

A simple new equation for the reversible temperature dependence of photosynthetic electron transport: a study on soybean leaf

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Abstract. The temperature response of J_{\max} , the irradiance-saturated potential rate of photosynthetic electron transport in the absence of Rubisco limitation, has usually been modelled by a complicated, modified Arrhenius type of equation. Light saturation can be difficult to achieve and reduces the precision of fluorescence measurements. Consequently, we calculated the rate of electron transport at 1200 μmol photosynthetically active radiation (PAR) quanta $\text{m}^{-2} \text{s}^{-1}$ from chlorophyll fluorescence measurements on intact soybean leaves [*Glycine max* (L.) Merr] as temperature increased from 15 to 43°C with 1250 $\mu\text{mol mol}^{-1}$ ambient [CO_2]. Electron transport rate was maximal around 37°C and the decline in rate following further increases in leaf temperature to 43°C was found to be completely reversible immediately upon return to lower temperatures. We report a convenient, new equation for the temperature dependence of the rate of electron transport under high irradiance:

$$J(T_L) = J(T_0)e^{-\left(\frac{T_L - T_0}{\Omega}\right)^2},$$

where T_L is the leaf temperature (°C), $J(T_0)$ is the rate of electron transport at the optimum temperature T_0 , and Ω is the difference in temperature from T_0 at which J falls to e^{-1} (0.37) of its value at T_0 . Increased supply of nitrate increased $J(T_0)$. Acclimation to growth temperature was observed, with T_0 increasing from 35.4°C to 39.2°C for soybean plants grown at 20/15°C and 32/27°C (day/night), respectively. The average value of Ω was $18 \pm 0.6^\circ\text{C}$ and was unaltered by growth conditions. A comprehensive review of the literature revealed a slight tendency for Ω to increase with T_0 across species.

Keywords: chlorophyll fluorescence, CO_2 , gas exchange, growth temperature, nitrogen, photosynthesis model.

Introduction

Most leaves experience large diurnal and seasonal fluctuations in temperature. This has direct effects on the underlying biochemistry of photosynthesis in both the short- and long-term. For photosynthetic electron transport, the rate increases with increasing temperature up to a maximum and then declines with further temperature increases.

An equation that describes such a temperature response through parameters for enzyme activation and deactivation modified from Johnson *et al.* (1942) was incorporated into photosynthesis models by Tenhunen *et al.* (1976*b*) and Farquhar *et al.* (1980). Tenhunen *et al.* (1976*a*) measured the rate of CO_2 assimilation of *Phaseolus vulgaris* L. leaves under high CO_2 , low O_2 and high irradiance and fitted the equation to their data. Farquhar *et al.* (1980) fitted the equation to data obtained by Nolan and Smillie (1976) who

measured the response of photosystem II electron transport to temperature by uncoupled thylakoids isolated from *Hordeum vulgare* L. Farquhar *et al.* (1980) also derived the temperature optimum, T_0 , by differentiating the equation. The more rapid decline in rate above than below T_0 required an equation that produced an asymmetric curve. Subsequently, the equation has been fitted to gas-exchange data for soybean (Harley *et al.* 1985), cotton (Harley *et al.* 1992) and many other species (reviewed by Medlyn *et al.* 2002).

While this type of equation describes the temperature response very well, it is unnecessarily complex (being repeatedly misprinted in various publications). Several papers state that one parameter was held constant in order to be able to fit the equation (e.g. Harley *et al.* 1992; Dreyer *et al.* 2001). The justification or need for having different activation and deactivation energies has not been explored.

Abbreviations used: J , rate of electron transport; PAR, photosynthetically active radiation; T_L , leaf temperature; T_0 , optimum temperature.

As progressively more species have been characterised, it is apparent that the parameters describing the temperature-response function vary considerably. Variation due to differing capacity can be simply removed by normalising the data at a common temperature. However, the remaining variation is still considerable, particularly with respect to the optimum temperature. Growth temperature is known to alter the temperature optimum for photosynthesis in some species but not others (Billings *et al.* 1971; Slatyer 1977; Berry and Björkman 1980). Analysis of electron transport capacity has shown that optimum temperature can vary depending on the growth temperature in *Nerium* (Björkman *et al.* 1978), *Abutilon* (Ziska 2001) and *Nicotiana* (Bernacchi *et al.* 2003). However, the optimum temperature for photosynthetic electron transport does not always acclimate to growth temperature. For example, the optimum temperature for whole-chain electron transport remained around 41°C for *Larrea divaricata* Cav. plants grown at either 20 or 45°C, while the temperature at which the onset of damage was evident increased from 45 to 50°C (Armond *et al.* 1978).

Our objectives were 3-fold: (i) to parameterise the response of electron transport rate under high irradiance to temperature for soybean leaves grown under a range of conditions, (ii) to see whether the decrease in photosynthetic rate observed at temperatures above the optimum was due to damage or whether the rate could be immediately restored following return to lower temperatures, and (iii) to develop a simpler formulation of the temperature dependence.

