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Reversibility of Photosynthetic Inhibition in Cotton after Long-Term Exposure to Elevated CO_2 Concentrations[1]

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ABSTRACT

Cotton (*Gossypium hirsutum* L. cv Stoneville 213) was grown at 350 and 1000 microliters per liter CO_2. The plants grown at elevated CO_2 concentrations contained large starch pools and showed initial symptoms of visible physical damage. Photosynthetic rates were lower than expected based on instantaneous exposure to high CO_2.

A group of plants grown at 1000 microliters per liter CO_2 was switched to 350 microliters per liter CO_2. Starch pools and photosynthetic rates were monitored in the switched plants and in the two unswitched control groups. Photosynthetic rates per unit leaf area recovered to the level of the 350 microliters per liter CO_2 grown control group within four to five days. To assess only nonstomatal limitations to photosynthesis, a measure of photosynthetic efficiencies was calculated (moles CO_2 fixed per square meter per second per mole intercellular CO_2). Photosynthetic efficiency also recovered to the levels of the 350 microliters per liter CO_2 grown controls within three to four days.

Recovery was correlated to a rapid depletion of the starch pool, indicating that the inhibition of photosynthesis is primarily a result of feedback inhibition. However, complete recovery may involve the repair of damage to the chloroplasts caused by excessive starch accumulation. The rapid and complete reversal of photosynthetic inhibition suggests that the appearance of large, strong sinks at certain developmental stages could result in reduction of the large starch accumulations and that photosynthetic rates could recover to near the theoretical capacity during periods of high photosynthate demand.

Studies of short-term exposure to elevated CO_2 concentration have shown that increased starch levels are closely correlated with inhibition of photosynthesis (21, 26). Although the specific mechanisms of inhibition are not well understood (22), photosynthesis can be affected by factors such as enzyme regulation and activity (26, 28), insufficient pools of metabolites, such as Pi (11, 24), or changes in physiological processes which reduce quantum yield (1, 27).

Long-term exposure to elevated CO_2 concentrations often introduces effects that would not be predicted from short-term exposure studies. Some of these effects influence photosynthetic rates either directly or indirectly. Anatomical changes can occur which change internal leaf structure (15). Changes in degree and rate of leaf expansion and in total leaf area production affect photosynthetic rates on an area basis and on a whole-plant basis (13).

Long-term exposure to elevated CO_2 can also result in excessive accumulation of starch in the leaves (2, 16, 17, 31). Large, unusually shaped starch grains have been postulated to cause damage, either through contortion of the chloroplast grana or through actual disruption of chloroplasts (2, 31). Visible damage to the plants such as chlorosis, necrotic spots, and early leaf senescence occur after continued and excessive starch accumulation. However, the extent of starch accumulation varies at different development stages. For example, when sink strengths are high, demand for photosynthates reduces starch pools (18, 23), even in plants grown at high CO_2 concentrations. Conversely, reduction of sink strength increases starch accumulation (3, 23).

Elsewhere, we have described the inhibition of photosynthesis in cotton after long-term exposure to elevated CO_2 (4). When cotton grown continuously at 350 $\mu l \cdot l^{-1}$ CO_2 is exposed to 1000 $\mu l \cdot l^{-1}$ CO_2, photosynthetic rates per unit leaf area increase by 50%. However, in cotton grown continuously at 1000 $\mu l \cdot l^{-1}$ CO_2, photosynthetic rates per unit leaf area are only 15% greater than photosynthetic rates of 350 $\mu l \cdot l^{-1}$ CO_2 grown plants measured at 350 $\mu l \cdot l^{-1}$ CO_2. Stomatal conductance of cotton grown continuously at 1000 $\mu l \cdot l^{-1}$ CO_2 is 40% lower than cotton grown at 350 $\mu l \cdot l^{-1}$ CO_2 but exposed to 1000 $\mu l \cdot l^{-1}$ CO_2. By determining the relationship between photosynthetic rate and intercellular CO_2 concentration, it is possible to assess the stomatal limitations to photosynthesis (19). At 1000 $\mu l \cdot l^{-1}$ CO_2, stomatal limitations reduced potential net photosynthesis per unit leaf area less than 5%, regardless of growth conditions. Thus, reduced stomatal conductance at elevated CO_2 concentrations was not responsible for the reduction of photosynthesis; rather, there was an increase in mesophyll resistance, better expressed as an increase in biochemical resistance to CO_2 fixation. Plants grown at elevated CO_2 concentrations showed greatly increased starch accumulation compared to plants grown at ambient CO_2 levels. Furthermore, plants grown at elevated CO_2 levels showed initial symptoms of damage such as reduced Chl content, brittleness, and leaf curling.

