Chapter 18

FEEDBACK CONTROL AND STRESS EFFECTS ON PHOTOSYNTHESIS

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INTRODUCTION

In 1868, Boussingault first proposed the hypothesis "that the accumulation of assimilates in an illuminated leaf may be responsible for a reduction in the net photosynthetic rate of that leaf." To date, this hypothesis has not been conclusively proven nor disproven. Considerable activity continues to address the issue.

In this review, I want first to develop our current concept of the possible cause of feedback inhibition of photosynthesis in higher plants and then to discuss the photosynthetic response of cotton to environmental stress. The stress response will be related to direct versus indirect effects on the photosynthetic process.

The photosynthetic process consists of three distinct but integrated aspects, namely (1) the photochemical conversion of radiant energy to chemical energy, (2) the physical process controlling the transfer of CO_2 from the atmosphere to the illuminated chloroplast and (3) the biochemical reactions involved in CO_2 reduction. Therefore, if the products of the photosynthetic process inhibit the subsequent rate of carbon assimilation, the manifestations of the inhibition must reside in one of these three areas.

In 1968, Neales and Incoll reviewed the experimental evidence relevant to proving the end product inhibition hypothesis. They stated that in order to prove the hypothesis two conditions must be satisfied. First, a negative correlation between the measured photosynthetic rate and the concentration of assimilates in the leaf must exist, and second, a mechanism to explain the inhibition must be proposed. They concluded that experimental evidence was difficult to envisage which unequivocally confirmed the accumulation of assimilate in a leaf caused a decrease in the subsequent photosynthetic rate. Although evidence existed to indicate a negative correlation between assimilate concentration and photosynthetic rate, no mechanism to explain the response had been demonstrated.

Guinn and Mauney (1980) reviewed the literature developed largely since the 1968 review. They also concluded that demonstration of end-product inhibition is

difficult. The demonstration of a negative correlation between photosynthetic rate and assimilate concentration in a leaf is complicated by the fact that assimilates are the products of photosynthesis. Therefore, as photosynthetic rate increases, the production of assimilate increases and possibly results in a positive rather than a negative correlation. Failure to demonstrate a negative correlation does not prove the absence of end-product inhibition when accumulation of assimilate and photosynthetic rate are not separated in time.

The mechanisms proposed by Guinn and Mauney (1980) largely involved a physical disruption of normal chloroplast activities due to starch accumulation, and biochemical disruptions due to both simple and phosphorylated sugars (see Chapter 17). A hormonal control mechanism of photosynthesis was also suggested. The hormones were either produced by the leaves or translocated to the leaf tissue from other tissue such as developing fruits or roots. No definitive evidence was available to support the hormonal control hypothesis. To date, direct evidence is still non-existent, but much circumstantial evidence exists to suggest that the hormonal activity of a tissue may directly or indirectly influence the photosynthetic rate of green leaves (Geiger, 1976).

The current concept of plant growth rate being regulated by photosynthetic activity involves source-sink relationships. This concept implies that the source's ability to produce assimilate is directly related to the sink's ability to utilize assimilate for growth. Therefore, during the course of plant development, growth rates may be limited due to inadequate source activity, or due to inadequate sink activity, depending upon plant species, growth stage, and environmental conditions differentially affecting the source or the sink (see Chapter 16).

Efforts to demonstrate feedback inhibition of photosynthesis have largely been concerned with manipulations of source-sink ratios or with disruption of the translocation pathways. Many of these studies involved physical disruption of the normal source or sink size and, thus, wounding of the tissue. The results of these studies must be carefully interpreted with respect to subsequent metabolic activity on a whole plant basis. Alteration of normal source-sink ratios by nondestructive methods allows evaluation of not only the immediate responses of photosynthesis, but also the recovery potential upon return to the normal state. Techniques of nondestructive manipulation of source-sink ratios include reduction of effective leaf area by shading leaves and monitoring the unshaded leaves, altering sink activity by low temperature treatments, increasing external CO_2 concentrations, and lengthening the photoperiod to alter total daily assimilate production. Other experiments did not manipulate the plant but monitored the diurnal or seasonal changes in photosynthetic rate and correlated this with assimilate concentration in the leaf as a function of normal source-sink activity changes. The preponderance of evidence, at this point in time, suggests that source activity is highly variable and subject to control by sink demand, strongly implying some type of feedback inhibition.

If end product inhibition of photosynthesis does exist, then the products must be interfering with one or more of the individual steps in the integrated process. Intermediates and products of the photosynthetic process include O_2, ATP, NADPH, simple and phosphorylated sugars, and starch. As previously stated, the photosynthetic process consists of three distinct aspects: (a) the photochemical conversion of radiant energy to chemical energy, (b) the physical processes controlling the transfer of CO_2 from the atmosphere to the illuminated chloroplast and (c) the biochemical conversion of CO_2 to CH_2O and its disposition. Current information concerning feedback inhibition will be discussed with respect to possible mechanisms involving each of these three components of the photosynthetic process.

THE PHOTOCHEMICAL CONVERSION OF LIGHT TO CHEMICAL ENERGY

The light reaction involves the capture of photosynthetically active radiation by chlorophyll in the grana thylakoids and the subsequent transfer of electrons from H_2O to NADPH. The photooxidation of H_2O produces O_2 and protons (H^+), and the transfer of the electrons and protons across the thylakoid membrane results in an electrochemical gradient which provides the driving force for ATP synthesis (Chapter 15).

Guinn and Mauney (1980) emphasized the possible inhibitory effects of large starch granules in the chloroplast. The proposed mechanisms for feedback inhibition by starch were based on physical, rather than chemical effects. Accumulation of starch in the chloroplast could result in distortion of the grana, thereby altering the light absorption characteristics. However, Nafziger and Koller (1976) discounted this possibility because of the lack of response of photosynthesis to increasing irradiance when starch concentrations were high. Physical damage to the chloroplasts such as deterioration of the thylakoid membrane, absence of microbodies and leakage of chlorophyll results from large accumulations of starch (Ackerson and Herbert, 1981; De Silva *et al.*, 1974). However, excessive accumulation of starch is required to cause chloroplast damage.

