EFFECTS OF CARBON DIOXIDE ON THE PHYSIOLOGY AND BIOCHEMISTRY OF PHOTOSYNTHESIS IN SOYBEAN

BY

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It is not too much to say that a comparatively sudden increase of carbon dioxide in the air to an extent of but two or three times the present amount, would result in the speedy destruction of nearly all our flowering plants.

H. T. Brown and F. Escombe (1902)

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KEY TO ABBREVIATIONS

C	Stromal concentration of CO_2
CA	Carbonic anhydrase
Ca	CO_2 concentration ambient to leaf
Ci	CO_2 concentration in air in leaf intercellular spaces ($\mu l\ l^{-1}$)
Ci'	Percent of CO_2 in air in leaf intercellular spaces (v/v)
C_{in}	CO_2 concentration of air entering leaf chamber
C_{out}	CO_2 concentration of air leaving leaf chamber
$^{A}CO_2$	Activator CO_2 in Rubisco activation
DAP	Days after planting
DHAP	Dihydroxyacetone Phosphate
diPGA	1,3-diphosphoglycerate
DTT	Dithiothreitol
E	Enzyme
E4P	Erythrose 4-phosphate
EDTA	Ethylenediaminetetraacetic acid
FBP	Fructose 1,6-bisphosphate
F6P	Fructose 6-phosphate
GAP	Glyceraldehyde 3-phosphate
Kc	Michaelis constant for CO_2
K_{cat}	Enzyme turnover number (s^{-1})
K_m	Michaelis constant
Ko	Michaelis constant for O_2
LAI	Leaf area index
M	Metal cation for enzyme activation

O	Stromal concentration of O_2
P	Atmospheric pressure
PCO	Photorespiratory carbon oxidation
PCR	Photosynthetic carbon reduction
PGA	3-phosphoglycerate
P-GLY	2-phosphoglycolate
Pi	Inorganic phosphate
pK'	First ionization constant
Pn	Net photosynthetic rate
PVP-40	Polyvinylpyrrolidone
R5P	Ribose 5-phosphate
R_{leaf}	Total leaf resistance to water vapor diffusion
RuBP	Ribulose 1,5-bisphosphate
Ru5P	Ribulose 5-phosphate
S7P	Sedoheptulose 7-phosphate
SBP	Sedoheptulose 1,7-bisphosphate
SLW	Specific leaf weight
Tris	Tris (hydroxymethyl) aminomethane
Tris-HCl	Hydrochloride of Tris
V	Standard molar gas volume
Vc	V_{max} of carboxylation reaction
Vo	V_{max} of oxygenation reaction
v_c	Velocity of carboxylation reaction
V_{max}	Theoretical maximum velocity of enzyme catalyzed reaction
v_o	Velocity of oxygenation reaction
X5P	Xylulose 5-phosphate
α	Solubility coefficient in water

Abstract of Dissertation Presented to the Graduate School
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Chairman: L. H. Allen, Jr.
Major Department: Agronomy

In three consecutive years (1983, 1984, and 1985) soybeans (Glycine max L. Merr. cv Bragg) were grown from seed to maturity in six outdoor environmentally controlled plant growth chambers under natural solar irradiance. The CO_2 concentrations inside the chambers were controlled to various levels during these studies. Both field and laboratory measurements were made to investigate the effects of CO_2 concentration on photosynthesis. Emphasis was placed on the response to CO_2 of ribulose 1,5-bisphosphate (RuBP) and RuBP carboxylase (RuBPCase), the substrate and enzyme of the carbon fixation reaction in soybean.

Following growth at 330 (atmospheric concentration) or 660 $\mu l\ CO_2\ l^{-1}$, leaflet photosynthetic rates were always greater for the elevated CO_2 grown plants when measured over a wide range of CO_2 concentrations. This enhanced capacity for photosynthesis was possibly a result of changes in internal leaf anatomy, or to greater

assimilate demand, or both, in the high CO_2 grown plants. The RuBP concentration decreased with increasing CO_2, but still appeared to be greater than the active site concentration of RuBPCase. The RuBPCase activity, expressed on an area basis, was not affected by growth CO_2 concentration. It appears that RuBPCase and RuBP are thus not involved significantly in the enhanced photosynthetic capacity.

Evaporative cooling kept leaf temperatures from reaching the higher air temperatures during studies on temperature effects on soybean grown at atmospheric and twice atmospheric concentrations of CO_2. Although air temperatures were increased by approximately 5 and 10°C, leaf temperatures were usually not increased more than approximately 2.5 and 4.5°C, respectively. These leaf temperature increases were not great enough to affect canopy photosynthesis or RuPBCase activity (on a chlorophyll basis) in either CO_2 treatment. Canopy photosynthesis was, however, greater at the higher CO_2 concentration. The concentration of RuBP was reduced at higher temperatures.

Increasing growth CO_2 concentrations (from 160 to 990 μl CO_2 l^{-1}) resulted in decreasing RuBPCase activities and RuBP levels, when both were expressed on a chlorophyll basis. At the higher CO_2 concentrations, the concentration of RuBP appeared to approach the concentration of RuBPCase active sites. Both the apparent $K_m(CO_2)$ and V_{max} of RuBPCase showed small, but statistically significant, decreases with increasing CO_2.

CHAPTER I
INTRODUCTION: A REVIEW OF PHOTOSYNTHETIC CARBON ASSIMILATION IN C_3 PLANTS

Photosynthesis is the process in which green plants and certain bacteria assimilate inorganic carbon into organic compounds. Light is the source of energy for this process and is absorbed in the plant by various pigments. The photochemical reactions involved in absorbing and transferring light energy are referred to as the "light reactions" while reactions responsible for the fixation of inorganic carbon and its subsequent metabolism are often referred to as the "dark reactions." Since several of the enzymes of photosynthetic carbon assimilation are light-activated, the "dark reactions" are not completely independent of light.

Under conditions of high quantum flux density, several processes can be identified as being potentially involved in regulation of photosynthetic carbon assimilation. One of the more marked of these processes is the CO_2 fixation reaction. Characteristics of this reaction have been used to assign plants to various photosynthetic categories. Terrestrial plants have been divided into four photosynthetic categories based on the path of carbon during photosynthesis, physiological characteristics, and leaf anatomy. In C_3 plants the initial product of the carbon fixation reaction is a three-carbon phosphorylated compound, whereas in C_4 plants it is a four-carbon organic acid. Crassulacean acid metabolism (CAM) is a

photosynthetic pathway in which the initial carbon fixation product is a four-carbon compound, however, most of the carbon fixation occurs at night. Characteristics of these three pathways are reviewed by Black (1973). The fourth category, C_3-C_4 intermediates, exhibit physiological and anatomical characteristics intermediate between C_3 and C_4 species. Holaday and Chollet (1984) have recently reviewed the photosynthetic characteristics of plants in this category.

One of the main objectives of the research described in the following chapters was to investigate the CO_2 fixation reaction in soybean, a C_3 type plant, by examining the enzyme and substrates involved. Prior to discussing specific objectives and the general experimental approach, CO_2 fixation in C_3 type plants is reviewed. This review covers CO_2 fixation and the subsequent regeneration of the CO_2 acceptor, the competitive photorespiratory cycle, and the enzyme responsible for catalyzing the initial reactions in both pathways. Proposed sites of regulation other than the carboxylation reaction are also discussed.

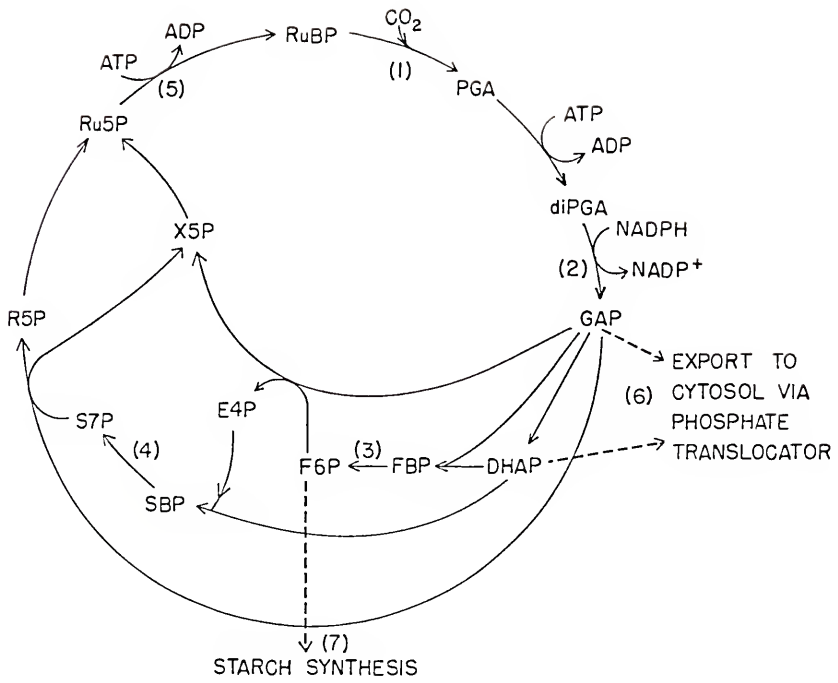
Photosynthetic Carbon Reduction Cycle

Description of the Cycle

The photosynthetic carbon reduction (PCR) cycle (also known as the reductive pentose phosphate or Calvin cycle) is the biochemical pathway in which CO_2 is converted to a number of sugar phosphates including the regeneration of the CO_2 acceptor ribulose 1,5-bisphosphate (RuBP) (Bassham et al., 1954). This biochemical pathway is apparently present in all photosynthetic green plants (Bassham,

1979). The 13 enzyme-catalyzed reactions of this cycle occur in the chloroplast. These reactions are catalyzed by 11 different enzymes, as it is currently believed that the two aldolase reactions are catalyzed by the same enzyme as are the two transketolase reactions (Robinson and Walker, 1981; Latzko and Kelly, 1979). A non-stoichiometric schematic diagram of the PCR cycle is presented in Figure 1.1. Carbon enters the cycle when CO_2 is combined with RuBP to produce two three-carbon compounds. This carboxylation reaction is catalyzed by the enzyme RuBP carboxylase (RuBPCase). Carbon passes through the cycle to regenerate the CO_2 acceptor. At two key points in the cycle carbon compounds may be removed and either utilized in starch synthesis or exported from the chloroplast to be metabolized in the cytosol. Both of these pathways represent net carbon gain for the photosynthetic cell. The ATP and NADPH consumed in the PCR cycle are generated during photosynthetic electron transport, and production of both requires light energy (Arnon et al., 1954). In addition to combining with CO_2, RuBP can combine with O_2 in an oxygenation reaction catalyzed by RuBP oxygenase (Bowes et al., 1971). The carboxylation and oxygenation reactions are catalyzed by the same enzyme RuBP carboxylase/oxygenase (Rubisco), which functions both as a carboxylase and an oxygenase. Further discussion of this enzyme and its regulation is presented later.

Fig. 1.1. A non-stoichiometric diagram of the PCR cycle in C_3 chloroplasts (after Bassham, 1979). Abbreviations: RuBP, ribulose 1,5-bisphosphate; PGA, 3-phosphoglycerate; diPGA, 1,3-diphosphoglycerate; GAP, glyceraldehyde 3-phosphate; DHAP, dihydroxyacetone phosphate; FBP, fructose 1,6-bisphosphate; F6P, fructose 6-phosphate; E4P, erythrose 4-phosphate; SBP, sedoheptulose 1,7-bisphosphate; S7P, sedoheptulose 7-phosphate; X5P, xylulose 5-phosphate; R5P, ribose 5-phosphate; Ru5P, ribulose 5-phosphate. Sites of potential metabolic regulation are: (1) RuBP carboxylase; (2) GAP dehydrogenase; (3) fructose 1,6-bisphosphatase; (4) sedoheptulose 1,7-bisphosphatase; (5) phosphoribulokinase; (6) pathway for starch synthesis in the chloroplast; (7) phosphate translocator facilitating exchange of certain metabolites between chloroplast and cytosol.


ADP
RuBP
CO_2
ATP
(5)
(1)
PGA
Ru5P
ATP
ADP
diPGA
X5P
NADPH
(2)
$NADP^+$
R5P
GAP
EXPORT TO
(6) CYTOSOL VIA
PHOSPHATE
TRANSLOCATOR
E4P
S7P
(4)
(3)
F6P
FBP
DHAP
SBP
(7)
STARCH SYNTHESIS

Regulation of the PCR Cycle

Five of the PCR cycle enzymes have been identified as being light-activated. These are RuBPCase, glyceraldehyde 3-phosphate (GAP) dehydrogenase, fructose 1,6-bisphosphatase (FBPase), sedoheptulose 1,7-bisphosphatase (SBPase), and phosphoribulokinase (Buchanan, 1980). These enzymes are located at positions 1 through 5, respectively, in Figure 1.1. A number of roles for light in enzyme activation have been proposed for PCR cycle enzymes. The chloroplast stroma becomes more alkaline in the light, as compared to the dark, as a result of proton transport across the thylakoid membranes (Heldt et al., 1973). The change in pH favors carbon assimilation and is sufficient to increase CO_2 fixation from zero to high rates (Werden et al., 1975). In exchange for protons moving out of the stroma, Mg^{2+} ions act as counter-ions and enter the stroma thus raising the total Mg^{2+} concentration (Portis and Heldt, 1976). Portis et al. (1977) have shown that the light-dependent changes in stromal Mg^{2+} concentration can control FBPase and SBPase activity. The activation of RuBPCase *in vitro* has also been shown to require Mg^{2+} (Laing and Christeller, 1976; Lorimer et al., 1976). Other mechanisms of light-activation of PCR cycle enzymes include the ferredoxin/thioredoxin system (Buchanan, 1980) and the light effect mediator (LEM) system (Anderson, 1979a). These two mechanisms are similar in that both use light energy to reduce disulfide (oxidized) containing compounds to the sulfhydryl (reduced) state. In the reduced state they are able to activate certain enzymes. One difference between the two mechanisms is that the ferredoxin/thioredoxin system requires a soluble protein factor

whereas the LEM system does not. Very recent evidence from Salvucci et al. (1985) has shown an apparently different chloroplast protein to be involved in the activation of RuBPCase. Activation, while suggested to be catalyzed by the protein, is regulated by the energization status of the thylakoids (Salvucci et al., 1986b) and is thus light-dependent. Light effects on some PCR cycle enzymes can also be mediated by effectors such as ATP and NADPH, both of which are generated in the light. The relative saturation of the adenylate pool with phosphate (i.e., ATP levels relative to ADP and AMP levels) regulates the activity of phosphoribulokinase and 3-phosphoglycerate (PGA) kinase (Pradet and Raymond, 1983). Both of these enzymes catalyze reactions requiring ATP (Figure 1.1). Also related to light are electron transport rates. Dietz et al. (1984) report, however, that even at high light intensity and saturating CO_2, electron transport rates do not play a direct role in limiting photosynthetic rates.

Five potential control points associated with the PCR cycle have been identified by Anderson (1979b) to be possible regulatory sites. Two of these points are the export of the triose phosphates GAP and dihydroxyacetone phosphate (DHAP) by the phosphate translocator and the pathway from fructose 6-phosphate (F6P) to starch. These points are discussed later. The remaining three points are the enzymes RuBPCase, FBPase, and SBPase. Dietz and Heber (1984) found even at high light and CO_2, FBPase did not limit photosynthesis. Likewise, Latzko and Kelly (1979) report all PCR cycle enzymes have been found to possess activity sufficient to support observed rates of CO_2

fixation with the exception of SBPase. Knowles (1985) has suggested that transketolase may regulate carbon flow through the PCR cycle by restricting regeneration of RuBP. Evidence from Dietz and Heber (1984) also indicates that at some point during the regeneration of RuBP from F6P and triose phosphate, CO_2 fixation appears to be limited under conditions of high CO_2 and high light intensity. The activity of RuBPCase has been suggested to be a limiting factor in photosynthesis even at high CO_2 concentrations (Dietz and Heber, 1984).