Materials and methods

Plant material

Seeds of indeterminate soybean [*Glycine max* (L.) Merr. cv. Stephen] were sown in 12-L plastic pots containing a mixture of sand and vermiculite (1:1, v/v) and grown in a controlled environment chamber with a 14-h photoperiod of 700 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, 60/70% relative humidity day/night and three different temperature regimes: 20/15, 25/20, 32/27 °C day/night at a $[\text{CO}_2]$ of 350 $\mu\text{mol mol}^{-1}$. The lowest and highest temperature regimes were repeated with atmospheric CO_2 enrichment to 700 $\mu\text{mol mol}^{-1}$.

The source of light used in the growth chamber was a metalarc lamp (General Electric Lighting, Louisville, KY), MVR 1000/U. Plants were well spaced (30 cm apart at sowing) to avoid mutual shading. Rhizobial inoculation was not provided for the plants. Each pot was flushed every second day with full-strength Herridge's solution (0.50 mM MgSO_4 , 0.25 mM CaCl_2 , 0.25 mM KCl , 0.125 mM KH_2PO_4 , 0.125 mM K_2HPO_4 , 25 μM Ferric monosodium salt of EDTA, 12 μM H_3BO_3 , 3.6 μM MnCl_2 , 77 μM ZnCl_2 , 76 nM CuCl_2 , 25 nM NaMoO_4 ; Herridge 1977) and watered twice daily on days when nutrients were not given. To obtain variation in leaf nitrogen content, three different concentrations of KNO_3 were added to the nutrient solution (2, 5 and 16 mM). Nutrient solutions were added to each pot, until they drained at the base (2.5–3.0 L per pot).

Measurements

Fluorescence

Chlorophyll fluorescence measurements were made using a fibre-optic-based modulation fluorometer (PAM; H. Walz, Effeltrich,

Germany). Attached leaves were enclosed in a temperature-controlled clamp-on cuvette connected to a water bath (to control the leaf temperature) and to a CO_2 supply of 1250 $\mu\text{mol mol}^{-1}$ (to minimise photorespiration and the limitation by Rubisco). Leaf temperature was measured with a copper–constantan thermocouple touching the lower surface of the leaf and controlled by circulating water from a cooling/heating water bath to the water jacket of the leaf chamber. The humidity of the air stream was controlled by passing CO_2 -free air through a gas-washing bottle and an anodised aluminium block condenser. The temperature of the condenser was controlled by a cooling/heating water bath. Leaf-to-air vapour pressure difference was held at approximately 1.25 kPa by changing the temperatures of the water baths for the leaf chamber and for the condenser. Actinic light of 1200 $\mu\text{mol PAR quanta m}^{-2} \text{s}^{-1}$ was given and periodically saturating pulses (9500 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ for 0.8 s) of white light were given with a halogen lamp (KL 1500; Schott, Mainz, Germany) to generate trap closure. Measurements were made as temperature increased from 15–16°C to 43–45°C on the third trifoliolate leaves, which were between 12 and 16 d old. The time taken for the increase from 15 to 43°C was approximately 20–25 min and it stayed at 43°C for about 0.5 min. After reaching 43–45°C, the temperature was brought back down to 25°C. It took 5–10 min to cool down from 43–45°C to 25°C. Photochemical efficiency of PSII, ϕ_{PSII} , was calculated according to Genty *et al.* (1989). The temperature dependence of ϕ_{PSII} was measured in intact soybean leaves grown at different temperatures, CO_2 concentrations and nitrate nutrition. For each nitrate treatment, two or three plants were used as replicates. The rate of electron transport, J , as shown in Fig. 1 and Table 1, was calculated by:

$$J = \phi_{\text{PSII}} I a (1 - f) / 2, \quad (1)$$

where I is light that reaches the upper surface of the leaf (1200 $\mu\text{mol PAR quanta m}^{-2} \text{s}^{-1}$), a is leaf absorptance, which depends on chlorophyll content (see Evans and Poorter 2001). We obtained $a = 0.9$ for high-nitrogen leaves, 0.7 for medium-nitrogen leaves and 0.65 for low-nitrogen leaves from measurement using a Taylor's sphere. f is a factor correcting for spectral imbalance of light ($f \approx 0.15$; Evans 1987). The factor 2 is due to the requirement for one quanta to be absorbed by each photosystem for whole-chain electron transport. Uncertainty in $I a (1 - f) / 2$ affects only the magnitude of calculated electron transport rate for a particular growth condition, but not the optimum temperature or the shape of the temperature response. In this paper, we focused on the relative change with temperature rather than with an absolute comparison to rates calculated independently from gas exchange.

