

Reduction of photosynthesis in sun leaves of *Gossypium hirsutum* L under conditions of high light intensities and suboptimal leaf temperatures

M Königer *, K Winter *

Lehrstuhl für Botanik II, Julius-von-Sachs Institut für Biowissenschaften, Mittlerer Dallenbergweg 64,
D-97082 Würzburg, Germany

(Received 20 December 1992; accepted 30 April 1993)

Summary — Attached sun leaves of cotton (*Gossypium hirsutum* L var Delta Pine 61) were enclosed in a gas exchange cuvette and exposed for 4 d to either 1 000 or 1 800 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ and a series of air temperatures (35, 30, 25 and 20°C) during the 12-h light periods. The temperature during the 12-h dark periods was maintained at 25°C. The highest CO_2 assimilation rates (A_{max} : $\approx 30 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) were reached at 1 800 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ and air temperatures of 30 and 35 °C. At 30 °C and 1 000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, A_{max} was reduced by 20%. At both light intensities A_{max} and stomatal conductance declined with decreasing temperatures. The reduction in A_{max} was most severe at 1 800 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ and 20°C. The leaves exposed to these conditions also exhibited marked decreases in rates of CO_2 -saturated photosynthetic O_2 evolution under light saturation (PS_{max}) and light limitation (\emptyset) and in F_v/F_M . These 3 parameters were less severely affected by treatment with medium light intensity (1 000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$). The data show that when sun leaves of cotton are exposed for short periods of time to temperatures only slightly suboptimal for photosynthesis, high light intensities can cause marked photoinhibition.

Gossypium hirsutum = cotton / photoinhibition of photosynthesis / temperature

Résumé — Réduction de la photosynthèse chez les feuilles héliophiles de *Gossypium hirsutum* L sous des conditions de haute intensité de lumière et de température foliaire suboptimale. Des feuilles héliophiles de cotonnier, attachées à la plante (*Gossypium hirsutum* L var Delta Pine 61) ont été mises dans une cuvette d'échange gazeux et exposées pendant 4 jours à 1 000 ou 1 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ et à une série de températures d'air (35°, 30°, 25°, 20°C) pendant les périodes d'éclaircissement de 12 h. La température, pendant les périodes obscures de 12 h, a été maintenue à 25°C. Les plus hauts taux d'assimilation de CO_2 (A_{max} : env 30 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$) ont été atteints à 1 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ et des températures de l'air de 30° et 35°C. À 30°C et 1 000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ A_{max} a diminué de 20%. Aux 2 intensités de lumière, A_{max} et la conductivité stomatique ont diminué avec la baisse des températures. La réduction en A_{max} a été très sévère à 1 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ et 20°C. Les feuilles exposées à ces conditions ont aussi montré des baisses importantes dans les taux d'évolution d' O_2 photosynthétique, à saturation en CO_2 , sous conditions de saturation en lumière (PS_{max}), de limitation en lumière (\emptyset) et en F_v/F_M . Ces 3 paramètres ont été moins sévèrement affectés par le traitement de lumière à moyenne intensité (1 000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Les données montrent que, lorsque les feuilles héliophiles du cotonnier sont exposées pendant de courtes périodes de temps à des températures seulement légèrement suboptimales pour la photosynthèse, les hautes intensités de lumière peuvent provoquer une photo-inhibition importante.

Gossypium hirsutum = coton / photo-inhibition de la photosynthèse / température

* Present address: Smithsonian Tropical Research Institute, PO Box 2072, Balboa, Republic of Panama.

Abbreviations: A_{max} , maximum CO_2 assimilation rate measured at ambient CO_2 during a given light period; F_v/F_M , ratio of variable to maximum fluorescence yield; p_i , intercellular CO_2 partial pressure; p_a , ambient CO_2 partial pressure; PFD , photon flux density (400–700 nm); PS_{max} , photosynthetic capacity, maximum rate of O_2 evolution measured under saturating light and CO_2 conditions; \emptyset , photon use efficiency of photosynthetic O_2 evolution measured in saturating CO_2 and based on absorbed red light (630–700 nm).

INTRODUCTION

Photosynthesis, especially of tropical species, is known to be adversely affected by chilling temperatures, which are considered as those between 0 and 12°C (Öquist *et al.*, 1987). In combination with high light intensities these low temperatures may result in severe photoinhibition of photosynthesis (Taylor and Rowley, 1971; Baker *et al.*, 1983; Long *et al.*, 1983; Powles *et al.*, 1983; Martin and Ort, 1985; Greer, 1988; Somersalo and Krause, 1990). Photoinhibition is characterized by reductions in both light-limited and light-saturated rates of photosynthesis (Walker and Osmond, 1986) and the fluorescence parameter F_V/F_M , a measure of the photochemical efficiency of PS II (Kitajima and Butler, 1975). Some investigations on plants grown under low to moderate light intensity (Ögren *et al.*, 1984; Greer *et al.*, 1986; Greer, 1988; Gong and Nilsen, 1989; Rosenqvist *et al.*, 1991) and even high light intensity (Ögren and Sjöström, 1990) suggest that photoinhibition can also be induced by temperatures higher than those in the typical chilling range.

