and activities of certain enzymes associated with photosynthesis were also measured. An attempt is made to relate these two kinds of measurements using cotton, one of the more thermophilic of the temperate zone crops, which has been reported to grow at temperatures as high as 45 C (10).

MATERIALS AND METHODS

Preparation of Plant Material. Four controlled environment cabinets in the Commonwealth Scientific and Industrial Research Organization Canberra Phytotron were used for the preparation of plant material. Seedlings of cotton (*Gossypium hirsutum* L., var. Deltapine Smooth Leaf) were started in perlite-vermiculite at 30/25 C, and then transferred at the cotyledon stage to water culture (initially half-strength, then full strength aerated Hoagland's solution) in the appropriate cabinets. One week after transfer the plants were thinned to one per pot, six plants of uniform appearance remaining in each cabinet for measurement. The culture solution, and the position of each pot in a cabinet, were changed weekly.

Conditions were maintained as follows: 60% relative humidity, 16-hr photoperiod, 6.0 to 6.4 $\times$ 10^4 erg cm^{-2} sec^{-1} light intensity and day/night temperatures of 20/15, 25/20, 30/25, and 40/35 C. Higher light intensities were difficult to maintain and attempts to grow the cotton plants at 15/10 C and at 18/13 C were not successful.

Measurements were commenced when the plants had attained a convenient size and leaf number 5 (a fully expanded leaf) counted from the most recently formed leaf, was large enough and had a sufficiently long petiole for insertion in a leaf chamber. The morphology and growth rates of the plants varied considerably with the growth temperature so that it was not appropriate to use plants of the same age. In every case, measurements were made on young plants which were still producing new leaves, and well before flowering. Plants grown at 25/20, 30/25, and 40/35 C were measured at 4 weeks, and plants grown at 20/15 C were measured at 7 weeks after transplantation.

Determination of Leaf Gas Exchange Characteristics. Leaf gas exchange characteristics were measured as described by Slatyer (18). A light response curve was obtained first in each case. The concentration of CO_2 in the air supplied to the chamber was maintained constant at 1900 μg CO_2 liter^{-1} air, and the light intensity at which the photosynthetic rate became constant (*i.e.*, light-saturated) was found for leaf temperatures of 20, 25, 30, and 40 C. A curve relating net photosynthesis to a range of ambient CO_2 concentrations (P/c_a) at saturating light in-

tensity, was then obtained for each of the four leaf temperatures. From each point on this curve, a second curve, relating net photosynthesis to the estimated CO_2 concentration at the mesophyll cell walls (P/c_w) was then obtained by accounting for the effects of stomatal and boundary layer resistance in the CO_2 pathway (18). Particular attention was given to points lying on the plateau of the P/c_w curve, giving values of P_{max} (the maximum rate of photosynthesis obtainable at saturating light intensity and at saturating CO_2 concentration) and to points lying close to the axis P=O, in order to obtain values for the intracellular resistance (r_i) calculated as the reciprocal of the slope of the initial, CO_2-limited, part of the CO_2-response curve.

Many authors report photosynthetic rates obtained by providing normal air at the inlet of their leaf chamber. However, the concentration of CO_2 surrounding the leaves may be significantly lower than that in the inlet airstream. In these experiments the term P_{amb} is used to describe rates of photosynthesis when the air surrounding the leaves contained 0.03% CO_2 by volume at 25 C.

Since stomatal aperture, and hence stomatal resistance, r_i, varied in plants from different growth temperature regimes, values of P_{amb} were adjusted to give estimates, P^*_{amb}, of this parameter at the minimum value for r_i which was obtained at each growth temperature.

The basis of these calculations was:

$$P^*_{amb} = \frac{P_{amb} \Sigma r}{\Sigma r - r_t + r_t min}$$

where P^*_{amb} is the estimated value of P_{amb} at the minimum stomatal resistance, r_i^{min}; Σr is the sum of the resistances to CO_2 transfer, obtained from the slope of the response curve relating P to ambient CO_2 concentration, and r_i is the actual stomatal resistance at the temperature of measurement. It is apparent that this correction is strictly valid only on the linear part of the CO_2-response curve. However, it provides an adjustment in the right direction, when r_i is limiting photosynthesis.