New equation

We used a new equation for the dependence of electron transport rate, J , on leaf temperature (T_L), which describes the data well (June *et al.* 1998). The equation is:

$$J(T_L) = J(T_o) e^{-\left(\frac{T_L - T_o}{\Omega}\right)^2}, \quad (2)$$

where $J(T_o)$ is the rate of electron transport at the optimum temperature, T_o , and Ω is the difference in temperature from T_o at which J falls to e^{-1} (0.37) of its value at T_o . A smaller value of Ω means a narrower peak. This equation effectively assumes that the reversible processes are symmetrical around the optimum temperature. The equation was fitted with the non-linear curve fitting function in Origin using all of the data for a given leaf.

The photosynthesis model (Farquhar *et al.* 1980) defined a parameter J_{max} , as the light saturated potential rate of electron transport. In practice, light saturation may not be achievable with a given light source, or the leaf may suffer damage if held under saturating light for a prolonged period, or measurement errors are greater at saturating light. Consequently, measurements are generally made under high irradiance. J_{max} could then be estimated from an

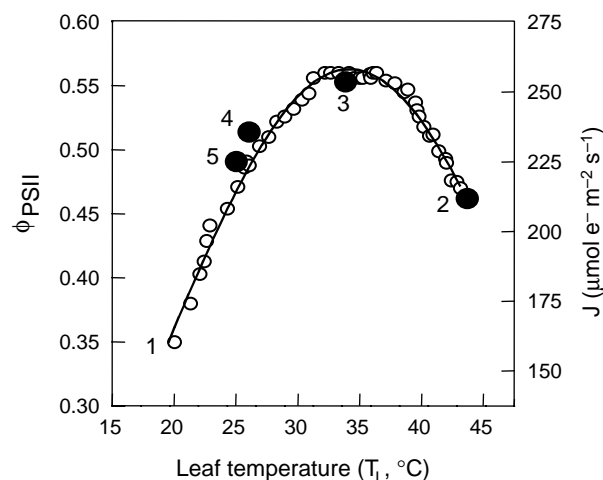


Fig. 1. The temperature dependence of photochemical efficiency (ϕ_{PSII}) and calculated electron transport rate, J . This leaf was grown under $700 \mu\text{mol mol}^{-1} \text{CO}_2$, $20/15^\circ\text{C}$ day/night temperature and 16 mM NO_3^- . Order of measurement is indicated by numbers: 1 to 2 increasing temperature at $0.5^\circ\text{C per minute}$ (open symbols) then decreasing from 2 to 3 (10 min) to 4 (5 min) and to 5 (3 min), as indicated by solid symbols. The response was fully reversible after reaching the highest temperature near number 2. Solid curve is the fit to eqn 2 with $J(T_0) = 257 \mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$, $T_0 = 33^\circ\text{C}$ and $\Omega = 23.8^\circ\text{C}$.

irradiance-response curve at each temperature. The temperature response of J at high irradiance should be very similar to that of J_{max} , whereas at low irradiance, J is likely to be almost independent of temperature (e.g. see Fig. 3B in Oberhuber and Edwards 1993).

Results

Reversibility

The temperature response of photosynthetic electron transport was inferred for each leaf by monitoring chlorophyll fluorescence. Each leaf was monitored from approximately $15\text{--}20^\circ\text{C}$ as it was heated to $43\text{--}45^\circ\text{C}$ (Fig. 1). Leaves were subsequently cooled below the optimum temperature in order to assess whether any damage had occurred. Decreasing the temperature from 43 to 35°C over 10 min resulted in the electron transport rate returning to the same rate achieved by the leaf when it had passed through this temperature during the heating part of the cycle. No loss of activity was ever observed. Thus, the decline in rate between the temperature optimum and 43°C on this time scale was fully reversible. The responses to temperature were well described by eqn 2, with the rate declining symmetrically both above and below the optimum temperature.

$J(T_0)$, the rate of electron transport at the optimum temperature

The rate of electron transport differed between leaves from plants supplied with different nitrate treatments. Decreasing the nitrate concentration in the nutrient solution applied to the plants resulted in lower rates of electron

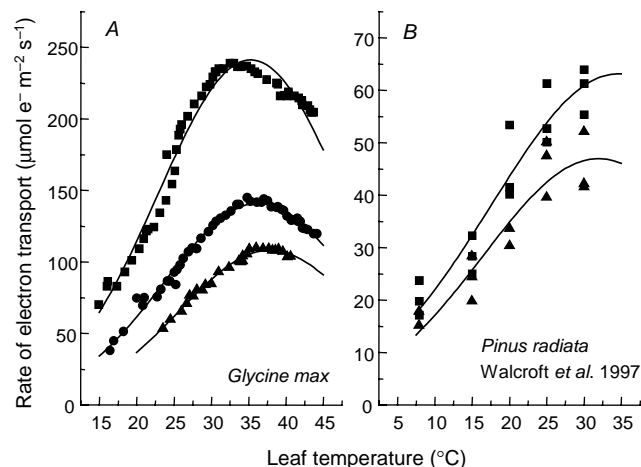


Fig. 2. Temperature dependence of electron transport for leaves grown with different nutrient treatments. (A) Soybean with 2 (triangles), 5 (circles) and 16 (squares) mM nitrate and a growth temperature of $20/15^\circ\text{C}$. (B) *Pinus radiata* (Walcroft *et al.* 1997) with 33% (triangles) and 100% (squares) nutrient. Solid lines are the fit to eqn 2. Fitted parameters are shown in Table 1 and 2.