This study was designed to determine the effects of large starch accumulations in cotton after long-term exposure to elevated CO_2 concentrations but before severe, visible damage occurs. We were interested in the reversibility of feedback inhibition due to starch as well as the repair of any damage to the chloroplasts caused by large starch grains. Depletion of the large starch pools should eliminate feedback inhibition of photosynthesis, while complete recovery of photosynthesis requires repair of any damage. Plants grown continuously at 1000 $\mu l \cdot l^{-1}$ CO_2 were switched to ambient CO_2 concentrations in order to monitor changes in the starch pools and the photosynthetic rates of individual leaves. Understanding the reversibility of photosynthetic inhibition is important in predicting long-term growth and yield because of the varying sink strengths during development. The ability to reduce accumulations of starch and to relieve photosynthetic inhibition can significantly increase photosynthetic rates and increase the amount of photosynthate available for the sinks.

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MATERIALS AND METHODS

Plant Material. Cotton (*Gossypium hirsutum* L. cv Stoneville 213) was grown from seed in 10-cm pots in a 1:1 (v/v) mixture of vermiculite and gravel. Plants were watered to saturation each morning with a modified half-strength Hoagland solution (5) and each afternoon with deionized H_2O.

Growth Conditions. Plants were continuously exposed to CO_2 concentrations of 350 or 1000 $\mu l \cdot l^{-1}$ CO_2 in controlled growth chambers in the Duke University Phytotron (14). The concentration of CO_2 was automatically monitored and controlled (10). The CO_2 used was produced by a process which does not generate ethylene, and measurements of ethylene concentrations in the growth chambers show no abnormal ethylene concentrations that could alter the responses (20). Plants were grown under a 12-h photo- and thermal-period. A photosynthetic photon flux density of 950 ± 50 $\mu mol \cdot s^{-1} \cdot m^{-2}$ was provided by a combination of sodium and multivapor high-intensity discharge lamps. Temperatures were maintained at 26/20°C day/night, and vapor pressure deficits were maintained at 1 kPa during the day. Plants were grown for 4 weeks until they were approximately 40 cm tall and had produced four to five fully expanded leaves.

Switching Experiment. One group of plants grown at 1000 $\mu l \cdot l^{-1}$ CO_2 was transferred at the start of the photoperiod to the 350 $\mu l \cdot l^{-1}$ CO_2 chamber where it received the same conditions as those remaining at 350 or 1000 $\mu l \cdot l^{-1}$ CO_2. Although depletion of the starch pool might have occurred faster by placing the plants in total darkness, they would not have maintained their normal diurnal activities, making photosynthetic measurements suspect.

Gas Exchange Techniques. Net photosynthesis was measured in the two control groups grown continuously at 350 or 1000 $\mu l \cdot l^{-1}$ CO_2 and in the 1000 switched to 350 $\mu l \cdot l^{-1}$ CO_2 group. Five plants from each group were measured using a clamp-on leaf cuvette in an open IR gas analysis system. All measurements were made at 350 $\mu l \cdot l^{-1}$ CO_2, 26°C and at saturating irradiance. The most recently fully expanded leaf was selected and followed throughout the experiment. Leaves were carefully selected in all three groups to be of comparable age and stage of development. Stomatal conductance and internal CO_2 concentration were calculated based on simultaneous measurements of transpiration (25). Measurements were made on each plant at the same time each measurement day, between 1 and 4 h into the photoperiod.