Enzyme Assays. The biochemical assays and gas exchange measurements were conducted on equivalent plants at the same time in order to allow direct comparison between the measurements. Whole plants were removed from the Phytotron for biochemical assays. The activities of phosphoenolpyruvate carboxylase, ribulose 1,5-diphosphate (RuDP)[1] carboxylase, carbonic anhydrase, NADH-malate dehydrogenase, and glycolate oxidase were obtained on the basis of leaf area. Chlorophyll ($a + b$) content was measured and activities on the basis of chlorophyll content were calculated. The mean value of the activity of each enzyme for a pair of plants from each growth temperature regime was calculated.

Discs punched from leaf number 5 were allowed to fall into ice-cold extracting buffer in a glass mortar in an ice bath. They were extracted by grinding with carborundum (5). The extracting buffer was modified to contain 20 mM 2-mercaptoethanol, 20 mM dithiothreitol, and 8 mg per ml Sigma PVP-10 (polyvinylpyrrolidone). Chlorophyll ($a + b$) was estimated by adding acetone to a small volume of the uncentrifuged extract, giving a final concentration of 80% acetone (v/v) and reading the absorbance of the supernatant at 652 nm (4).

The activities of RuDP carboxylase, glycolate oxidase, and NADH-malate dehydrogenase were measured as described previously (5). Carbonic anhydrase was assayed by a modification of the Wilbur-Anderson veronal indicator method (21),

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[1] Abbreviation: RuDP: ribulose 1,5-diphosphate.

subsequently modified by Rickli et al. (17). All reagents, syringes and tubes were maintained at 0 C in ice baths and the measurements were carried out in a cold room at about 2 C. Carbonic anhydrase activity was expressed as units of activity calculated from the formula given by Everson (7).

A comparison was made of the carbonic anhydrase activity measured when half the number of leaf discs were extracted in the same volume of buffer. The activities, expressed in terms of units cm^{-2} leaf area, agreed within 5%. It was concluded that the ratio of leaf material to buffer used in the extractions was satisfactory, and that the extraction could not be improved by reducing this ratio, in contrast to results reported by Björkman (2).

RESULTS

Figure 1 shows the values of P_{max}, at different measurement temperatures for plants from each growth temperature regime. The value of r_i, the stomatal resistance to CO_2 diffusion corresponding to each value of P_{max}, is also shown.

In general, P_{max} tended to reach a peak at the temperature corresponding to the daytime growth temperature; the only obvious exception to this generalization was for the 20/15 C regime, where the 40 C values were slightly higher than those at 20 C. The lack of temperature sensitivity in this regime may suggest a photochemical limitation to photosynthesis, observed elsewhere in thermophilic species grown at low temperatures (11).
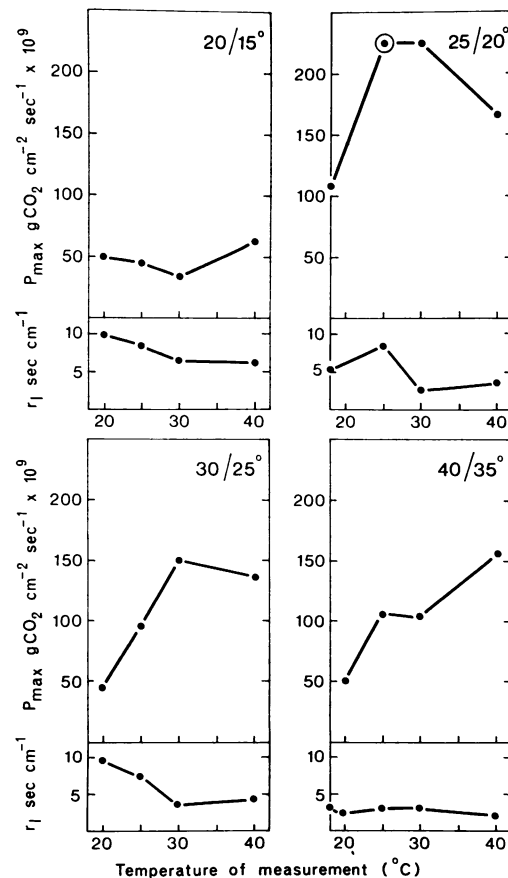