transport (Fig. 2A, Table 1). Nitrate treatment did not affect the breadth of the temperature response (Ω), or T_0 . The effect of nitrogen treatment on $J(T_0)$ can also be seen in the data collected on *Pinus radiata* (Fig. 2B; Walcroft *et al.* 1997).

Table 1. Parameters for soybean grown under various treatments fitted with eqn 2

The values are the mean of two replicate leaves (\pm s.e.), grown under three different day/night temperature regimes, two atmospheric CO_2 treatments (A = ambient CO_2 , E = CO_2 enriched to $700 \mu\text{mol mol}^{-1}$), and three nitrate nitrogen concentrations

Growth conditions	$J(T_0)$ ($\mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$)	T_0 ($^\circ\text{C}$)	Ω ($^\circ\text{C}$)
20/15 $^\circ\text{C}$			
A, 16 mM	240 ± 1.4	35.4 ± 0.1	17.6 ± 0.2
5 mM	135 ± 6	36.1 ± 0.2	18.3 ± 0.4
2 mM	113 ± 6	37.7 ± 0.1	18.7 ± 1.5
E, 16 mM	255 ± 0.9	32.9 ± 0.1	24.1 ± 0.3
5 mM	175 ± 17	34.4 ± 0.8	22.7 ± 0.6
2 mM	145 ± 10	36.4 ± 2.5	19.4 ± 1.1
25/20 $^\circ\text{C}$			
A, 16 mM	219 ± 8.7	37.8 ± 1.3	16.7 ± 0.3
2 mM	150 ± 8	38.1 ± 0.2	16.2 ± 0.9
32/27 $^\circ\text{C}$			
A, 16 mM	183 ± 0.5	39.3 ± 0.5	19.8 ± 0.6
5 mM	111 ± 13	39.6 ± 1.2	17.1 ± 1.9
2 mM ^A	110	39.1	18.0
E, 16 mM ^B	199 ± 8	38.9 ± 1.0	18.2 ± 0.8
5 mM	115 ± 6	37.6 ± 0.5	14.5 ± 1.3
2 mM	82 ± 8	39.8 ± 1.7	15.7 ± 0.9

^A1 replicate only. ^B3 replicates.

T_o , the optimum temperature

Growth temperature affected the short-term temperature response of photosynthetic electron transport. Increasing both the day and night temperatures by 12°C from 20/15 to 32/27°C day/night increased T_o by 3.8°C in soybean (Fig. 3A). *Abutilon* was even more responsive, with a 16°C increase in growth temperature increasing T_o by 9°C (Fig. 3B; Ziska 2001). There was no consistent effect of growth temperature on either $J(T_o)$ or Ω across species.

Ω , the breadth of the peak

In order to assess whether there was any systematic difference in Ω across species or in association with other parameters, we analysed a wide range of published responses. It was apparent that significant variation in Ω existed and there was a tendency for Ω to increase as T_o increased (Fig. 4). For some datasets, although eqn 2 could be fitted, the temperature range of the data did not include the derived value of T_o . These data (open symbols in Fig. 4) were omitted from the regression. The majority of the data presented for any given curve was generally obtained below the optimum. The value of Ω did not differ systematically between plant functional types.

Discussion

We have presented data for the temperature response of photosynthetic electron transport of soybean leaves grown under a range of conditions. The relationships were well described by a new equation defined by three parameters.

The short-term temperature response and reversibility

The temperature-response function originally put forward for the photosynthesis model by Farquhar *et al.* (1980) was based on data for electron transport through PSII by

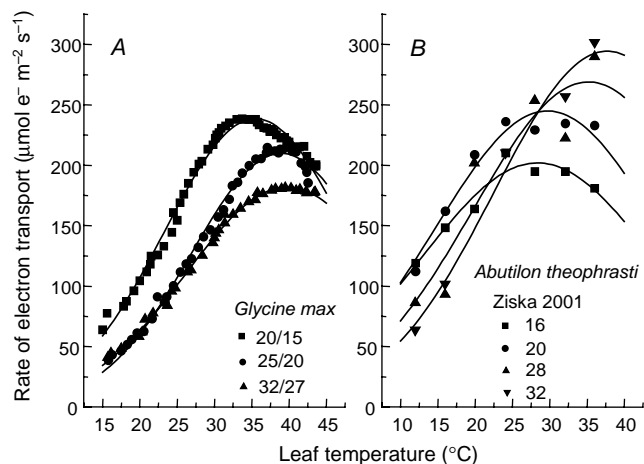


Fig. 3. Temperature dependence of electron transport for leaves produced under different growth temperatures. (A) Soybean. (B) *Abutilon theophrasti* (Ziska 2001). Solid lines are the fit to eqn 2. Fitted parameters are shown in Tables 1 and 2.