A potentially serious problem associated with the photochemical conversion of solar energy to chemical energy is photoinhibition. Photoinhibition is defined as the photodestruction of the photosynthetic apparatus when photochemical energy cannot be dissipated in an orderly manner during normal CO_2 fixation (Osmund *et al.*, 1980). In order for photoinhibition to be extensive, the internal CO_2 concentration must be close to the CO_2 compensation point and photorespiration must be inhibited (<1 percent O_2). Photosystem II activity appears to be extremely sensitive to photoinhibitory damage; however, it is very unlikely that photoinhibition becomes a major limitation under normal environmental conditions.

THE PHYSICAL PROCESS CONTROLLING THE TRANSFER OF CO_2 FROM THE ATMOSPHERE TO THE ILLUMINATED CHLOROPLAST

The total conductance of CO_2 can be partitioned into individual components as discussed by Nobel (1974). Stomatal conductance is similar for water vapor and CO_2 with a diffusion coefficient difference of 1.6 in favor water vapor at 20C. Several reports suggest that stomatal conductance is altered by treatments which result in the accumulation of assimilate in the source leaf. Setter *et al.* (1980) reported that increased stomatal resistance was the cause of the reduced carbon exchange rate (CER) in soybeans which had been treated by altering the translocation system or by reducing sink size to increase assimilate concentration in the leaves. The stomatal response to the treatment was very rapid (0.5 hour with petiole girdling and 5 hours with pod removal) and coincident with CER reductions. These stomatal responses could also reflect alterations in the normal concentrations and activities of the leaf hormones (Geiger, 1976), especially abscisic acid (ABA) which is involved in stomatal control (Boveys and Kriedmann, 1974). Potter and Breen (1980) used an extended photoperiod treatment to alter sugar: starch concentrations in soybean and sunflower leaves. They observed stomatal conductance changes directly associated with CER reductions in both species. Older, essentially fully expanded, sunflower leaves were more responsive than were young, rapidly expanding leaves. Large accumulations of starch and soluble sugars were observed in all leaves but were not highly correlated with the photosynthetic rate changes. Peet and Kramer (1980) reduced source size by shading various soybean leaves. They reported that photosynthetic rates of the unshaded leaves increased, and the rate increase was associated with increases in both stomatal and mesophyll conductances. Thorne and Koller (1974), using similar techniques, found no change in soybean stomatal conductance as CER was increased. They did report that large reductions in mesophyll resistance accompanied the increased CER as sink demand increased relative to source activity. Additionally, the chloroplast starch concentrations were reduced tenfold; whereas, the soluble sugar concentrations were increased as CER increased. It is rather difficult to envision how solute accumulations can cause stomatal conductance changes, unless it is associated with altered hormonal relations of the leaf.

The increased length of the diffusion pathway for CO_2 around large starch grains has been proposed as an explanation for the increased mesophyll resistance by starch accumulation in the chloroplast. Based upon this assumption several studies evaluated CER in leaves with various starch concentrations generated in CO_2—enriched atmospheres. As the external CO_2 concentration increases the effects of increased mesophyll resistance should lessen due to the steeper gradient from the external atmosphere to the site of carboxylation. Mauney *et al.* (1979) reported a negative correlation between net photosynthesis and starch concentrations when CER was measured at normal CO_2 levels (330μ l l^{-1} in cotton) (Figure

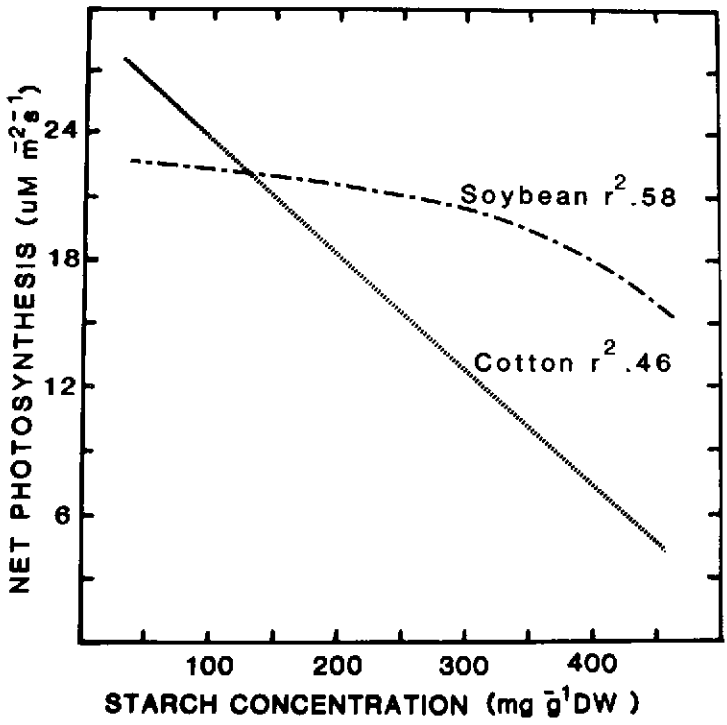

Figure 1. Net photosynthesis of cotton and soybean as a function of leaf starch concentration (adapted from Mauney *et al.*, 1979; Nafziger and Koller, 1976).

