Plant Physiol. (1982) 70, 689–694
0032-0889/82/70/0689/06/$00.50/0

Insensitivity of Water-Oxidation and Photosystem II Activity in Tomato to Chilling Temperatures[1]

Received for publication March 1, 1982 and in revised form April 13, 1982

BJORN MARTIN AND DONALD R. ORT
Department of Botany, United States Department of Agriculture, Agricultural Research Service, University of Illinois, Urbana, Illinois 61801

ABSTRACT

Chilling tomato plants (*Lycopersicon esculentum* Mill. cv. Rutgers and cv. Floramerica) in the dark resulted in a sizable inhibition in the rate of light- and CO_2-saturated photosynthesis. However, at low light intensity, the inhibition disappeared and the absolute quantum yield of CO_2 reduction was diminished only slightly. The quantum yield of photosystem II (PSII) electron flow was 18% lower when measured in chloroplasts isolated from chilled leaves than in chloroplasts isolated from unchilled leaves. Even though the maximum rate of PSII turnover in these chloroplasts was 12% lower subsequent to chilling, it was in all cases two or more times that required to support the light- and CO_2-saturated rate of photosynthesis measured in the attached leaf. The concentration of active PSII centers in chloroplasts isolated from leaves either before or after chilling was determined by measurement of the products of water oxidation from a series of saturating flashes short enough to turnover the electron transport carriers only a single time. There was no significant change in the concentration of active PSII centers due to dark chilling.

It was concluded that PSII activity and water oxidation capacity are not significantly impaired in tomato by chilling in the dark and therefore are not primary aspects of the inhibition of CO_2 reduction observed in attached leaves.

The tomato is representative of a fairly diverse group of plants in which brief exposure to low temperature ($0 < T < 12°C$) impairs photosynthesis for a prolonged period of time. A single cool night (in this case 1°C for 16 h) is sufficient to lower maximum rates of photosynthesis in tomato measured at atmospheric CO_2 levels by 60% on the subsequent day, whereas full recovery requires several days (12). The decline in photosynthesis subsequent to chilling in the dark cannot be assigned to a single cause. We recently reported results (12) from experiments with attached tomato leaves that allowed us to identify two separate components of the inhibition. By measuring and controlling the intercellular CO_2 concentration, we were able to demonstrate that slightly less than one-third of the decline could be attributed to reduced availability of CO_2, a consequence of reduced stomatal aperture. The larger effect of the chilling stress on photosynthesis which was not relieved by saturating intercellular CO_2 levels results from a direct effect on chloroplast activity. It is likely that this is a general pattern of the effect of dark chilling on photosynthesis in sensitive plants because there is evidence for both stomatal and nonstomatal causes for chilling impairment of photosynthesis in attached leaves of *Xanthium strumarium* (3) and *Zea mays* (14).

There is a good deal of experimental evidence indicating that chloroplasts isolated from prechilled leaves of sensitive plants have impaired electron transport. Kaniuga and associates (8, 9) have identified water oxidation as the chill-labile process in tomato chloroplasts supporting similar conclusions drawn earlier by other workers (*e.g.* 10, 11, 16, 17) for a variety of plants. Consequently, it is a widely held belief that the reduced capacity for water oxidation observed in isolated chloroplasts is a major cause of the reduced photosynthesis measured in attached prechilled leaves. We began to question this explanation for chilling inhibition of photosynthesis when in a previous study with attached tomato leaves we found that dark chilling caused no significant effect on net photosynthesis measured at light intensities below about 200 $\mu E\ m^{-2}\ s^{-1}$. This observation is not in accord with the notion that impaired water oxidation capability is a significant element of prechilling injury to photosynthesis for reasons which are detailed in the Discussion of this paper.

In this paper, we report on a detailed study of the effect of dark chilling on PSII that demonstrates injury to water oxidation is not a primary element of prechilling inhibition of photosynthesis in tomato.

MATERIALS AND METHODS

Tomato plants (*Lycopersicon esculentum* Mill. cv. Rutgers and cv. Floramerica) were grown in a controlled environment chamber as described earlier (12). The third leaf (almost fully expanded) of 5- to 6-week-old plants was used for gas exchange measurements or for chloroplast isolations.

Chilling Treatment. A potted tomato plant was enclosed in a cardboard box in a darkened incubator at 1°C for 16 h. The RH in the incubator was maintained at 100%. When the plant was removed from the incubator, it was placed for 30 min in a darkened plastic bucket lined with moistened filter paper to prevent wilting during acclimation to room temperature.

Measurement of CO_2 Fixation. The third leaf, still attached to the plant, was enclosed in an assimilation chamber. The rate of light-saturated CO_2 fixation was measured in a closed compensating system at 300 $\mu l/l$ or 1,500 μ/l ambient CO_2 as described earlier (12).