FIG. 1. Effects of measurement temperature on P_{max} in leaves of plants from different growth temperature regimes. The regime (C) is shown in the top right-hand corner of each graph. r_i: stomatal resistance to CO_2 diffusion; ⊙: This value represents an underestimation of P_{max} indicated by high stomatal resistance.
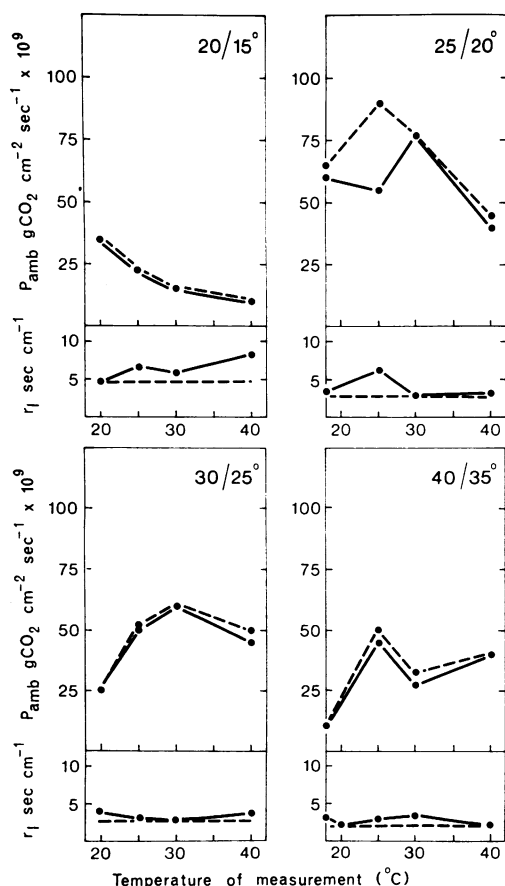
FIG. 2. Effects of measurement temperature on P_{amb} (solid lines) for leaves of cotton plants from different growth temperature regimes. The regime is shown in the top right-hand corner of each graph. Dashed lines join values of P_{amb}^*, (values of P_{amb} calculated for minimal stomatal resistance). r_i = stomatal resistance to CO_2 diffusion. Dashed horizontal line shows minimum value of r_i in leaves from each growth temperature regime.

A similar picture emerges when P_{amb} values are examined (Fig. 2) although the actual rates of photosynthesis were significantly lower than those of Figure 1, and each temperature response curve flatter. The results for the 40/35 C regime appear to be somewhat exceptional in that the value of P_{amb} at 25 C was higher than at 40 C.

In general, the P_{amb}^* and P_{amb} values were in close agreement, suggesting that changes in stomatal resistance at different measurement temperatures did not unduly influence the shape of the curves in Figure 2. The 25/20 C growth treatment tested at 25 C was exceptional in that high stomatal resistance clearly depressed the value of P_{amb}.

Figure 3 shows the effect of growth temperature regime on P_{max} measured at various temperatures. Although the shape of the curve differs for each measurement temperature, the highest P_{max} values tend to be obtained from plants grown at a 25/20 C growth regime, suggesting that this is close to the preferred temperature for the genetic material used.

P_{max}, by definition, represents the light-saturated value of net photosynthesis when CO_2 is not limiting the reaction rate. Another widely used index of photosynthetic efficiency is the intracellular resistance to CO_2 transfer, r_i. In Figure 4, values of r_i are plotted against temperature of measurement for plants from each growth temperature. These data provide strong confirmatory evidence of the results previously presented, showing that the minimum value of r_i tends to be obtained at the meas-

urement temperature which corresponds to the growth temperature. Furthermore, the general level of r_i was lower in the 25/20 C treatment than in all others.

In Figure 5, values of P_{max} and r_i for all treatments are plotted against one another. A close inverse relationship is seen to exist between them (r = 0.82; $P < 0.001$). This is to be expected since both are influenced by resistances in the biochemical carbon pathway. In addition, r_i is influenced by the resistance to CO_2 transfer from the surface of the mesophyll cells to the sites of carboxylation. As the relative importance of this resistance increases, a less obvious relationship can be expected.

Figure 6 shows the activities of RuDP carboxylase, glycolate oxidase, and NADH-malate dehydrogenase, measured at a standard temperature of 30 C, and of carbonic anhydrase activity, measured at 0 C, for plants from each growth temperature regime.

