Electron transport is the functional limitation of photosynthesis in field-grown Pima cotton plants at high temperature

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ABSTRACT

Restrictions to photosynthesis can limit plant growth at high temperature in a variety of ways. In addition to increasing photorespiration, moderately high temperatures (35–42 °C) can cause direct injury to the photosynthetic apparatus. Both carbon metabolism and thylakoid reactions have been suggested as the primary site of injury at these temperatures. In the present study this issue was addressed by first characterizing leaf temperature dynamics in Pima cotton (*Gossypium barbadense*) grown under irrigation in the US desert south-west. It was found that cotton leaves repeatedly reached temperatures above 40 °C and could fluctuate as much as 8 or 10 °C in a matter of seconds. Laboratory studies revealed a maximum photosynthetic rate at 30–33 °C that declined by 22% at 45 °C. The majority of the inhibition persisted upon return to 30 °C. The mechanism of this limitation was assessed by measuring the response of photosynthesis to CO_2 in the laboratory. The first time a cotton leaf (grown at 30 °C) was exposed to 45 °C, photosynthetic electron transport was stimulated (at high CO_2) because of an increased flux through the photorespiratory pathway. However, upon cooling back to 30 °C, photosynthetic electron transport was inhibited and fell substantially below the level measured before the heat treatment. In the field, the response of assimilation (*A*) to various internal levels of CO_2 (C_i) revealed that photosynthesis was limited by ribulose-1,5-bisphosphate (RuBP) regeneration at normal levels of CO_2 (presumably because of limitations in thylakoid reactions needed to support RuBP regeneration). There was no evidence of a ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) limitation at air levels of CO_2 and at no point on any of 30 *A*–*C*$_i$ curves measured on leaves at temperatures from 28 to 39 °C was RuBP regeneration capacity measured to be in substantial excess of the capacity of Rubisco to use RuBP. It is therefore concluded that photosynthesis in field-grown Pima cotton leaves is functionally limited by photosynthetic electron transport and RuBP regeneration capacity, not Rubisco activity.

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INTRODUCTION

High temperature reduces plant growth and can limit crop yields. Estimates range up to a 17% decrease in yield for each degree Centigrade increase in average growing season temperature (Lobell & Asner 2003). Photosynthesis is one component of crop growth that is most sensitive to high temperature and photosynthetic rates usually peak at about 30 °C with significant declines in assimilation for each additional degree increase.

The effects of high temperature on photosynthesis have been investigated for some time. For many years photosystem II (PSII) was considered the most temperature-sensitive step in photosynthesis (Santarius 1975; Berry & Björkman 1980) but it appears from numerous reports that PSII inhibition does not occur until leaf temperatures are quite high, usually 40 °C and above (cf. Havaux 1993; Al-Khatib & Paulsen 1999). Recently, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) has been shown to deactivate at temperatures that cause no harm to PSII (Feller, Crafts-Brandner, & Salvucci 1998) and this deactivation has been proposed to be the primary constraint to photosynthesis in this temperature range (Crafts-Brandner & Salvucci 2000). The deactivation is presumed to result from loss of activity of Rubisco activase (Salvucci *et al.* 2001) or perhaps binding of activase to the thylakoid membrane (Rokka, Zhang, & Aro 2001). However, there are many reports of moderate heat damage to components of photosynthetic electron transport other than PSII, especially increased thylakoid proton conductance (summarized in Schrader *et al.* 2004). Treatments that might be expected to strengthen thylakoid membranes such as inducing high levels of zeaxanthin (Havaux *et al.* 1996) or pro-

viding isoprene (Sharkey, Chen, & Yeh 2001a) protect photosynthesis from moderately high temperatures that do not damage PSII. This opens the possibility that the deactivation of Rubisco is an adaptation or protective mechanism in response to high-temperature sensing by the thylakoid membrane.

One source of variability in the reported effects of heat stress on photosynthesis is the method of stressing leaves. Often, leaf pieces are floated on water. This ensures good thermal contact and good control over leaf temperature, but it means that one of the leaf surfaces will not have access to CO_2. This heat stress will be very constant due to the large heat capacity of water. However, leaves in a natural environment, oak leaves for example, can experience rapid temperature fluctuations, in some cases exceeding 10 °C variation within 1 min (Singsaas & Sharkey 1998; Singsaas *et al*. 1999). Although oak-leaf temperatures often exceed air temperature by 10 °C (Singsaas & Sharkey 1998), cotton is one of few plants that transpire enough water to substantially cool its leaves below air temperature. The large water loss cools the cotton leaves (Radin *et al*. 1994; Lu *et al*. 1997) but it is not known if it also causes cotton leaf temperature to be more stable than oak leaf temperature. It is important to understand leaf temperature dynamics during warm weather to assess how heat damages photosynthesis and to know precisely how plants respond to heat stress. Measuring leaf temperature is technically challenging and often only time-averaged leaf temperatures are reported (Martin *et al*. 1999). If rapid, severe leaf temperature changes are common, time-averaged leaf temperature will not accurately describe the heat stress that leaves experience.