Individual reaction rates may be influenced by the accumulation or depletion of reaction products and substrates. Some enzymes are also affected by other chloroplast metabolites. These may be modulated in a positive or negative manner by the binding of a positive or negative allosteric effector at a site on the enzyme distinct from the catalytically active site (Robinson and Walker, 1981).

Triose Phosphate Export and Starch Synthesis

Export of triose phosphate from the chloroplast via the phosphate translocator and the synthesis of starch are processes which utilize fixed carbon from the PCR cycle (Figure 1.1). The phosphate translocator is the most powerful of several transport systems facilitating exchange between the chloroplast and the cytosol (Heber and Heldt, 1981). It is located at the inner chloroplast envelope membrane and is capable of transporting triose phosphates (DHAP and GAP), PGA, and inorganic phosphate (Pi) (Flugge and Heldt, 1984). The

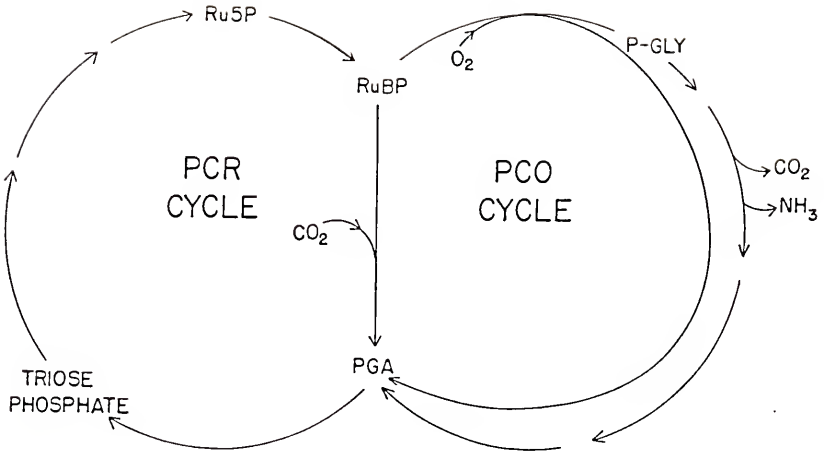
stoichiometry of the transporter is such that export of one molecule of triose phosphate or import of one molecule of PGA is accompanied by the counter transport of one Pi (Heber and Heldt, 1981). Thus, the total amount of phosphate in the stroma is kept constant. The export of triose phosphate is the mechanism whereby carbon fixed in the chloroplast can be transported to the cytosol, where it is metabolized and subsequently translocated to other locations within the plant. The availability of cytosolic Pi to be transported into the chloroplast can affect photosynthesis and starch metabolism (Walker and Sivak, 1986). Triose phosphate can be metabolized to sucrose and Pi in the cytosol, with Pi becoming available for transport back into the chloroplast in exchange for additional triose phosphate. Low rates of sucrose synthesis during photosynthesis may result in decreased cytosolic Pi for transport and therefore a build-up of some PCR intermediates in the chloroplast (Huber and Israel, 1982). Inorganic phosphate is ultimately required for formation of chloroplastic sugar phosphates. When the rate of CO_2 fixation is greater than the availability of cytosolic Pi for chloroplast import, triose phosphates will not be formed and PGA may accumulate (Heber and Heldt, 1981). The subsequent high PGA/Pi ratio in the chloroplast has been shown to result in starch synthesis (Preiss, 1982). Starch synthesized in the chloroplast is usually degraded during the following night period (Heber and Heldt, 1981). The possibility that photosynthesis may be limited by (1) the inability of the plant to use sucrose at a rate similar to the rate at which it is produced, or

(2) accumulation of starch in the chloroplast, is discussed in Chapter II.

Photorespiratory Carbon Oxidation Cycle

Photorespiration may be defined as the oxygen and light-dependent release of CO_2 from certain plants (Somerville and Ogren, 1982). The rate of photorespiration is often greater than the rate of dark respiration (Zelitch, 1971). Summarized briefly, RuBP combines with O_2 to produce 2-phosphoglycolate (P-GLY) and PGA in a reaction catalyzed by Rubisco, the same enzyme responsible for catalyzing CO_2 fixation in the PCR cycle (Bowes et al., 1971; Ogren and Bowes, 1971). The P-GLY produced in the photorespiratory carbon oxidation (PCO) cycle undergoes a series of reactions in the chloroplast, peroxisome and the mitochondrion where photorespiratory CO_2 is released (Ogren, 1984; Chollet and Ogren, 1975). The ratio of oxygenase to carboxylase activity is dependent on the relative concentrations of O_2 and CO_2, Rubisco kinetics (Laing et al., 1974) and temperature. Temperature affects both the kinetics of Rubisco (Jordan and Ogren, 1984) and the relative solubilities of O_2 and CO_2 (Jordan and Ogren, 1984; Ku and Edwards, 1977). Figure 1.2 shows a non-stoichiometric schematic diagram demonstrating the integration of the PCR and PCO cycles by the common enzyme Rubisco and the common substrate RuBP. Besides CO_2, NH_3 is also released in the PCO cycle. Keys et al. (1978) have shown that NH_3, like CO_2, is released in the mitochondrion during the conversion of glycine to serine and NH_3 is then reassimilated into glutamine in the cytosol.

Fig. 1.2. A non-stoichiometric diagram of the integration of the PCR and PCO cycles in C_3 chloroplasts (after Lorimer, 1981). The initial reaction in both cycles is catalyzed by Rubisco and utilizes RuBP. Triose phosphate represents GAP and DHAP. P-GLY is 2-phosphoglycolate. Other abbreviations are as in Fig. 1.1.


Ru5P
RuBP
O_2
P-GLY
PCR
CYCLE
PCO
CYCLE
CO_2
NH_3
CO_2
PGA
TRIOSE
PHOSPHATE

Because the PCO cycle results in a loss of CO_2 and energy it is often regarded as a wasteful process. Much research has been aimed at understanding photorespiration. Although various roles have been proposed for the PCO cycle, it appears that other than the subsequent metabolism of any P-GLY produced during RuBP oxygenase activity, there is no known requirement for photorespiration (Ogren, 1984). It has been suggested that photorespiration is an unavoidable result of both the nature of the Rubisco active site chemistry and the concentrations of CO_2 and O_2 at the active site (Andrews and Lorimer, 1978). Mutants of Arabidopsis lacking activity of different PCO cycle enzymes have been found to have inhibited photosynthesis in air and are not viable (Somerville, 1986). However, under conditions of high CO_2 or low O_2 normal photosynthesis was observed. This led Somerville and Ogren (1982) to the conclusion that once carbon enters the PCO cycle it must continue to be metabolized to prevent photosynthetic inhibition. Thus, apparently the only way photorespiration can successfully be reduced is by reducing the oxygenase/carboxylase activity ratio.

RuBP Carboxylase/Oxygenase

Introduction

Under saturating light conditions, the amount and degree of activation of RuBPCase regulates CO_2 assimilation (Jensen and Bahr, 1977). This emphasizes the importance of Rubisco (used here Rubisco refers to the enzyme RuBP carboxylase/oxygenase while RuBPCase and RuBP oxygenase refer to the carboxylation and oxygenation activities, respectively). This enzyme represents up to 65% of the total soluble

leaf protein (Ellis, 1979). It is located in the chloroplast stroma in concentrations of approximately 0.4 to 0.5 mM (Jensen and Bahr, 1977). In higher plants the enzyme is composed of eight large subunits (containing one active binding site per large subunit) and eight small subunits whose function is not yet known (Miziorko and Lorimer, 1983). Thus, the binding site concentration in the chloroplast is approximately 3 to 4 mM. The prodigious amount of this enzyme is countered by its slow rate of catalysis. The turnover number of fumarase (a tricarboxylic acid cycle enzyme) is 50 times greater than spinach RuBPCase (Seemann and Berry, 1982). Compared to spinach carbonic anhydrase (Pocker and Miksch, 1978) spinach RuBPCase is four orders of magnitude slower. Because of its central role in CO_2 assimilation and agricultural productivity, Rubisco has been previously and is currently the object of intense investigation.

Reactions of Rubisco

The two competitive reactions catalyzed by Rubisco are the carboxylation and the oxygenation of RuBP (Bowes et al., 1971; Ogren and Bowes, 1971). As previously described, oxygenation of RuBP is the initial step in photorespiration while carboxylation of RuBP initiates photosynthesis. The ratio of photosynthesis to photorespiration can be described in terms of enzyme kinetics by the equation of Laing et al. (1974),

$$v_c/v_o = VcKoC/VoKcO, \qquad [1.1]$$

where v_c and v_o are the rates of carboxylation and oxygenation, and Vc, Vo, Kc and Ko are the V_{max} (theoretical maximum rate of reaction)

and K_m (Michaelis constant) values for carboxylation and oxygenation, respectively. The concentration of CO_2 and O_2 at the reaction site are represented by C and O. At atmospheric conditions of CO_2 and O_2 and 25°C, the ratio of carboxylation/oxygenation is approximately 4/1 (Ogren, 1984). In spite of much research to identify factors which can alter the v_c/v_o ratio, only the substitution of Mn^{2+} for Mg^{2+} during the enzyme reaction and temperature have proven effective (Ellis, 1979). The $K_m(O_2)$ is decreased when activation and catalysis involves Mn^{2+} rather than Mg^{2+} (Lorimer, 1981). Temperature has been found to differentially affect Rubisco kinetics. This was shown using the substrate specificity factor defined by Jordan and Ogren (1984),

$$VcKo/VoKc, \qquad [1.2]$$

where the variables are defined as in equation [1.1]. At given concentrations of CO_2 and O_2 the specificity factor determines the relative rates of carboxylation and oxygenation. A high value indicates a high carboxylase to oxygenase ratio. As temperature increases Vc, Vo, and Kc increase, however, Ko is not temperature dependent. The overall effect of the temperature increase is a decrease in the specificity factor (Jordan and Ogren, 1984). Jordan and Ogren (1984) found the specificity factor of purified enzyme to drop to less than one-third of its value as the temperature increased from 5 to 40°C. A similar response was observed by Brooks and Farquhar (1985) using gas exchange techniques on intact leaves.

Activation of RuBP Carboxylase

Prior to becoming catalytically competent, RuBPCase undergoes an activation process. The proposed model for activation involves CO_2 and Mg^{2+} in the following manner (Lorimer et al., 1976; Laing and Christeller, 1976),

$$\underset{\text{(inactive)}}{E + {}^{A}CO_2} \rightleftharpoons \underset{\text{(inactive)}}{E - {}^{A}CO_2} + M \rightleftharpoons \underset{\text{(active)}}{E - {}^{A}CO_2 - M}, \qquad [1.3]$$

where E is enzyme, $^{A}CO_2$ is activator CO_2 (distinct from substrate CO_2), and M is a divalent metal cation, usually Mg^{2+}. The formation of the E - C form (E - $^{A}CO_2$) is slow while formation of the E - C - M form (E - $^{A}CO_2$ - M) is rapid. In intact chloroplasts activation has been shown to depend on light and CO_2 (Bahr and Jensen, 1978). Activation and catalysis are separate phases in the RuBPCase reaction. Lorimer et al. (1977) have described methods for the activation of the enzyme in vitro. Activation of Rubisco is necessary for both carboxylase and oxygenase activities (Lorimer, 1981). Inhibition of RuBPCase by substrate RuBP (Jordan and Chollet, 1983; Laing and Christeller, 1976) and by HCO_3^- (Machler and Nosberger, 1980) have been reported in in vitro studies. In the light and in air-CO_2 levels, RuBPCase (in vivo) is typically activated to a substantial degree (Perchorowicz et al., 1981). Herein lies an enigma in that conditions believed to exist in the stroma in the light (5 to 10 μM CO_2, 5 to 10 mM Mg^{2+} and pH 8.0) are not sufficient to activate RuBPCase in vitro (Miziorko and Lorimer, 1983). A number of metabolites have been shown to affect RuBPCase activation and/or activity. This group of metabolites has been reported to include

NADPH, 6-phosphogluconate, ribose 5-phosphate, 3-phosphoglycerate, fructose 1,6-bisphosphate and several other compounds (Jordan et al., 1983; Badger and Lorimer, 1981; Hatch and Jensen, 1980; Lorimer et al., 1978; Chollet and Anderson, 1976; Chu and Bassham, 1975). These effectors were suggested to act at allosteric regulatory sites (Chu and Bassham, 1975) but more recent evidence indicates that the effectors bind competitively at the same active site as does RuBP (Jordan et al., 1983; Badger and Lorimer, 1981; McCurry et al., 1981). It has been suggested that the concentration of these effectors in the stroma (Lorimer et al., 1978) and the magnitude of their induced responses (Akazawa, 1979) are inadequate to be physiologically important in vivo. Somerville et al. (1982) have identified a mutant of Arabidopsis thaliana in which RuBPCase is present in a nonactivatable form in vivo. This implies that a factor necessary for activation is absent in the mutant. Recently, Salvucci et al. (1985) have discovered two polypeptides missing from the same Arabidopsis mutant and have linked these polypeptides to a soluble chloroplast enzyme designated Rubisco activase. These data suggest activase may be involved in light-activation of RuBPCase in vivo and that activation is a catalyzed and not a spontaneous process (Salvucci et al., 1986a).

An additional regulatory aspect of light on RuBPCase was discovered independently by Vu et al. (1983), McDermitt et al. (1983), and Ku et al. (1982). They found crude extracts of RuBPCase from leaves collected in the dark to be less catalytically active than from leaves collected in the light. This light/dark modulation has been found in a number of different species from different photosynthetic

categories (Vu et al., 1984a). Restoration of catalytic ability by ammonium sulfate fractionation of the crude extract of dark collected leaves indicated the potential involvement of an inhibitory compound (Vu et al., 1984b). Subsequent work by Seemann et al. (1985) and Servaites (1985) have shown the inhibitor to be a phosphorylated compound which binds to the active site of RuBPCase. Berry et al. (1986) have identified the inhibitor as carboxyarabinitol 1-phosphate.

Non-Catalytic Roles of Rubisco

Due to its high concentration in the chloroplast, Rubisco has been suggested to function as a storage protein (Huffaker and Miller, 1978; Huffaker and Peterson, 1974). It also has been suggested to be a major source of protein for animals for the same reason (Huffaker and Peterson, 1974). Another function, that of a metabolite buffer, has been proposed by Ashton (1982). The ability of compounds such as fructose 1,6-bisphosphate (FBP) to bind to Rubisco and the relative concentrations of FBP and Rubisco binding sites imply that greater than 98% of the FBP could theoretically be bound to Rubisco in illuminated chloroplasts (Ashton, 1982). The physiological significance of this effect is apparently speculative.

Experimental Approach

The CO_2 in the atmosphere surrounding a leaf, or other photosynthetic organ, is the source of carbon for terrestrial photosynthesis. Manipulation of the CO_2 concentration and observation of the resulting photosynthetic responses provide insight into the

control and mechanism of CO_2 fixation. This approach has been carried out by a number of investigators (see reviews by Kimball, 1983; Lemon, 1983; Strain and Cure, 1985), not only to learn more about photosynthesis but to study the effects of CO_2 supply on yield and what effect rising atmospheric levels of CO_2 might have on vegetation. In the experiments reported in the following chapters, soybeans were grown in outdoor, naturally sunlit, controlled environment chambers, in which CO_2 concentration and dry bulb and dew point temperatures were controlled to pre-selected values. Gas exchange techniques were used to measure leaf and canopy photosynthetic rate response to different CO_2 concentrations. Leaf tissue samples were collected for analysis of RuBP and RuBPCase, the substrate and enzyme involved in CO_2 fixation.