1). No significant correlation was found between soluble sugar concentrations and net photosynthetic rates in any of the four species examined. Nafziger and Koller (1976) suggested a curvilinear response of photosynthetic rate of soybean with leaf starch concentrations (Figure 1) as contrasted to a linear response proposed by Mauney *et al.* (1979) for cotton. In soybean, starch concentrations in excess of 1.5 mg cm^{-2} were required to reduce the photosynthetic rate. In cotton, approximately a 10 percent reduction in net photosynthesis was observed for each 10 percent increase in starch concentration. Apparently the sensitivity of photosynthesis to leaf starch concentration is species and leaf condition dependent. Both groups indicated that starch concentrations greatly in excess of that normally observed in leaves were required to cause a significant reduction in the photosynthetic rate.

Nafziger and Koller (1976) also determined the CO_2 compensation point to differentiate between mesophyll resistance and carboxylation resistance. They found the CO_2 compensation point was not altered by increased starch concentration. They concluded that the intracellular transport of CO_2 (mesophyll resis-

tance) was the factor limiting photosynthesis when starch concentration was high.

The rate of diffusion of the CO_2 molecule from the atmosphere to the site of carboxylation is reduced by accumulation of assimilate in the leaf. The primary cause of the increased resistance may be either stomatal or mesophyllic depending upon species, leaf age or stage of development. The increased stomatal resistance observed in some species may be the result of altered hormonal relations in the leaf due to the type of treatment imposed to induce large concentrations of assimilate in the leaf. The mesophyll resistance increases are largely of a physical nature and excessive starch concentrations are required to significantly affect the photosynthetic rate.

THE BIOCHEMICAL CONVERSION OF CO_2 TO CH_2O AND ITS DISPOSITION

The reduction of CO_2 to carbohydrate in the chloroplast occurs via the C_3 photosynthetic carbon reduction (PCR) pathway essentially as described in the 1950's (Chapter 15). Although we recognize the existence of other pathways (C_4 and CAM) responsible for the initial conversion of CO_2 to organic compounds, they are subservient to the C_3-PCR pathway. We now recognize that the C_3-PCR pathway does not totally describe the path of carbon in the chloroplast under natural atmospheric conditions. Photosynthetic carbon metabolism can best be described as the integrated sum of the activities of two mutually opposing but interlocking cycles, the C_3-PCR cycle and the C_2-photosynthetic carbon oxidation (PCO) cycle (Figure 2). The C_2-PCO cycle is known as photorespiration. Ribulose bisphosphate carboxylase/oxygenase (a bifunctional enzyme) serves to regulate carbon flow through these two competitive cycles through effects of CO_2 and O_2 concentrations and the kinetic properties of this enzyme (Latzko and Kelly, 1979). Several reviews address control of the activities of the two cycles and the resultant effect on net assimilation (Akazawa, 1979; Jensen and Bahr, 1977; Kelly and Latzko, 1976). Activation of RuBPC:Oase is a readily reversible process dependent on $[CO_2]$, $[Mg^{2+}]$, pH and levels of sugar phosphates. (Bahr and Jensen, 1978; Jensen and Bahr, 1977; Lorimar *et al.*, 1979). CO_2 is involved in both activation and catalysis. Several phosphorylated sugars are effectors of RuBPC:Oase. Fructose-6-P, ribulose-5-P, 6-P-gluconate, erythrose-4-P, and xylose-5-P, all at lmM concentrations, function as activators of the carboxylase *"in vitro"* (Buchanan and Sherman, 1973). Inhibition of carboxylase activity has been demonstrated using fructose 1-6-BP, fructose-1-P and glucose-1-P. The role of the sugar phosphates in activation versus catalysis is not clearly established; however, the effectors probably act at a single site, the catalytic site for RuBP. Effectors such as 6-P-gluconate and NADPH stabilize the active form of the enzyme and possibly function *in vivo*, especially early in the light period. However, as the PCR cycle increases in activity, 6-P-gluconate disappears and probably

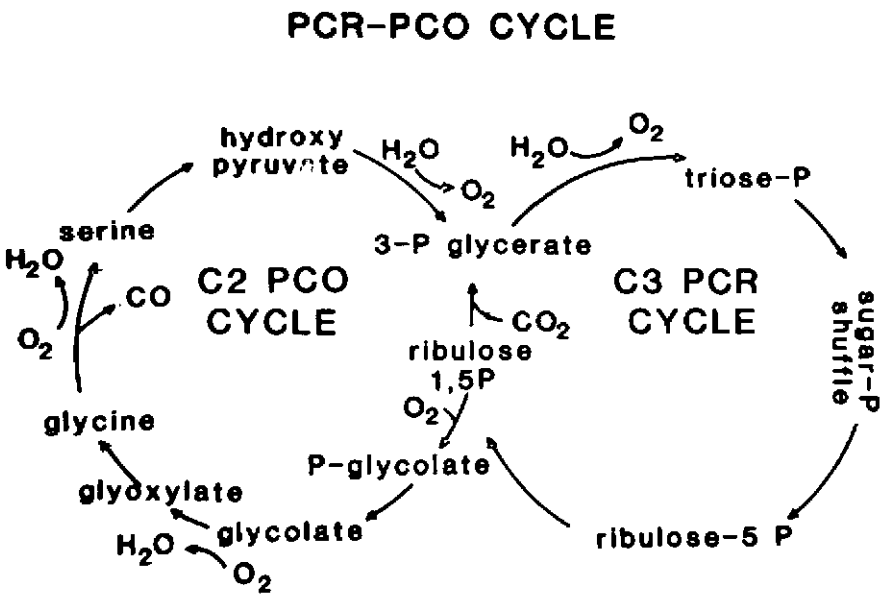

Figure 2. The photosynthetic carbon reduction-oxidation cycle existing in C_3 plants.

has no further effect on RuBPC:Oase activity. The magnitude of the inhibitory and stimulatory effects of metabolites is generally not very great and also not very consistent among different investigators. Extensive research by Chollett and Anderson (1976) demonstrated that none of the chloroplast metabolites examined differentially regulate carboxylase/oxygenase activities. These results were as expected since the same active site serves the two opposing reactions.