Measurements of the response of photosynthesis to varying CO_2 concentrations can be quite useful in determining underlying biochemical limitation(s). Because the concentration of Rubisco ribulose-1,5-bisphosphate (RuBP) binding sites in chloroplasts is typically much higher than the K_m of Rubisco for RuBP, photosynthesis in a leaf with fully activated Rubisco is typically limited by the regeneration of RuBP or the amount of Rubisco but not both. Whole-leaf C_3 photosynthesis tends to respond to CO_2 either according to Rubisco-limited kinetics or RuBP-regeneration-limited kinetics (Farquhar, Von Caemmerer, & Berry 1980). The limiting factor can be assessed using curves representing the response of photosynthesis (A) to CO_2 inside the leaf (to avoid stomatal effects) (C_i). Analysis of A–C_i curves has become an important tool for studying photosynthesis characteristics of plants in natural and agronomic situations (Wullschleger 1993; Long & Bernacchi 2003). If Rubisco activation constrains photosynthesis at moderately high temperature then the A–C_i curve should appear to be Rubisco-limited over a wide range of C_i. On the other hand, if thylakoid proton conductance or some other component of electron transport is the functional constraint on photosynthesis at moderately high temperature, then the A–C_i curve will be better described by equations that describe RuBP regeneration-limited photosynthesis.

In addition to stomatal conductance, there is a mesophyll conductance to CO_2 diffusion. Bernacchi *et al*. (2002) have recently shown that the mesophyll conductance increases with temperature faster than does maximum Rubisco activity. Nevertheless, they estimated that mesophyll conductance can play a larger role in limiting photosynthesis at high temperature than at low temperature. Mesophyll conductance can decline with temperature above 38 °C and so could account for declines in photosynthesis above this temperature but not below this temperature.

In this study, cotton leaf temperature was measured in the field during several hot days likely to cause damage to photosynthesis. Cotton was chosen because it normally is grown in conditions in which photosynthesis may be limited by high temperature and because stomatal conductance is normally quite high and has little effect on photosynthesis (Radin *et al*. 1994). Fine-wire thermocouples were attached to leaves and leaf temperature monitored throughout the day. Based on these data, a protocol measuring photosynthesis at 30 °C and comparing data from leaves at 42 °C was developed. Both laboratory and field-based measurements of A–C_i curves were then made to assess which component process most limits photosynthesis in cotton leaves at moderately high temperature.

MATERIALS AND METHODS

Plant material

For field studies, Pima cotton (*Gossypium barbadense* L. cv S-7) was planted on 22 April 2003 at the Maricopa Agricultural Center of the University of Arizona (as described in Radin *et al*. 1994). The plants were flood-irrigated once per week. Leaf temperatures, leaf incident irradiation, and photosynthesis were measured on 16–20 June 2003 as described below. For laboratory studies, Pima S-6 plants were grown as described in Schrader *et al*. (2004).

Measurements of leaf temperature and photosynthetic photon flux density in the field

Leaf temperatures and the photosynthetic photon flux density incident on the leaf surface were measured at 2-s intervals on six separate leaves of field-grown Pima cotton and recorded with a data logger (Model CR10; Campbell Scientific, Logan, UT, USA). Fine wire thermocouples (0.762 mm diameter: Omega Engineering, Stamford, CT, USA) as described in Singsaas & Sharkey (1998) and Hanson *et al*. (1999) were used. Two thermocouples were routed to the abaxial leaf surface. For one, the hot junction was approximately 2 cm from the leaf surface whereas the cold junction was connected to a cold-junction compensator; this measured air temperature (T_{air}). The other thermocouple had the hot junction about 2 cm from the leaf and the cold junction appressed to the abaxial surface. This measured the difference between leaf and air temperature (ΔT). T_{leaf} was then calculated as $T_{air} - \Delta T$.