The purpose of the experiments described in the following chapters was to examine the effects of CO_2, both in the short-term and the long-term, on the physiology and biochemistry of photosynthesis in soybean. It was hypothesized that long-term exposure (exposure during growth) to different CO_2 concentrations could result in a change in the capacity for photosynthesis in soybean. To examine this hypothesis, specific objectives were:

(1) to determine the leaflet photosynthetic rate response to CO_2 for soybeans grown at atmospheric and twice-atmospheric CO_2 concentrations,

(2) to examine the effects of CO_2 concentration (during short- and long-term exposures) on RuBP levels,

(3) to examine the effects of CO_2 concentration (during short- and long-term exposures) on RuBPCase activity,

(4) to determine the effects of growth in subatmospheric and superatmospheric concentrations of CO_2 on kinetics of RuBPCase,

(5) to examine the effects of growth air temperature on RuBP levels and RuBPCase activity, and

(6) to determine if either the RuBP level or RuBPCase activity may be limiting to photosynthesis under high quantum flux density and various CO_2 concentrations.

In Chapter II, experiments are described in which soybeans were grown at atmospheric and twice-atmospheric concentrations of CO_2. Short-term exposures (1 hr) to various CO_2 concentrations allowed leaflet photosynthetic rate response to CO_2 to be measured as well as RuBP levels and RuBPCase activities. In Chapter III, the effects of growth in various subatmospheric and superatmospheric concentrations of CO_2 on canopy photosynthetic rates are described. The effects of CO_2 concentration on levels of RuBP and the activity and kinetics of RuBPCase were also determined. The effects of three different day/night air temperature regimes on canopy photosynthesis, RuBP levels, and RuBPCase activity of soybean grown at atmospheric and twice-atmospheric CO_2 concentrations were investigated and are discussed in Chapter IV. In Appendix A, the photosynthetic rate response to light for leaflets and canopies is discussed. The effect of leaf tissue sample size on the in vitro assay of RuBPCase activity is discussed in Appendix B. Parameters from linear regression analyses are tabulated in Appendix C.

The long-range goal of research such as described herein is to reach a greater understanding of the fundamental process of photosynthesis. This knowledge may hopefully contribute to improvements in agricultural productivity.

CHAPTER II
THE EFFECTS OF SHORT-TERM EXPOSURES TO CO_2 ON LEAF PHOTOSYNTHETIC RATE, RuBP CARBOXYLASE ACTIVITY AND RuBP LEVEL

Introduction

That present day concentrations of atmospheric CO_2 are limiting to photosynthesis in C_3 plants is widely recognized (Pearcy and Bjorkman, 1983). It is well documented that photosynthetic rates increase when C_3 plants are exposed to higher than normal CO_2 concentrations (Tolbert and Zelitch, 1983; Osmond et al., 1980; Allen, 1979). The increase in CO_2 not only provides more substrate for carbon assimilation, but also alters the photosynthetic/photorespiration ratio by reducing photorespiration (Ogren, 1984). Investigations into the effects of CO_2 on photosynthesis have proceeded in several directions including long-term and short-term exposures of plants to various CO_2 concentrations. Often times long-term exposure involves growing plants from seed to maturity at elevated CO_2 concentrations. Experiments of this type, in which plants were grown at both atmospheric and elevated CO_2 concentrations, have yielded mixed results when leaf photosynthetic rates were measured at the respective growth CO_2 concentrations. In some experiments, plants grown at atmospheric CO_2 had greater photosynthetic rates than high CO_2 grown plants (Peet et al., 1986; von Caemmerer and Farquhar, 1984; Hofstra and Hesketh, 1975). In other experiments the reverse was found, leaf photosynthetic rates

were greater in high CO_2 grown plants when both were measured at their growth CO_2 concentration (Peet et al., 1986; Havelka et al., 1984; Huber et al., 1984; Downton et al., 1980; Wong, 1979; Ho, 1977).

In other experiments in which various C_3 species were grown either from seed or for long periods of time at different CO_2 concentrations, photosynthesis was measured over a range of CO_2 concentrations. Results from these experiments suggest leaf photosynthetic rate responses appear to fit into one of three categories. These categories may in fact represent a continuum of possible responses that depend on species, growth conditions, stage of growth, and other factors, including experimental conditions. These categories may be described as follows: (1) leaf photosynthetic rates are greater in plants grown at higher rather than lower CO_2 when measured at all CO_2 concentrations, (2) leaf photosynthetic rates are greater in plants grown at lower rather than higher CO_2 when measured at all CO_2 concentrations, and (3) leaf photosynthetic rates are greater in plants grown at lower CO_2 when measured at low CO_2 but higher in plants grown in high CO_2 when measured at high CO_2. Hicklenton and Jolliffe (1980a), working with young tomato plants, found leaf photosynthetic rates, on a fresh weight basis, to be greater in plants grown at 1000 μl CO_2 l^{-1} than those grown at 300 μl CO_2 l^{-1} when measured over a range of CO_2 concentrations. With older plants the difference in photosynthetic rate response of the leaves was less. Plants grown at 5000 μl CO_2 l^{-1}, however, were always found to have leaf photosynthetic rates lower than 300 μl CO_2 l^{-1} grown tomato plants. Mauney et al. (1979) grew soybeans at 330 and 630 μl CO_2 l^{-1}

and found that when leaf photosynthetic rates were measured at the lower CO_2 concentration the rates were the same but at high CO_2 concentration the rates were greater in the 630 μl CO_2 l^{-1} grown plants. The majority of the data in the literature shows leaf photosynthetic rates, when expressed on an area basis, to be greater in plants grown at lower rather than higher CO_2 when measured over a range of CO_2 concentrations. This type of relationship has been reported for experiments run under a variety of environmental conditions with various species such as cotton (Delucia et al., 1985; Mauney et al., 1979), Phaseolus vulgaris (Ehret and Jolliffe, 1985; von Caemmerer and Farquhar, 1984), sunflower (Mauney et al., 1979), tomato (Ho, 1977), Nerium oleander and Larrea divaricata (Downton et al., 1980), and waterhyacinth (Spencer and Bowes, 1986). Plants in which the relative rates of leaf photosynthesis shift between low and high CO_2 grown plants, depending on the CO_2 concentration during measurement, make up the third response category. Examples of this type of response have been reported with cotton (Wong, 1979), grape (Kriedemann et al., 1976), and Amorphophallus konjac (Imai and Coleman, 1983).

The different responses to CO_2 may be explained in part by the species chosen. However, the species alone cannot account for all of the variation in photosynthetic rates since some species demonstrated more than one type of response. For example, Ho (1977) and Hicklenton and Jolliffe (1980a) both worked with tomato but observed different types of photosynthetic behavior. While their experiments were similar in regard to CO_2 concentrations, differences existed in plant

age, growth photoperiod, growth temperature, and whether the plants were grown from seed or were transferred to a particular CO_2 concentration at an early age. In addition, Mauney et al. (1979) obtained different results working with the same species under apparently similar experimental conditions in two consecutive years. Measurement protocol as well as growth and measurement conditions, and possibly other factors, apparently influence leaf photosynthetic rate response to CO_2 (Woo and Wong, 1983).

There are numerous reports where long-term growth in high CO_2 resulted in declining leaf photosynthetic rates, which ultimately became lower than rates of plants maintained at atmospheric CO_2 concentrations (Kramer, 1981). This reduction in photosynthetic rate has sometimes been shown to be reversible when plants are switched from high to low CO_2 conditions (Sasek et al., 1985; Kriedemann and Wong, 1984). Sasek et al. (1985) suggest that feedback inhibition of photosynthesis by starch accumulation is responsible for these types of observations, but according to Raven (1981), there is little evidence for feedback inhibition of photosynthetic rates by photosynthetic product accumulation. Growing plants in air enriched with CO_2 has often been shown to increase the amount of starch present in the leaf (Cave et al., 1981; Mauney et al., 1979; Hofstra and Hesketh, 1975; Madsen, 1968). These high starch levels have sometimes been linked to chloroplast disruption (Cave et al., 1981; Carmi and Shomer, 1979). Neales and Incoll (1968) have reviewed reports that suggest chloroplast disruption may include reduction of light incident to the grana and interference with CO_2 diffusion inside the leaf. The

relationship between high starch levels and changes in leaf photosynthetic rates is equivocal. There are a number of examples where high levels of starch have been correlated to reduced photosynthetic rates (Delucia et al., 1985; Sasek et al., 1985; Azcon-Bieto, 1983; Mauney et al., 1979; Nafziger and Koller, 1976; Hofstra and Hesketh, 1975), and a number of examples where starch was not observed to affect photosynthetic rates (Potter and Breen, 1980; Carmi and Shomer, 1979; Mauney et al., 1979; Little and Loach, 1973). In fact, Mauney et al. (1979) and Little and Loach (1973) showed positive correlations between starch levels and leaf photosynthetic rates. It has been suggested (Milford and Pearman, 1975) that starch may not inhibit photosynthesis until a threshold level, which is not normally attained under field conditions, is reached. Accumulation of starch in the leaf may be related to, among other things, the assimilate demand of the plant. The role of assimilate demand in leaf photosynthesis has been reviewed by Neales and Incoll (1968) and Geiger (1976). The possible mechanisms involved have been discussed by Herold (1980). Most of the data in the literature suggest high assimilate demand results in high photosynthetic rates (Geiger, 1976). King et al. (1967), however, have reviewed several reports showing both positive and negative influences on photosynthetic rates. Positive correlations between leaf photosynthetic rates and increased assimilate demand have been demonstrated in a variety of depodding and leaf shading experiments (Wittenbach, 1983; Clough et al., 1981; Mondal et al., 1978; Thorne and Koller, 1974; King et al., 1967).

In addition to the above mentioned effects on photosynthesis, age or the developmental stage of a plant may influence CO_2 assimilation rates. The podfilling stage in soybeans can be a period of high photosynthetic activity (Enos et al., 1982; Hesketh et al., 1981; Woodward and Rawson, 1978; Dornhoff and Shibles, 1970), however, Sinclair (1980) has pointed out that there are substantial differences, among cultivars, in the ability to maintain high photosynthetic rates late in the season. Differences in RuBPCase activity in soybean have been noted between expanding and mature leaves (Vu et al., 1983). Changes in the relative photosynthetic rate responses to CO_2 in atmospheric concentrations and high CO_2 grown leaves have been shown to occur as plants become older (Peet et al., 1986; Ehret and Jolliffe, 1985; Hicklenton and Jolliffe, 1980a). Baysdorfer and Bassham (1985) have found that as alfalfa progressed from seedling to mature crop, photosynthesis shifted from being source-limited to sink-limited.

Different leaf photosynthetic rate responses to CO_2 have been obtained with a variety of species and under wide ranging environmental and experimental conditions, which may account for much of the variation in results. Additionally, the diversity of interpretation of the results implies that regulation of photosynthesis is not, as yet, well understood. A confounding possibility is the suggestion (Maggs, 1964) that leaves usually operate below their maximum level.

The objectives of this study were to measure leaflet photosynthetic rate response to CO_2 for soybean grown at atmospheric

and twice atmospheric concentrations of CO_2. In addition, the effects of the two CO_2 growth treatments and short-term response to a range of CO_2 concentrations on the activity of RuBPCase and the level of RuBP were investigated. The photosynthetic rate response to CO_2 and the response of RuBPCase and RuBP to CO_2 were examined to determine what role the biochemical parameters may have in regulating leaflet photosynthesis under conditions of high quantum flux density and various concentrations of CO_2.

Materials and Methods

Plant Material and Growth Conditions

Soybeans (Glycine max L. Merr. cv Bragg) were planted in outdoor, computer-managed, environmentally controlled plant growth chambers located at the University of Florida's Irrigation Research and Education Park, in Gainesville, on 30 Aug. 1983. The upper part of each growth chamber was constructed of clear acrylic and polyester film, allowing the plants to receive 88% of the natural solar irradiation. The chamber tops measured 2 m by 1 m in cross section by 1.5 m in height. The lower steel part of the chamber was of the same cross section and 1 m in depth. It was filled with a reconstructed Arredondo fine sand profile, which was sealed from the upper aerial part following seedling emergence to prevent the mixing of the soil and aerial atmospheres. The dry bulb temperature of the chamber atmosphere was controlled to 31°C during the day and to 23°C at night. The dewpoint temperature was controlled to 16°C. The CO_2 concentration of the chamber atmosphere was controlled, from the date

of planting until final harvest, to either 330 μl CO_2 l^{-1} or 660 μl CO_2 l^{-1}. A general description of growth chamber operation may be found in Jones et al. (1985b), while Jones et al. (1984b) provide a detailed description of the growth chamber design and the computer control system.

For the experiments described here, two of six plant growth chambers were used. Within each of these plant growth chambers were placed two leaf chambers, each capable of accommodating one fully expanded soybean leaflet. The leaf chambers were constructed of an acrylic frame covered with a clear polyester film which transmitted 88% of the incident solar radiation. The internal volume of each leaf chamber was 0.375 liters. Chilled water, circulating through the chamber frame, maintained the temperature of the air in the leaf chamber close to the air temperature in the plant chamber. The leaf chambers were controlled by a computer system similar to but separate from the system controlling the plant chambers.

The origin of the air circulating through the leaf chamber system was the respective plant chamber. Air was circulated, by diaphragm pumps, from the plant chamber through homogenizing containers and then through the leaf chamber system. The leaf chamber system consisted of two IR gas analyzers (Beckman, model 865), two dewpoint hygrometers (General Eastern, model 1100 DP), one thermocouple (0.25 mm diameter) placed in each leaf chamber to monitor air temperature and three thermocouples (0.076 mm diameter) wired in parallel and placed in contact with the abaxial leaflet surface to monitor leaflet temperature. Each IR gas analyzer and hygrometer was dedicated to two

two leaf chambers. Air lines were heated and insulated to help prevent condensation. Air flow rates through the leaf chambers were between 0.318 and 0.468 m^3 per hour (5.3 and 7.8 liters per minute). The dry bulb and dewpoint temperatures and the CO_2 concentration in the leaf chambers were similar to conditions in the respective plant chambers.

The plants completed germination approximately 4 days after planting (4 DAP). On October 18, 49 DAP, the plants were thinned to a density of 30 plants per m^2. Throughout the experiments, shadecloth (approximately 50% shading) was attached to the outside of the plant chamber at a height equal to the top of the canopy to approximate a closed canopy and reduce the solar irradiance on the sides of the canopy.

CO_2 Concentration Experiments

A series of short-term experiments were performed from October 25 to October 30 (56 to 61 DAP), during which time all plants were at the beginning seed or R5 stage of development (Fehr and Caviness, 1977) . During this period the CO_2 concentrations in the plant chamber, and thus also in the leaf chamber, were controlled to various levels different than the normal CO_2 growth concentrations. These additional CO_2 concentrations (110, 220, 330, 550, 660, and 880 μl CO_2 l^{-1}) were imposed at midday and were maintained for approximately 1 hour. During these exposure times photosynthetic rate data were collected, and immediately following these measurements leaf tissue samples were rapidly collected for subsequent laboratory analysis. Supplementary

CO_2 concentrations (160, 440, and 990 μl CO_2 l^{-1}) were imposed in the plant and leaf chambers after plant tissue sampling to expand the CO_2 range over which photosynthetic rate measurements were collected. In all cases, when the CO_2 was changed from one concentration to another, steady state conditions were allowed to return inside the plant and leaf chamber prior to collecting data for analysis. This always represented a period of not less than 10 minutes. All data collected during these CO_2 experiments were obtained between 1100 and 1430 Eastern Standard Time (EST). During each day this was a cloud-free high irradiance period when the quantum flux density (400 to 700 nm) was measured to be at least 1000 μmol quanta m^{-2} s^{-1} at the leaf level, which in these experiments was saturating for leaflet photosynthesis. Quantum flux density was measured with a quantum sensor (Li-Cor, model LI-190S) and corrections were made for the transmittance through the plant and leaf chambers.