Expressed on a leaf area basis there was little change in RuDP carboxylase activity in leaves grown at different temperatures, glycolate oxidase activity appears to decline with increasing growth temperature, and the activity of NADH-malate dehydrogenase does not follow any obvious pattern with change in growth temperature regime. Apart from plants grown at 30/25 C, there appears to be an increase in activity of this enzyme with increase in growth temperature regime. Carbonic anhydrase activity shows a pronounced peak in plants from the 25/20 C growth regime, and the over-all profile of changes in activity of this enzyme resembles the comparable P_{max} curves of Figure 1.

Chlorophyll ($a + b$) content per unit leaf area increased considerably with increase in growth temperature; plants grown at 20/15 C contained about one-third the chlorophyll content of plants grown at the more moderate temperatures of 25/20 and 30/25 C. Plants grown at 40/35 C had very dark green leaves, containing four times the chlorophyll content of plants
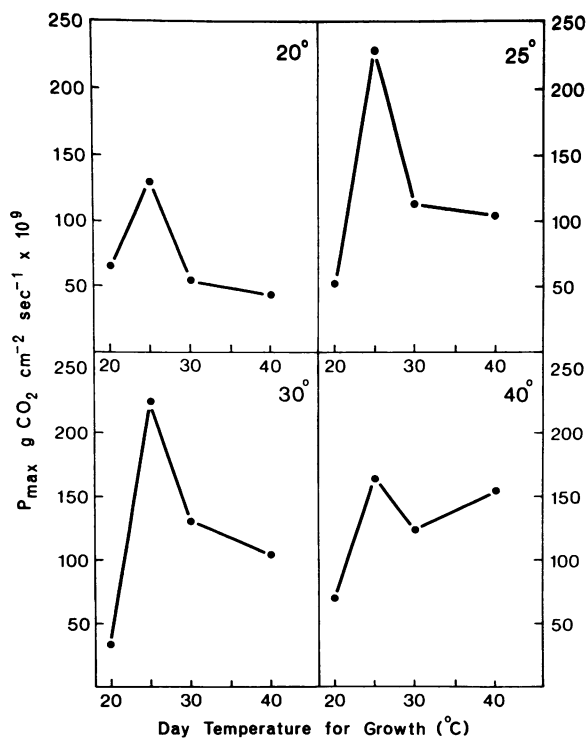

FIG. 3. P_{max} of plants from different growth temperature regimes. The measurement temperature (C) is shown in the top right-hand corner of each graph.

grown at 20/15 C. In consequence, when enzyme activities are expressed on a chlorophyll basis, they all tend to show a progressive decline with increasing growth temperature. In these experiments, therefore, where comparison of enzyme activity and photosynthetic characteristics is intended, leaf area appears to provide a more realistic basis of expression.

DISCUSSION

The results of Figures 1 to 4 provide evidence of the substantial degree to which plants can acclimate to the temperature regime in which they are grown, supporting, in general terms, the work of Mooney and West (14) and others. Apart from their intrinsic interest, the data also suggest that users of controlled environment facilities should exercise a good deal of caution in the extrapolation of results from one growth temperature to another. Also, caution is needed in the interpretation of the many temperature response curves now published in the literature. It is clear that, to a considerable degree, the photosynthetic temperature response may reflect the temperature at which a plant has been grown as well as the inherited tendency for a preferred temperature.

The interpretation of temperature effects on photosynthesis in terms of enzyme reactions raises many difficulties. Although several workers have shown reasonably close correlations between photosynthetic parameters and levels of RuDP carboxylase (1, 12, 15, 16), the present data (Fig. 6) show little correlation between them, suggesting that RuDP carboxylase activity is not a primary factor in mediating the temperature response.

A much closer correlation is found between the pattern of change in carbonic anhydrase activity, in relation to growth temperature, and that of P_{max} and r_i. Since the role of this enzyme is to facilitate CO_2 transfer, it could be expected that,
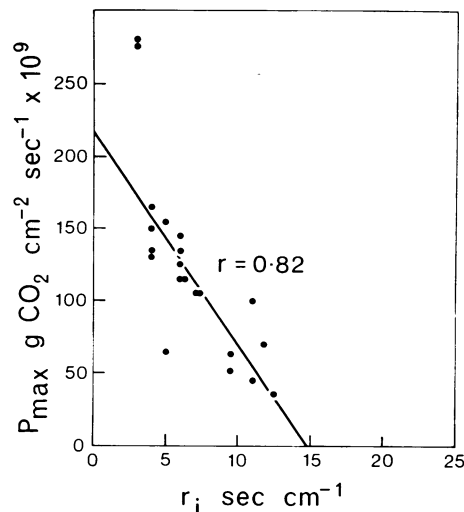