Gas exchange in the laboratory

Measurements of photosynthesis in the laboratory were made using a system described in Loreto & Sharkey (1990). Attached, intact leaves were clamped in an aluminium cuvette with a clear, glass top. Cuvette temperature was maintained by six Peltier blocks embedded in the cuvette base and controlled to ±0.1 °C with an electronic controller (Newport Electronics, Santa Anna, CA, USA). The air inside the cuvette was stirred with fans to reduce boundary layer resistance. Partial pressures of CO_2, O_2 and N_2 were established with mass flow controllers (Datametrics no. 825; Edwards High Vacuum, Wilmington, MA, USA). CO_2 partial pressure and dew point in the air supplied to and exiting from the cuvette were measured with a Li-Cor model 6262 infrared gas analyser (Li-Cor Inc., Lincoln, NE, USA). Light (1200 μmol photons m^{-2} s^{-1}) was provided by a 2.5-kW xenon arc lamp and measured with a Li-Cor model 190SB quantum sensor. The equations of von Caemmerer & Farquhar 1981) were used to calculate photosynthetic rates.

During gas exchange measurements chlorophyll fluorescence was measured with a Hansatech FMS2 field-portable, pulse modulated chlorophyll fluorimeter (Hansatech Institute, Kings Lynn, Norfolk, UK). The instantaneous quantum yield (Φ_{PSII}) was calculated using the equation of Genty, Briantais & Baker (1989).

Gas exchange in the field

Photosynthesis was measured on field-grown plants using a CIRAS-1 portable photosynthesis system (PP Systems, Amesbury, MA, USA). The response of photosynthesis to varying concentrations of CO_2 was recorded by measuring photosynthesis in approximately 100 p.p.m. steps from 1000 p.p.m. external $[CO_2]$ down to 30 p.p.m.

RESULTS

Leaf temperatures of field-grown Pima cotton were measured over the course of 5 d in mid-June, 2003. Early in the morning leaf temperature was within a few degrees of and usually below air temperature (typical data shown in Fig. 1). As air temperature increased, leaf temperature increased until the air temperature exceeded about 35 °C. At this point, the leaf temperature became erratic and sometimes substantially below air temperature. Over one 15-min period leaf temperature was as low as 26.3 and as high as 38.2 °C (Fig. 2). Leaf temperature often changed by more than 1 °C per second. A 5-min running average of leaf temperature (heavy line in Fig. 2) does not capture the fact that this leaf was exposed to potentially damaging temperatures frequently during the day.

When measured in the laboratory, Pima cotton photosynthesis had a broad temperature optimum centred around 30 °C, which was reduced by 22% at 45 °C. The high-temperature inhibition persisted upon returning leaves to 30 °C (Fig. 3).
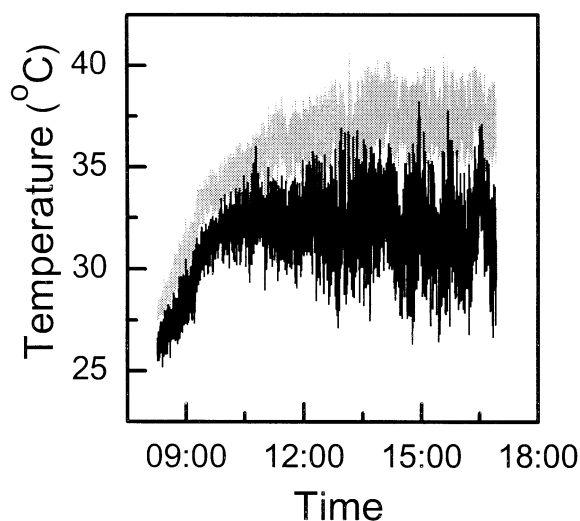

Figure 1. Leaf temperature dynamics in field-grown cotton (*Gossypium barbadense*). Leaf temperature (black lines) and air temperature (grey lines) were measured every 2 s for 8 h on 18 June 2003.