Leaf Photosynthesis Measurements

The leaf chamber system was used to collect leaf gas exchange data, at 5-minute intervals, continuously during the photosynthesis experiments. Measurements of CO_2 concentration and dewpoint temperatures were made on air entering and leaving the leaf chambers. In addition, measurements were made of the dry bulb temperature of the air inside the leaf chambers, the leaflet temperature, and the air flow rate. The net photosynthetic rate (Pn) of the leaflet was calculated using the following equation from Gaastra (1959),

$$Pn = \frac{C_{in} - C_{out}}{A} * \text{flow rate}, \qquad [2.1]$$

where C_{in} and C_{out} are the CO_2 concentrations of the air stream entering and leaving the leaf chamber, respectively, A is the area of the leaflet, and flow rate is the rate of the air-stream flowing through the leaf chamber system.

The concentration of CO_2 in the air in the leaf intercellular space (Ci), was calculated based on the method of Farquhar and Sharkey (1982),

$$Ci = Ca - (Pn * R_{leaf} * 1.6), \qquad [2.2]$$

where Ca is the CO_2 concentration of the air ambient to the leaflet, R_{leaf} is the total leaf resistance to diffusion of water vapor and 1.6 is the ratio of the binary diffusivities of water vapor/air and CO_2/air (Farquhar and Sharkey, 1982). The product of R_{leaf} * 1.6 is the leaf resistance to diffusion of CO_2. This method of estimating Ci was found by Sharkey et al. (1982) to be in close agreement with measured values of the intercellular concentration of CO_2. The calculation of R_{leaf} was based on the equations of Neumann and Thurtell (1972), using measured values of dewpoint and dry bulb air temperatures, air flow rates, and leaflet area. Photosynthetic rates for leaflets grown at each CO_2 concentration are the pooled values from two leaflets.

Plant Sampling Procedure

Leaf tissue samples were collected via access doors located on the rear (north side) of the plant chambers. Inside each door was

positioned a curtain of polyester film that reduced disturbance of the atmosphere within the plant chamber during plant tissue sampling. This procedure was found to result in small and only brief disturbances of the atmosphere during sampling events. The plant tissue collected was from the upper canopy and consisted of 20 to 25 fully expanded, non-shaded, and visibly healthy leaflets. These leaflets were selected in part based on visual similarity to the leaflets used in the leaf chambers for photosynthetic rate measurements. Leaflet lamina were removed at the petiolule and immediately plunged into liquid N_2. This process was completed in approximately 1 second. The leaf tissue was then ground in a liquid N_2 chilled mortar and the resulting leaf powder was stored in a container in liquid N_2. The leaf tissue was kept at liquid N_2 temperature from the time of harvesting until laboratory analysis which occurred at a later date. Vu et al. (1984a) have shown this method to preserve enzymatic activity for prolonged periods of time.

RuBP Carboxylase Assay

A quantity of frozen leaf powder (100 to 170 mg dry weight) was removed from liquid N_2 storage and placed in a pre-chilled Ten Broeck tissue homogenizer. Added to the leaf powder was 10 ml of extraction buffer consisting of 50 mM Tris (pH 8.5), 5mM DTT, 0.1 mM EDTA, and 1.5% (w/v) PVP-40. The leaf tissue was homogenized for approximately 60 seconds at 0°C, at which point an aliquot of the homogenate was reserved for chlorophyll determination, and the remainder was centrifuged at 12,000 g for 3 minutes. The supernatant of the crude

extract was either assayed immediately or else following a 5-minute activation period at 30°C in 10 mM $NaHCO_3$ and 10 mM $MgCl_2$. Assays were carried out in triplicate at 30°C, with continuous shaking (125 strokes min^{-1}), in 5-ml glass vials with screw-on septum caps. The assay buffer consisted of 50 mM Tris (pH 8.5), 5 mM DTT, 0.1 mM EDTA, 10 mM $MgCl_2$, 0.5 mM RuBP, and 20 mM $NaH^{14}CO_2$ (7.54 GBq mol^{-1}). The sealed vials were purged with N_2 for 10 minutes prior to the addition of the Tris buffer and the $NaH^{14}CO_2$. The total assay volume was 1 ml. Assays of enzyme activity were initiated with the injection, through the septum cap, of 0.1 ml of either nonactivated or HCO_3^-/Mg^{2+} activated crude extract to determine initial or total activity, respectively (Perchorowicz et al., 1981). Assays were terminated after 45 seconds with the injection of 0.1 ml of 6N HCl. A 0.9 ml aliquot of the assay mixture was transferred to a 20-ml glass scintillation vial which was placed on a warm heating plate under an air-stream, and remained there until the contents were dried. When dry, 2.5 ml of water and 5 ml of scintillation cocktail were added to the vials and acid-stable ^{14}C products were determined by liquid scintillation spectrometry.

RuBP Determination

The determination of RuBP was based on the method of Latzko and Gibbs (1974) with modifications by Vu et al. (1983). A quantity of frozen leaf powder (85 to 150 mg dry weight) was removed from liquid N_2 storage and placed in a pre-chilled Ten Broeck tissue homogenizer. Added to the leaf powder was 10 ml of 0.5N HCl at 0°C. The leaf

tissue was homogenized for approximately 60 seconds at 0°C, an aliquot was reserved for pheophytin determination and the remainder was then centrifuged at 12,000 g for 5 minutes. To 5 ml of the supernatant was added 0.75 ml 2M Tris base and 0.44 ml 4N KOH. The neutralized supernatant (pH 8.3) was then stored on ice. Assays were carried out in triplicate in 5-ml glass vials with screw-on septum caps at 30°C with continuous shaking (125 strokes min^{-1}). The assay buffer consisted of 50 mM Tris (pH 8.5), 5 mM DTT, 10 mM $MgCl_2$, 20 mM $NaH^{14}CO_2$ (7.54 GBq mol^{-1}), and 0.5 ml of the neutralized leaf extract supernatant. The total assay volume was 1 ml. The RuBP determination was initiated with the injection of 0.1 ml of activated crystallized RuBPCase from tobacco (equivalent to approximately 55 μg protein). The tobacco enzyme had been prepared previously according to the method of Kung et al. (1980), and was reactivated by dissolving the enzyme in 50 mM Tris (pH 8.5), 10 mM $MgCl_2$, 10 mM $NaHCO_2$ and 100 mM NaCl and incubating for 25 minutes at 50°C (Kung et al., 1980). After 60 minutes the assay was terminated with the injection of 0.1 ml 6N HCl. An aliquot (0.9 ml) of the assay mixture was transferred to a 20-ml glass scintillation vial which was dried on a warm heating plate under an air-stream. When dried, 2.5 ml water and 5 ml scintillation cocktail were added to each vial and acid-stable ^{14}C products were determined by liquid scintillation spectrometry.

Chlorophyll, Protein, and Specific Leaf Weight Determinations

Chlorophyll determinations were performed on sample aliquots reserved during the RuBPCase assays. Chlorophyll was extracted in 80%

acetone and calculations were by the method of Arnon (1949). The chlorophyll in sample aliquots reserved during RuBP determinations was converted to pheophytin during extraction with acid, therefore the original chlorophyll concentration was determined using the method of Vernon (1960). In addition, chlorophyll was determined in leaf disks of known surface area, collected and assayed at the same time that leaf tissue was collected for RuBPCase and RuBP assays. Soluble protein determinations were performed on aliquots of the same supernatant from the crude extracts used to initiate the RuBPCase assays. The dye binding spectrophotometric method of Bradford (1976) was used. Protein standards were prepared from crystallized and lyophilized BSA (bovine serum albumin) dissolved in the same buffer used in extraction of RuBPCase from leaf tissue. Specific leaf weight (SLW) was determined by drying freshly harvested leaves of known surface area, collected 49 DAP from the unshaded upper canopy, to constant weight in a 70°C oven.

Pod Load and Leaf Area Measurements

On October 18, 1983 (49 DAP), 12 plants were removed from each chamber for determination of pod weight (grams dry weight) and leaf area. To measure pod weight, all viable pods were removed from the plants and dried to constant weight in a 70°C oven. To determine leaf area, all green leaves were removed from the plants and the surface area (one side of each leaf) was measured with an area meter (Lambda, model LI 3000).

Analysis of Statistical Significance

To determine the statistical significance of experimental results, simple linear regressions were performed using the short-term CO_2 concentrations to which plants were exposed as regressor. Comparisons of slopes and intercepts between CO_2 growth treatments, and comparison of slopes to zero, were used as tests to determine if there were significant differences between treatments and also if there were significant responses to the various short-term CO_2 concentrations. In addition to simple linear regression, a quadratic regression was also performed on the RuBP data. Both types of regressions gave very similar results regarding the significance of RuBP response to CO_2. In cases where data were collected following growth at the two CO_2 treatments (but prior to exposure to the various short-term CO_2 concentrations) t-tests were used to determine the significance of the growth CO_2 treatments on certain plant characteristics. In all cases, all tests of significance were made at the 5% level unless otherwise noted. Regression parameters are tabulated in Appendix C.

Results

Response of Leaf Characteristics to CO_2

Soybean plants were grown from seed at atmospheric and twice atmospheric CO_2 concentrations. As shown in Table 2.1, specific leaf weight increased significantly at elevated CO_2. Chlorophyll and total soluble protein (expressed on a leaf area basis) were not significantly different in the two CO_2 treatments. Pod weight, leaf

Table 2.1. Effects of growth CO_2 concentration on leaf characteristics. Specific leaf weight determined on samples collected 49 DAP. All other samples collected 56 to 60 DAP. Mean values ± SD are presented.

	Growth CO_2 concentration $\mu l\ CO_2\ l^{-1}$	
	330	660
Specific leaf weight[1] g dry wt. m^{-2}	17.00 ± 0.10	23.70 ± 0.04
Chlorophyll[2] g m^{-2}	0.475 ± 0.005	0.520 ± 0.017
Total soluble protein[3] g m^{-2}	4.03 ± 0.04	4.26 ± 0.14
Protein/Chlorophyll ratio	8.5	8.2

[1]
[2] $t = 67.03$, df = 2 Ho: $\mu 660 - \mu 330 = 0$ rejected at 5% level.
[3] $t = 3.39$, df = 2 Ho: $\mu 660 - \mu 330 = 0$ not rejected at 5% level.
$t = 2.06$, df = 2 Ho: $\mu 660 - \mu 330 = 0$ not rejected at 5% level.

area per plant, and the ratio of pods to leaf area all increased with CO_2 (Table 2.2), however, the differences in pod weight and leaf area were not significant. These morphological and biochemical differences reflect the effects of increased CO_2 concentration which also affects leaf photosynthetic rate response.

Leaf Photosynthetic Rate

The effects of CO_2 concentration on leaf photosynthesis were examined following long-term and short-term exposures to different CO_2 concentrations. Intercellular CO_2 concentrations (Ci) were calculated as the CO_2 concentration ambient to the leaf (Ca) was varied from 80 to 1000 μl CO_2 l^{-1}. In Figure 2.1, Ci is plotted against Ca for leaves grown at both CO_2 concentrations. Linear regression analysis of the data yields slopes, and hence Ci/Ca ratios, of 0.72 (r=0.985) and 0.55 (r=0.965), respectively, for the 330 and 660 μl CO_2 l^{-1} grown leaves. The difference in the Ci/Ca ratio was found to be significant. Because the Ci/Ca ratio was lower in high CO_2 grown leaves, the Ci calculated at any ambient CO_2 concentration was greater in leaves grown at 330 μl CO_2 l^{-1}.

Leaf photosynthetic rates were greater in high CO_2 grown plants at all CO_2 concentrations in which they were measured (Figure 2.2). When plotted against Ci, plants grown at high CO_2 had greater maximum leaf photosynthetic rates. Plotting leaf photosynthetic rate against Ci allows evaluation of the CO_2 assimilation rate response to CO_2 concentration independent of stomatal influences. Each point in Figure 2.2 represents one photosynthetic rate measurement made at a

Table 2.2. Effects of growth CO_2 concentration on pod weight and total green leaf area per plant on samples collected 49 DAP. Mean value ± SD are presented for leaf area. Pod weight represents total dry weight of pods divided by the number of plants.

	Growth CO_2 concentration $\mu l\ CO_2\ l^{-1}$		
	330	660	660/330
Pod weight[1] g dry wt. plant^{-1}	0.084 ± 0.023	0.125 ± 0.052	1.48
Leaf area[2] m^2 plant^{-1}	0.1475 ± 0.0546	0.1855 ± 0.0432	1.25
Pod/Leaf area g m^{-2}	0.569	0.674	1.18

[1] $t = 1.23$, df = 4 Ho: $\mu 660 - \mu 330 = 0$ not rejected at 5% level.
[2] $t = 1.89$, df = 32 Ho: $\mu 660 - \mu 330 = 0$ not rejected at 5% level.

Fig. 2.1. Intercellular CO_2 concentration versus ambient CO_2 concentration for leaves grown at two CO_2 concentrations. In leaves grown at 330 µl CO_2 l^{-1} (+), Ci/Ca = 0.72 (r = 0.985). In leaves grown at 660 µl CO_2 l^{-1} (△), Ci/Ca = 0.55 (r = 0.965).

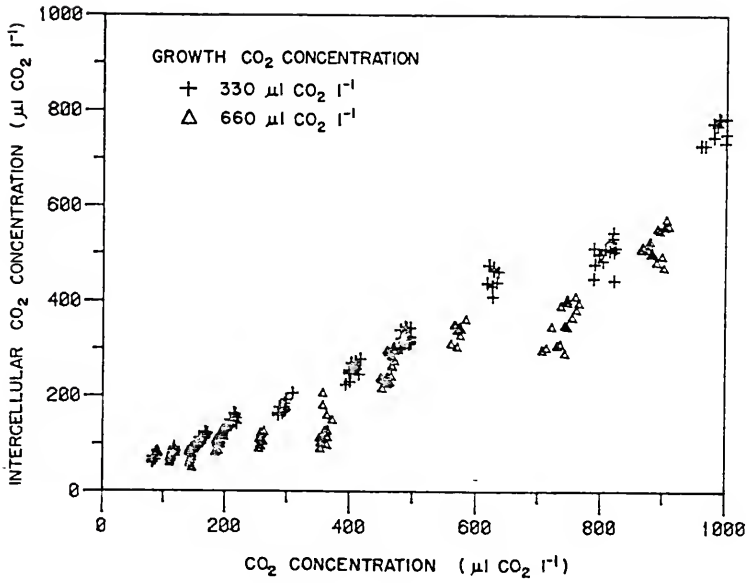
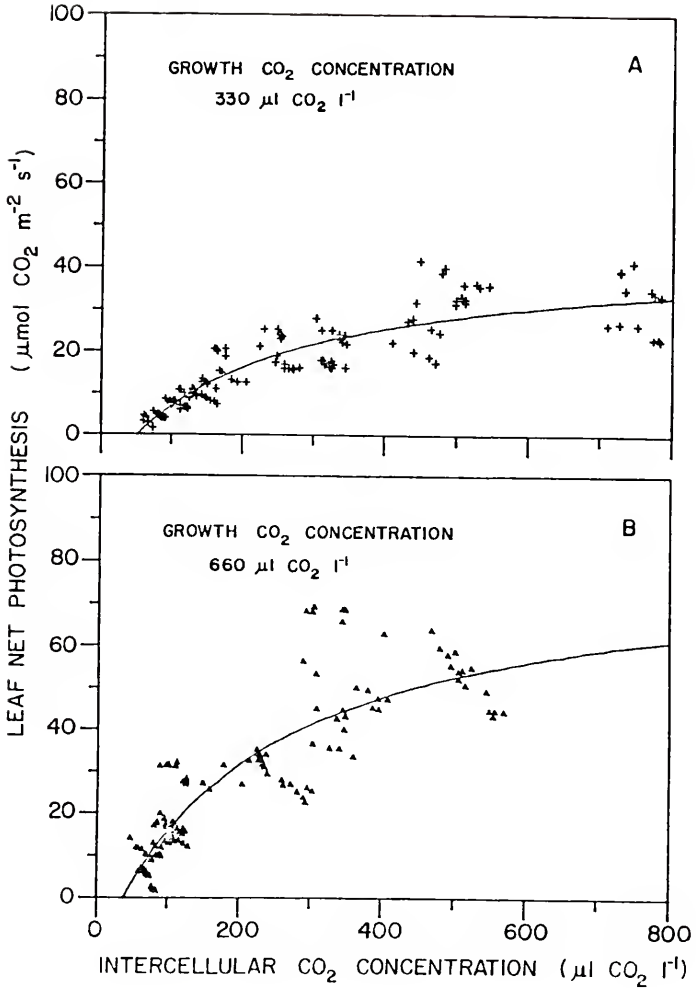