We have previously reported with cotton that temperatures far above the chilling range, when combined with high photon flux densities equivalent to full sunlight, led to severely reduced dry matter production (Königer and Winter, 1991; Winter and Königer, 1991). Although there was evidence that these reductions were partially related to high irradiance stress, it was difficult in these long-term studies with whole plants to differentiate between direct photoinhibitory effects and other possible inhibitory factors such as end-product inhibition of photosynthesis (Harris *et al.*, 1983; Stitt, 1991). Therefore we initiated a series of short-term experiments with individual cotton leaves to determine whether reductions in photosynthesis at only slightly suboptimal temperatures were directly correlated with parameters that reflected photoinhibition.

MATERIAL AND METHODS

Plant material

Cotton seedlings (*Gossypium hirsutum* L var Delta Pine 61) were grown in 18-l pots in a mixture of clay and high moor peat (Einheitserde zum Pikieren, Typ P, Gebrüder Patzer KG, Sinntal-Jossa, FRG) up to a size when the primary leaf was 20 cm² and the first fo-

liar leaf was 6 cm². These plants were then exposed in controlled-environment chambers (for details see Winter and Königer, 1991) for 16 d in the following conditions: 33/25°C (day/night temperature) and 1 500 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ during 12-h light periods. The soil was kept well watered and pots were flushed every other day with 2 l nutrient solution containing 12 mM NO_3^- (Wong, 1979). Experimental treatments were initiated following these 16-d cultivation periods.

CO₂ exchange

Leaf gas exchange was determined continuously throughout the 4-d treatments using an open gas exchange system (Winter and Schramm, 1986). Measurements were made on recently expanded attached single leaves (4th leaf following the primary leaf). They were enclosed in a gas-exchange cuvette (GWK 3, 19.5·12.0·9.5 cm³; Fa Walz, Effeltrich, FRG) through which air containing 35 Pa CO₂ and 21 kPa O₂ and with a dew-point of 5°C was supplied at a rate of 6.6 l min⁻¹. During photosynthetic CO₂ uptake, the CO₂ partial pressure in the air surrounding the leaf decreased by at most 3 Pa. The rest of the plant was maintained at \approx 30/25°C (day/night temperature) throughout all treatments. Net CO₂ assimilation rate, conductance to water vapor transfer and internal CO₂ partial pressure were calculated according to Von Caemmerer and Farquhar (1981).

O₂ exchange

Photosynthetic O₂ evolution from leaf discs (10 cm²) was determined at 25°C and 5% CO₂, 20% O₂, 75% N₂ with an LD-2 O₂ electrode unit and an LS-2 light source (Hansatech, King's Lynn, Norfolk, UK). Photosynthetic capacities (PS_{max}) were measured at 1 600 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. Photon use efficiencies (Φ) were based on absorbed red light (RG 630; Schott Glaswerke, Mainz, FRG). The light source was calibrated with a quantum sensor (LI-190 SB, Li-Cor, Lincoln, NE, USA). Absorbance (630–700 nm) was determined with an integrating sphere (LI-1800-12, Li-Cor) connected to a spectroradiometer (LI-1800, Li-Cor).

Fluorescence

Chl a fluorescence of leaf discs excised from the treated (attached) leaves was measured at room temperature using a pulse amplitude modulation fluorometer (PAM 101; Fa Walz; Schreiber *et al.*, 1986). Samples were taken at the end of 12-h dark periods and enclosed in a brass cuvette. The upper leaf surface was pressed against a Perspex window, which formed the bottom of the chamber lid, in which the fiber-optic

probe of the fluorometer was fixed. Fluorescence was excited with a measuring beam of weak light from a pulsed light-emitting diode to obtain F_0 , the fluorescence level when all PS II reaction centers are open. Maximum fluorescence, F_M , was determined by applying a 1-s pulse of saturating light. The variable fluorescence, F_V , is given by the difference between F_M and F_0 . The photochemical efficiency of PS II, expressed as F_V/F_M , was calculated according to Kitajima and Butler (1975).

Pigments and carbohydrates

Contents of total chlorophyll (a+b) and total carotenoids of leaf discs were determined in 80% acetone according to Röbbelen (1957) including the suggested changes of Metzner *et al* (1965). Soluble and insoluble sugars were determined using the anthrone test (Morris, 1948).

RESULTS

Gas exchange

Leaves that had developed in high light (1 500 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) and at 33/25°C (day/night temperature) were exposed for 4 d to either 1 800 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (high PFD) or 1000

$\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (medium PFD) and day/night temperatures of either 35/25, 30/25, 25/25 or 20/25°C. Figures 1 to 4 show representative day courses of leaf temperatures and various gas exchange parameters for cotton leaves during these 4-d treatments. Leaf temperatures were maintained within $\pm 1^\circ\text{C}$ of the air temperatures between 20 and 30°C and were ≈ 33 at 35°C air temperature. Leaves exposed to 1 800 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ and 30°C (fig 1) or 35°C (not shown), conditions which are close to those the plants experienced during their development, showed maximum CO_2 assimilation rates (A_{max}) of $\approx 30 \mu\text{mol m}^{-2}\text{s}^{-1}$, which were reached 2–5 h after onset of the light. Photosynthetic rates thereafter gradually declined by $\approx 25\%$. This pattern of CO_2 gas exchange was similar each day for 30 and 35°C throughout the 4-d treatment.