Field measurements taken at a variety of carbon dioxide concentrations allowed for the construction of A–C_i curves and a computation of assimilation rates at both normal and high mesophyll CO_2 concentrations. Eleven A–C_i curves were constructed over 3 d and a second-order polynomial was fitted to each data set. The equation of that line was then used to calculate assimilation at a C_i of 22 or 60 Pa. A plot of those values of assimilation versus leaf temperature (Fig. 4) revealed that the approximately 20–25% inhibition of photosynthesis seen at high temperatures in laboratory plants (Fig. 3) was also evident in field-grown Pima cotton. In addition, although high CO_2 stimulated assimilation and
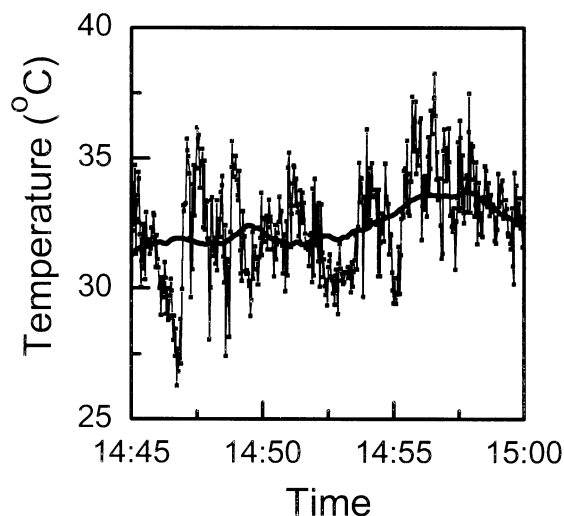

Figure 2. Leaf temperature and averaged leaf temperature during a 15-min time period in mid-afternoon. Leaf temperatures from the data used for Fig. 1 (points connected by thin lines) plus the running 5-min average of leaf temperature (thick line).
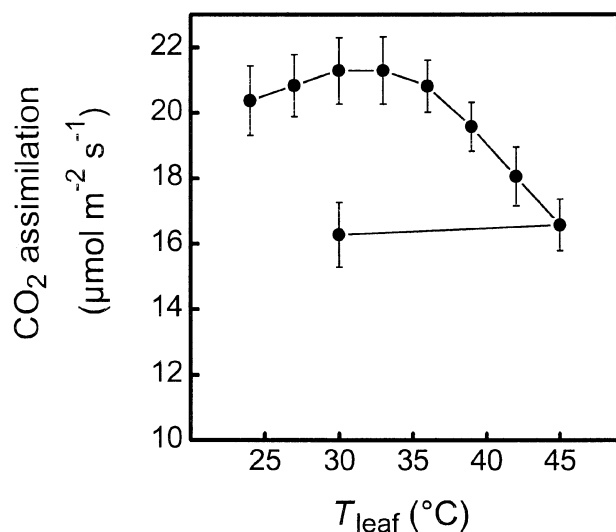
Figure 3. Temperature dependency of photosynthesis measured in growth-chamber-grown Pima cotton (plants grown at 32 °C). Photosynthesis was measured in intact, attached leaves from 24 to 45 °C, in 3° increments at a C_a of 36 Pa. Leaves were then returned to 30 °C for a final measurement. Approximately 3 min elapsed between measurements at the various temperatures; therefore, each profile took about 30 min (mean ±SE, $n = 8$).

shifted the temperature optimum about 3 °C higher, the high-temperature inhibition was not eliminated.

The relationship between assimilation, electron transport, CO_2 concentration, and rapid changes in leaf temper-
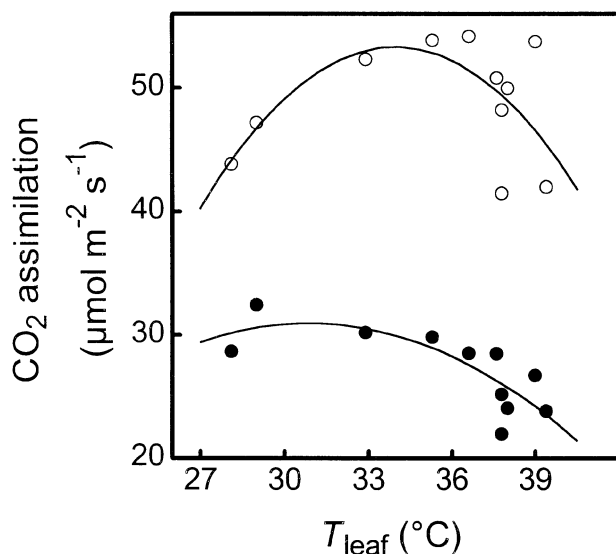