uncoupled thylakoids isolated from *H. vulgare* (Nolan and Smillie 1976). The rate declined more rapidly at temperatures above the optimum than below. This feature was captured in the function, which had different parameters for activation and deactivation. It is important to note that the data were collected from uncoupled thylakoids and not intact chloroplasts. The asymmetry in their results, compared with our data from whole, intact leaves, could be due to uncoupling or to the lack of protective systems normally active in the intact leaf. Indeed, evidence for a more rapid decline in the rate once the leaf temperature has exceeded the optimum is scarce. Using soybean leaves, we were able to exceed the optimum temperature by at least 10°C without causing any damage (Fig. 1). We suspect that there is no damage to the thylakoid membrane within the temperature range and over the time scale that we used. By damage we mean an irreversible loss of capacity due to injury to the photosynthetic system in contrast to reversible deactivation.

The temperature above which damage occurs does not necessarily relate to the optimum temperature. Armond *et al.* (1978) grew *L. divaricata* at a range of temperatures from 20 to 45°C and while the optimum temperature for whole-chain electron transport by thylakoids was around 41°C regardless of growth temperature, the temperature at which F_o began to rise (indicating damage) increased from 45 to 50°C. Seemann *et al.* (1984) surveyed a range of species grown under different temperature regimes. While the F_o rise temperature varied between species (41–49°C), little acclimation was observed for any given species (2.5°C change in F_o rise temperature for a 20°C increase in growth temperature). For both *Tilia cordata* Mill. and *Populus*

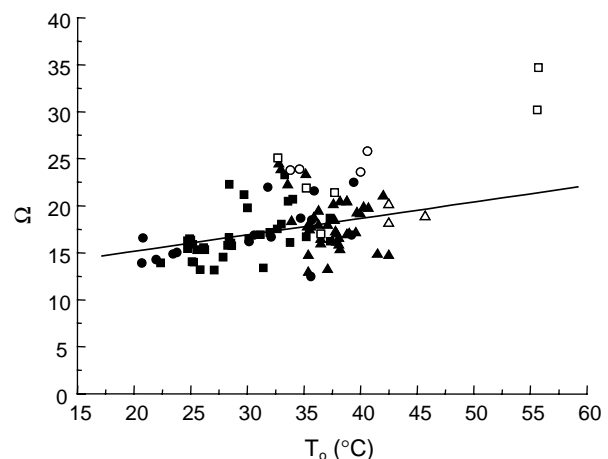


Fig. 4. Relationship between fitted values of Ω and T_o . $\Omega = 11.6 (\pm 1.6) + 0.18 (\pm 0.05) T_o$ ($r^2=0.13$). Data are taken from Tables 1 and 2. Additional points were calculated by fitting eqn 2 to the temperature response functions presented by Wohlfahrt *et al.* (1999). Open symbols indicate where the temperature range did not reach T_o and these points were omitted from the regression. Closed triangles indicate *Glycine max*, closed circles indicate tree species, and closed squares indicate herbaceous species.

tremula L., the rise in F_0 began around 45°C, whereas the optimum temperatures for photosynthetic electron transport were 40 and 35°C, respectively (Niinemets *et al.* 1999).

Georgieva and Yordanov (1993) measured F_0 of pea seedlings (*Pisum sativum* cv. Ran) within the temperature range of 2–50°C and found that F_0 increased dramatically after 42°C. They suggested irreversible damage to thylakoid membranes after 44°C but not before that. Methy *et al.* (1997), working with two Mediterranean evergreen tree species, *Pinus halepensis* Mill. (conifer) and *Quercus ilex* L. (evergreen oak), found that changes in F_0 started at a temperature of 48.5°C. It is interesting to note, however, the experiment of Yamane *et al.* (1997) with rice and spinach. They found that the increase in F_0 after 5 min at 40°C was reversible as F_0 returned to the initial level within 5 min of returning to lower temperature. Thus, an increase in F_0 does not necessarily mean irreversible damage to PSII.

Reversibility with a longer time of recovery has been found by Faria *et al.* (1996) working with *Quercus suber* L. (cork oak). They showed a midday depression of quantum yield of non-cyclic electron transport in the plants, which recovered by the evening. This midday depression of quantum yield of electron transport has also been observed by others (Adams *et al.* 1988; Ögren 1988; Bolhar-Nordenkamp *et al.* 1991) as well as recovery in the evening on the same day (Ögren 1988). Ögren and Evans (1992) suggested that this midday depression may be due to photoinhibition. Working with six species of *Eucalyptus*, they observed photoinhibition at 37°C under a light intensity of 3000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, but no photoinhibition was observed for a leaf held at 44°C for 1 h and exposed to 100 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ in the presence of 5 kPa CO_2 .

