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A gas exchange–fluorescence analysis of photosynthetic performance of a cotton crop under high-irradiance stress [Extended abstract.]

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The main objectives of the investigation were to determine: (1) the extent to which, if any, cotton leaves exposed to intense sunlight in the field suffer photoinhibitory damage to the photosynthetic system. A potential for such damage is present whenever the light energy absorbed by the leaves greatly exceeds the rate with which de-excitation can take place via photosynthesis; (2) the extent to which water stress may induce or exacerbate photoinhibition by reducing the rate of photosynthesis and hence increasing the amount of excessive light energy; and (3) the possible operation of protective mechanisms that would prevent an accumulation of excessive excitation energy at the photosynthetic reaction centres.

A crop of upland cotton (*Gossypium hirsutum* L. cv. Acala SJ2) grown in the San Joaquin Valley of California was chosen as the principal material for these studies. The main reasons for this choice are that this region receives extremely high insolation during the period of canopy development and the diaheliotropic leaf movements exhibited by this species of cotton further increase the daily radiation receipt by exposed leaves to *ca.* 40% above that received by horizontally oriented leaves.

The field studies were done at the University of California's West Side Field Station near Five Points, California, during the period May–October 1987. Irrigated cotton was grown under agricultural practices standard for the region. Studies were also made during the gradual development of water stress in a crop that received no irrigation other than an initial irrigation before sowing. Concurrent field measurements of CO_2 and water vapour exchange, and pulse-modulated chlorophyll fluorescence in fully exposed cotton leaves, were made during five 10–14 day periods over the entire growing season, as were also daily courses of plant water status. Micrometeorological parameters such as irradiance, temperature and humidity were recorded continuously. Photon (quantum) yields of photosynthetic O_2 evolution at CO_2 saturation and strictly rate-limiting photon flux densities (PFDs) were determined in the field station laboratory on leaf discs taken from fully exposed leaves in the field. The fraction of the incident PFD absorbed by the leaves was determined with an integrating sphere system.

Fully sunlit leaves of cotton, grown under standard furrow irrigation, exhibited very high rates of net CO_2 uptake (*ca.* 44 μmol CO_2 m^{-2} s^{-1}) throughout the growing season. There was no significant change in this rate during the period in which the mean dry weight of each plant increased from 0.5 to 500 g. The net CO_2 uptake rates found in the present study are at least 40% higher than previously published values for cotton.

The intrinsic photon yield of photosynthetic O_2 evolution of cotton leaves developed in full sunlight also remained high and constant throughout the season with a mean value of 0.104 ± 0.006 O_2 per photon. This value is very near the maximum photon yield reported for any species of vascular plants and identical to that of cotton leaves developed under a low light régime (Björkman & Demmig, *Planta* **170**, 489–504 (1987)). Similarly, early morning values for the ratio of variable to maximum chlorophyll fluorescence, F_V/F_M, which is a measure of the efficiency of the photochemistry of photosystem II, showed no signs of photoinhibitory effects (seasonal mean $F_V/F_M \pm$ s.d. $= 0.822 \pm 0.014$). We therefore conclude that photoinhibitory damage was absent in irrigated cotton grown under normal agricultural practices, even though these solar tracking leaves receive extremely high daily photon irradiances (*ca.* 90 mol quanta m^{-2}).

The midday water potential (ψ) of unirrigated cotton plants gradually decreased from about -1.1 to -3.0 MPa over a two-month period. Net CO_2 uptake rate (A) for fully sunlit leaves gradually fell from 44 to *ca.* 6 μmol m^{-2} s^{-1}. The decline in A was essentially linear with respect to the decline in ψ and was accompanied by a decreased leaf conductance to water vapour loss (g_L). A linear relation was obtained between A and g_L during this stress period. Hence, the ratio A/g_L, a measure of photosynthetic water-use efficiency by the leaves, was more or less independent of leaf water status. Apparently, stomatal conductance adjusted so that the intercellular CO_2 pressure was maintained in the range 190–205 μbar†.