Figure 4. Temperature dependency of photosynthesis at a C_i of 22 Pa (closed circles) and 60 Pa (open circles) measured in field-grown Pima cotton. Photosynthesis was measured on 11 separate leaves at C_a values of 110 down to 3 Pa. Second-order polynomials were fitted to those $A–C_i$ curves and the equation of those curves were used to calculate assimilation at C_i values of 22 and 60 Pa. All leaves were at or above 1200 μmol photons m^{-2} s^{-1} (irradiance data not shown).
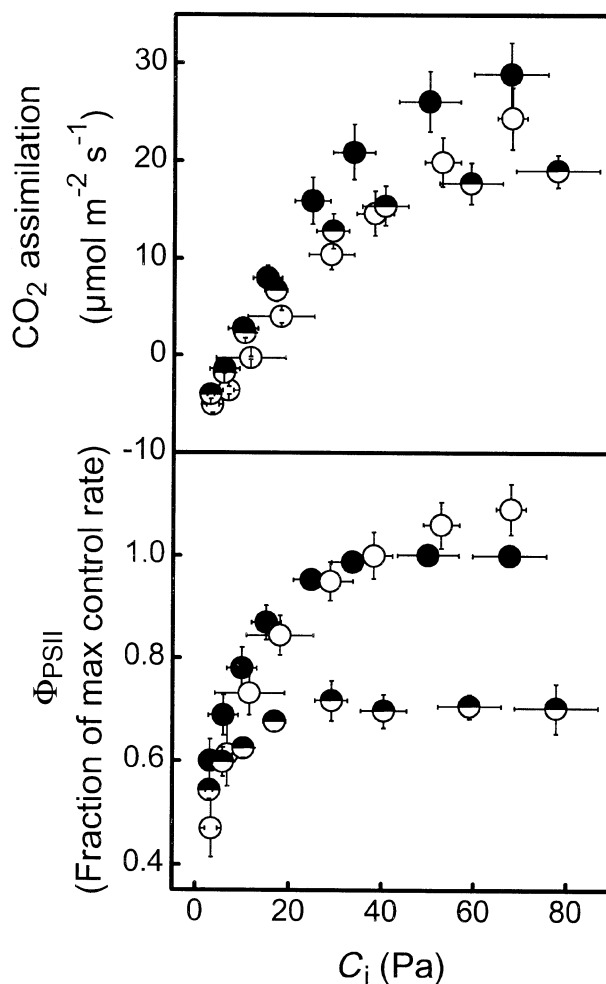

Figure 5. $A–C_i$ and $\Phi_{PSII}–C_i$ curves before (closed symbols), during (open symbols), and after (half-closed symbols) heat stress. Leaves were first measured at 30 °C and eight different [CO_2], then rapidly heated to 42 °C for 5 min before commencing 12 min of measurement at the various C_a values. Leaves were then allowed to recover for 25 min at 30 °C prior to the final measurement at the various [CO_2] (mean ±SE, $n = 3$).

ature was further investigated in laboratory-grown plants. Attached leaves were measured at 30 °C, then 45 °C, then again at 30 °C. The $A–C_i$ response and the response of Φ_{PSII} to C_i are shown in Fig. 5. Increasing C_i stimulated assimilation in all three cases. However, during and after the heat spike, photosynthesis was less at all values of C_i than before the spike.