GROWTH CO_2 CONCENTRATION
+ 330 µl CO_2 l^{-1}
Δ 660 µl CO_2 l^{-1}
INTERCELLULAR CO_2 CONCENTRATION (µl CO_2 l^{-1})
CO_2 CONCENTRATION (µl CO_2 l^{-1})
0
200
400
600
800
1000

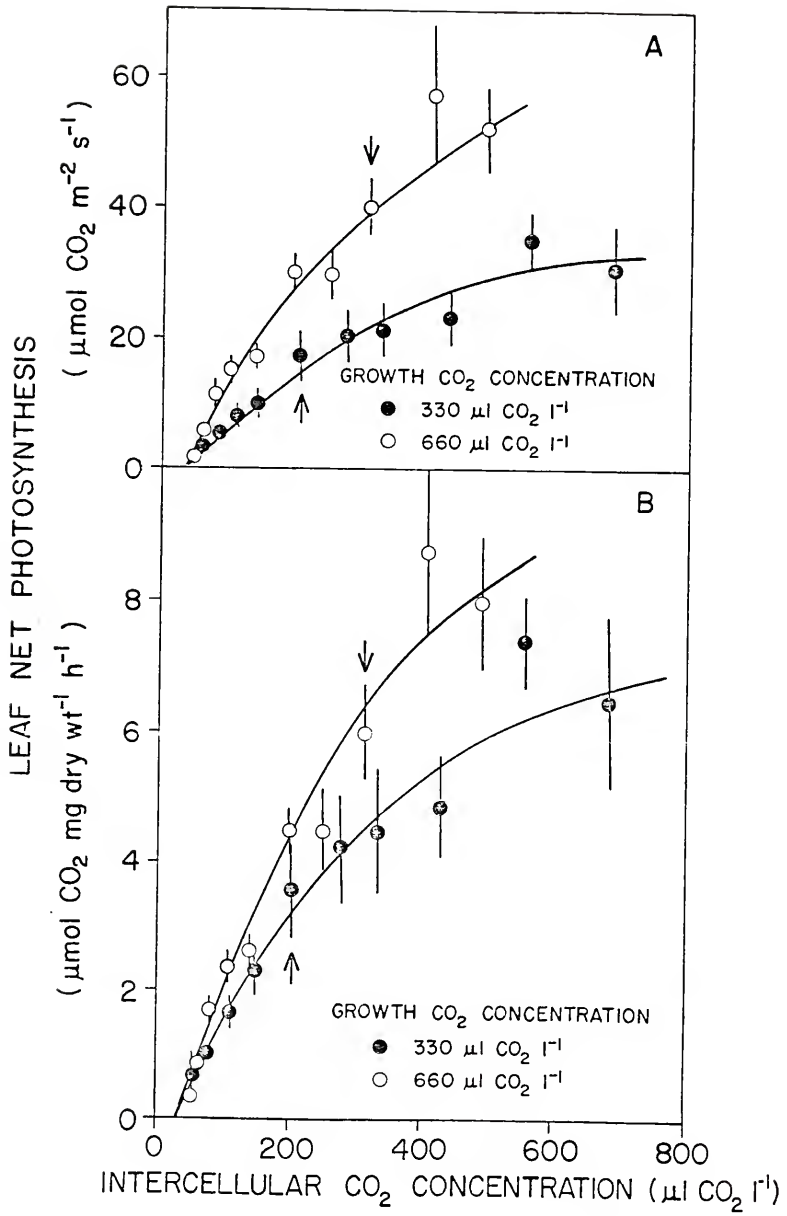
Fig. 2.2. Leaf photosynthetic rate versus intercellular CO_2 concentration for leaves grown at 330 μl CO_2 l^{-1} (A) and 660 μl CO_2 l^{-1} (B). Each data point represents one measurement made at 5 minute intervals. The solid curves were generated by non-linear regression analysis of the data. The regression model was P=Pmax*Ci/(KCi+Ci)+R; where P is leaf net photosynthetic rate, Pmax is the maximum value of P-R, Ci is intercellular CO_2, KCi is the Michaelis constant for Ci and R is the estimated respiration rate at Ci=0. For 330 μl CO_2 l^{-1} grown leaves: Pmax=55.5, KCi=206 and R=-11.0. For 660 μl CO_2 l^{-1} grown leaves: Pmax=96.1, KCi=223, and R=-13.8. Pmax and R are in μmol CO_2 $m^{-2}s^{-1}$ and KCi is in μl CO_2 l^{-1}. Photosynthetic measurements were made 56 to 60 DAP.


GROWTH CO_2 CONCENTRATION
330 µl CO_2 l^{-1}
A
GROWTH CO_2 CONCENTRATION
660 µl CO_2 l^{-1}
B
LEAF NET PHOTOSYNTHESIS (µmol CO_2 m^{-2} s^{-1})
INTERCELLULAR CO_2 CONCENTRATION (µl CO_2 l^{-1})
0
20
40
60
80
100
200
400
600
800

5-minute interval, and are the pooled values from two leaflets at each CO_2 treatment. The highest rate measured for a leaf grown at 330 μl CO_2 l^{-1} was 41 μmol CO_2 m^{-2} s^{-1}, and for a 660 μl CO_2 l^{-1} grown leaf 69 μmol CO_2 m^{-2} s^{-1} (Figure 2.2, A and B). At low Ci, high CO_2 grown leaves showed greater rate response to increases in CO_2. The solid curves in Figure 2.2 (A and B) were generated by non-linear regression analysis of the data points.

The data in Figure 2.2 were divided into 10 discrete groups based on CO_2 concentration, and the mean Ci and mean leaf photosynthetic rate were calculated. The Ci values in each group varied less than 5% from the mean. These means are plotted in Figure 2.3 (A). Comparison of Figure 2.2 (A and B) with Figure 2.3 (A), indicates that plotting the means of the data did not affect the relationship between photosynthetic rates nor the relationship between photosynthetic rate and Ci. Since there was a difference in SLW between leaves grown at the two CO_2 concentrations, mean leaf photosynthetic rates were also calculated based on dried leaf weight and are plotted against Ci in Figure 2.3 (B). The difference in photosynthetic rates between high CO_2 and atmospheric CO_2 concentration grown leaves was less when expressed on a dry weight basis, particularly at lower CO_2 concentrations. However, leaf photosynthetic rates were still greater in the high CO_2 grown leaves at all CO_2 concentrations, suggesting that the increase in SLW in the high CO_2 grown leaves did not account for all of the increase in leaf photosynthetic rate. Arrows in Figure 2.3 (A and B) indicate the mean photosynthetic rates obtained when measured at the respective ambient growth CO_2 concentrations. The

Fig. 2.3. Mean leaf photosynthetic rate versus mean intercellular CO_2 concentration for leaves grown at 330 μl CO_2 l^{-1} (●) and 660 μl CO_2 l^{-1} (▲). Photosynthetic rates are expressed on a leaf area basis (A) and a leaf dry weight basis (B). Data are from Figure 2.2. Arrows indicate mean photosynthetic rates measured at the respective ambient CO_2 growth concentrations. Vertical lines represent ± SD.


A
B
LEAF NET PHOTOSYNTHESIS
(μmol CO2 m-2 s-1)
(μmol CO2 mg dry wt-1 h-1)
GROWTH CO2 CONCENTRATION
330 μl CO2 l-1
660 μl CO2 l-1
INTERCELLULAR CO2 CONCENTRATION (μl CO2 l-1)
0
20
40
60
2
4
6
8
200
400
600
800

photosynthetic rates of leaves grown and measured at 660 μl CO_2 l^{-1} were greater than in leaves grown and measured at 330 μl CO_2 l^{-1}.

RuBP Carboxylase Activity

Assays of RuBPCase activity were performed on leaf tissue sampled from plants at their growth CO_2 concentration and also following short-term exposure to a range of CO_2 concentrations. Both initial (nonactivated) and total (HCO_3^-/Mg^{2+} activated) activities were assayed in samples (collected under high light conditions) that were extracted without added Mg^{2+}. The results of these assays are shown in Figure 2.4 (A and B). Each data point is the mean of triplicate assays. Enzyme activity in Figure 2.4 is expressed on a leaf area basis so a more meaningful comparison can be made with leaf photosynthetic rates. Figure 2.4 (A) shows that initial activity of RuBPCase did not significantly respond to short-term exposure to different CO_2 concentrations. There was no significant difference between the two growth CO_2 concentrations. Total activity was also independent of short-term CO_2 concentrations [Figure 2.4 (B)]. It also did not significantly respond to increases in CO_2. The catalytic rates were quite similar (not significantly different) between the two growth CO_2 concentrations whether measured as initial or total enzyme activity at all CO_2 concentrations. On a leaf area basis there was less than a 5% difference between the activities (both initial and total) of RuBPCase when sampled at the respective growth CO_2 concentrations. Initial and total enzyme activities were also calculated on a chlorophyll basis and these data are presented in

Fig. 2.4. Leaf RuBPCase activity versus CO_2 concentration for samples collected following 1 hour exposures to six different CO_2 concentrations. Plants grown at 330 μl CO_2 l^{-1} (●) or 660 μl CO_2 l^{-1} (▲). Both initial activity (A) and total activity (B) were assayed. Mean values of triplicate assays are presented.

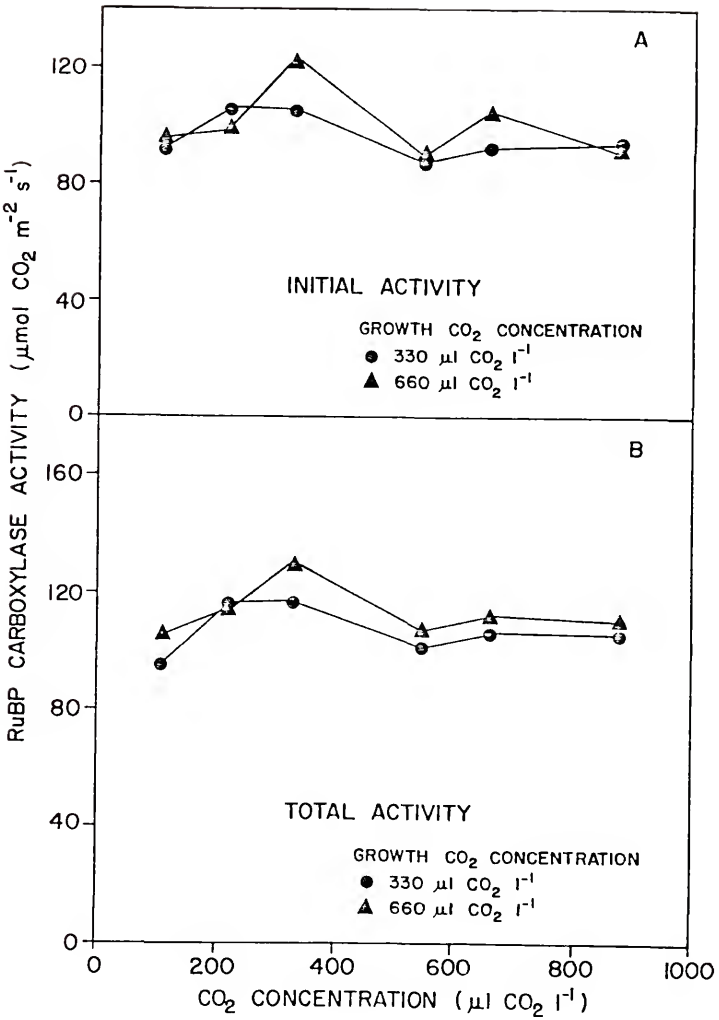

A
B
INITIAL ACTIVITY
TOTAL ACTIVITY
GROWTH CO_2 CONCENTRATION
330 µl CO_2 l^{-1}
660 µl CO_2 l^{-1}
RuBP CARBOXYLASE ACTIVITY (µmol CO_2 m^{-2} s^{-1})
CO_2 CONCENTRATION (µl CO_2 l^{-1})
0
40
80
120
160
200
400
600
800
1000

Table 2.3. Due to the difference in the amount of chlorophyll per unit leaf area, the relative enzyme activities shift somewhat when expressed on a different basis. When expressed on a chlorophyll basis, leaves grown and sampled at 330 μl CO_2 l^{-1} had initial and total activities 10 and 13% greater than leaves grown and sampled at 660 μl CO_2 l^{-1}. However, the response to CO_2 of both initial and total RuBPCase activities was not significantly different between the two growth CO_2 treatments. The activation state of RuBPCase in vivo may be estimated by initial activity/total activity * 100%. As would be expected based on the independence of initial and total enzyme activities from CO_2 concentration (Figure 2.4), the activation was also independent of CO_2 (Figure 2.5). The response of activation to CO_2 concentration was insignificant (at the 1% level) for both CO_2 treatments. There was no significant effect of exposure to different short-term CO_2 concentrations or to long-term growth CO_2 concentration on activation.

RuBP Levels

Steady state RuBP levels were measured in the same tissue samples collected for RuBP carboxylase assays. Samples were collected at growth CO_2 concentrations and also following the short-term exposures to the various CO_2 concentrations. RuBP data are reported on a leaf area basis in Figure 2.6. Each data point represents the mean of triplicate assays. There was a significant response of RuBP levels to CO_2 concentration. In both growth CO_2 treatments, below a CO_2 concentration of 330 μl CO_2 l^{-1}, RuBP levels increased as CO_2

Table 2.3. Effects of two growth CO_2 concentrations on RuBPCase activity in leaves collected following 1-hour exposures to six different CO_2 concentrations. Both initial and total enzyme activity were assayed. Mean values of triplicate assays ± SD are presented.

	RuBP carbosylase activity			
Ambient CO_2	330 μl CO_2 l^{-1}		660 μl CO_2 l^{-1}	
Concentration	μmol CO_2 mg Chl^{-1} hr^{-1}			
μl CO_2 l^{-1}	Initial	Total	Initial	Total
110	700 ± 13	731 ± 53	651 ± 26	726 ± 10
220	803 ± 20	880 ± 6	627 ± 18	793 ± 5
330	803 ± 28	890 ± 24	847 ± 9	903 ± 14
550	665 ± 5	771 ± 29	614 ± 18	735 ± 32
660	706 ± 6	800 ± 16	720 ± 19	771 ± 8
880	715 ± 7	809 ± 17	624 ± 18	757 ± 6

Fig. 2.5. Activation status of RuBPCase versus CO_2 concentration for leaves grown at 330 μl CO_2 l^{-1} (●) or 660 μl CO_2 l^{-1} (▲). Mean values of triplicate assays are presented. Percent activation calculated from data in Figure 2.4.