In practice it is rare that one needs to be able to predict the rate of electron transport at temperatures that exceed T_0 by more than 5°C. It is also difficult to drive the leaf temperature much above 40°C because of transpirational cooling. To avoid leaf-to-air vapour pressure differences increasing rapidly as leaf temperature increases, one needs to increase the relative humidity in the leaf chamber. This increases the potential for condensation of water in the gas-exchange system, which would interfere with subsequent measurement. If vapour pressure difference is not held as small as possible, then the temperature difference between leaf and chamber increases. To force leaf temperatures above 40°C may require chamber temperatures that cause damage to surrounding parts of the leaf through the gasket.

In reviewing many temperature responses, only two cases emerged in which the rate declined more rapidly above the optimum than below it. For *Phaseolus vulgaris*, the rate declined very abruptly above 44°C (Tenhunen *et al.* 1976a). For *T. cordata* and *Populus tremula* (Niinemets *et al.* 1999), the rates measured at 49°C were below that predicted by eqn 2. However, 49°C was sufficient to cause damage evident from the rise in F_0 , so an irreversible decline could

be expected. An abrupt deactivation was suggested for the data from *Juglans regia* L. (Dreyer *et al.* 2001), but eqn 2 fits equally well to the data. All of the response curves obtained here with soybean were well explained by eqn 2.

Consequently, given the fact that the response curve is reversible up to 40°C, eqn 2 is a simpler function to use for the temperature response of photosynthetic electron transport. Rather than ascribing biochemical significance to the activation, deactivation and entropy terms, one simply needs T_0 and Ω . The range of normalised temperature responses seen between species are readily fitted by eqn 2. Most of the variation is simply associated with variation in T_0 (Fig. 5). The remaining variation is largely accounted for by Ω . While this parameter differs between the individual responses, there is no apparent linkage to either species or growth condition apart from a tendency for Ω to increase with T_0 (Fig. 4).

T_0 , the optimum temperature

The parameter explaining most of the variation in response curves was T_0 . For some species, it is clear that T_0 can be influenced by the growth temperature. Increasing growth temperature from 20 to 45°C for *Nerium oleander* L. increased T_0 from 36 to 45°C (Björkman *et al.* 1978). For *Abutilon theophrasti* Medik., increasing growth temperature from 16 to 32°C increased T_0 from 28 to 38°C (Fig. 3B; Ziska 2001). Smaller responses to growth temperature were found for soybean (Fig. 3A) and tobacco (Bernacchi *et al.* 2003). Generally, the change in T_0 is considerably less than the change in growth temperature. Bernacchi *et al.* (2003) present an equation to predict the rate of photosynthetic electron transport on the basis of growth and measurement temperatures. They also used a Gaussian-type of curve, but with a more complex form than eqn 2. Overall, eqn 2 gives a significantly better fit to their data than their eqn 12 (Table 3). Equation 2 does particularly well for the 14/10°C

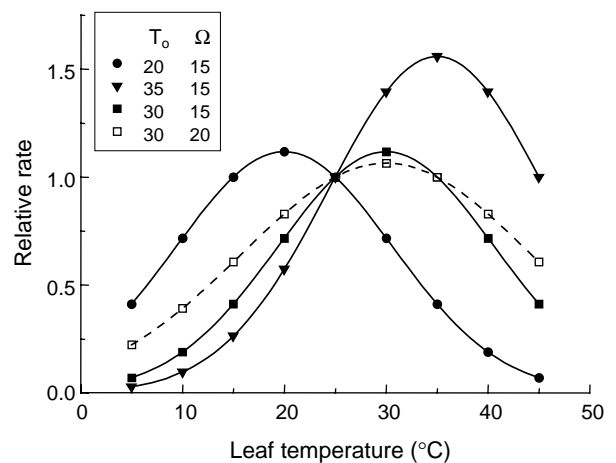


Fig. 5. Temperature response curves normalised to 1 at 25°C. The three solid curves differ only in T_0 , while the dashed curve has a greater value for Ω .

treatment. While the function used by Bernacchi *et al.* (2003) includes a term for the growth temperature, it is unreliable because the relationship between the optimum and growth temperatures is poorly described by their function.

For many of the published temperature responses, data were not collected above T_o . Consequently, when fitting eqn 2 to these data, T_o is actually an extrapolated estimate (e.g. tobacco at 25 and 35°C, Table 2). In the absence of an optimum, eqn 2 can still be fitted closely to the data. However, it is probably unwise to interpret the derived values of T_o and Ω . For example, constraining the value of Ω in tobacco to that observed for the 14/10°C treatment where a clear T_o was obtained (i.e. $\Omega = 20.7$), lowers the fitted estimate of T_o from 55°C to approximately 40°C. This value is more consistent with the T_o we estimated for soybean grown at 25 or 32°C, but the fit to the data is not as good. Despite the excellent fit that eqn 2 made to different

datasets, if rates need to be calculated outside the calibration range of the original data, they should be used with caution.