Carbohydrate Determination. Samples for starch analysis were taken with a circular hole puncher from plants in each group. At each sampling time, four punches (0.34 cm^2 each) of mesophyll tissue were taken from the central portion of the leaf, avoiding large veins. Samples were taken 2 h before the end of the photoperiod, by which time peak starch accumulations had occurred, based on diurnal measurements (4). Tissue was quick-killed in a microwave oven for 15 s, dried for 24 h at 60°C, and stored dessicated. The dried samples were weighed and then homogenized in a 12:5:3 (v/v) mixture of methanol, chloroform, and H_2O. The starch fraction was separated from the soluble sugars and pigments by four sequential centrifugations and resuspensions in methanol, chloroform, and H_2O (9). The starch was digested to glucose for 30 min at 55°C using amyloglucosidase from *Asperillus oryzae* (23) (Sigma A-9268, grade V, dialyzed and diluted to approximately 10 units/ml in 50 mM Na-acetate buffer, pH 4.5). Glucose concentrations were determined using a glucose oxidase-iodide assay (7).

RESULTS

Plants grown at 1000 $\mu l \cdot l^{-1}$ CO_2 had lower photosynthetic rates per unit leaf area than 350 $\mu l \cdot l^{-1}$ CO_2 grown plants, when measured at 350 $\mu l \cdot l^{-1}$ CO_2 (Fig. 1). During the course of the experiment, control plants maintained at either 350 or 1000 $\mu l \cdot l^{-1}$ CO_2 showed a slight decline in photosynthetic rates, probably associated with aging. However, plants grown at 1000 $\mu l \cdot l^{-1}$ CO_2 then switched to 350 $\mu l \cdot l^{-1}$ CO_2 showed a gradual rise in photosynthetic rate over several days, eventually reaching the rate of the 350 $\mu l \cdot l^{-1}$ CO_2 grown controls. Stomatal conductance of the switched plants similarly increased to the level of the plants

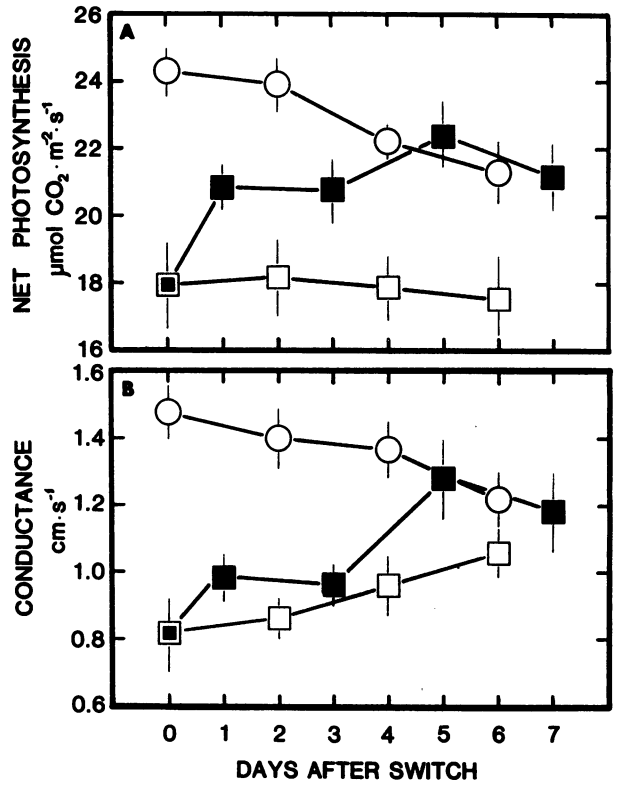

FIG. 1. Net photosynthetic rates per unit leaf area (A) and stomatal conductance (B) measured at 350 $\mu l \cdot l^{-1}$ CO_2 for cotton grown at 350 (○) or 1000 (□) $\mu l \cdot l^{-1}$ CO_2, or for cotton grown at 1000 $\mu l \cdot l^{-1}$ CO_2 but switched to 350 $\mu l \cdot l^{-1}$ CO_2 on d 0 (■). Each point represents the mean of measurements on five plants. Error bars indicates 2× mean SE.