Reducing the light intensity from 1 800 to 1 000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (fig 2) as well as lowering the temperature from 30° to 25° or 20°C at either light intensity (figs 3 and 4) resulted in an immediate and marked decline in CO_2 assimilation rates, which were then maintained throughout the remainder of the experimental period. There was a closer correlation between changes in CO_2 assimilation rates and leaf conductance in the high-temperature than in the low-temperature treatments. Irrespective of light

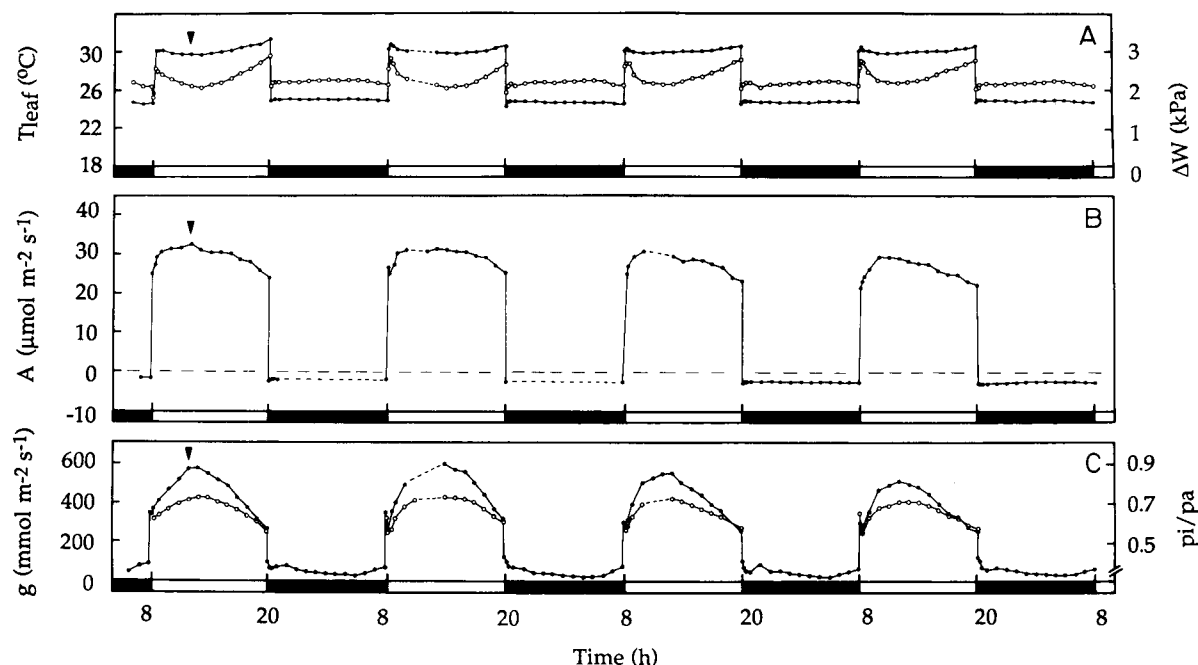


Fig 1. Day courses of leaf temperature (T_{leaf} , •), leaf-air vapour pressure deficit (ΔW , o), CO_2 assimilation rate (A , ▲), conductance (g , •) and the ratio of internal to external CO_2 partial pressure (p_i/p_a , o) of sun leaves of *Gossypium hirsutum*. Leaves were exposed for 4 d to 1 800 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ and 30°C during the 12-h light periods and 25°C during the dark periods.