Carbon dioxide and O_2 compete for the same active site on this enzyme and control the ratio of carboxylation:oxygenation and thus the flow of carbon through the PCR versus the PCO cycles. Temperature plays a major role in determining CO_2:O_2 concentrations in the chloroplast (Azcon-Bieto *et al.*, 1981; Bykov *et al.*, 1981; Tenhunen *et al.*, 1979). Since O_2 is a product of the photochemical reaction, its concentration could be greater than that due to normal atmospheric conditions. Increases in net photosynthesis of 30 to 50 percent are observed in C_3 species when the O_2 content of the atmosphere is reduced from 20 percent to 1-2 percent (Laining *et al.*, 1974). In cotton, we estimated photorespiration as the difference between short time CO_2 fixation rates and net carbon exchange rates (CER) of single leaves (Perry and Krieg, 1981; Perry *et al.*, 1983). At 25C photorespiration is minimal but increases rapidly with increasing temperature, reaching a maximum of 50 percent of net photosynthesis at 35-37C (Figure 3). Using a 2 percent O_2 and 340 μl l^{-1} CO_2 gas mixture, we ascertained

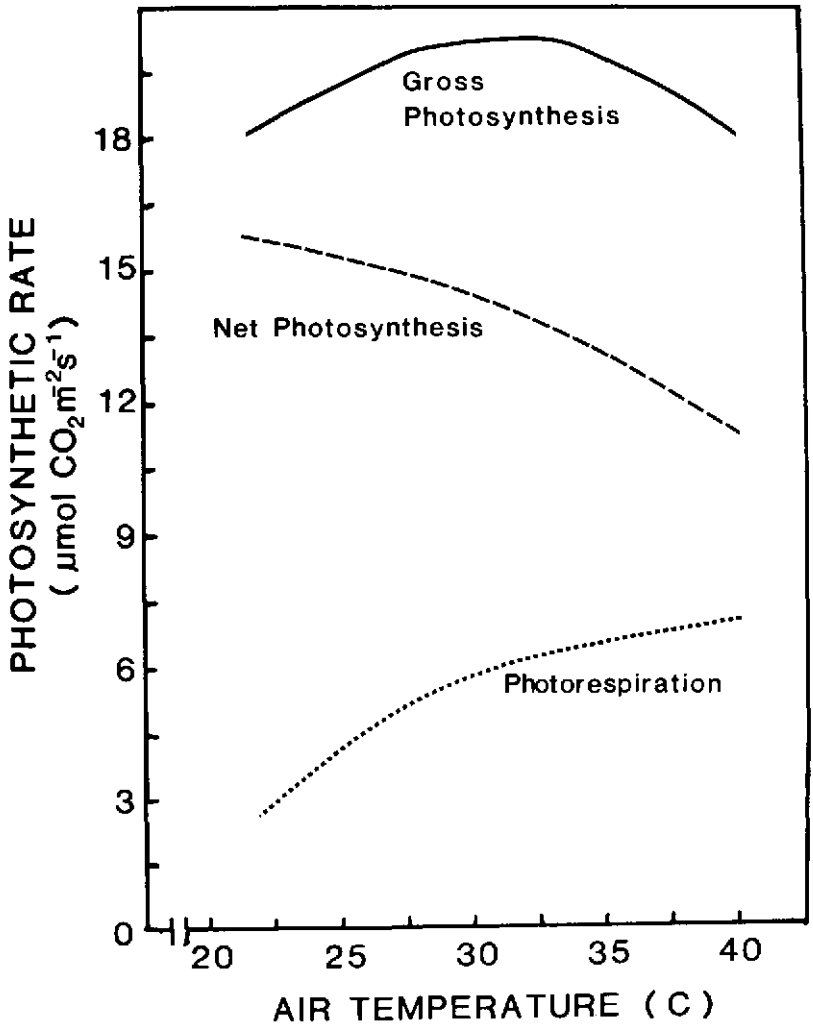

Figure 3. Photosynthetic responses of individual cotton leaves to increasing air temperature.

that essentially all of the difference between gross and net photosynthesis can be attributed to photorespiratory CO_2 evolution. Dark respiration rates of 2-3 mg CO_2 evolved dm^{-2} hr^{-1} were measured on fully expanded leaves. The current opinion is that glycolytic and Kreb cycle activity are greatly reduced in the light due to the cytoplasmic energy charge and due to the high concentration of phosphorylated sugars favoring sucrose synthesis and inhibiting dark respiration.

CO_2 compensation concentrations increase with temperature, also reflecting the increased photorespiratory activity (Bykov *et al.*, 1981). This reduced the CO_2 concentration gradient and, thus, the rate of diffusion of CO_2 to the chloroplast. If stomatal or mesophyll resistance are simultaneously increased due to starch accumulations, net photosynthesis would be severely reduced. Nofziger and Koller (1976) indicated that at a given temperature, the CO_2 compensation point was not altered and attributed the reduction in net photosynthesis to increased mesophyll resistance. Based on the current evidence this response is as expected.

Zelitch (1979) proposed that certain metabolites such as L-glutamate, L-aspartate, phosphoenolpyruvate and glyoxylate are effective inhibitors of photorespiration. He suggested that alterations in the pool sizes of certain common metabolites can increase photosynthesis by inhibiting glycolate synthesis and thus photorespiration, probably by a feedback mechanism. Chemical or genetic regulation of some commonly occurring metabolites could possibly produce plants with higher rates of net photosynthesis through effects on photorespiratory inhibition, although the worth of this response remains to be demonstrated.