The quantum yield for photosynthetic CO_2 fixation in attached leaves (or in one case a detached leaf) was measured at 1,500 $\mu l/l$ ambient CO_2 with the same equipment as was used for measurement of light-saturated rates. In the case of the detached leaf degassed water was supplied to the cut petiole throughout the experiment through a silicon rubber tube. All light intensities used for quantum yield determinations were at or below the light compensation point. CO_2 evolution was calculated from the rates at which CO_2 accumulated in the closed assimilation system. We observed that dark respiration frequently drifted, particularly after a chilling treatment. Consequently, it was necessary to estimate

[1] Supported by United States Department of Agriculture/Competitive Research Grants Office Grant 79-59-2171-1-1-309-1 to D. R. O.

the dark respiration corresponding to each light intensity by interpolation between dark respiration measurements made every second light intensity. Total photosynthesis was calculated by subtracting the rate of CO_2 accumulation in the light from the rate of dark respiration. Absorbed light was calculated by subtracting transmitted and reflected light from the incident light. Reflected light was measured 1 cm above the leaf surface at an angle of 45° from the perpendicular and transmitted light was measured normal to the plane and directly below the leaf. An LI-190S Quantum Sensor (Lambda Instruments, Lincoln, NE) was used for all light measurements. In more than 100 determinations made on tomato leaves, the amount of incident light absorbed was 91.1 ± 1.7% with reflected light of 4.5 ± 0.9% and transmitted light of 4.3 ± 1.2%. The measurements were performed at 25°C and 90% RH. The light intensity was varied by inserting metal screens and diffusive glass plates between the assimilation chamber and a quartz iodide lamp (1,000 w, 3200 K filament, General Electric[2]) above the chamber.

Isolation of Chloroplasts. The third almost fully expanded leaf from two tomato plants was rinsed in distilled H_2O, deveined, and cut into pieces. The pieces were ground in 80 ml grinding medium for 5 s in a Waring blendor. The grinding medium consisted of 50 mM Mes-KOH (pH 6.5), 0.3 M NaCl, 5 mM $MgCl_2$, 10 mM KCl, 2 mM EDTA, and 10 mM ascorbic acid. The slurry was filtered through 8 layers of cheesecloth and centrifuged at 2,200*g* for 20 s. The pellet was resuspended in 30 ml resuspension medium using a soft paint brush. The resuspension medium consisted of 50 mM Mes-KOH (pH 6.5), 0.4 M sorbitol, 5 mM $MgCl_2$, 10 mM KCl, 2 mM EDTA, 5 mM ascorbic acid, and 0.2% (w/v) BSA. The suspension was centrifuged at 200*g* for 2 min after which the supernatant was collected and centrifuged at 2,200*g* for 20 s. The pellet was resuspended and stored in the same resuspension medium as above, at a final Chl concentration of 1 to 2 mg/ml (1). The entire isolation procedure was performed at 0 to 4°C.

Measurement of Electron Flow through PSII of Isolated Chloroplasts. The quantum requirement for PSII electron transport was measured spectrophotometrically with an Aminco DW-2a (American Instrument Company, Silver Spring, MD) dual wavelength spectrophotometer following the photoreduction of ferricyanide (420 nm minus 450 nm) mediated by the lipophilic oxidant diiminodurene (5). The reaction mixture was illuminated by red light from a tungsten halogen lamp (Osram 64655, 24 v, 250 w) filtered through two Corning filters (CS 2-58, CS 1-75) and a reflective heat filter (Melles Griot 03 MHG 007). The irradiance was varied by inserting neutral density filters in the light beam. Absorbed light was calculated by subtracting transmitted and reflected light from the incident light.

Maximum rates of PSII electron transport were measured from the rate of aerobic oxidation of photoreduced dimethyl-methylenedioxy-*p*-benzoquinone (18) employing a Clark-type polarographic O_2 electrode.

Measurement of the Concentration of Active PSII Centers. The chloroplasts were excited with a series of 100 saturating single turnover flashes from a xenon lamp (model FX-193 Flashtube; EG & G, Salem, MA, 6 μs half width, 3 Hz). The acidification of the medium was measured with a glass electrode (Orion 9103) and a Keithley 610C electrometer. Calibration was performed for each reaction mixture by adding a known amount of HCl as an internal standard.

[2] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the United States Department of Agriculture or the University of Illinois and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