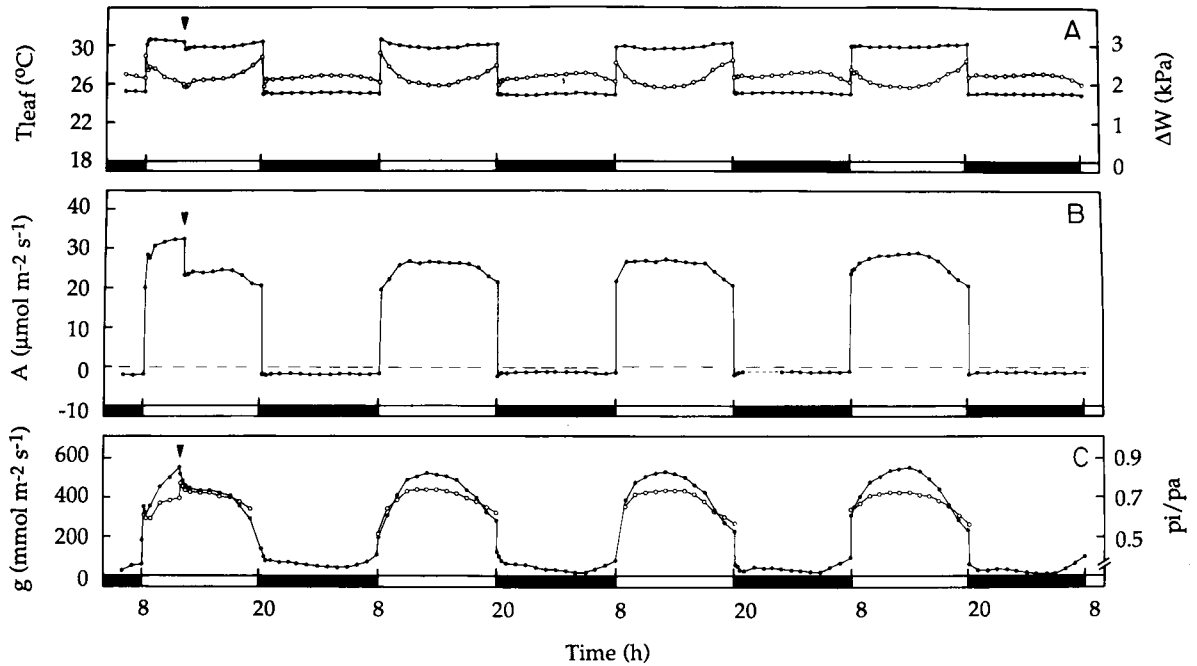


Fig 2. As in figure 1, but after the initial 4 h of the light period on d 1 at $1\,800\ \mu\text{mol photons m}^{-2}\text{s}^{-1}$ and 30°C , the light intensity was reduced to $1\,000\ \mu\text{mol photons m}^{-2}\text{s}^{-1}$ (arrow). The temperature during the 12-h dark periods was 25°C .

and temperature conditions during a given treatment the ratio of internal/external CO_2 partial pressure (p_i/p_a) paralleled the changes in conductance and reached the highest values in the middle of the light periods.

Figure 5 summarizes gas exchange parameters during all 4-d treatments expressed as percentage of the values at the beginning of the experiments (d 1, 12.00 h). A_{max} , the maximum rate of net CO_2 assimilation during a given light

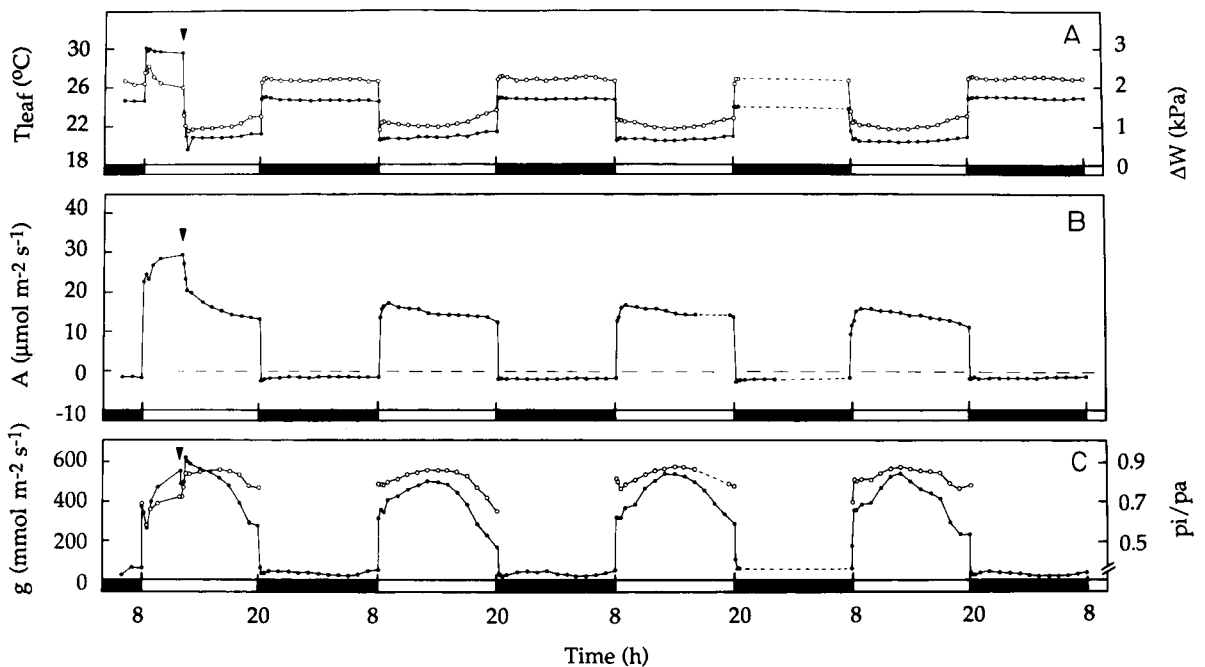


Fig 3. As in figure 1, but after the initial 4 h of the light period on d 1 at $1\,800\ \mu\text{mol photons m}^{-2}\text{s}^{-1}$ and 30°C , the temperature during the light period was reduced to 20°C (arrow). The temperature during the 12-h dark periods was 25°C .