Before heating, Φ_{PSII} increased with C_i up to 35 Pa but then did not increase any further. During heating, Φ_{PSII} increased with C_i over the entire measurement range and at high C_i exceeded the maximum rate observed before heating. Following the heat stress, Φ_{PSII} increased with C_i only up to 20 Pa and the maximum rate was significantly lower than either before or during the heat stress. CO_2 assimilation at a C_i of 22 Pa during the heat spike was reduced 54%, from 15.6 to 7.1 μmol CO_2 m^{-2} s^{-1} whereas electron transport under the same conditions was reduced
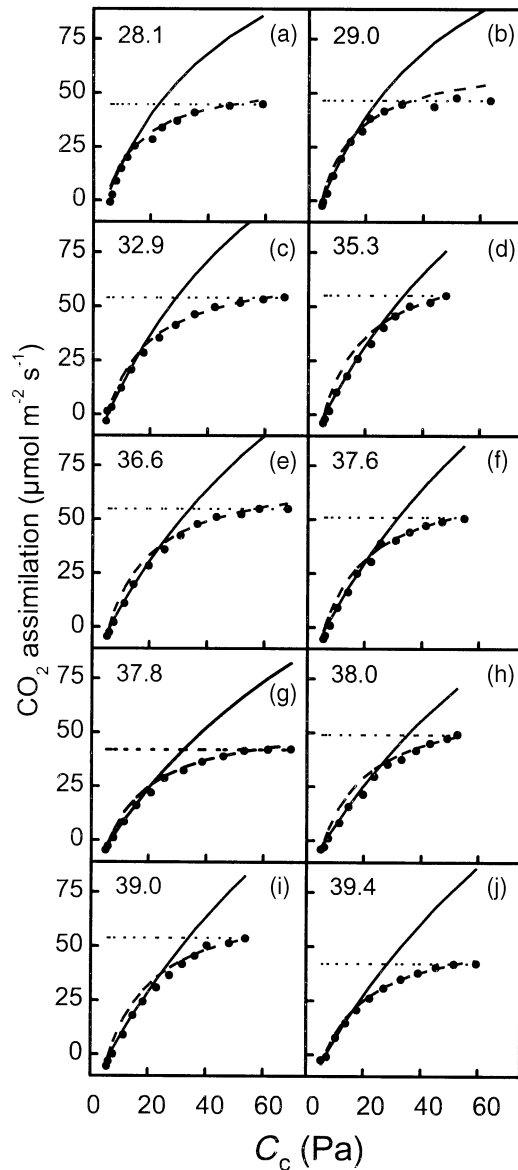
Figure 6. The response of assimilation to chloroplast CO_2 at leaf temperatures ranging from 28 to 39 °C measured in field-grown Pima cotton on 18–20 June 2003. Chloroplast CO_2 was estimated using a mesophyll conductance at 25 °C of 0.625 μmol m^{-2} s^{-1} bar^{-1} as estimated from data in Fig. 5 and Loreto *et al.* (1992) plus the temperature dependence of mesophyll conductance published by Bernacchi *et al.* (2002). Closed circles represent the raw data. The solid line indicates the predicted photosynthetic rate if Rubisco kinetic properties were the only limiting factor [using the parameters of Bernacchi *et al.* 2001, 2002 in the Farquhar model (Farquhar *et al.* 1980)]. The dashed line was predicted using electron transport as the sole limiting factor. The dotted line was predicted using triose-phosphate utilization as the sole limiting factor. (a) T_{leaf} = 28.1 °C, time of day (TOD) = 0659–0719 h. (b) T_{leaf} = 29.0 °C, TOD = 0804–0833 h. (c) T_{leaf} = 32.9 °C, TOD = 0901–0926 h. (d) T_{leaf} = 35.3 °C, TOD = 1234–1254 h. (e) T_{leaf} = 36.6 °C, TOD = 1042–1105 h. (f) T_{leaf} = 37.6 °C, TOD = 1119–1152 h. (g) T_{leaf} = 37.8 °C, TOD = 1314–1331 h. (h) T_{leaf} = 38.0 °C, TOD = 1614–1639 h. (i) T_{leaf} = 39.0 °C, TOD = 1404–1429 h. (j) T_{leaf} = 39.4 °C, TOD = 1456–1516 h.

only 5%, from 94 to 89% of the maximum control rate. This large loss of CO_2 uptake in the presence of a much smaller decline in electron transport is consistent with the well-described stimulating effect of heat on photorespiration (Oberhuber & Edwards 1993). Modelling confirmed that this decline in CO_2 uptake could be caused mostly by photorespiration but increased mitochondrial respiration as described in Bednarz & Van Isreal (2001) could also have contributed.

Ten *A*–*C*$_i$ curves were measured on field-grown Pima S-7 plants to determine what limits photosynthesis under natural conditions (Fig. 6). All 10 *A*–*C*$_i$ curves were measured when ambient light levels exceeded 1200 μmol photons m^{-2} s^{-1}. Leaf temperatures were not regulated and largely reflected the ambient air temperature but the temperature of a single leaf did not vary by more than 2 °C during the measurements. Curves were analysed by fitting the data to the Farquhar model of photosynthesis (Farquhar *et al.* 1980) plus end product limitation (Sharkey 1985). The model was parametrized using the data of Bernacchi *et al.* (2001, 2002). The mesophyll conductance at 25 °C was taken to be 0.625 mol m^{-2} s^{-1} based on data in Fig. 5 and Loreto *et al.* (1992). The CO_2 concentration in the stroma was calculated using *A* and *C*$_i$ data from the gas exchange measurements plus equations in Bernacchi *et al.* (2002). Data were fitted by adjusting V_{cmax}, J_{max}, and triose phosphate use rate upward until none of the data points were significantly below the predicted line. In most curves all three limitations could be seen but in some cases it was not clear that photosynthesis was ever limited by Rubisco (e.g. 39.4 °C data) or by triose phosphate use (e.g. 38.0 °C data). Nineteen additional curves measured on Pima S-2 and S-3 plants gave the same result (data not shown).