Only two other enzymes of the PCR pathway are subject to metabolic control and, thus, possible regulators of the rate of carbon flow through the cycle (Portis *et al.*, 1977). These are fructose 1-6 bisphosphatase (FBPase) and sedoheptulose 1-7 bisphosphatase (SBPase). These two enzymes hydrolyze the respective bisphosphates at the C-1 position to yield an inorganic phosphate and fructose-6-P or sedoheptulose-7-P, respectively. FBPase has been studied most intensively. It has an alkaline pH optimum (8.5), is largely inactive below 7.8, and is highly dependent on Mg^{2+}. The enzyme is activated by light largely due to pH and Mg^{2+} changes in the stroma favoring activity. The activity of FBPase increases in a sigmoidal manner with increased FBP concentrations. One possible mechanism of control by this enzyme involves the sigmoidal dependence of FBPase activity on FBP concentrations. If the glyceraldehyde-3-P concentration were to decline due to export from the chloroplast, then the chloroplast levels of DHAP and FBP would also decline and affect FBPase activity. SBPase responds similarly to effectors and metabolite concentrations. An interesting side note is that at a pH of 8.8, FBPase dissociates into two halves which retain catalytic activity and are reported to acquire SBPase activity (Buchanan and Sherman, 1973). It remains to be clarified whether the SBPase of the PCR cycle is a specific SBPase or a dissociated FBPase. The correlation between FBPase activity and rates of CO_2 fixation with intact chloroplasts is close (Portis *et al.*, 1977), but whether FBPase or SBPase is rate-limiting the CO_2 fixation is still a valid question.

The triose phosphates produced by the PCR cycle can either be exported from the chloroplast and produce sucrose for export in the cytoplasm, or they can be converted to hexose phosphates in the chloroplast and stored as starch. Why would starch accumulate in the chloroplast, if it is inhibitory to photosynthesis through increased mesophyll resistance? Why aren't the triose phosphates exported to minimize starch accumulation and possible damage to the chloroplasts?

We now recognize that co-transport occurs in moving assimilate from the chloroplast to the cytoplasm (Bassham, 1979). An inorganic phosphorus (Pi) must be imported for each triose-P exported from the chloroplast. Therefore, if the translocator were not able to function as rapidly as triose phosphates were being produced, fixed carbon would be diverted to starch synthesis inside the chloroplast. The rate of translocation across the chloroplast membrane may be limited by translocator activity or due to inadequate pool size of Pi in the cytoplasm. The unavailability of Pi in the cytoplasm may result from sequestering by soluble sugars or by reduced sucrose synthesis. Starch synthesis is stimulated by low concentrations of Pi in the chloroplast and by increased concentrations of 3-PGA.

STRESS EFFECTS ON PHOTOSYNTHESIS

The photosynthetic process is subject to control by both environmental and genetic factors. Complex control mechanisms (largely undefined) probably evolved to enable the plant to maintain a high degree of homeostasis when environmental conditions change or the demand from processes within the plant change. Lack of adequate supplies of soil water or excessive atmospheric demand for water frequently result in plant water deficits which constitute a growth-limiting stress to the cotton plant under field conditions. Usually accompanying the water stress is a high temperature stress which further confounds the plant response, especially the photosynthetic rate response. Several excellent books have addressed the developmental and physiological responses of plants to environmental stresses of water and temperature (Mussells and Staples, 1979; Turner and Kramer, 1980). Likewise, the photosynthetic process and the response to water and temperature stresses have been the subject of several recent reviews (Boyer, 1976a,b; Krieg, 1983 a,b). The water relations of the cotton plant and the response of select developmental and physiological processes to water deficits have been reviewed by Jordan (1983).

This effort is directed toward defining the possible control of the photosynthetic process in cotton due to end-product inhibition or the accumulation of assimilates. In order to accomplish this task one must be able to define and differentiate the responses of the source leaves, the translocation system and the various sinks to a range of stress intensities. No comprehensive experiments of this nature have been reported by my knowledge. Based upon the existing evidence, I will present my concept of the source-sink relations of the cotton plant and the possible control of photosynthetic rates by feedback inhibition.

As previously described the photosynthetic process consists of three major components, namely the photochemical process of energy conversion, the physical processes of diffusion controlling transfer of CO_2 from the external environment to the site of carboxylation, and lastly the biochemical process responsible for CO_2 reduction to CH_2O and its subsequent disposition. Environmental stresses can directly affect the photosynthetic rate or they can indirectly affect the assimila-

tion process by directly affecting the sinks ability to process the assimilate, thus creating the possibility of feedback inhibition.

DIRECT EFFECTS OF STRESS

Water stress was reported to directly affect the photochemical activity of isolated cotton chloroplasts (Fry, 1970, 1972). Hill reaction activity, as measured by ferricyanide reduction, was reduced approximately 2 percent per bar decline in osmotic potential of the bathing medium. This rate of reduction was fairly consistent using chloroplasts from cotton tissue subjected to stress in a number of different ways.

The literature is replete with examples of water stress inducing stomatal closure and thus greatly reducing gas exchange (Bielorai and Hopmans, 1975; Brown *et al.*, 1975; Davis, 1977; El Sharkawy and Hesketh, 1964; McMichael 1980; Troughton, 1969). Stomatal control is a function of guard cell water relations, and as stress increases and leaf water potential (ψ_L) declines, the stomata begin to close. A linear correlation between stomatal conductance and photosynthetic rate has been reported for cotton between leaf water vapor conductance values of 0.1 and 0.7 cm s^{-1} (McMichael, 1980). However, leaf conductances of field-grown cotton are often greatly in excess of 1.0 cm s^{-1}, and values in excess of 2.0 cm s^{-1} are not uncommon (Ackerson and Herbert, 1981; Ackerson and Krieg, 1977; Cutler *et al.*, 1977; Hutmacher and Krieg, 1981, 1983). Additionally, the stomatal response to declining leaf water potential is considerably different in field-grown cotton as compared with plants grown in controlled environment chambers (Ackerson and Herbert, 1981; Bielorai and Hopmans, 1975; Brown *et al.*, 1976; Davis, 1977; Troughton, 1969). Under field conditions, we observed the photosynthetic rate to be slightly more sensitive to decreasing leaf water potential than the stomatal conductance response (Figure 4). The leaf water potential at which reductions begin to occur is altered by leaf age and stress history. Older leaves and leaves developing under the influence of soil water deficits required lower leaf water potentials to initiate the inhibition. Numerous reports in the literature suggest nonstomatal control of photosynthesis, even though stomatal changes are evident as leaf water potential declines (Ackerson *et al.*, 1977; Hutmacher and Krieg, 1983; Karami *et al.*, 1980; Krieg, 1983, Sung and Krieg, 1979).