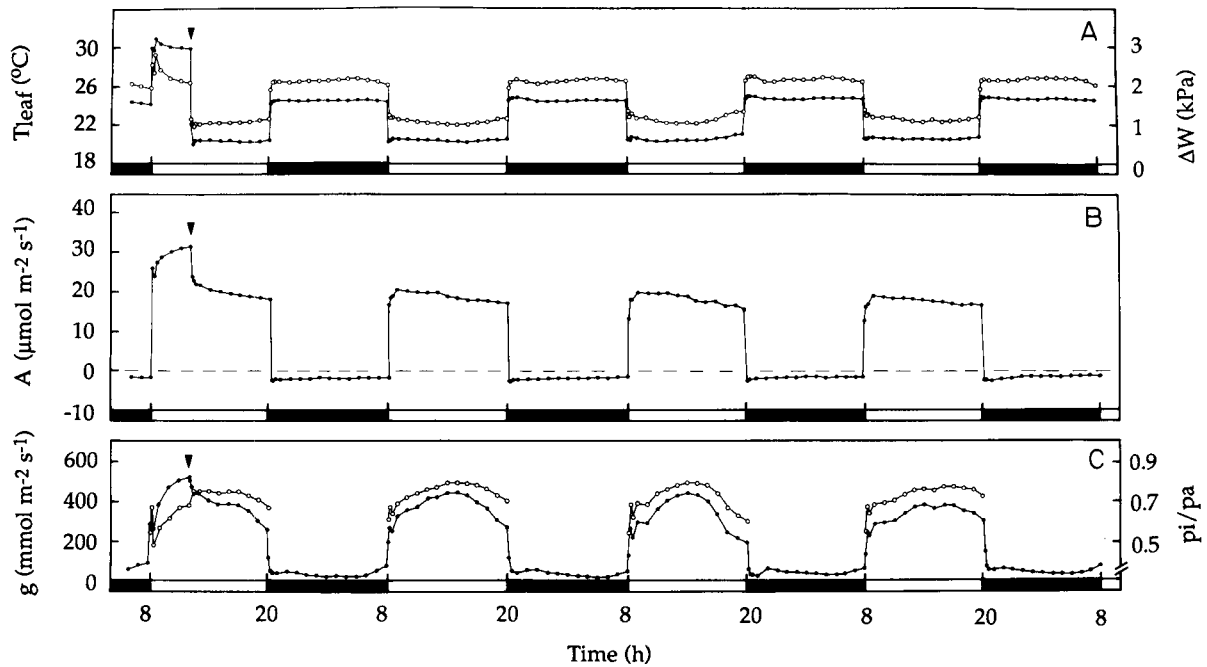


Fig 4. As in figure 1, but after the initial 4 h of the light period on d 1 at $1\,800\ \mu\text{mol photons m}^{-2}\text{s}^{-1}$ and 30°C , the temperature and light intensity were reduced to 20°C and $1\,000\ \mu\text{mol photons m}^{-2}\text{s}^{-1}$, respectively (arrow). The temperature during the 12-h dark periods was 25°C .

period, remained high throughout the 4 d at $1\,800\ \mu\text{mol photons m}^{-2}\text{s}^{-1}$ and 30 and 35°C . Decreasing the temperature to 20°C for high light treated plants resulted in a much larger percentage inhibition of CO_2 assimilation rate than in plants under medium light, and rates in high light dropped below those in medium light. The reduction in A_{max} at low temperatures could not be explained by stomatal closure, because the ratio of internal to external CO_2 partial pressure increased slightly with decreasing temperatures. Changes in A_{max} were linearly correlated with total carbon gain during 12 h light periods for all treatments (fig 6).

Photosynthetic oxygen evolution and F_V/F_M

Photosynthetic competence as shown by PS_{max} , \emptyset and F_V/F_M was determined on leaf samples harvested at the end of the dark period of d 4 (fig 7). At 30°C in high light, PS_{max} was high and \emptyset and F_V/F_M were close to those generally observed in healthy, non-photoinhibited C_3 leaves (Björkman and Demmig, 1987).

With decreasing temperatures in high light, PS_{max} , \emptyset and F_V/F_M decreased by 18, 24 and

5% (25°C) and 31, 38 and 39% (20°C), respectively, whereas an increase in temperature to 35°C had only little impact on these parameters. After 4 d in medium light at 30°C the values were not markedly different from those measured in high light. PS_{max} , \emptyset and F_V/F_M were reduced by 4, 5 and 8%, respectively, as treatment temperature was lowered to 20°C .

Leaves exposed to $1\,800\ \mu\text{mol photons m}^{-2}\text{s}^{-1}$ and 20°C showed signs of photooxidation (table I). Total chlorophyll content was reduced by $\approx 26\%$ in comparison to the 30°C treatment in high light.

Carbohydrates

For 2 of the experimental treatments ($1\,800\ \mu\text{mol m}^{-2}\text{s}^{-1}$, 30 and 20°C), carbohydrate contents were measured to investigate the possible role of feedback inhibition of photosynthesis in these treatments. On d 4, at the end of light and dark periods, there was no clear difference in the total carbohydrate content between leaves exposed to the 2 temperature treatments (table II).