The intrinsic photon yield remained essentially unaffected by water stress above leaf water potentials of -2.2 MPa. Significantly reduced photon yields were only found under quite severe water stress levels ($\psi < 2.4$ MPa). The mean photon yield for leaves at -2.8 MPa was 0.0742 ± 0.0101 O_2 per photon, some 30% below the value for unstressed leaves. Similarly, predawn *in situ* measurements of chlorophyll fluorescence showed that the F_V/F_M ratio was little affected by water stress above -2.2 MPa, but exposed leaves of severely water-stressed plants showed a significant decrease in this ratio. These results clearly show that severe water stress, combined with high irradiance levels, can cause a significant reduction in the intrinsic efficiency of photosynthetic O_2 evolution and PSII photochemistry of cotton leaves. Photoinhibitory damage to PSII could well be the cause of this reduction; however, it seems more likely that it is a result of a sustained increase in an energy dissipation process that competes with the reaction centres for excitation energy and serves to protect these centres from over-excitation (see below).

We conclude that photoinhibition has no significant effect on the daily carbon gain in a cotton crop even under water stress. Firstly, the intrinsic photosynthetic photon yield and PSII photochemistry are only affected under extreme conditions that would be rare in a crop situation. Secondly, the reduction in the photon yield observed under very severe stress is insufficient to exert a significant influence on photosynthetic rate other than during the brief periods of the day when the PFD is low and rate limiting.

As it is clear that leaves of field-grown cotton are able to tolerate large excesses of excitation energy, much of the work was directed toward elucidating the underlying causes of this tolerance. Field and laboratory analyses of chlorophyll fluorescence proved to be a very valuable approach to this problem. Laboratory studies enabled us to determine quantitative relations between components of fluorescence quenching and photosynthesis in intact cotton leaves, and these relations were applied in the field to assess the contribution of different processes to de-excitation of the energy absorbed by the chlorophyll.

Our estimates show that in leaves of irrigated cotton photosynthesizing in full sunlight (1830 μmol quanta m^{-2} s^{-1} absorbed by the leaf), 25% of the excitation energy was used for CO_2 fixation. An additional 19% was used up in photorespiration in which O_2 is the terminal electron acceptor. The remainder, 56%, must therefore be de-excited by other means. Our fluorescence analysis indicates that all of this excessive energy was dissipated via a process of non-radiative dissipation (NRD), thereby preventing a potentially harmful accumulation of excitation energy at the PSII reaction centres. Only about 27% of the PSII reaction centres were in the reduced state in these leaves at peak solar radiation. By maintaining most of the PSII centres in the oxidized state the generation of triplet state chlorophyll, singlet oxygen

† 1 μbar $= 10^{-1}$ Pa.

and other potentially destructive radicals that are thought to cause photoinhibitory damage, was presumably prevented.

In cotton plants growing under intermediate water stress levels ($\psi = -1.8$ MPa) the net rate of CO_2 uptake in full sunlight was *ca.* 27 μmol m^{-2} s^{-1}. In this case, 18% of the excitation energy was used in CO_2 fixation and 16% was dissipated via photorespiration, raising the excess to 66% of the total. The cotton leaves compensated by increasing NRD to this percentage. The fraction of reduced PSII centres was higher than in leaves of irrigated plants but at most 38% were in the reduced state.

In severely water-stressed plants ($\psi = -2.8$ MPa) only about 13% of the excitation energy was used for CO_2 fixation and about the same percentage was dissipated in photorespiration. Because the intrinsic photon yield and the efficiency of PSII photochemistry of these leaves were below normal, even after overnight recovery, it is difficult to obtain accurate values for NRD and the reduction state of the PSII centres. Rough estimates show that NRD increased still further, perhaps to as much as 73% of the total and that fewer than half the PSII centres were in the reduced state at peak solar radiation.

We conclude that the high tolerance of leaves of field-grown cotton to light levels greatly in excess of what can be used in photosynthesis is in large part attributable to their high capacity to dissipate excessive excitation energy via non-radiative energy dissipation. This process evidently is well regulated so that normally little or no energy is dissipated when photosynthesis is light-limited but the fraction of energy dissipated via this process gradually increases as the ability of photosynthesis to keep up with the absorbed excitation energy decreases.