Throughton (1969) indicated that the mesophyll resistance to CO_2 transport became a major factor when the relative water content (RWC) of the leaf declined below 75 percent. In field-grown cotton the leaf water potential-relative water content relationships during dehydration indicate that at a ψ_L of -20 bars the RWC would be approximately 75 percent (Cutler and Rains, 1978; Cutler *et al.*, 1977a). Whether the increased mesophyll resistance is due to increased starch concentrations is not know at this time. However, under stress conditions, the leaf tissue normally has less starch than the non-stressed leaves (Eaton, 1955; Eaton and Ergle, 1948; Tollervey, 1970.

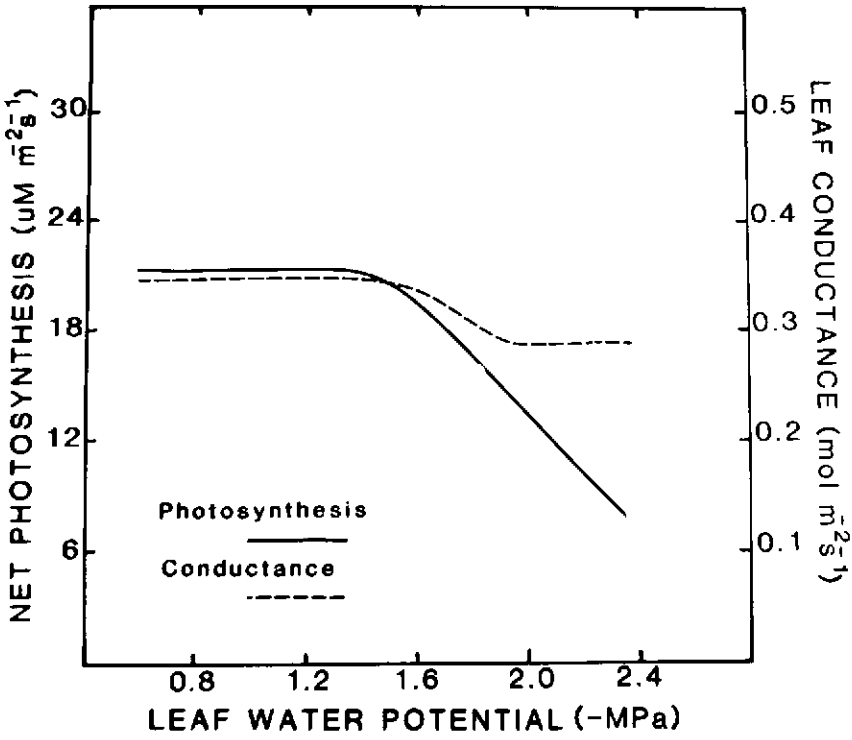

Figure 4. The influence of leaf water potential on photosynthetic rate and leaf conductance of water vapor for individual cotton leaves.

Direct effects of low water potential on carboxylation enzyme activity are not known at present. However, enzyme activities are determined *"in vitro"* in artificial environments. It is extremely difficult to determine the microenvironmental changes in the chloroplast as a result of low water potential and how these changes affect enzyme activity. Total RuBP carboxylase/oxygenase activity is subject to control by both biophysical and biochemical factors, and much remains to be done in defining the relative changes in these factors as stress progresses. Techniques are needed which will accurately reflect the "active" enzyme concentration in relation to "total" enzyme concentration.

The relative activity of RuBP carboxylase/oxygenase controls the rate of carboxylation versus oxgenation of RuBP and thus determines net photosynthesis. Our data indicate that the ratio of carboxylation to oxygenation is unaffected by increasing water stress to leaf water potentials of -24 bars (Perry and Krieg, 1983). Both gross and net photosynthesis begin to be affected as ψ_L declined from -20 bars (Figure 5). The constant ratio of gross photosynthesis to photorespiration in cotton implies stress effects on total enzyme activity. Previous reports of water