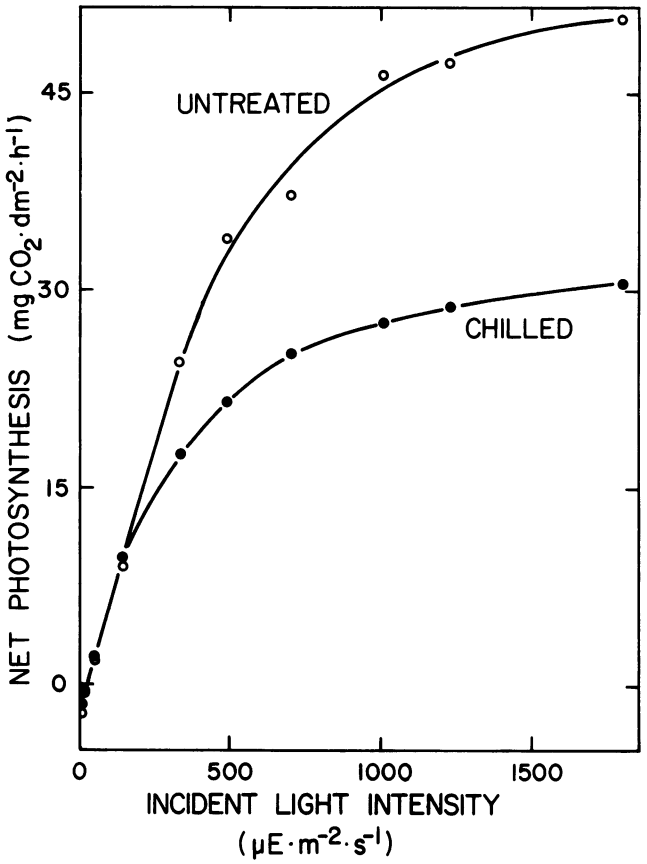

FIG. 1. The dependence of CO_2-saturated photosynthesis before and after chilling an attached leaf on the incident light intensity. The ambient CO_2 concentration was 1,500 μl l^{-1}, the ambient temperature was 25°C and the RH 60%.

RESULTS

The Effect of Chilling on Maximum Rate and Quantum Yield of CO_2 Reduction. As we reported previously (12), the maximum inhibition of CO_2-saturated photosynthesis by chilling is not observed at limiting light intensities (Fig. 1). In fact, at incident irradiances below 200 μE m^{-2} s^{-1}, net photosynthetic rates in the plant before and after 16 h of chilling were identical. That is, the apparent quantum yield for CO_2 reduction was not significantly altered by chilling even though the maximum rate was depressed nearly 40%.

The insensitivity of the quantum yield of CO_2 reduction to chilling was examined in greater detail. The absolute quantum yield of CO_2 reduction was calculated from the slope of the dependence of the CO_2 reduction rate on the amount of absorbed light (Fig. 2). All measurements were made at saturating intercellular CO_2 levels and irradiances near or below the light compensation point thus circumventing entirely any involvement of changing stomatal conductance (13). The data show a decrease in quantum yield of only 10% (from 0.056 prior to chilling to 0.051 subsequent to chilling), whereas the maximum rate (both CO_2- and light-saturated) was inhibited by nearly 25%.

Similar experiments were conducted on leaves which were detached from the plant prior to chilling. In this case, detached leaves were chilled between moistened filter paper resting on ice with degassed water supplied to the petiole through a silicon rubber tube. The rate of light- and CO_2-saturated photosynthesis continued to decline as the period of prechilling was prolonged (Fig. 3). After 20 h of prechilling, the maximum rate was depressed 32% and after 40 h the maximum rate was depressed 50%. In spite of the continual decline in maximum rate with time of prechilling,

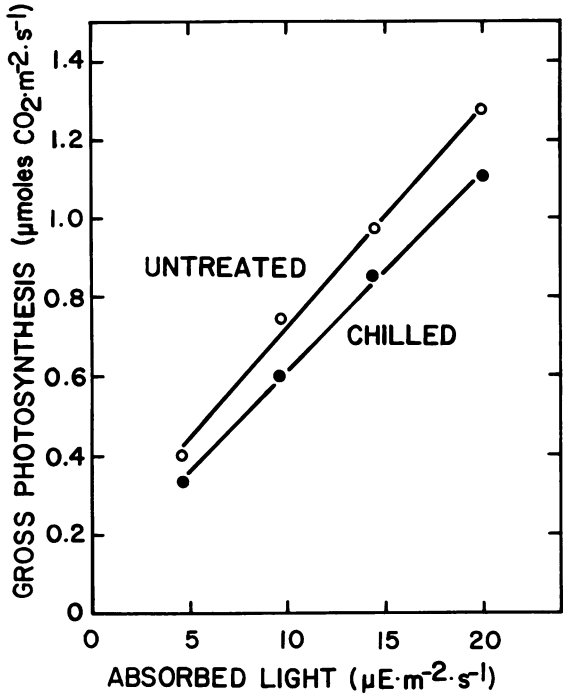

FIG. 2. The effect of chilling in the dark on the quantum yield for photosynthetic CO_2 fixation in an attached tomato leaf. All conditions were identical to those given for Figure 1 except the RH was 90%. The quantum yield was 0.056 prior to chilling and 0.051 after the chilling treatment. The light- and CO_2-saturated rate of photosynthesis was 53 mg $CO_2/dm^2 \cdot h$ prior to chilling and decreased to 42 mg $CO_2/dm^2 \cdot h$ after the chilling treatment.

there was no further decrease in the quantum yield after 20 h. The drop in quantum yield after 20 h of prechilling was approximately twice as severe in the detached leaves as in attached leaves (*cf.* Figs. 2 and 3).