Fig. 7 shows the values of V_{cmax} and J_{max} used to generate the theoretical lines shown in Fig. 6. It can be seen that the value for J_{max} required to explain the temperature dependency of the electron transport limitation shown in Fig. 6 is in the range of 200–300 μmol m^{-2} s^{-1} over the entire tem-
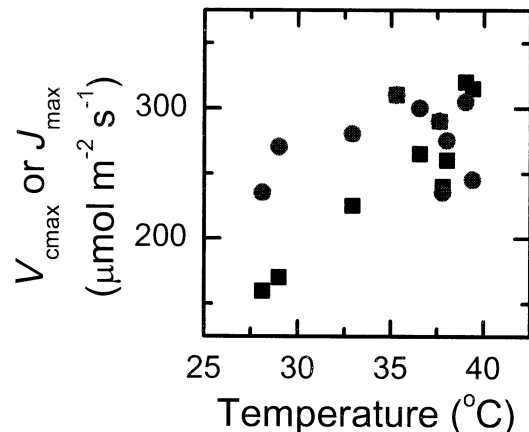

Figure 7. Theoretical V_{cmax} (squares) and J_{max} (circles) versus leaf temperature. These are the values of V_{cmax} and J_{max} used to generate the curves shown in Fig. 6.

perature range whereas the value for V_{cmax} needed to explain the temperature-dependency of the Rubisco limitation shows no maximum. Theoretical values of J_{max} needed to fit the data did not increase with temperature. End product synthesis often limited photosynthesis at high CO_2 as evidenced by the lack of response to increasing CO_2, but only at high $[CO_2]$.

DISCUSSION

Cotton leaf temperature can be substantially below air temperature (Fig. 1) and the ability to reduce leaf temperature is correlated with increased yield in this crop (Radin *et al.* 1994; Lu *et al.* 1997). The correlation between leaf temperature and yield is evidence that photosynthesis is limited by heat stress in cotton. The substantial under-temperatures of cotton are possible because of the large leaf size (reducing boundary layer conductance and so sensible heat gain) and very high stomatal conductance (allowing high rates of water loss for evaporative cooling). Furthermore, because the sky was cloudless on the days that leaf temperature was measured there was the potential for substantial long-wave radiant heat loss to the sky. Leaf temperature varied rapidly and many short, high-temperature episodes were experienced by the leaves. These rapid temperature changes have been reported for oak trees (Singsaas & Sharkey 1998) and moss (Singsaas & Sharkey 1998; Hanson *et al.* 1999), which typically have low stomatal conductances and a leaf temperature that is higher than air temperature. Although the high rates of evaporation of cotton kept leaf temperatures below air temperature (Fig. 1) its leaves still suffered frequent large heat flecks (Fig. 2).

High temperatures limit photosynthesis in both cultivated (Al-Khatib & Paulsen 1999) and native species (Hamerlynck & Knapp 1996). In Pima cotton, temperatures above 33 °C inhibited photosynthesis and that inhibition persisted upon the return to 30 °C (Fig. 3). Cotton grown under normal agronomic practices experiences temperature that is high enough to inhibit photosynthesis (Figs 1 & 2). Because of this, it is important to know how photosynthesis is limited following exposure to a dynamic and episodic high-temperature stress, not just during the first high temperature experienced by a leaf as is normally done in laboratory experiments.

Photosynthesis can be limited biochemically by one of three basic mechanisms: (1) Rubisco activity; (2) the rate of RuBP regeneration (which depends on the photosynthetic electron transport system); or (3) the rate of triose phosphate use. End product synthesis limitations could be seen in the field data but only at non-physiological CO_2 concentrations (Fig. 6). We therefore discount their involvement in high-temperature limitations to photosynthesis at ambient CO_2 concentrations. It has also been suggested that the mesophyll resistance to CO_2 diffusion can limit photosynthesis at high temperature (Bernacchi *et al.* 2002).

If Rubisco activity were the sole factor limiting whole-leaf photosynthesis following a heating episode, then it should be possible to remove that limitation by increasing the supply of substrate (CO_2) to the enzyme. If increasing carbon dioxide does not remove or alleviate the inhibition, or has much less of an effect than in control plants, then the limitation lies (at least partially) in electron transport. The same thing is true for a limitation by mesophyll resistance (or its inverse, mesophyll conductance). If the limitation is not reversed by high CO_2 then the problem cannot be low mesophyll conductance.