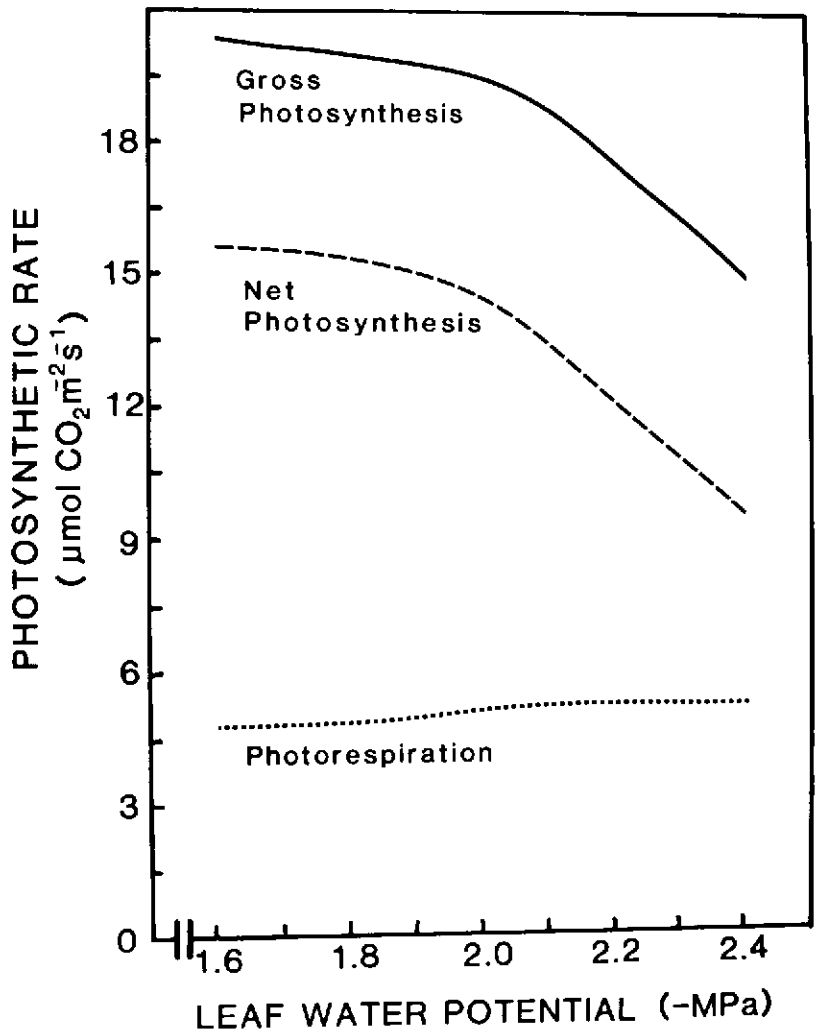

Figure 5. The influence of leaf water potential on photosynthesis-photorespiration rate responses of individual cotton leaves.

stress increasing photorespiratory activity of C_3 plants may be confounded by the effects of temperature changes coincident with the leaf water potential.

The ratio of gross photosynthesis to net photosynthesis is very temperature-sensitive. Gross photosynthesis of cotton had a temperature optimum of 32 to 33C; whereas, net photosynthesis declined almost linearly from 25C to 37C (Perry and Krieg, 1983). Photorespiration represented approximately 50 percent of net photosynthesis at 35C and occurred at a rate of 11 to 12 mg CO_2 dm^{-2} hr^{-1}.

Using 2 percent O_2 to essentially eliminate photorespiration, the difference between gross and net photosynthesis disappeared; thus the light-derived CO_2 evolution was from photorespiration. Temperature has a profound influence on gas solubility in aqueous solutions with CO_2 solubility reduced to a greater extent than O_2 solubility with increasing temperature (Tenhunen *et al.*, 1979). The CO_2 compensation point also increases linearly with temperature, reflecting increased photorespiratory activity (Bykov *et al.*, 1981). The increased CO_2 compensation point was a reflection of reduced total enzyme activity rather than a differential effect on carboxylase or oxygenase as evidenced by the response with different O_2 concentrations.

Cotton chloroplasts accumulate a rather large volume of starch during the course of the day which is attributed to carbon assimilation exceeding transport capacity (Eaton, 1955; Eaton and Ergle, 1948; Mason and and Maskell, 1928a). Starch synthesis is involved in reducing the osmotica in the chloroplast and maintaining a more favorable water status (Ackerson, 1981). Cotton also contains a number of intrachloroplastic bodies that appear to be lipid (Berlin *et al.*, 1981a). The number and total volume of the intrachloroplastic granules increase under high light conditions, under water stress conditions and with increasing leaf age. At present, no reason for their occurrence is known; however, one possibility is discussed below.

Osmond *et al.* (1980) hypothesized that photorespiration in C_3 plants provides a means to dissipate excess biochemical energy when CO_2 reduction is impaired due to stomatal closure. In many temperate C_3 plants, such as soybean, photorespiration represents about 50 percent of net photosynthesis at temperatures as low as 25C (Laing *et al.*, 1974) In cotton, photorespiration is minimal at 25C but increases with temperature, representing about 50 percent of net photosynthesis at 35C (Perry and Krieg, 1983). If mesophyll resistance is increased due to starch accumulation or to declining tissue water content, the CO_2 concentration at the carboxylation site would be low and not capable of utilizing all the biochemical energy provided, unless the photochemical reactions were affected as suggested by Fry (1970, 1972). However, if lipids were synthesized rather than starch, mesophyll resistance changes would be minimized and excess energy could be dissipated in further reduction of the carbon compared with that in carbohydrate. A major problem in this scheme is that CO_2 is a catalyst for malonyl CoA synthesis from acetyl CoA in the initiation of fatty acid synthesis (Stumpf, 1976). As previously stated, this hypothesis is proposed as a means of dissipating excess biochemical energy when CO_2 reduction can't utilize the available supply. Therefore, circumvention of this basic prerequisite for CO_2 in malonyl CoA synthesis must occur. Shannon and others (deVillis *et al.*, 1963; Shannon *et al.*, 1963) indicated that the following reaction occurs in plant tissue:

$$\text{Oxaloacetate} + \tfrac{1}{2}O_2 \xrightarrow[\text{Mn}]{\text{Peroxidase}} \text{Malonate} + CO_2$$

The malonic acid can form malonyl CoA by using ATP and CoASH, bypassing the need for CO_2 catalysis. Cutler *et al.* (1977a) indicated that malate concentrations increase by 60 percent during the course of the daylight hours, and the concentration is higher in water stressed tissue than in non-stressed tissue. These results support the idea that the substrate for production of malonic acid is present in rather significant quantities.