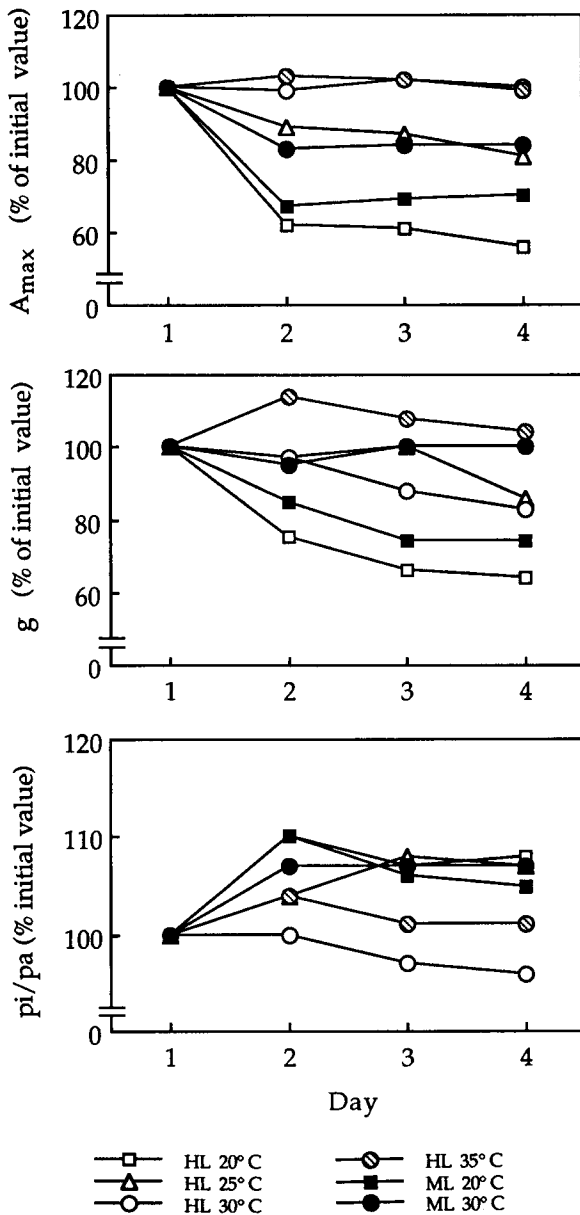


Fig 5. Changes in gas exchange parameters of sun leaves of *Gossypium hirsutum* during 4-d treatments. Leaves were exposed to 1 800 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (HL) or 1 000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (ML) at air temperatures of 35, 30, 25 and 20°C, respectively. Maximum CO_2 assimilation rates (A_{max}) of each photoperiod are expressed as percent of the initial value (12.00 h on d 1). Corresponding values for conductance (g) and the ratio of internal to external CO_2 partial pressure (p_i/p_a) are also expressed as percent of the initial value. Data for d 2, 3 and 4 are the means of 2–3 experiments. The SD, which did not deviate from $\pm 13\%$, is not shown. 100% $A_{max} = 30.45 \pm 1.95 \mu\text{mol m}^{-2}\text{s}^{-1}$ ($n = 12$), 100% $g = 494.89 \pm 63.23 \text{ mmol m}^{-2}\text{s}^{-1}$ ($n = 12$), 100% $p_i/p_a = 0.68 \pm 0.03$ ($n = 12$).

In contrast, when whole cotton plants were exposed to high light (1 800 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) and day/night leaf temperatures of $\approx 20/25^\circ\text{C}$, for example, conditions similar to those used in one of the single leaf experiments, the total carbohy-

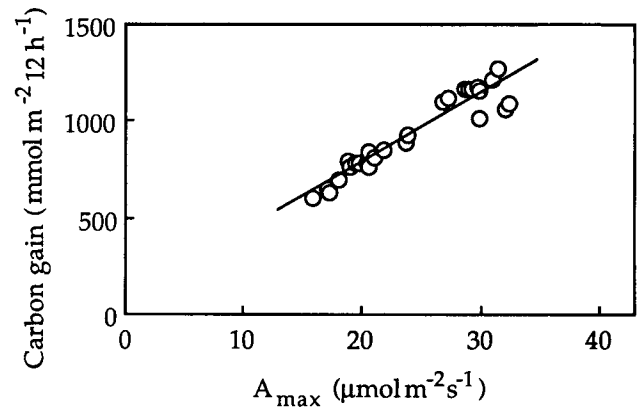


Fig 6. Relationship between maximum CO_2 assimilation rates during 12-h light periods and corresponding values of integrated carbon gain for leaves of cotton exposed to 4-d treatments at 1 000 or 1 800 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ and 35, 30, 25 and 20°C.

drate content increased (table III). This was most evident in samples taken at the end of the dark period. F_V/F_M (end of dark period) was reduced from 0.781 ± 0.021 to 0.672 ± 0.028 after d 1, and did not show any further decrease during the 3 consecutive d (data not shown).