FIG. 5. The relationship between P_{max} and intracellular resistance, r_i, including results for leaves from all growth temperature regimes and at all measurement temperatures ($r = 0.82$; $p < 0.001$).
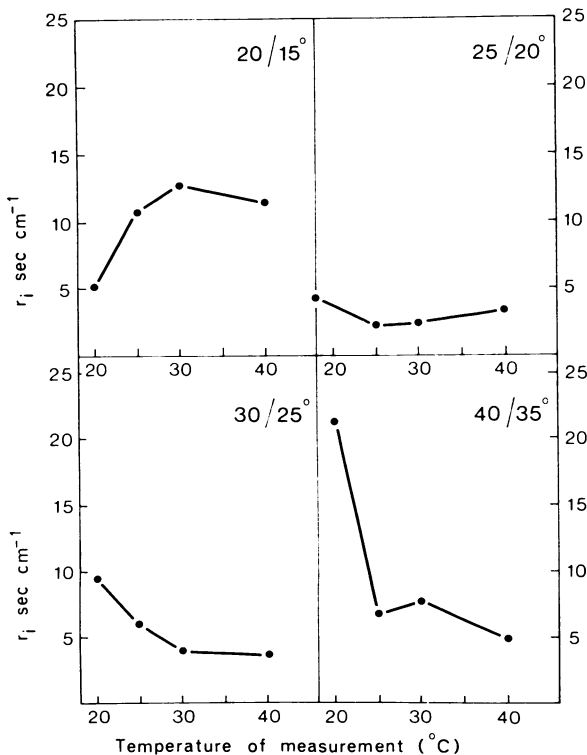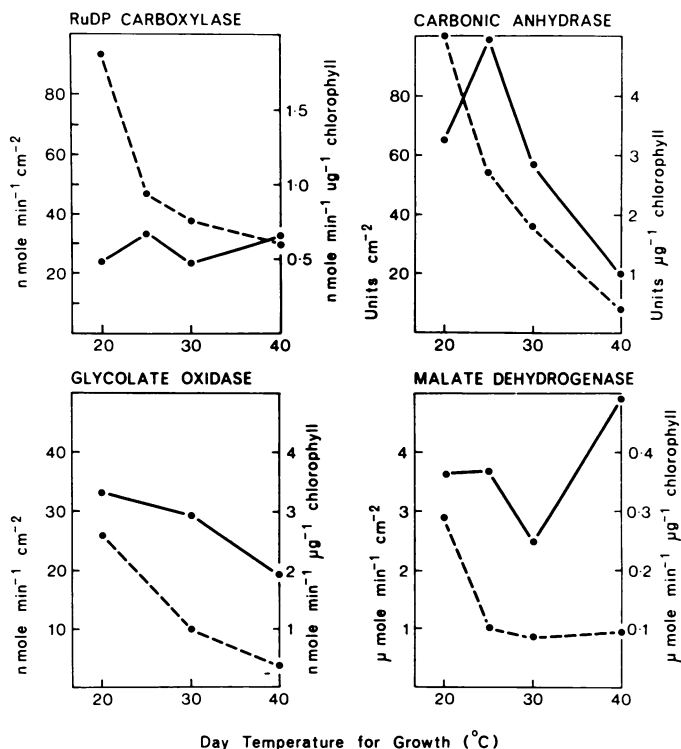

Day Temperature for Growth (°C)

FIG. 6. The profile of changes in activity of RuDP carboxylase, glycolate oxidase, NADH-malate dehydrogenase (all measured at 30 C), and carbonic anhydrase (measured at 0 C) in extracts of leaves from different growth temperature regimes. Solid lines: changes in activities on the basis of leaf area; dashed lines: changes in activities on the basis of chlorophyll content.

under the saturating CO_2 conditions that are used for P_{max} determinations, only a weak correlation would be found (cf. 6–8). The explanation may lie in an effect of carbonic anhydrase in increasing the local availability of CO_2 at the carboxylation reaction site, regardless of the level of ambient CO_2, as suggested by Graham and Reed (9).

FIG. 4. Effects of measurement temperature on intracellular resistance, r_i, for plants from different growth temperature regimes. The regime (C) is shown in the top right-hand corner of each graph.

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