Three different sets of experiments reported here support RuBP regeneration (which reflects electron transport) as the limiting factor. First, Fig. 4 demonstrates that at temperatures above about 33 °C, high $[CO_2]$ did not relieve the high-temperature-induced limitation to photosynthesis seen in field-grown Pima cotton. Second, when laboratory plants were supplied with high $[CO_2]$ after a brief heating episode, the enhancement to photosynthesis was markedly less than before heating (compare Fig. 5a, closed circles to Fig. 5a, half-open circles). Third, the application of theoretical limits derived from robust models of C_3 photosynthesis indicate that Rubisco limitations cannot explain the data collected in the field, whereas electron transport limits can (Fig. 6). In fact, the models require ever-increasing kinetic parameters for Rubisco catalysis as temperature increases (Fig. 7) that greatly exceed photosynthetic rates measured in the field (Fig. 6). We therefore conclude that Rubisco activity is not the sole factor limiting assimilation either in the field or after a rapid heating spike in the laboratory and that electron transport plays an overriding role in limitations to whole leaf photosynthesis following a heat-stress episode. This analysis does not preclude the possibility that Rubisco deactivates at high temperature when its capacity is in excess of the capacity for RuBP regeneration.

Bernacchi, Pimentel, & Long (2003) found that J_{max} increases exponentially over the temperature range of 10–40 °C. We found no consistent trend for increasing J_{max} in these plants over the range from 30 to 40 °C and note that Bernacchi *et al.* (2003) found the same thing for tobacco grown at 14 °C. The constant J_{max} is indicative of damage to some component of RuBP regeneration at these high temperatures. This coupled with increasing photorespiration would lead to declining photosynthesis. On the other hand, the parameter V_{cmax} is already compensated for temperature in the parameterization of Bernacchi *et al.* (2002) and so should have been constant with temperature. It is unclear why the estimated V_{cmax} increased with temperature, but in any case there was no evidence for a limitation by Rubisco capacity or activation state at high temperature in the field.

In support of the limitation being at the level of electron transport, Fig. 5 shows that increasing $[CO_2]$ on a control leaf stimulates electron transport up to a C_i of about 35 Pa. Beyond that point, the curve becomes insensitive to increasing $[CO_2]$ as electron transport becomes limiting. After heating (Fig. 5b, open symbols) the electron transport limitation drops to a C_i of around 20 Pa and the slope at the lower $[CO_2]$ is much shallower than in the control plants. We therefore conclude that this large decrease in sensitivity of electron transport to $[CO_2]$ is indicative of an

electron-transport-based limitation to assimilation after heating. The mesophyll conductance estimated using the constant J method (Loreto *et al.* 1992) was 0.6 μmol m^{-2} s^{-1} indicating that mesophyll conductance was not limiting after the heating episode.

Given the large number of severe heat flecks experienced by cotton leaves (Fig. 2), the after-effects of heat are critical to the functioning of photosynthesis in the field. Therefore, the damage to photosynthetic electron transport seen *after* the first experience of moderately high temperature (Fig. 5b, half-closed circles) may be more important to normal leaf functioning than the fact that electron transport is not damaged *during* the first heating experienced by leaves (Fig. 5b, open circles).

It has been reported that Rubisco deactivates at high temperature (Feller *et al.* 1998; Crafts-Brandner & Law 2000). Although we saw substantial deactivation of Rubisco in growth-chamber-grown cotton plants experiencing their first-ever high-temperature exposure (Schrader *et al.* 2004), at no point could photosynthesis in field-grown cotton be shown to be limited by Rubisco activity (Fig. 6). One explanation for this apparent discrepancy is that the repeated exposure to heat flecks in field-grown plants (as opposed to the first-ever exposure in the laboratory) brings Rubisco activity down to the minimal level needed to use the available products of photosynthetic electron transport. Thus the functional limitation appears to be in thylakoid reactions, with Rubisco activity regulated to not greatly exceed what can be supported by RuBP regeneration. This could be an adaptive response that reduces subsequent damage to photosynthetic electron transport. In support of this notion, other studies have shown that deactivation of Rubisco reduces the after-effect of heat stress on photosynthesis (Sharkey *et al.* 2001b). The source of this limitation at the level of the thylakoid membrane is explored in a separate study (Schrader *et al.* 2004).

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