DISCUSSION

The data show that even leaves that have developed under conditions close to full sunlight are markedly photoinhibited by high light at temperatures far above the chilling range. Photoinhibitory effects under these conditions have previously been shown primarily for plants grown under low light intensities (100–300 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$; Ögren *et al*, 1984; Greer *et al*, 1986; Greer, 1988; Gong and Nilsen, 1989; Rosenqvist *et al*, 1991). It is not surprising that such low-light acclimated plants would be highly susceptible to excess light. It is therefore intriguing that in these sun-acclimated leaves of cotton, even a 4-d treatment in high light at 25°C, only 5°C below the optimum temperature for photosynthesis, led to significant reductions in the CO_2 saturated rates of O_2 evolution under light saturation (18%) and light limitation (24%), while F_V/F_M was less affected (5%) (fig 7). Photoinhibition was also reported for sun leaves of willow when exposed for a few h to high light intensities and temperatures $< 23^\circ\text{C}$ (Ögren and Sjöström, 1990).

At these relatively high temperatures it is unlikely that phenomena like membrane disruption

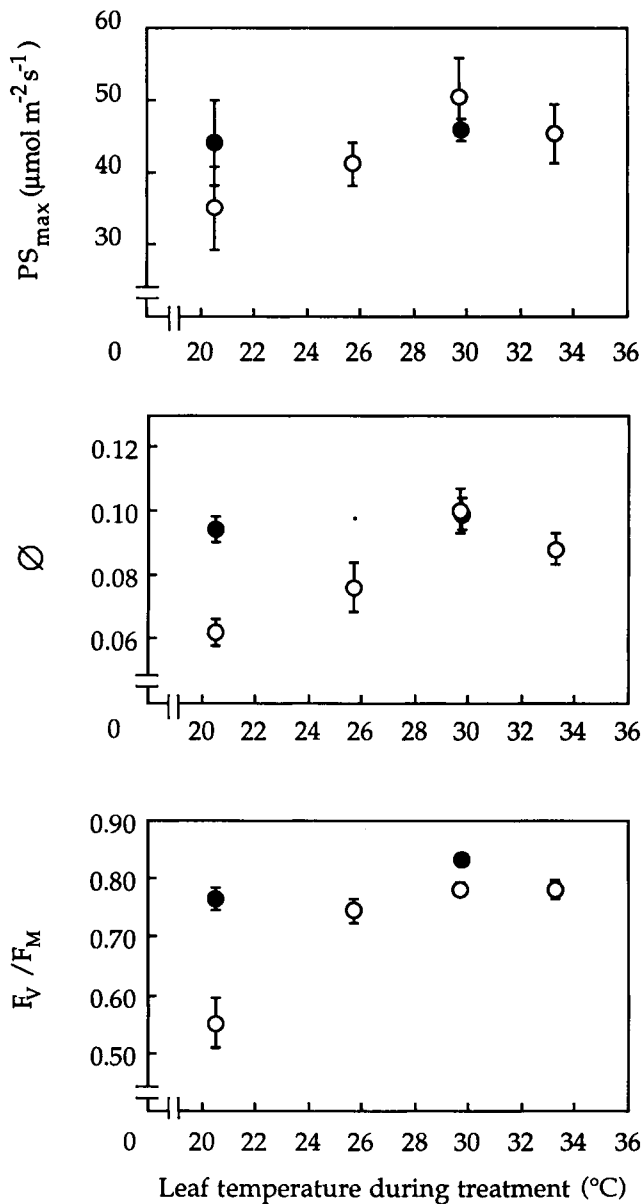


Fig 7. Effects of 4-d treatments at light intensities of 1 800 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (o) and 1 000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (•) at various temperatures on photosynthetic competence of cotton leaves. PS_{max} , light and CO_2 saturation rates of O_2 evolution; Φ , photon use efficiency of CO_2 saturation rates of O_2 evolution; F_V/F_M , photosystem II photon use efficiency. Data are means \pm SD ($n = 3 - 5$).

(Rikin *et al*, 1979; Powles, 1984) and changes in the chloroplast ultrastructure (Taylor and Craig, 1971; Musser *et al*, 1984; Wise and Naylor, 1987), which have been discussed with regard to chilling injury, are relevant to the results reported here. It is interesting to note that upon a decrease in leaf temperature, CO_2 assimilation rates decreased relatively rapidly and remained

constant throughout the 4-d experimental periods (figs 3, 4). We do not know whether the decreases in the parameters reflecting photosynthetic competence (PS_{max} , Φ , F_V/F_M), which were determined after 4 d only, showed similar kinetics, although the experiment with whole cotton plants demonstrated that F_V/F_M was reduced to its final level within 1 d. It is clear that any restriction in carbon metabolism will decrease the use of the products of the light reactions, thus restricting electron flow. In principle, the reductions in Φ , F_V/F_M reflect either photodamage to PS II reaction centers or photoprotective mechanisms, *ie* controlled increase in the rate of dissipation of excitation energy as heat (Demmig-Adams, 1990; Krause and Weis, 1991). We do not know the extent to which these different mechanisms of photoinhibition contribute to the observed decrease of Φ and F_V/F_M . The decrease in chlorophyll content after 4 d at 20°C and high light suggests that some photodamage had occurred.