$J(T_o)$, the rate of electron transport at the optimum temperature

The photosynthesis model (Farquhar *et al.* 1980) defined a parameter J_{max} for the light-saturated potential rate of electron transport. For a given irradiance, the actual rate of electron transport, J , is less than J_{max} . The irradiance dependence of J is well described by a non-rectangular hyperbolic function (Farquhar and Wong 1984; Ögren and Evans 1993). To reach J_{max} often requires an irradiance that is not readily achieved or could be damaging if the leaf is exposed to it for an extended period while another variable is altered. When estimating J from fluorescence, the measurement error increases at high irradiance. Consequently, it is often preferable to make measurements at an irradiance that is not saturating, i.e. is not J_{max} . For the measurements

Table 2. Temperature responses of J from the literature fitted with eqn 2

Growth temperature of *N. tabacum* = 14/10, 25/20, 35/28°C day/night; LN = 33%, HN = 100% nutrient; EW = elevated CO₂ and warmed, W = warmed, EC = elevated CO₂ and cool, C = cool. Data for I reported as J_{max} , but typically measured under high irradiance

Species	I ($\mu\text{mol quanta m}^{-2} \text{s}^{-1}$)	$J(T_o)$	T_o	Ω	Reference
Herbaceous					
<i>Abutilon theophrasti</i>	1640	202	28.4	22.3	Ziska (2001)
	20	244.7	29.7	21.2	
	28	268.8	35.2	21.9	
	32	294.1	37.7	21.4	
<i>Glycine max</i>	to I_{sat}	82	35.2	16.7	Harley <i>et al.</i> (1985)
<i>Gossypium hirsutum</i>	1200	296.5	33.6	20.5	Weis and Berry (1988)
	1500	229.2	36.5	17.0	Harley <i>et al.</i> (1992)
	1800	258	36.6	13.9	Crafts-Brandner and Salvucci (2000)
<i>Hordeum vulgare</i>		23	32.7	25.1	Labate <i>et al.</i> (1990)
<i>Nicotiana tabacum</i>	14/10	271.2	34	20.7	Bernacchi <i>et al.</i> (2003)
	25/20	408	55.6	30.2	
	35/28				
		386	55.7	34.7	
<i>Phaseolus vulgaris</i>			37.3	18.7	Tenhunen <i>et al.</i> (1976a)
Trees					
<i>Acer pseudoplatanus</i>	> 1000	167.8	30.6	16.9	Dreyer <i>et al.</i> (2001)
<i>Betula pendula</i>	> 1000	161.7	35.9	21.6	Dreyer <i>et al.</i> (2001)
<i>Eucalyptus pauciflora</i>	1350		31.8	22.0	Kirschbaum and Farquhar (1984)
<i>Fagus sylvatica</i>	> 1000	166.9	34.7	18.7	Dreyer <i>et al.</i> (2001)
<i>Fraxinus excelsior</i>	> 1000	189	32.1	16.7	Dreyer <i>et al.</i> (2001)
<i>Juglans regia</i>	> 1000	143.2	35.7	18.5	Dreyer <i>et al.</i> (2001)
<i>Pinus radiata</i>	LN	> 1000	47.9	33.8	Walcroft <i>et al.</i> (1997)
	HN	–	63.1	34.6	
<i>Pinus sylvestris</i>	EW	to I_{sat}	155.7	21.9	Wang <i>et al.</i> (1996)
	W	–	161.8	23.8	
	EC	–	145.7	20.7	
	C	–	155.7	21.9	
<i>Populus tremula</i>	1650	191.4	35.6	12.5	Niinemetts <i>et al.</i> (1999)
<i>Prunus persica</i>	1000	175	40.0	23.6	Walcroft <i>et al.</i> (2002)
<i>Quercus petraea</i>	> 1000	231.6	39.4	22.5	Dreyer <i>et al.</i> (2001)
<i>Quercus robur</i>	> 1000	226.3	40.6	25.8	Dreyer <i>et al.</i> (2001)
<i>Tilia cordata</i>	1650	52.2	39.2	16.9	Niinemetts <i>et al.</i> (1999)

reported here, an irradiance of 1200 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ was used.

In addition to the fluorescence data presented here, the rate of electron transport was calculated from gas-exchange measurements. The response of J to irradiance was measured under a range of temperatures (June 2002). We calculated the ratio of J measured at 1200 and 2000 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (0.82 ± 0.01). The value was independent of measurement and growth temperature despite variation in J_{max} and the curvature factor θ . Therefore, we are confident that the temperature-response functions presented here represent an unbiased estimate.