The Effect of DCMU on Maximum Rate and Quantum Yield of CO_2 Reduction. Photosynthesis of an attached tomato leaf was inhibited at the level of PSII by brief submersion in an aqueous solution of DCMU. The treatment was sufficient to bring about a 23% reduction in the rate of light- and CO_2-saturated photosynthesis. Unlike chilling inhibition of photosynthesis, DCMU inhibition resulted in a large decrease (40%) in the quantum yield (Fig. 4). Thus, the effect of PSII inhibition was nearly twice as severe on the quantum yield as on the maximum rate.

Measurement of the Quantum Yield of PSII Electron Transport in Chloroplasts Isolated from Chilled and Unchilled Leaves and Chloroplasts Inhibited with DCMU. The quantum yield of PSII electron transport was determined from the dependence of the photoreduction rate of ferricyanide (mediated by the lipophilic oxidant diiminodurene) on the amount of absorbed light. In this reaction, electron flow beyond plastoquinone was prevented by dibromothymoquinone and electrons were intercepted from the reducing side of PSII prior to plastoquinone by the diiminodurene present in the membrane (5). The diiminodurene was then rapidly oxidized by ferricyanide present in the suspension media. Figure 5A depicts data from a typical measurement, in this case with chloroplasts isolated from an unchilled leaf. Figure 5B summarizes data obtained from four separate isolations from unchilled leaves and five separate isolations from chilled leaves. The lines represent the average quantum yields and the shaded areas show the standard deviation for each line. The average values indicate that the quantum yield of PSII electron flow is 18% lower for chloroplasts isolated from chilled leaves. The light- and CO_2-saturated photosynthetic rate of these leaves prior to disruption was reduced by an average of 33% (Table I). The average quantum yield of 0.36

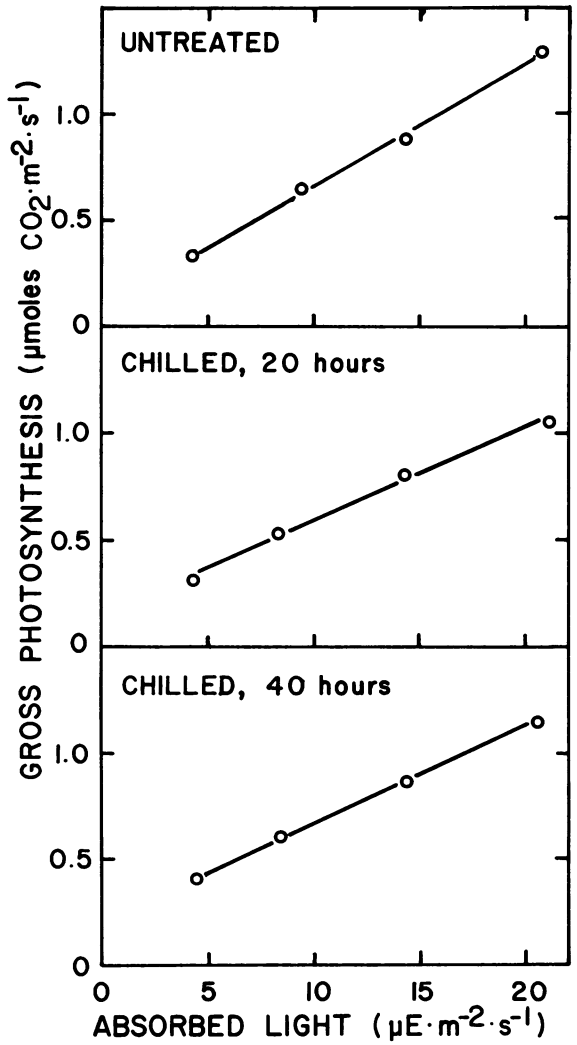

FIG. 3. The effect of chilling in the dark on the quantum yield for photosynthetic CO_2 fixation in a detached tomato leaf. The quantum yield was measured after 20 h of chilling in the dark and again after 40 h of chilling in the dark. All experimental conditions were identical to those given in Figure 2. The quantum yields dropped from 0.058 to 0.044 after 20 h of chilling but did not decrease further as the chilling was prolonged (0.046 after 40 h). The rate of light- and CO_2-saturated photosynthesis was 60 mg $CO_2/dm^2 \cdot h$ prior to chilling, 42 mg $CO_2/dm^2 \cdot h$ after 20 h of chilling, and 29 mg $CO_2/dm^2 \cdot h$ after 40 h of chilling.