The production of lipid as compared with starch represents the use of one additional ATP and NADPH for each carbon stored. I suggest that the cotton plant might employ lipid synthesis in the chloroplast to minimize photoinhibition of the light harvesting apparatus due to undissipated biochemical energy and to maximize carbon conservation by minimizing photorespiration. This mechanism implies that changes in the microenvironment of the chloroplast enhance lipid synthesis and minimize starch synthesis. These changes would apparently be influenced by temperature or tissue water potential.

One must immediately ask "why would the chloroplast store starch (or lipid) rather than export the assimilate initially? Is the sucrose synthesis process rate-limiting? Or is the sink(s) activity, and thus the ability to process the assimilate, rate-limiting?" Based upon currently available information, one is led to the conclusion that the growth processes are more sensitive to environmental constraints such as water and high temperature stress and, therefore, are unable to process the assimilate into growth products as rapidly as they can be produced (see Chapters 7 and 10). Leaf expansion in cotton occurs at a greater rate in the dark than in the light (Bunce, 1977a; Krieg, 1981; Yimbo, 1980). This growth response is often attributed to inadequate turgor pressure in the expanding leaf during the day due to reduced leaf water potential. Simultaneously, growth rates of cotton fruit appear to be limited by internal processes rather than assimilate supply (Anderson and Kerr, 1943; Kirk and Krieg, 1981; Krieg and Sung, 1979; McArthur *et al.*, 1975). Boll growth rates have an optimum mean daily temperature of 27C and decline rapidly as the mean temperature increases (McArthur *et al.*, 1975). In much of the cotton-growing regions, the mean daily temperature is at least 27C during the boll development phase and in the semi-arid southwestern U.S. normally exceeds the optimum by several degrees. At present we do not know which assimilatory processes result in reduced boll growth rates when affected by temperature.

If assimilate demand is reduced by stress having a direct effect on the sink rather than the source, then photosynthate could accumulate and possibly inhibit its subsequent synthesis. Recent evidence indicates that cotton plants subjected to several cycles of mild water stress sufficient to reduce growth do have considerably greater leaf starch concentrations (Ackerson, 1981; Ackerson and Herbert, 1981). At high water potentials, the photosynthetic rates of the stress-adapted plants were lower than the nonadapted plants. The reduced photosynthetic rates could not be attributed to stomatal conductance differences, thus nonstomatal inhibitions are indicated. After 48 hours in the dark, the starch was depleted and

the plants were "de-adapted." Subsequent physiological responses of the de-adapted plants were identical to the control plants. These data suggest that starch accumulates under mild stress conditions due to sink activity being reduced more than source activity in the short term. Prolonged stress should result in a more steady-state condition where production is in accord with demand. Another interesting aspect of these studies on drought adaptation was the apparent absence of lipid-like bodies in the chloroplast and extremely large starch granules. The photosynthetically active radiation level was 800 μE m^{-2} sec^{-1}. Under high light conditions, less starch and more intrachloroplastic lipid-like bodies were observed (Berlin *et al.*, 1981). This observation again supports the contention that lipid synthesis is a means of dissipating excess photochemical energy and simultaneously minimizing the need for photorespiration.

SUMMARY

Crop growth represents a highly coordinated and integrated set of systems involved in assimilation of CO_2, H_2O and minerals from the environment and their reduction and use in various growth processes which result in biomass. The primary assimilatory tissue is separated from the major centers of growth, so a translocation system is necessary. Both the source of assimilates and the sinks where assimilates are utilized are subject to genetic and environmental controls as to rate of activity. There is a high probability that the assimilation capacity exceeds the system's ability to process the assimilate in most green plants. This statement must be qualified with respect to species, growth stage and environmental conditions during plant development. The mechanisms responsible for regulating source-sink activities, or supply and demand, are many and can involve both biophysical and biochemical components. The controls imposed can be directly on the assimilatory process, or they may be indirect such as through some type of feedback inhibition.

The mechanisms involved in control of photosynthetic activity through feedback inhibition continues to escape thorough definition. Although one can artificially create conditions whereby large starch accumulations exist and restrict CO_2 diffusion rates, the question of the significance of this type of inhibition under natural environmental conditions is still debatable.

Although sound experimental evidence is rare, I am of the opinion that the primary regulator of CO_2 reduction resides in RuBP carboxylase activity. The activity of this enzyme is subject to numerous controls including inorganic and organic effectors. The active enzyme exists as a complex of eight large subunits and eight small units in the appropriate three-dimensional structure. Association and dissociation are a function of the chemical environment and the order of addition of activators. The complexity of this enzyme and its function in carboxylation and oxygenation make it the logical candidate for regulation of the rate of carbon reduction in green leaves. Techniques to measure "active" enzyme con-

centrations and microenvironmental conditions in the chloroplast stroma are rapidly evolving (Jensen and Bahr, 1977; Lorimar *et al.*, 1977; Sicher *et al.*, 1981) and should provide insight into regulation of RuBPC:Oase in the near future.

With respect to source or sink limitations in the cotton plant, my current opinion is that most commercially adapted cotton varieties are sink limited during the first half or more of the growing season. During the latter stages of development, during rapid boll filling, the plant probably becomes source limited. Source limitations during this stage are much more apparent if the plant experienced water stress which reduced leaf area more than fruit load. Complications due to nitrogen deficiencies, hormonal changes and other factors are apparent and greatly confuse the cause-and-effect interpretations.

It is imperative that we develop a thorough understanding of the major physiological limitations to cotton growth, development and productivity, and the control of the limiting systems. Efforts to define the physiological limitations to cotton productivity are underway at numerous locations throughout the cotton growing regions of the worlds. Through cooperative efforts and well-designed experimentation, good progress can be made toward this goal of minimizing the rate-limiting processes and maximizing productivity.

ACKNOWLEDGEMENTS

Our research was supported by the USDA through both the Competitive Research Grants Program and cooperative agreements and from the State of Texas through Texas Tech University and the Texas Agricultural Experiment Station.