These findings on single leaves corroborate previous results from long-term studies on whole cotton plants, in which high light conditions combined with leaf temperatures slightly below 30°C led to significant reductions in dry matter production (Königer and Winter, 1991; Winter and Königer, 1991). Because of the complexity of the whole plant system we could not definitively relate the reduction in growth to photoinhibition of photosynthesis. This is emphasized by the data shown in table III, which indicate a possible feedback inhibition of photosynthesis by accumulation of carbohydrates, when whole plants are transferred to reduced temperatures in high light. The low day temperature could decrease the carbohydrate demand in whole plants and inhibit carbohydrate translocation during the night. However, by using single leaves we were able to demonstrate that the reduction in photosynthetic competence in high light and slightly suboptimal leaf temperatures were independent of major changes in carbohydrates (table II).

In conclusion, changes in the velocity of photosynthesis in sun leaves of cotton caused by mild temperature stress in high light over a period of several d, can lead to pronounced photoinhibition of photosynthesis. However, photoinhibition does not seem to be the only factor that contributes to a reduction in growth in high light and temperatures < 30°C (Winter and Königer, 1991; Königer and Winter, 1991).

Table I. Pigment composition of the 4th foliar leaf of *Gossypium hirsutum*.

Pigments ($\mu\text{g cm}^{-2}$)	$1\,500\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$		$1\,000\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$		$1\,800\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$		
	Control	30°C	20°C	35°C	30°C	25°C	20°C
Chlorophyll (total)	39.33 ± 1.90	49.16 ± 2.18	38.00 ± 1.13	42.40 ± 2.57	39.37 ± 2.17	40.97 ± 6.28	28.94 ± 1.36
Carotenoids (total)	8.77 ± 0.94	9.79 ± 0.25	9.24 ± 1.59	8.77 ± 0.57	9.64 ± 0.44	10.47 ± 2.01	7.89 ± 1.20

Plants were cultivated for 16 d at 33/25°C (day/night temperature) and $1\,500\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$. Control values were determined at the end of the dark period on d 16. Leaves were then enclosed in a gas-exchange cuvette and exposed for 4 d to air temperatures of 35, 30, 25 and 20°C at either $1\,000$ or $1\,800\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$. Data are means ± SD ($n = 4 - 6$).

Table II. Carbohydrate contents ($\mu\text{mol glucose equivalents g}^{-1}$ DW) of the 4th foliar leaf of *Gossypium hirsutum*.

Carbohydrates ($\mu\text{mol g}^{-1}$ DW)	30/25°C		20/25°C	
	End of light period	End of dark period	End of light period	End of dark period
Total	1 170 ± 224	434 ± 187	1 258 ± 295	608 ± 157
Soluble	370 ± 19	171 ± 41	522 ± 103	369 ± 163
Insoluble	800 ± 212	263 ± 160	736 ± 199	263 ± 160

Plants were cultivated for 16 d at 33/25°C (day/night temperature) and $1\,500\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$. Leaves were then exposed to $1\,800\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$ and 30/25°C or 20/25°C during 12-h light/12-h dark cycles. Carbohydrates were measured at the end of the 4-d treatments. Data are means ± SD ($n = 3$).

Table III. Carbohydrate contents ($\mu\text{mol glucose equivalents g}^{-1}$ DW) of the 4th foliar leaf of *Gossypium hirsutum*, determined at the end of light and dark periods, respectively, during a 4-d treatment.

Carbohydrates ($\mu\text{mol g}^{-1}$ DW)	33/25°C ($1\,500\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$)		15*/25°C ($1\,800\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$)		
	Control	Day 1	Day 2	Day 3	Day 4
End of light period					
Total	1 181 ± 60	1 413 ± 77	1 455 ± 276	1 523 ± 45	1 682 ± 269
Soluble	403 ± 50	547 ± 99	611 ± 125	505 ± 24	571 ± 112
Insoluble	778 ± 44	867 ± 26	844 ± 204	1 018 ± 35	1 111 ± 159
End of dark period					
Total	301 ± 18	571 ± 50	765 ± 359	848 ± 336	1 275 ± 340
Soluble	124 ± 22	165 ± 6	244 ± 100	222 ± 59	422 ± 114
Insoluble	177 ± 5	406 ± 49	521 ± 260	626 ± 277	853 ± 245

* Due to the relatively small air movement in the environmental-growth chamber, air temperatures had to be set at 15°C during the light period to maintain a leaf temperature of $\approx 20^\circ\text{C}$. Plants were cultivated for 16 d at 33/25°C (day/night temperature) and $1\,500\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$. On d 16 carbohydrate contents were determined (control). Four h after onset of the following light period, whole plants were exposed in an environmental-growth chamber to an air temperature of 15°C (resulting in leaf temperatures of $\approx 20^\circ\text{C}$) and a light intensity of $1\,800\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$. Data are means ± SD ($n = 3$).

ACKNOWLEDGMENTS

This study was supported by the Deutsche Forschungsgemeinschaft (SFB 251, Universität Würzburg). We thank M Lesch for technical assistance and G Harris for critical comments on the manuscript.

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