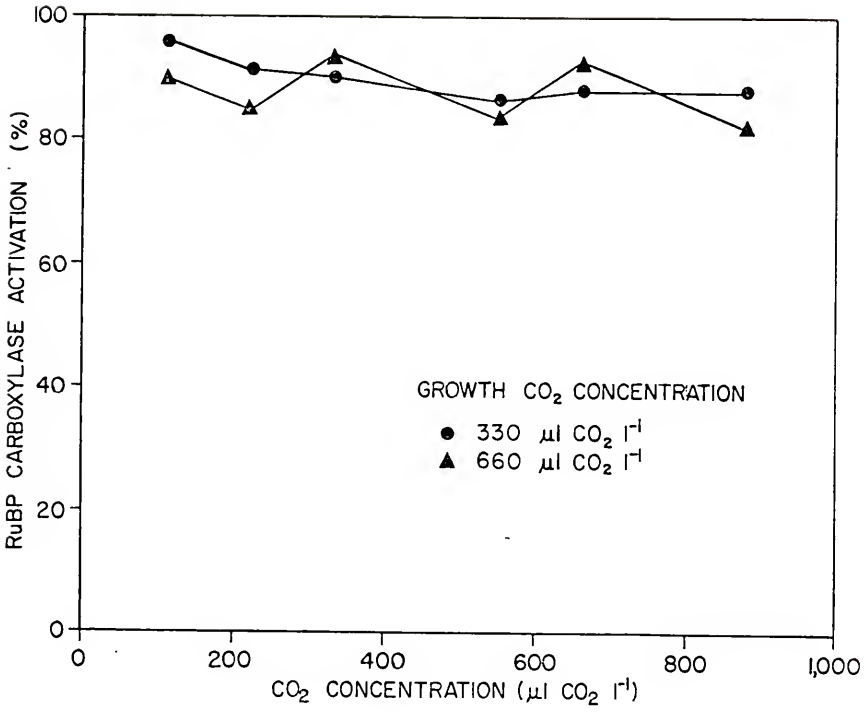
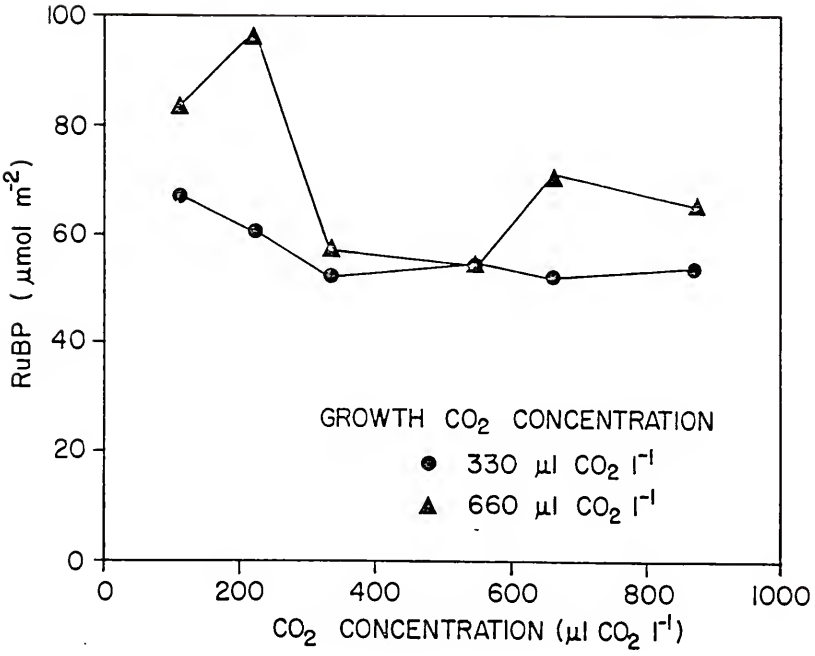

RuBP CARBOXYLASE ACTIVATION (%)
100
80
60
40
20
0
0
200
400
600
800
1,000
CO2 CONCENTRATION (µl CO2 l-1)
GROWTH CO2 CONCENTRATION
330 µl CO2 l-1
660 µl CO2 l-1

Fig. 2.6. Leaf RuBP levels versus CO_2 concentration in samples collected following 1-hour exposures to six different CO_2 concentrations. Leaves were grown at 330 μl CO_2 l^{-1} (●) or 660 μl CO_2 l^{-1} (▲). Mean values of triplicate assays are presented.


100
80
60
40
20
0
RuBP (μmol m-2)
0
200
400
600
800
1000
CO2 CONCENTRATION (μl CO2 l-1)
GROWTH CO2 CONCENTRATION
330 μl CO2 l-1
660 μl CO2 l-1

decreased. Above this concentration RuBP was rather insensitive to CO_2. The levels of RuBP were higher in leaves grown at high CO_2 regardless of the different short-term CO_2 concentrations. The RuBP levels showed significant responses to both the short-term CO_2 concentrations and to growth CO_2 treatment. Due to the difference in chlorophyll content, RuBP levels were also calculated on a chlorophyll basis. The concentration of RuBP in the chloroplast stroma was calculated assuming RuBP is present only in the chloroplast (Heber, 1974) and that the stromal volume is equivalent to 25 μl mg chlorophyll^{-1} (Sicher and Jensen, 1979). These data are shown in Table 2.4. As was the case on an area basis, the RuBP levels on a chlorophyll basis were significantly higher in the high CO_2 grown leaves. The RuBP level decreased significantly with increasing CO_2 concentration when expressed on either a chloropyll basis or as the stromal concentration of RuBP.

Discussion

Soybean leaflet photosynthetic rates increased with increasing CO_2 concentration in plants grown at both atmospheric and twice atmospheric CO_2 concentrations. There are relatively few examples of high CO_2 grown plants having greater leaf photosynthetic rates than atmospheric CO_2 grown plants, when both are measured over the same range of CO_2 concentration. However, at all CO_2 concentrations in which photosynthesis was measured, rates were greater in leaflets grown at the higher CO_2 concentration (Figures 2.2 and 2.3). Thus, these results agree with those of Hicklenton and Jolliffe (1980a) and

Table 2.4. Effects of growth CO_2 concentration on RuBP levels in leaves collected following 1-hour exposures to six different CO_2 concentrations. Levels of RuPB are expressed both on a chlorophyll basis and a chloroplast concentration basis. Mean values of triplicate assays ± SD are presented.

	RuBP			
Ambient CO_2 Concentration $\mu l\ CO_2\ l^{-1}$	Growth CO_2 Concentration $\mu l\ CO_2\ l^{-1}$			
	330	660	330	660
	nmol $mgChl^{-1}$		mM	
110	141 ± 2	158 ± 1	5.6 ± 0.08	6.3 ± 0.04
220	128 ± 1	186 ± 1	5.1 ± 0.04	7.4 ± 0.04
330	108 ± 5	106 ± 2	4.3 ± 0.20	4.2 ± 0.08
550	115 ± 1	103 ± 1	4.6 ± 0.04	4.1 ± 0.04
660	109 ± 1	135 ± 5	4.3 ± 0.04	5.4 ± 0.20
880	113 ± 1	123 ± 1	4.5 ± 0.04	4.9 ± 0.04

are similar to the results of Mauney et al. (1979) with soybean. The implication of this type of relationship between leaf photosynthesis and CO_2 with regard to control of leaf photosynthetic rate is discussed below.

Intercellular CO_2 concentrations were calculated and leaflet photosynthetic rates were then plotted against Ci. Figure 2.1 shows the relationship between Ci and Ca to be linear and therefore the ratio of Ci/Ca was found to be constant across a range of CO_2 concentrations from 80 to 1000 μl CO_2 l^{-1}. Whereas Goudriaan and van Laar (1978) found Ci/Ca to be constant in Phaseolus vulgaris only when Ca was below and not above 300 μl CO_2 l^{-1}, the results reported here are consistent with those of most other researchers (Spencer and Bowes, 1986; Sharkey et al., 1982; Wong et al., 1979). While the Ci/Ca ratios were constant at all CO_2 concentrations, the ratio was significantly lower (by 23%) in plants grown at higher CO_2. This could be due to the higher photosynthetic rates or differential stomatal response in the high CO_2 grown leaves. Either factor might lower the Ci. However, another factor may be responsible for the Ci/Ca ratio difference. Growth of soybean at elevated CO_2 concentrations can result in thicker leaves with more palisade cells per unit leaf area (J.C.V. Vu, personal communication; Thomas and Harvey, 1983), and therefore an increased mesophyll cell surface area/external leaf surface area ratio. An increased internal surface area would permit greater uptake of CO_2 from the leaf intercellular air spaces and result in a lower Ci value. Nobel et al. (1975) and Nobel (1980) have discussed the influence of several environmental

variables, other than CO_2, on the internal to external surface area ratio. The effects of increased mesophyll cell surface area on leaf photosynthesis are discussed below. Wong et al. (1985) and Spencer and Bowes (1986) did not find a difference in Ci/Ca ratios with different growth CO_2 concentrations.

In plants grown at both atmospheric and elevated CO_2, RuBPCase activity (on a chlorophyll basis) was not significantly greater in the leaves grown and sampled at the lower rather than the higher CO_2 concentration (Table 2.3). An apparently significant effect of CO_2 has been reported in the literature for a variety of C_3 plants including cotton (Wong, 1979), Nerium oleander, and Atriplex triangularis (Downton et al., 1980), Phaseolus (von Caemmerer and Farquhar, 1984; Porter and Grodzinski, 1984), soybean (Vu et al., 1983), tomato (Hicklenton and Jolliffe, 1980a), and waterhyacinth (Spencer and Bowes, 1986). When RuPBCase activity is expressed on a leaf area basis (Figure 2.4) there is also no significant difference between CO_2 treatments in the enzyme response to CO_2 concentration. In plants that were grown at a particular CO_2 concentration and then exposed for short periods of time to concentrations of CO_2 ranging from 110 to 880 μl CO_2 l^{-1}, prior to sampling leaves, there was no significant effect of the short-term exposures on initial or total enzyme activity (Figure 2.4). The independence of initial activity from short-term exposure to CO_2 in the light has also been reported in Arabidopsis (Salvucci et al., 1986a) and white clover (Schnyder et al., 1984). When the CO_2 concentration was raised to 5000 μl CO_2 l^{-1}, Schnyder et al. (1984), however, found a 50% decrease in activity

compared to the activity at the CO_2 compensation point. The percent activation of RuBPCase, an estimation of the in vivo enzyme activation status, like the initial and total activities was essentially not affected by CO_2 (Figure 2.5). Perchorowicz and Jensen (1983) and Schnyder et al. (1984) reported similar results with wheat and white clover, respectively. Although CO_2 is necessary in the activation of RuBPCase (Bahr and Jensen, 1978; Lorimer et al., 1976), there was no indication that even at CO_2 concentrations as low as 110 μl CO_2 l^{-1} (and corresponding Ci value of 60 to 75 μl CO_2 l^{-1}) the enzyme suffered a significant decrease in activation. This indicates that a high CO_2 concentration inside the leaf is not required for a high level of RuBPCase activation at high light intensity.

Unlike the apparent lack of effect of CO_2 concentration on RuBPCase activity in vitro, steady state RuBP levels were found to respond to CO_2. Plants grown at both CO_2 concentrations had the highest levels of RuBP following exposure to low CO_2 concentrations. The RuBP levels declined as CO_2 increased (Figure 2.6). Work by other researchers has yielded similar results (Badger et al., 1984; Dietz and Heber, 1984; Mott et al., 1984; Collatz, 1978). The results of Dietz and Heber (1984) indicated approximately two times the concentration of CO_2 was required with spinach, as compared with the soybean data in Figure 2.6, prior to the onset of the decline in RuBP. Hitz and Stewart (1980) did not find changes in RuBP levels in soybean during steady state photosynthesis in 21% O_2 and CO_2 concentrations ranging from 50 to 500 μl CO_2 l^{-1}. Levels of RuBP decreased as leaf photosynthetic rate increased with increasing CO_2 regardless of growth

at 330 or 660 μl CO_2 l^{-1} (Figure 2.6). The lower levels of RuBP (as CO_2 concentration was increased) are presumably a result of greater consumption due to higher photosynthetic rates associated with the increased CO_2 concentration. Although both photosynthesis and RuBP levels were greater in leaves grown at high CO_2, the turnover time for the pool of RuBP was about the same for leaves grown at either CO_2 concentration when calculated at both low and high ambient CO_2 (110 and 880 μl CO_2 l^{-1}). This suggests coordination between leaf photosynthetic rate and RuBP levels. Turnover times were calculated based on the rate of photorespiration being 15% of the rate of photosynthesis (Canvin, 1979), one mole RuBP consumed per mole CO_2 assimilated (Bassham, 1979), and two moles RuBP consumed per mole CO_2 released during photorespiration (Ogren, 1984). This stoichometry, the leaf photosynthetic rates, and the measured steady state levels of RuBP yielded turnover times of 11.5 and 10.8 seconds for leaves grown at 330 and 660 μl CO_2 l^{-1}, respectively, when measured at 110 μl CO_2 l^{-1}, and 1.1 and 0.8 seconds when measured at 880 μl CO_2 l^{-1}. The RuBP concentrations (Table 2.4) were always greater than the estimated RuBPCase binding site concentration for RuBP of 3 to 4 mM (Jensen and Bahr, 1977), indicating that RuBP was probably at saturating concentrations. The similarity of turnover times and the concentration of RuBP greater than the estimated binding site concentration, suggest that RuBP was probably not limiting leaf photosynthetic rates in these experiments.

Initial RuBPCase activity [Figure 2.4 (A)] was greater at all CO_2 concentrations than the leaf photosynthetic rate [Figure 2.3 (A)] when

both were expressed on a leaf area basis. Results of this nature have previously been reported (Bjorkman, 1981; Singh et al., 1974). There are a number of reasons why leaf photosynthetic rate measured in situ would be less than RuBPCase activity measured in vitro. The enzyme assays are performed under saturating inorganic carbon concentrations which not only provides more CO_2 than is normally available within the leaf chloroplast in the field, but also essentially eliminates the competitive oxygenase reaction. The effects of dark respiration are not measured in the enzyme assay. Also, extraction of the enzyme from its intrachloroplastic location prior to assay will presumably remove metabolic regulation that may normally function in the intact photosynthetic cell. Furthermore, the assay procedure used to determine RuBPCase activity measures both the E-C and E-C-M forms of the enzyme while in the intact leaf only the E-C-M form will be active (Seftor et al., 1986). If the E-C form is present in significant quantities the in vitro enzyme assay will tend to overestimate the active species of RuBPCase in vivo.

Farquhar et al. (1980) have proposed a model suggesting leaf photosynthetic rate is limited by RuBPCase at low Ci and by RuBP regeneration at high Ci. Results supporting this model have been reported by von Caemmerer and Farquhar (1981), while Makino et al. (1985) have indicated their results suggest RuBPCase was always limiting to leaf photosynthesis. Results reported here show no significant effect of Ci on RuBPCase activity and suggest that RuBP levels were probably saturating for RuBPCase binding sites at all Ci

values. These data, therefore, do not appear to support the model of Farquhar et al. (1980).

Since the leaflet photosynthetic rates were greater in leaves grown at twice the atmospheric concentration of CO_2, yet the difference in RuBPCase activity between the two CO_2 growth treatments were not significant, and RuBP appeared to be at saturating levels, three possibilities are suggested which may account for the greater leaflet photosynthetic rates of the high CO_2 grown plants. First, as already described, growth at elevated CO_2 concentration can result in an increase in the mesophyll cell surface area/leaf surface area ratio. Nobel et al. (1975) have shown an increase in this ratio to result in higher photosynthetic rates. This may have occurred in the high CO_2 treatment. Second, leaflet photosynthetic rates were measured during the pod filling stage, and plants grown at high CO_2 had a greater pod weight per plant and per unit leaf area. Long-term growth in high CO_2 has been shown to increase the number of fruit per plant in several cases (Havelka et al., 1984; Baker and Enoch, 1983; Cooper and Brun, 1967), and these increases represent an increase in assimilate demand. An increase in assimilate demand has often been associated with increased photosynthesis (Gifford and Evans, 1981; Geiger, 1976; King et al., 1967). Plants grown at high CO_2 had greater leaf photosynthetic rates as well as greater pod weights per plant. Enos et al. (1982) have also reported higher photosynthetic rates in soybean plants with heavier pods. Third, the CO_2-saturated RuBPCase activity *in vitro* may not be an accurate representation of activity *in vivo*. There may be differential regulation of RuBPCase *in*

vivo in soybean grown at different CO_2 concentrations, however, no evidence of this was observed.