While we did not make concurrent calculations of electron transport rate from fluorescence and gas-exchange measurements, this has been done repeatedly elsewhere (e.g. Genty *et al.* 1989; Hudson *et al.* 1992; Oberhuber and Edwards 1993; Bernacchi *et al.* 2003). The two estimates have been found to be strongly correlated. This indicates strong feedback links between the rate of consumption of RuBP assessed from CO_2 assimilation and photochemical efficiency of PSII inferred from fluorescence. There are several potential areas that could weaken the correlation. Gas exchange integrates over the entire leaf area enclosed in the chamber, whereas fluorescence samples predominantly from the mesophyll tissue nearest the surface probed by the modulated light source. Photosynthetic electron transport to electron acceptors other than the cycles of RuBP carboxylation and oxygenation have been postulated, but appear to be very minor given the close correlation between rates of electron transport calculated from gas exchange and fluorescence.

The electron transport capacity of a leaf varies depending on nutrient treatment (Fig. 2), leaf age, growth irradiance (von Caemmerer and Farquhar 1981) and growth temperature (Fig. 3). Variation in electron transport capacity at a common temperature between leaves is linearly related to the nitrogen content per unit leaf area (Evans 1996; Walcroft *et al.* 1997; Niinemets *et al.* 1999). Since the temperature response is unrelated to the electron transport capacity, it is convenient to use a single temperature-response function multiplied by a scaling factor for each leaf. The scaling factor is likely to be directly proportional to the nitrogen content per unit leaf area, which provides a convenient starting point for modelling.

Table 3. Comparison between two functions relating electron transport rate to leaf temperature for tobacco grown under three different temperatures

Functions were compared with the data [Bernacchi *et al.* (2003), Fig. 2] and chi squared values determined for each growth temperature

Growth temperature	Bernacchi eqn 12	June eqn 2
14/10	1873	24
25/20	307	135
35/28	96	44

Mechanism for the decline in rate of electron transport above the optimum temperature

The photosynthetic electron transport rate measured on an intact leaf reflects many processes that are tightly coupled. Dissecting out the mechanisms that result in a decline in the rate of electron transport at supraoptimal temperatures has not been easy. Evidence supporting three different mechanisms has been obtained. Direct measurements of uncoupled whole-chain or PSII electron transport decline above an optimal temperature [*Hordeum* T_0 32°C (Nolan and Smillie 1976); *Larrea* T_0 42°C (Armond *et al.* 1978); *Nerium* T_0 45–47°C (Björkman *et al.* 1978)]. However, Kobza and Edwards (1987) proposed that Rubisco activation state rather than electron transport limited the rate at high temperatures. In addition to the decline in Rubisco activity, the pool sizes of PGA fell and RuBP rose in these wheat leaves as temperatures increased above 25°C. Subsequent work with cotton and wheat has supported the suggestion. The decline in photosynthesis at supraoptimal temperatures correlated strongly with Rubisco activity (Law and Crafts-Brandner 1999). Rubisco activase is the most temperature-labile enzyme (Salvucci *et al.* 2001) and the decline in Rubisco activity has been related to the loss of Rubisco activase activity (Crafts-Brandner and Salvucci 2000). However, this is not the only mechanism at work. Experiments with plants lacking virtually all Rubisco activase still show a decline in photosynthesis at high temperature without any change in the carbamylation state of Rubisco (Sharkey *et al.* 2001). At supraoptimal temperatures, rather than RuBP pools always increasing (Kobza and Edwards 1987; Law and Crafts-Brandner 1999; Sharkey *et al.* 2001), they have also been found to fall (e.g. sunflower Oja *et al.* 1988). Oja *et al.* (1988) suggested that this could be due either to a shortage of ATP at supraoptimal temperatures, or metabolite drainage from the chloroplast resulting in inhibition of Rubisco by free phosphate. For the soybeans measured here, photosynthetic electron transport rate calculated from gas exchange at a leaf temperature of 40°C was still responsive to increases in irradiance approaching 2000 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (June 2002). This suggests that photosynthesis in soybean was not simply limited by Rubisco activity or RuBP regeneration, but was controlled by the rate of electron transport.

The resultant curve of J with respect to leaf temperature changes shape depending on the concentration of CO_2 . At low CO_2 , the curve is most likely to reflect the change in kinetic properties of Rubisco with respect to temperature except at high temperature, where the decline reflects decreasing carbamylation associated with loss of Rubisco activase activity. At high CO_2 , the increase with temperature probably is a true reflection of the increasing rate of electron transport. However, the reversible decline in rate above T_0 may be due to one of several processes including a fall in electron transport capacity, a loss of Rubisco activity or a

loss of Calvin cycle intermediates. Elucidating the mechanism(s) behind the decline in electron transport at supra-optimal temperatures remains a challenge, but regardless of the mechanism, it is hoped that eqn 2 will prove useful for calculating photosynthesis whenever variable temperatures are encountered.

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