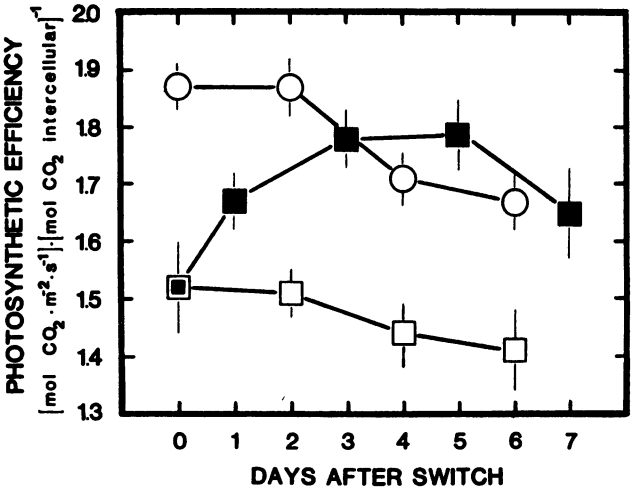

FIG. 2. Photosynthetic efficiency (mol CO_2 fixed $\cdot m^{-2} \cdot s^{-1}$/mol intercellular CO_2) based on net photosynthetic measurements at 350 $\mu l \cdot l^{-1}$ CO_2 and calculated internal CO_2 concentrations. Cotton was grown at 350 (○) or 1000 (□) $\mu l \cdot l^{-1}$ CO_2, or at 1000 $\mu l \cdot l^{-1}$ CO_2 then switched to 350 $\mu l \cdot l^{-1}$ CO_2 on d 0 (■). Each point represents the mean of measurements of five plants. Error bars indicate 2× mean SE.

maintained at 350 $\mu l \cdot l^{-1}$ CO_2. The increase in photosynthetic rate was termed as recovery from inhibition.

In order to factor out stomatal effects on photosynthesis, the results were also expressed in terms of internal CO_2 concentrations. This allows comparisons of nonstomatal limitations to photosynthesis. Photosynthetic rates were expressed in terms of a photosynthetic efficiency, the ratio of net CO_2 fixation per unit leaf area to the internal intercellular concentration of CO_2 (Fig. 2). This ratio represents the efficiency with which CO_2 is fixed once inside the leaf. In the two control groups, photosynthetic efficiency decreased slightly during the course of the experiment; however, the plants grown at 1000 $\mu l \cdot l^{-1}$ CO_2 then switched to 350 $\mu l \cdot l^{-1}$ CO_2 showed an increase in photosynthetic efficiency over several days, eventually reaching the rate of the 350 $\mu l \cdot l^{-1}$ CO_2 grown control group.

Prior to the switching, maximum diurnal starch levels in the 1000 $\mu l \cdot l^{-1}$ CO_2 grown plants were more than three times greater than in the 350 $\mu l \cdot l^{-1}$ CO_2 grown plants (Fig. 3). The levels in the unswitched control group remained high throughout the experiment, whereas in the plants switched to 350 $\mu l \cdot l^{-1}$ CO_2, the starch levels dropped very quickly and reached the level of the 350 $\mu l \cdot l^{-1}$ CO_2 grown control group within 2 to 3 d.

DISCUSSION

As in our previous study (4), cotton grown continuously at elevated CO_2 concentrations had large accumulations of starch and showed initial symptoms of chloroplast damage. They also had lower photosynthetic rates than would be expected based on instantaneous exposures to elevated CO_2 concentrations. By calculating internal CO_2 concentrations, we determined that the inhibition of photosynthesis at elevated CO_2 concentrations was predominantly due to nonstomatal limitations to CO_2 fixation (4, 8). This allowed consideration of the importance of feedback inhibition and starch accumulation damage separately from stomatal effects.

Since increased starch levels are associated with reduction of photosynthetic rates, depletion of the large starch pools in 1000 $\mu l \cdot l^{-1}$ CO_2 grown plants minimizes the contribution of feedback inhibition. Thus, after switching 1000 $\mu l \cdot l^{-1}$ CO_2 grown plants to 350 $\mu l \cdot l^{-1}$ CO_2, the recovery of photosynthetic rate per unit leaf area as the starch pool was depleted should indicate the extent of feedback inhibition. However, as starch pools are reduced, repair of any damage that may have occurred to the chloroplasts begins, also contributing to the recovery of photosynthetic rates. Severe or permanent damage, requiring lengthy repair processes, would delay or prevent complete recovery of the photosynthetic rates.