electrons $photon^{-1}$ obtained for chloroplasts from unchilled leaves compares favorably with the theoretical value of 0.5 (The theoretical value assumes an even distribution of excitation energy between the two photosystems but requires no assumptions about the stoichiometry of PSI to PSII.) Measurements from intact leaves (*e.g.* Fig. 2) give lower values in the range of 0.25 electrons $\cdot$ $photon^{-1}$. This lower value apparently cannot be accounted for by photorespiratory CO_2 evolution inasmuch as no enhancement of CO_2 uptake was observed when the O_2 level was reduced from 21% to 1%. The discrepancy may indicate that our measurements of absorbed light are overestimated because of difficulty in evaluating leaf absorptivity due to internal scattering and lower absorptivity in leaf margins associated with the dissected shape of the tomato leaf. If so, such a systematic error should be identical before and after chilling and therefore not affect any of the conclusions drawn from the data. An alternative possibility is that other energy requiring processes (*e.g.* nitrate assimilation) may compete with CO_2 reduction for ATP, thereby lowering the quantum yield of CO_2 reduction.

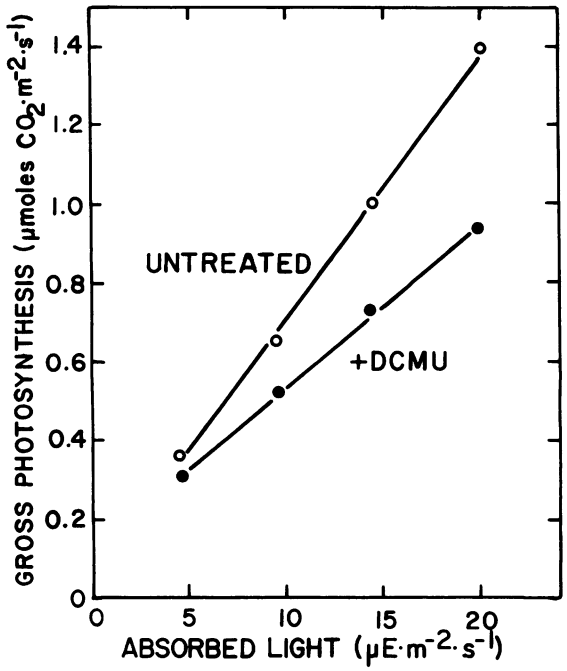

FIG. 4. The effect of DCMU on the quantum yield for photosynthetic CO_2 fixation in an attached tomato leaf. After completion of the control measurements, the attached leaf was immersed in 15 μM DCMU for 5 min at 25°C. It was then stored overnight at 25°C in the dark for measurements the following day. Experimental conditions were identical to those given for Figure 2. The quantum yield was reduced from 0.066 to 0.041 by the DCMU treatment. The rate of light- and CO_2-saturated photosynthesis was 40 mg $CO_2/dm^2 \cdot h$ prior to treatment and 32 mg $CO_2/dm^2 \cdot h$ after the treatment with DCMU.

Inhibition of PSII in isolated chloroplasts by addition of DCMU (Fig. 6) displays the same pattern of inhibition as the DCMU treatment of attached leaves shown in Figure 4. That is, the effects of inhibiting PSII are more severe on the quantum yield of electron transport than on the maximum rate. In Figure 6, sufficient DCMU was added to the reaction mixtures to suppress the maximum rate of electron flow by 36%, which is close to the average decrease of the light- and CO_2-saturated photosynthesis of the intact leaf brought about by chilling. Attendant to this decrease in maximum rate was a 2-fold greater decrease in the quantum yield.

Competency of PSII of Chloroplasts Isolated from Chilled and Unchilled Leaves to Support CO_2 Reduction of Attached Leaves. The maximum rate of PSII turnover is not severely reduced in chloroplasts isolated from chilled leaves. Table I presents data from 12 trials in which there was a 12% difference in the mean values for chloroplasts isolated from unchilled *versus* chilled leaves. This difference is small enough and the variation in individual measurements large enough that we place no significance in it.

As Table I indicates, in all cases, the capacity of PSII-dependent electron flux measured in isolated chloroplasts substantially exceeds the requirement of electron transport for CO_2 reduction measured in the attached leaves. The PSII electron transport rates required to support the observed CO_2 fixation rates were calculated on the basis of a requirement of four electrons per CO_2 molecule reduced and 1.5 molecules of ATP produced for each molecule of NADP reduced. In the case of the chilled leaf, the capacity of PSII exceeds by a factor of nearly two the electron transport requirement of CO_2 fixation.