An additional factor needs to be addressed with regard to photosynthetic rates; the effects of leaf starch. Although starch was not measured quantitatively in these experiments, visual estimations of relative starch levels performed prior to enzyme assays indicated that leaves grown at high CO_2 contained more starch. In previous studies, where starch was measured quantitatively, it was found to be higher in soybean leaves grown at elevated CO_2 concentrations (Allen et al., 1983). In the experiments reported here the results are in agreement with those in the literature that indicate no evidence of photosynthetic rate inhibition by starch accumulation at high CO_2.

Based on the results presented here from soybean, it is shown that growth at twice the atmospheric concentration of CO_2 can result in an enhanced capacity for leaflet photosynthesis. Since the response of RuBPCase activities was not significantly different with growth CO_2 treatment and the levels of RuBP appeared to be saturating with regard to RuBPCase binding sites, the role of either in the enhanced photosynthetic capacity remains unsupported. The increased photosynthetic capacity following growth in elevated CO_2 may be due to either an increase in the internal/external leaf area ratio or greater assimilate demand or a combination of both.

CHAPTER III
RESPONSE OF PHOTOSYNTHETIC BIOCHEMISTRY AND PHYSIOLOGY TO LONG-TERM EXPOSURE TO SUBATMOSPHERIC AND SUPERATMOSPHERIC CO_2 CONCENTRATIONS

Introduction

Much of the interest in the effects of CO_2 on vegetation is based on the fact that the atmospheric concentration of CO_2 has been increasing for the last century (Baes et al., 1977). Research has focused on predicting how this continuing trend will affect future crop yields and water use. In addition to learning the answers to these questions, experiments with CO_2 concentrations can enhance our comprehension of plant processes such as photosynthesis. Since the response of plants to CO_2 is largely mediated by the photosynthetic process, understanding the effects of CO_2 on photosynthesis is paramount to understanding the effects on whole crop responses.

Almost all of the research on the long-term effects of CO_2 on plants has involved exposing plants to elevated concentrations of CO_2 (Lemon, 1983; Kramer, 1981). It appears that long-term research on plants grown at reduced rather than elevated CO_2 concentrations has previously just involved plants native to high altitudes where they normally grow at CO_2 partial pressures below those at or near sea-level (Mooney et al., 1966; Billings et al., 1961). Long-term exposure to elevated CO_2 results in a number of changes in plant characteristics. Leaf area on a whole plant basis has been shown to increase with CO_2 (Jones et al., 1984a; O'Leary and Knecht, 1981;

Cooper and Brun, 1967). Stomatal density (stomata mm^{-2}) increased, although not significantly, in soybean grown at high CO_2 (Thomas and Harvey, 1983). In Phaseolus fewer stomates were found on the abaxial surface of leaves grown at high CO_2, but the leaves were larger and thus the overall result was more stomates per leaf (O'Leary and Knecht, 1981). Increases in specific leaf weight (SLW) following growth at elevated CO_2 have been reported in tomato (Madsen, 1968), Nerium oleander (Downton et al., 1980), Phaseolus (Jolliffe and Ehret, 1985), and soybean (Havelka et al., 1984; Jones et al., 1984a; Thomas and Harvey, 1983; Hofstra and Hesketh, 1975). Chlorophyll content of leaves has been shown to either increase (Downton et al., 1980), decrease (von Caemmerer and Farquhar, 1984), or stay the same (Havelka et al., 1984) in plants grown at elevated CO_2. Other cytological responses to long-term high CO_2 exposure include increased cell water content (Madsen, 1968), and changes in cell volume (Gates et al., 1983; Madsen, 1968). In soybean, the presence of a third layer of palisade cells not found in plants grown at atmospheric CO_2 concentration was observed in high CO_2 grown plants (Thomas and Harvey, 1983). Carbon dioxide concentration has been shown to affect the concentration of proteins as well as enzyme activities. In soybean grown at elevated CO_2 seed protein was found to decrease as CO_2 increased (Rogers et al., 1984), but in another study there was no effect of CO_2 on pod nitrogen levels (Hardy and Havelka, 1976). The response of total soluble leaf protein to CO_2 varies. It has been shown to increase (Downton et al., 1980), decrease (Wong, 1979), and not change (Havelka et al., 1984; Porter and Grodzinski, 1984) with

long-term exposure to elevated CO_2. Most reports indicate that growth at high CO_2 results in reduced activity of RuBPCase when compared to plants grown at atmospheric CO_2 concentrations, when activity is expressed on either a chlorophyll basis (Spencer and Bowes, 1986; Vu et al., 1983; Downton et al., 1980) or a leaf area basis (von Caemmerer and Farquhar, 1984; Wong, 1979). However, Fair et al. (1973) have reported higher activity, when expressed on a fresh weight basis, in young barley plants grown at 10,000 to 50,000 μl CO_2 l^{-1}. The difference in activity became less as the plants aged. The proportion of leaf soluble protein composed of RuBPCase (mg RuBPCase/g soluble protein) decreased 22% in Nerium oleander when the growth CO_2 concentration was increased from 330 to 660 μl CO_2 l^{-1} (Downton et al., 1980). The effects of CO_2 on a variety of other enzymes have also been reported. Carbonic anhydrase activity increased in oat when grown at 80 μl CO_2 l^{-1} and decreased when grown at 600 μl CO_2 l^{-1} (Cervigni et al., 1971). In Phaseolus, carbonic anhydrase activity decreased following growth at 1200 μl CO_2 l^{-1} (Porter and Grodzinski, 1984). Phosphoenolpyruvate carboxylase activity decreased when waterhyacinth was grown at 600 μl CO_2 l^{-1} (Spencer and Bowes, 1986), as did nitrate reductase in barley grown at 10,000 to 50,000 μl CO_2 l^{-1} (Fair et al., 1973). There was no difference in fructose 1, 6-biphosphatase activity in Nerium oleander grown at atmospheric and twice atmospheric CO_2 concentrations (Downton et al., 1980). Glycolate oxidase activity decreased when grown at high CO_2 in both Phaseolus (Porter and Grodzinski, 1984) and barley (Fair et al., 1973), but in tomato no well-defined response to CO_2 was apparent

(Hicklenton and Jolliffe, 1980a). Catalase activity was lower in barley grown at high CO_2 (Fair et al., 1973). There were no significant differences in sucrose phosphate synthase activity in soybeans grown at atmospheric or elevated CO_2 (Huber et al., 1984) or in soybean proteolytic enzyme activity (Havelka et al., 1984). Whether the differences in the activities of these enzymes from plants exposed to various CO_2 treatments are always significant is not clear. The physiological significance of the responses to CO_2 of all of these enzymes is not always evident.

There are reports of plant damage, sometimes extreme, as a result of growth at high concentrations of CO_2. Accumulation of starch in plants grown at 1000 μl CO_2 l^{-1} was found to cause chloroplast disruption (Cave et al., 1981). Chlorosis occurred in Phaseolus grown at 1400 μl CO_2 l^{-1} (Ehret and Jolliffe, 1985) and in tomato (Thomas and Hill, 1949). Thomas and Hill (1949) also reported the appearance of necrotic areas on tomato leaves at high CO_2. Brown and Escombe (1902) reported a variety of disorders in plants grown at 1100 μl CO_2 l^{-1}. These included loss of leaves, reduced number of flowers and lack of fruit formation. According to Ehret and Jolliffe (1985), it has been suggested that the injuries reported by Brown and Escombe (1902) may have been due to the impurities in the air in the enclosed greenhouse. While ethylene contamination of compressed CO_2 cylinders was demonstrated by Morrison and Gifford (1984), presumably most reports of plant injury are not the result of tainted air. There are numerous examples of plants exposed to high CO_2 with no injurious effects, including exposure for 14 days to CO_2 as high as 50,000 μl

CO_2 l^{-1} (Hicklenton and Jolliffe, 1980b), suggesting that exposure to high CO_2 per se is not damaging to all plants.

Whole canopy photosynthetic rate responses to CO_2 of canopies grown at atmospheric and elevated CO_2 have been reported for soybeans by Acock et al. (1985) and Jones et al. (1984a). In both cases soybeans were grown in outdoor sunlit chambers for an entire season. Both Acock et al. (1985) and Jones et al. (1984a) showed greater photosynthetic rates, at all levels of solar irradiance, in canopies grown at elevated CO_2 when compared to canopies grown at 330 μl CO_2 l^{-1}. Jones et al. (1984a) reported maximum canopy photosynthetic rates, measured at the respective growth CO_2 concentration and approximately 1900 μmol quanta m^{-2} s^{-1}, were 50% greater in the canopy grown at 800 μl CO_2 l^{-1} compared to the 330 μl CO_2 l^{-1} grown canopy.

In Chapter II a study was described in which soybeans were grown continuously from seed at atmospheric and twice atmospheric concentrations of CO_2 to investigate the effects on photosynthesis. In the study presented here, the range of growth CO_2 concentrations was expanded. Soybeans were grown at three subatmospheric, atmospheric, and two superatmospheric concentrations of CO_2. The objective of this study was to investigate the effects of long-term growth in various concentrations of CO_2, ranging from subatmospheric to superatmospheric levels, on soybean. Specific objectives were to determine the effects on the activity and kinetics of RuBPCase and on the levels of RuBP. In addition, the effects of CO_2 growth concentration on several plant characteristics and on canopy photosynthesis were investigated.

Materials and Methods

Plant Material and Growth Conditions

Soybeans (Glycine max L. Merr. cv Bragg) were planted in six outdoor environmentally controlled plant growth chambers (described in Chapter II) on 14 Sept. 1984. The CO_2 concentration was controlled to 160, 220, 280, 330, 660, or 990 μl CO_2 l^{-1} in each chamber from the date of planting until harvest. The chamber dry bulb and dewpoint temperatures were controlled to 31 and 16°C, respectively. The chambers received natural solar irradiation. The quantum flux density (400-700 nm) values reported here are measurements made at the upper canopy level (the chambers transmit 88% of the incoming solar radiation). These values were integrated over 5 min intervals from data collected every 20 s.

Photosynthetic rate measurements and collection of all plant material for analysis were made on 18 October (34 DAP). At this time the plants had not yet started reproductive development and had been thinned to a density of 30 plants per m^2. The canopies were at the V7 to V8 stage of development (Fehr and Caviness, 1977). Leaf tissue for biochemical analysis was collected and stored in liquid N_2 as previously described. For each canopy, leaf area index (LAI) was estimated from the measured leaf area of four plants harvested from each chamber on 18 October.

Canopy Photosynthesis Measurements

Measurements of net photosynthetic rate of whole canopies were made based on a whole chamber carbon mass balance which was corrected for leakage of CO_2 from the system (Jones et al., 1985b). The desired CO_2 concentration in a chamber was maintained by injecting pure CO_2 into the chamber to replace the CO_2 assimilated by the canopy. The CO_2 injections were based on light response algorithms determined for each canopy. The algorithms were updated as the canopies developed. Corrections for drift in this procedure were made every 5 min by making chamber CO_2 concentration measurements with an IR gas analyzer (Jones et al., 1984a).

Canopy photosynthetic rate response to light was measured as the solar irradiation varied throughout the day. Measurements were made every 5 min over a 10.5 h period (0750 to 1800 EST) which was cloud free. During this time period, irradiance at the upper canopy level varied from 145 μmol quanta m^{-2} s^{-1} in the morning, to a midday maximum of 1370 μmol quanta m^{-2} s^{-1}, to 15 μmol quanta m^{-2} s^{-1} in the evening.

RuBP Carboxylase Assay and RuBP Determination

The methods for sampling leaf tissue and for the assay of RuBPCase activity and the determination of RuBP levels were the same as those described in Chapter II. For the determination of the Michaelis constant, $K_m(CO_2)$, and V_{max} of RuBPCase, the assay procedures were modified and are described in the following section.

Determination of Apparent $K_m(CO_2)$ and V_{max}

Assays of RuBPCase activity were performed to determine the Michaelis constant, $K_m(CO_2)$, and V_{max}. The $K_m(CO_2)$ reported here is actually the apparent $K_m(CO_2)$ as assays were performed on a crude extract from the leaf rather than the purified enzyme. The assay procedures were similar to those described in Chapter II with some modification and are described as follows. A quantity of frozen leaf powder (70 to 150 mg dry weight) was removed from liquid N_2 storage and placed in a pre-chilled Ten Broeck tissue homogenizer. Added to the leaf powder was 5 ml of extraction buffer consisting of 100 mM Tris-HCl (pH 8.0), 5 mM DTT, 10 mM isoascorbate, 5 mM $MgCl_2$, and 1.5% (w/v) PVP-40. The leaf tissue was homogenized for approximately 60 s at 0°C. An aliquot of the homogenate was reserved for chlorophyll determination, and the remainder was centrifuged at 12,000 g for 3 min. Following centrifugation the supernatant was activated and used to initiate the assays (described later) and the pellet was discarded. The buffer media used in the assay consisted of 50 mM Tris-HCl, 5 mM DTT, 5 mM $MgCl_2$, and 10 mM isoascorbate. The media was prepared CO_2-free by purging at pH 3.1 for 15 min with N_2 then raising the pH to 8.0 with CO_2-free NaOH solution. To a 5-ml assay vial was added the CO_2-free buffer media, 0.5 mM RuBP, and 54 Wilbur-Anderson units of carbonic anhydrase (CA) (from bovine erythrocytes). The carbonic anhydrase was added to prevent depletion of CO_2 during the assay, particularly at the lower concentrations of HCO_3^- (Bird et al., 1980). The vials were capped and then purged with N_2 for 10 min. Through the cap septum $NaH^{14}CO_2$ (7.54 GBq/mol) was added in eight different final

concentrations ranging from 0.25 to 10 mM. The consumption of substrate $H^{14}CO_3^-$ was always less than 20% and usually less than 10% during each of the assays. The assays were initiated by the injection of activated supernatant from the homogenized crude extract. The supernatant was activated at 0°C for 45 min in 50 mM Tris (pH 8.0), 5 mM DTT, 10 mM isoascorbate, 5 mM $MgCl_2$, and 10 mM $NaH^{14}CO_3$. Following activation, the supernatant was kept at 0°C while the assays were being performed. The injection of 25 µl of activated supernatant carried over 0.25 mM $H^{14}CO_3^-$ into the assay vials and this quantity was taken into consideration when the final $H^{14}CO_3^-$ concentration calculations were made. Assays were performed in triplicate, at 30°C, with continuous shaking (125 strokes min^{-1}), in a total volume of 1 ml. The assays were terminated after 45 s with 6N formic acid in methanol. An aliquot (0.4 ml) of the assay mixture was then transferred to a 5-ml plastic scintillation vial which was placed under an air-stream until all remaining ^{14}C not fixed into acid-stable products was driven off. This required leaving the vials in the air-stream overnight. To the approximately 0.4 ml remaining in each vial was added 4 ml of scintillation cocktail. Acid-stable ^{14}C products were determined by liquid scintillation spectrometry.