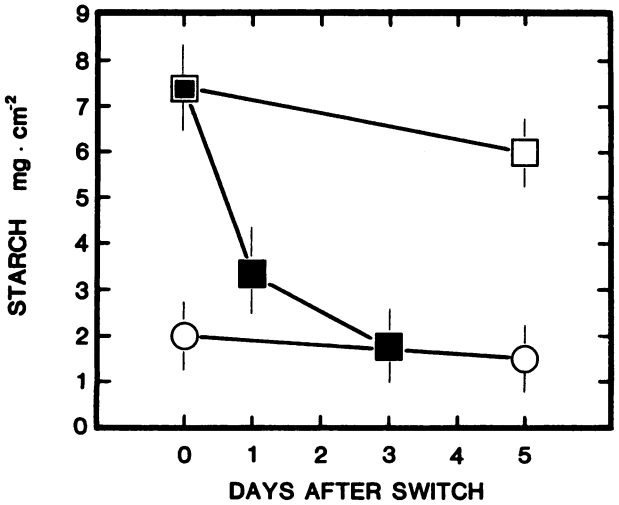

FIG. 3. Peak starch levels of cotton grown at 350 (○) or 1000 (□) $\mu l \cdot l^{-1}$ CO_2, or for cotton grown at 1000 $\mu l \cdot l^{-1}$ CO_2 then switched to 350 $\mu l \cdot l^{-1}$ CO_2 on d 0 (■). Each point represents the mean of samples taken from four plants 2 h before the end of the photoperiod. Error bars indicate 2× the mean SE.

Specific mechanisms of photosynthetic inhibition correlated to starch concentrations are still unresolved (22). Some hypotheses suggest physical mechanisms for inhibition due to starch accumulation, such as starch grain shading of light reaching the chloroplasts (29) or increases in diffusive path lengths or interference of intracellular CO_2 transport due to the large starch grains (21). High CO_2 concentrations cause partial stomatal closure, leading to hypotheses that photosynthesis could thereby be limited by low CO_2 concentrations inside the chloroplasts. However, using the analysis techniques of Farquhar and Sharkey (8), it is possible to determine the contribution of stomatal effects. In fact, it seems likely that partial stomatal closure does not significantly reduce photosynthesis, at least in cotton (4). It now appears most likely that various biochemical resistances to CO_2 fixation can act to inhibit photosynthesis.

Growth at elevated CO_2 concentrations has been shown to result in increased levels of RuBP but with reduced RuBPCase activity (6, 28, 30). This can be further complicated at high CO_2 concentrations by limitations to carboxylation due to RuBP regeneration capacity and electron transport capacity (26, 27). Inhibition can also occur by the chloroplast's production of triose-P exceeding the capacity of triose-P metabolism through starch and sucrose formation. This results in insufficient Pi pools in the chloroplasts (11, 24). This effect is further stimulated when photorespiration is reduced at high CO_2 concentrations since dephosphorylation of P-glycolate from photorespiration is reduced in the chloroplasts. Regulation also occurs in the biosynthetic enzymes such as those involved in sucrose and starch formation (12), which in turn leads to feedback pathways to carboxylation.

The results of this experiment indicate that the inhibition of photosynthesis observed after long-term exposure to high CO_2 can be reversed after several days of exposure to normal (350 $\mu l \cdot l^{-1}$) CO_2 concentrations. Photosynthetic recovery is correlated to a rapid depletion of the starch pool, presumably eliminating feedback inhibition of photosynthesis. The recovery is rapid and extensive, indicating that feedback inhibition is probably the predominant factor responsible for the photosynthetic inhibition. The increase in photosynthetic rates is largely independent of any stomatal effects, as evidenced by the increase in photosynthetic efficiency. We observed reduced Chl contents, changes in leaf texture, and very large, unusually shaped starch grains which disturbed normal chloroplast structure (micrographs not shown). Further research is in progress to detect specifically damage repair, such as Chl resynthesis and the repair of photosynthetic units and electron transport systems. Repair of minor damage caused by large starch grains could be occurring concurrently with the starch depletion.

The rapid reversibility of photosynthetic inhibition at elevated CO_2 concentrations would be important when large sinks appear during certain developmental stages, such as branching, flowering, and fruiting, when large starch accumulations could be reduced. This results in recovery of photosynthetic rates near the potential photosynthetic capacity, increasing productivity and yield.

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