The Effect of Chilling on the Concentration of Active PSII Centers. The concentration of active PSII centers in chloroplasts isolated from leaves either before or after chilling was determined

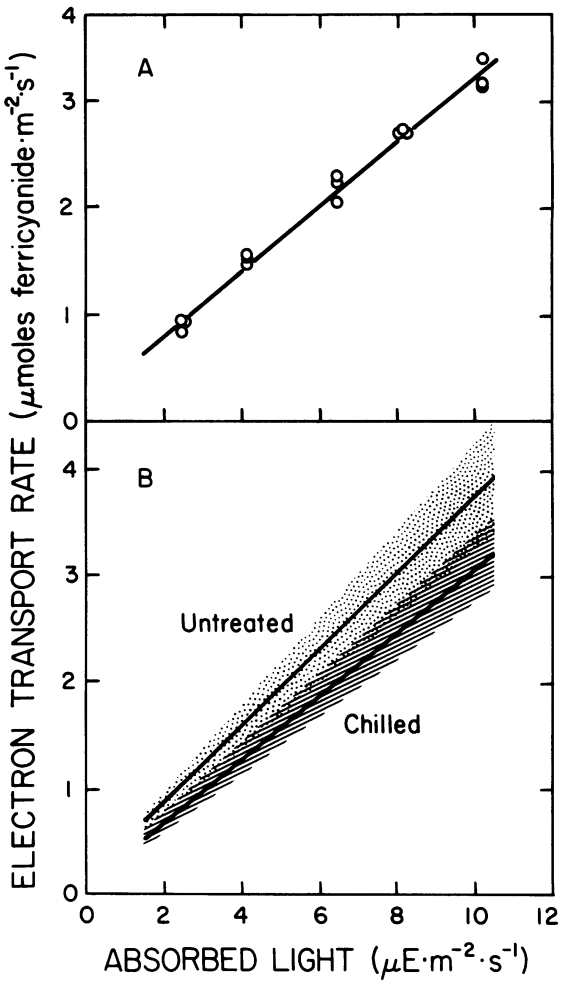

FIG. 5. The effect of chilling in the dark on the quantum yield of electron flow through PSII measured in chloroplasts isolated from untreated and chilled leaves. A, a typical result from an experiment with three measurements at each light intensity. B, the average quantum yields for four chloroplast isolations from untreated leaves and five chloroplast isolations from chilled leaves. The shaded areas give the standard deviation for the two lines. The measurements were done at 20°C and the reaction mixture contained 50 mM Hepes-KOH (pH 7.5), 0.1 M sorbitol, 5 mM $MgCl_2$, 10 mM KCl, 1.5 mM $K_3Fe(CN)_6$, 0.5 mM diaminodurene, 1 μM dibromothymoquinone, and 10 μg Chl/ml.

from measurements of the products of water oxidation following a series of very brief and intense flashes of light. Because each flash was intense enough to excite every reaction center yet short enough to ensure that each reaction center turned over only a single time, the number of protons released from water was equal to the number of active PSII centers present in the sample (4). In this case, H^+ production due to water oxidation was measured after a series of 100 flashes with an intervening dark time (333 ms) sufficient to allow for complete regeneration of the active PSII centers to the reduced state. We found no statistically significant change in the concentration of active PSII centers subsequent to chilling (Table I). The same result was obtained when the flash energy was increased or decreased by 25% showing that the flashes were saturating. The same result was also obtained when intervening dark time was increased to 1000 ms or decreased to 100 ms, showing the intervening dark time was adequately long.

DISCUSSION

Our intent in this study was to establish whether or not impaired water oxidation capability is a significant aspect of the inhibition

Table I. *Comparison of the Effect of Prechilling on CO_2 Reduction versus PS II Activity*

The experimental conditions for the measurement of CO_2 reduction are given in Figure 1. Rates of PS II electron flow were determined from O_2 consumption with a Clark-type O_2 electrode due to the aerobic oxidation of dimethyl-methylenedioxy-*p*-benzohydroquinone (0.25 mM) in a reaction mixture containing 50 mM Hepes-KOH (pH 8.0), 0.1 M sorbitol, 5 mM $MgCl_2$, 10 mM KCl, 0.1 mM $MnCl_2$, 1 μM dibromothymoquinone, and chloroplasts containing 10 μg Chl/ml.

	Ambient CO_2	Net Photosynthesis Rate	Rate of Electron Flux Required To Support Net Photosynthesis	Rate of PSII Electron Transport	Concn. of Active PSII Centers
	μl/l	*mg $CO_2/dm^2 \cdot h$*	*μeq/mg Chl · h*		*Chl molecules/active reaction center*
Untreated	300	31 ± 5, $n = 6$	460	980 ± 60, $n = 12$	620 ± 90, $n = 46$
	1,500	45 ± 6, $n = 10$	690		
Chilled	300	16 ± 3, $n = 8$	240	860 ± 110, $n = 12$	680 ± 40, $n = 6$
	1,500	31 ± 3, $n = 7$	460		

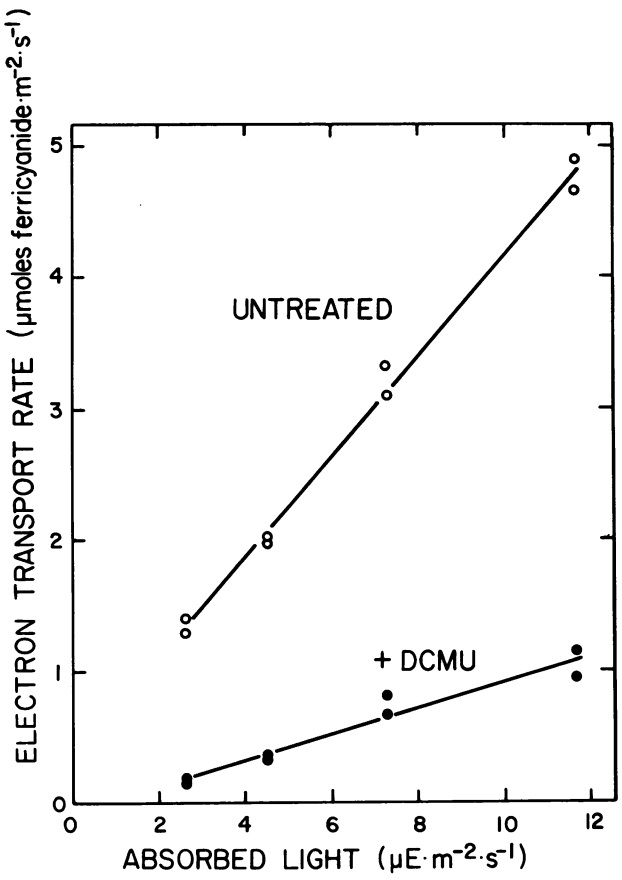

FIG. 6. The effect of DCMU on the quantum yield of PSII electron flow measured in isolated chloroplasts. Sufficient DCMU (0.1 μM) was added to reduce the maximum rate of basal electron transport by 36% (from 170 to 110 μeq mg^{-1} Chl h^{-1}). Conditions were identical to those in Figure 5 except for the omission of diaminodurene and dibromothymoquinone and the reduction of the ferricyanide concentration to 0.4 mM.

of photosynthesis by prechilling observed in thermophilic plants. We have taken three lines of investigation (a) measurement of quantum yields of CO_2 reduction in attached leaves and measurement of quantum yields of diiminodurene reduction by PSII of isolated chloroplasts; (b) measurement of maximum rates of PSII turnover in isolated chloroplasts; and (c) measurement of the concentration of active PSII centers in isolated chloroplasts. It is important to emphasize that we dealt only with the effects of dark chilling and it is likely the effects of cool temperatures coincident with illumination are distinctly different.

PSII electron transport from the oxidation of water to the reduction of the secondary quinone acceptor B is carried out by an integral membrane polypeptide complex (2, 15). Each of these operate independently with no electron shuttling between complexes but act to reduce a common plastoquinone acceptor pool (7). The association between PSII reaction centers and water oxidizing enzyme complexes is such that the failure of one water-oxidizing complex leads directly to the loss of photochemical activity of one PSII reaction center. The effect that the loss of photochemical activity of a reaction center will have on the quantum yield will depend on the extent to which energy transfer between PSII centers can occur. Joliot *et al.* (6) demonstrated that an inactive PSII center will not allow escape of exciton energy if its reaction center is in a "quenching form." The inhibition of water oxidation would cause the reaction center of the PSII complex to be in a quenching state (6). Consequently, loss of photochemical activity due to loss of water oxidation capability should have a direct effect on the quantum yield of PSII turnover. Furthermore, impaired water oxidation capacity would have a substantially more severe effect on the quantum yield than it would on the maximum rate of CO_2 reduction. The rate limiting step(s) in light- and CO_2-saturated photosynthesis are clearly not associated with PSII activity. Consequently, damage to a small fraction of the PSII centers would immediately be reflected in quantum yield measurements (where light is limiting), whereas any effect on light- and CO-saturated photosynthesis would be absent or greatly reduced. This prediction was born out in the experiments with both attached leaves and isolated chloroplasts in which PSII was partially inhibited by DCMU. In both cases, DCMU acted prior to the rate-limiting step and the effect of DCMU was more than 2-fold greater on the quantum yield values than on the maximum rates. The data in this paper (Figs. 1, 2, 4, 5, and Table I) show clearly that photosynthesis impaired by chilling does not conform to what is expected of impaired water-oxidation. Instead, any effect of chilling observed on the quantum yield was small compared to the effects on maximum rates.

The second line of evidence, maximum rates of PSII turnover in chloroplast isolated from unchilled or chilled leaves, demonstrates two points. First, the rate of PSII turnover was only marginally less in chloroplasts from chilled leaves compared to unchilled leaves. Secondly, in all cases, the rate of PSII turnover far exceeded the electron flux required to support the rate of light- and CO_2-saturated CO_2 reduction measured in attached leaves (Table I).

The third line of support, measurement of the concentration of active PSII centers, shows there was vanishingly little difference between chloroplasts isolated from chilled *versus* unchilled leaves. In this experiment, proton release from water oxidation was

measured and a decreased amount of protons released on the basis of the total Chl content is a necessary consequence of reduced water oxidation capacity. The fact that we observed very similar values for proton release in the two sorts of chloroplasts indicates that prechilling has a correspondingly small effect on water oxidation.

These lines of evidence are in agreement both qualitatively and quantitatively and lead to the conclusion that a significant reduction in the capacity of water oxidation does not occur in tomato due to prechilling and is not a primary element of the inhibition observed in attached leaves. Furthermore, the data demonstrate that no PSII reaction prior to the reduction of plastoquinone can account for the inhibition observed in attached leaves.

In an attempt to discover the source of the difference in our results and those of others (*e.g.* 8, 10, 17) who reported substantial inhibition of water oxidation by prechilling, we examined the effect of leaf detachment and prolonged chilling on the quantum yield of CO_2 reduction to reproduce the experimental conditions of the earlier work. We were, however, unable to verify any damage to PSII serious enough to cause the observed drop in CO_2 reduction. On the other hand, the fact that chilling subsequent to leaf detachment had a significantly more severe effect on the quantum yield of CO_2 reduction may reflect a correspondingly greater inhibition of water oxidation. Nevertheless, even though the maximum rate of CO_2 reduction continued to decline as chilling was prolonged from 20 to 40 h the quantum yield remained stable (Fig. 3), indicating even after leaf detachment an inhibition of PSII is not the primary cause of reduced CO_2 reduction.

Acknowledgments—The authors thank Drs. J S. Boyer and J. Whitmarsh for the use of their instrumentation as well as numerous valuable discussions.

LITERATURE CITED

1. ARNON DI 1949 Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1–15
2. DINER BA, FA WOLLMAN 1980 Isolation of highly active photosystem II particles from a mutant *Chlamydomonas reinhardtii*. Eur J Biochem 110: 521–526
3. DRAKE B, K RASCHKE 1974 Prechilling of *Xanthium strumarium* L. reduces net photosynthesis and, independently, stomatal conductance, while sensitizing the stomata to CO_2. Plant Physiol 53: 808–812
4. EMERSON R, W ARNOLD 1932 The photochemical reaction in photosynthesis. J Gen Physiol 16: 191–205
5. IZAWA S, JM GOULD, DR ORT, P FELKER, NE GOOD 1973 Electron transport and photophosphorylation in chloroplasts as a function of the electron acceptor. III. A dibromothymoquinone-insensitive phosphoylation reaction associated with photosystem II. Biochim Biophys Acta 305: 119–128
6. JOLIOT P, P BENNOUN, A JOLIOT 1973 New evidence supporting energy transfer between photosynthetic units. Biochim Biophys Acta 305:317–328
7. JOLIOT P, B. KOK 1975 Oxygen evolution in photosynthesis. *In* Govindjee, ed, Bioenergetics of Photosynthesis. Academic Press, New York, pp 388–412
8. KANIUGA Z, W MICHALSKI 1978 Photosynthetic apparatus in chilling-sensitive plants. II. Changes in free fatty acid composition and photoperoxidation in chloroplasts following cold storage and illumination of leaves in relation to Hill reaction activity. Planta 140: 129–136
9. KANIUGA Z, B SOCHANOWICZ, J ZABEK, K KRZYSTYNIAK 1978 Photosynthetic apparatus in chilling-sensitive plants. I. Reactivation of Hill reaction activity inhibited on the cold and dark storage of detached leaves and intact plants. Planta 140: 121–128
10. MARGULIES MM 1972 Effect of cold-storage of bean leaves on photosynthetic reactions of isolated chloroplasts. Inability to donate electrons to photosystem II and relation to manganese content. Biochim Biophys Acta 267: 96–103
11. MARGULIES MM, AT JAGENDORF 1960 Effect of cold storage of bean leaves on photosynthetic reactions of isolated chloroplasts. Arch Biochem Biophys 90: 176–183
12. MARTIN B, DR ORT, JS BOYER 1981 Impairment of photosynthesis by chilling temperatures in tomato. Plant Physiol 68: 329–334
13. MOHANTY P, JS BOYER 1976 Chloroplast response to low leaf water potentials. IV. Quantum yield is reduced. Plant Physiol 57: 704–709
14. RASCHKE K 1970 Temperature dependence of CO_2 assimilation and stomatal aperture in leaf sections of *Zea mays*. Planta 91: 336–363
15. SATOH K, WL BUTLER 1978 Low temperature spectral properties of subchloroplast fractions purified from spinach. Plant Physiol 61: 373–379
16. SMILLIE RM 1979 The useful chloroplast: A new approach for investigating chilling stress in plants. *In* Lyons JM, D Graham, JK Raison, eds, Low Temperature Stress in Crop Plants. The Role of the Membrane. Academic Press, New York, pp 187–202
17. SMILLIE RM, R NOTT 1979 Assay of chilling injury in wild and domestic tomatoes based on photosystem activity of the chilled leaves. Plant Physiol 63: 796–801
18. TREBST A, S REIMER, F DALLACKER 1976 Properties of photoreductions by photosystem II. Plant Sci Lett 6: 21–24