Since CO_2 is the form of inorganic carbon used as a substrate by RuBPCase (Cooper et al., 1969), it was necessary to calculate the concentration of dissolved CO_2 in the assay mixture based on the added quantities of $H^{14}CO_3^-$. At the assay temperature of 30°C the solubility coefficient of CO_2 (α) in water is 0.665 ml ml^{-1} (Umbreit et al., 1972) and the pK' of carbonic acid is 6.327 (Harned and

Bonner, 1945). Using these values, the gas space volume above the liquid in the assay vial, and the Henderson-Hasselbach equation, the partitioning of inorganic carbon between dissolved CO_2, CO_2 in the gas space and bicarbonate was calculated (Ogren and Hunt, 1978). No corrections were made for the effect of ionic strength on α or pK'. While the effect of salts on α appears to be minor in the concentration range encountered in these assays (Umbreit et al., 1972) the effect on pK' is more substantial (Harned and Bonner, 1945). However, since all assays had essentially the same salt concentration the relative effects on the kinetic values are insignificant. The concentration of $H^{14}CO_3^-$ in each vial was corrected for the consumption of $H^{14}CO_3^-$ during the assay. This required the assumption that the velocity of the reaction catalyzed by RuBPCase was constant during the 45 s assay. The corrected substrate concentrations and the reaction velocities were used to calculate $K_m(CO_2)$ and V_{max} values using Lineweaver-Burke plots and the least squares method (Cleland, 1979). These kinetic values were also calculated using Eadie-Hofstee plots (data not shown) and were found to be very similar to the values presented here.

Estimation of Dissolved Free CO_2 at the Cell Wall

The dissolved free CO_2 at the cell wall of the mesophyll tissue was assumed to be in equilibrium with the CO_2 in the air in the leaf intercellular spaces. Data from Figure 2.1 (Chapter II) yields a value of 0.72 for the ratio of the concentrations of intercellular to ambient CO_2, Ci/Ca, for plants grown at 330 μl CO_2 l^{-1}. The Ci/Ca

ratio for plants grown at 660 μl CO_2 l^{-1} differed from 330 μl CO_2 l^{-1} grown plants by 23% (Figure 2.1). Since Ci/Ca ratios were not determined for all the growth CO_2 concentrations used in this study, and the exact nature of the relationship between the Ci/Ca ratio and growth CO_2 concentration is not known, the value for 330 μl CO_2 l^{-1} grown leaves, Ci/Ca = 0.72, was used for all calculations. Other assumptions included an atmospheric pressure of 760 mm Hg and a solubility coefficient, α, for CO_2 in water of 0.665 ml ml^{-1}. All calculations were based on a temperature of 30°C. The calculation of free CO_2 dissolved in the cell wall was by the method of Umbreit et al., 1972),

$$CO_2 = \frac{P * \alpha * Ci' * 1000}{760 * V * 100}, \qquad [3.1]$$

where CO_2 is in units of moles $liter^{-1}$ (M, molar concentration), the term P/760 converts atmospheric pressure to standard conditions, Ci' is the intercellular CO_2 concentration in percent (v/v), the term 1000/V converts α from ml ml^{-1} to moles $liter^{-1}$, and 100 converts percent CO_2 to pCO_2 (partial pressure of CO_2 in mm Hg).

Chlorophyll, Protein, and Specific Leaf Weight Determinations

The measurements of chlorophyll, total soluble leaf protein, and specific leaf weight (SLW) were made using the same methods described in Chapter II, with the exception that leaves for the SLW determination were collected from either nodes 5 and 6 or 6 and 7. In each canopy leaves from these nodes represented two of the most recently fully-expanded leaves in the upper canopy. All plant

material used for these measurements was collected on 18 Oct. 1984 (34 DAP).

Analysis of Statistical Significance

Simple linear and quadratic regression analyses were performed to determine the statistical significance (at the 5% level) of experimental results. In this chapter, the CO_2 concentration during growth was used as regressor. The methods used are described further in Chapter II. Regression parameters are tabulated in Appendix C.

Results

Response of Plant Characteristics to CO_2

Continuous exposure during growth of soybeans to a range of CO_2 from 160 to 990 μl CO_2 l^{-1} resulted in changes in leaf and canopy characteristics. There was a significant, almost linear increase in SLW with increasing CO_2 (Table 3.1). The plants grown at the highest CO_2 concentration had leaves with SLW 50% greater than those grown at the lowest concentration. Below atmospheric concentration of CO_2 (330 μl CO_2 l^{-1}) there was a minor response of SLW to CO_2. The greatest response occurred as CO_2 was increased from 330 to 990 μl CO_2 l^{-1}. The LAI increased two-fold as CO_2 increased from 160 to 990 μl CO_2 l^{-1} (Table 3.1). The LAI generally increased with increasing CO_2, showing a significant response to CO_2 concentration. The LAI values were similar for canopies grown at 160 and 220 μl CO_2 l^{-1}, and although higher, similar for canopies grown at 280, 330, and 660 μl CO_2 l^{-1}.

Table 3.1. Effect of growth CO_2 concentration on SLW, LAI, chlorophyll, and total leaf soluble protein. The SLW was calculated based on fully-expanded leaves collected from the upper canopy level. Canopy LAI was estimated from the total leaf area of four representative plants. All measurements were made on leaf samples collected 18 October (34 DAP) when plants were in the V7 to V8 vegetative stage.

Growth CO_2 Concentration	Specific Leaf Weight	Leaf Area Index	Chlorophyll	Total Soluble Protein
$\mu l\ CO_2\ l^{-1}$	g dry wt. m^{-2}	$m^2\ m^{-2}$	$g\ m^{-2}$	$g\ m^{-2}$
160	20.3 ± 2.1[a]	1.63 ± 0.11	0.204 ± 0.001	2.53 ± 0.01
220	20.9 ± 1.7	1.61 ± 0.03	0.261 ± 0.002	3.23 ± 0.02
280	21.4 ± 2.4	2.40 ± 0.42	0.248 ± 0.002	2.58 ± 0.02
330	21.4 ± 2.4	2.54 ± 0.20	0.214 ± 0.001	2.31 ± 0.01
660	26.6 ± 5.2	2.40 ± 0.42	0.205 ± 0.004	2.28 ± 0.04
990	30.5 ± 5.2	3.25 ± 0.33	0.234 ± 0.001	2.29 ± 0.01

[a]Mean values ± SD.

The canopy grown at the highest CO_2 concentration had an LAI at least 28% greater than each of the other canopies.

On a leaf area basis, the chlorophyll and leaf soluble protein levels showed similar responses to CO_2 (Table 3.1). The general trend was a decrease in value with increasing CO_2, but the response to CO_2 of both chlorophyll and soluble protein was not significant. Because of the variation in SLW, chlorophyll and soluble protein are also expressed on a dry weight basis in Table 3.2. When expressed on this basis, the response of chlorophyll and soluble protein to CO_2 is significant. On a dry weight basis the levels of both chlorophyll and soluble protein in the 330 μl CO_2 l^{-1} grown leaves were approximately midway between the highest and lowest values, found in the 220 and 990 μl CO_2 l^{-1} grown canopies, respectively. Soluble protein on a dry weight basis decreased 50% as CO_2 was increased from 220 to 990 μl CO_2 l^{-1}. While the direction of responses to CO_2 was similar for both chlorophyll and soluble protein, the magnitude of these responses varied. This is shown in Figure 3.1 where the protein/chlorophyll ratio is plotted against CO_2 concentration. The ratio is highest at low CO_2. The response to CO_2 of the protein/chlorophyll ratio was found to be significant.

Canopy Photosynthetic Rate

Canopy photosynthetic rate responses to sunlight for plants grown at each of the six CO_2 concentrations are shown in Figure 3.2. Data points represent measurements made at 5 min intervals as solar irradiance varied throughout the day. When canopy photosynthetic

Table 3.2. Effect of growth CO_2 concentration on chlorophyll and total leaf soluble protein expressed on a dry weight basis. Values are calculated from data in Table 3.1.

Growth CO_2 Concentration	Chlorophyll	Total Soluble Protein
$\mu l\ CO_2\ l^{-1}$	mg (g dry wt.)$^{-1}$	mg (g dry wt.)$^{-1}$
160	10.05 ± 0.05a	124.7 ± 0.6
220	12.49 ± 0.09	154.5 ± 1.1
280	11.59 ± 0.09	120.5 ± 0.9
330	10.00 ± 0.05	107.9 ± 0.5
660	7.71 ± 0.15	85.7 ± 1.7
990	7.67 ± 0.03	75.1 ± 0.3

[a]Mean values ± SD.

Fig. 3.1. The soluble protein/chlorophyll ratio versus growth CO_2 concentration. Data were calculated from the mean values in Table 3.1. Vertical lines through data points represent ± SD.

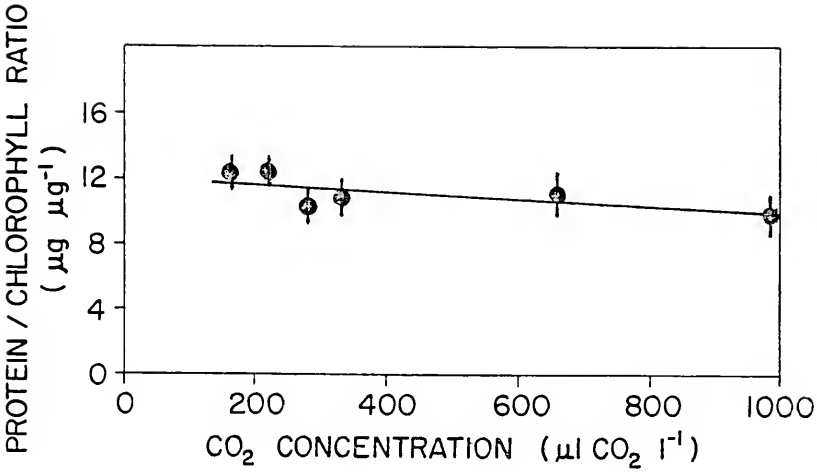
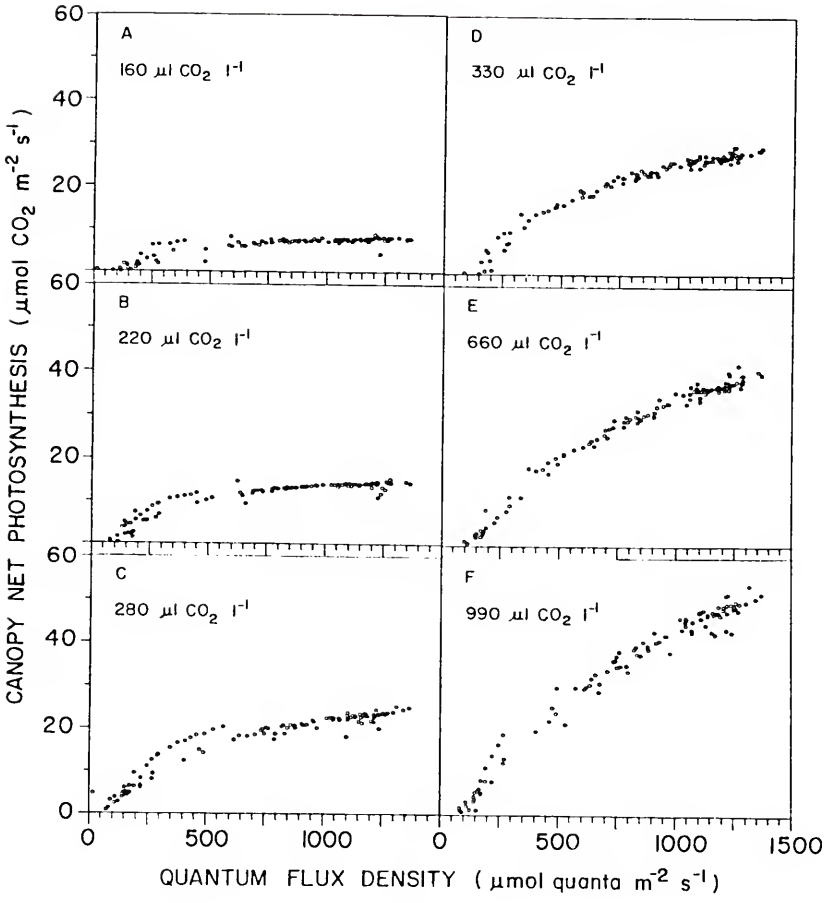

PROTEIN / CHLOROPHYLL RATIO
(µg µg⁻¹)
16
12
8
4
0
0
200
400
600
800
1000
CO2 CONCENTRATION (µl CO2 l⁻¹)

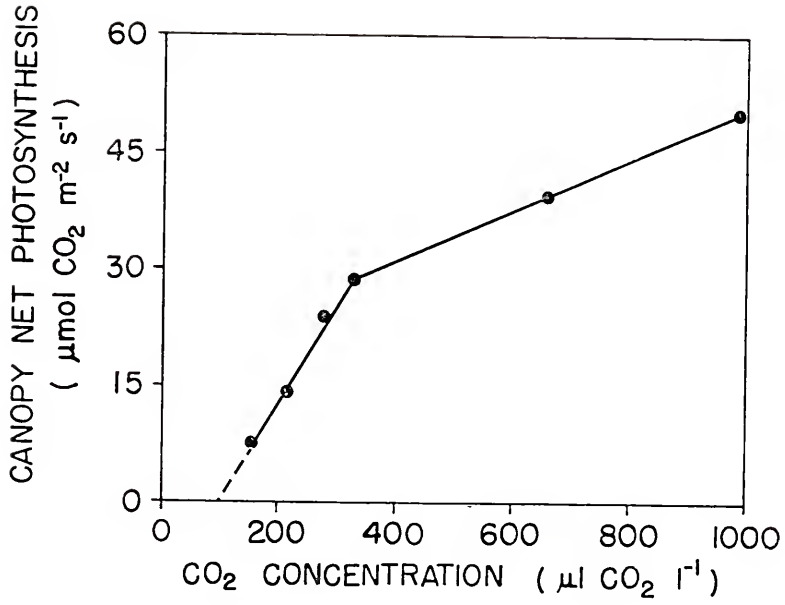
Fig. 3.2. A-F. Canopy net photosynthesis (on a land area basis) versus solar irradiance for canopies grown at 6 different CO_2 concentrations. A) 160 μl CO_2 l^{-1}, B) 220 μl CO_2 l^{-1}, C) 280 μl CO_2 l^{-1}, D) 330 μl CO_2 l^{-1}, E) 660 μl CO_2 l^{-1}, F) 990 μl CO_2 l^{-1}. Each data point represents a measurement made at a 5 min interval. Data were collected over a 10.5 h period (0750-1800 EST) on October 18 (34 DAP). Maximum solar irradiance occurred at midday when quantum flux density was approximately 1370 μmol quanta m^{-2} s^{-1}. Light levels are values for the upper canopy surface. Growth chambers transmit 88% of incoming solar irradiance. The canopy LAI's varied two-fold across the CO_2 concentration range.


A
160 µl CO2 l-1
D
330 µl CO2 l-1
B
220 µl CO2 l-1
E
660 µl CO2 l-1
C
280 µl CO2 l-1
F
990 µl CO2 l-1
CANOPY NET PHOTOSYNTHESIS (µmol CO2 m-2 s-1)
QUANTUM FLUX DENSITY (µmol quanta m-2 s-1)
0
20
40
60
500
1000
1500

rates were measured (at 34 DAP) canopies grown at 160 and 220 μl CO_2 l^{-1} were light saturated at light levels lower than 1000 μmol quanta m^{-2} s^{-1} (Figure 3.2 A and B). The canopy grown at 280 μl CO_2 l^{-1} (Figure 3.2 C) did not appear to light saturate at midday light levels of 1370 μmol quanta m^{-2} s^{-1}, but did not respond with increasing photosynthetic rates as high as the 330 μl CO_2 l^{-1} grown plants (Figure 3.2 D). The photosynthetic rate response increased continuously with increasing irradiance in plants grown at 330, 660, and 990 μl CO_2 l^{-1} (Figure 3.2 D, E, and F). At two and three times atmospheric CO_2 concentration the photosynthetic rate response to light was clearly still increasing, even at maximum midday irradiance, showing no indication of light saturation. Based on the visually estimated intercept of response curves in Figure 3.2, the canopy light compensation points did not appear to be strongly CO_2 dependent. Compensation points for each canopy were in the range of 50 to 150 μmol quanta m^{-2} s^{-1}.

The maximum photosynthetic rates of the canopies are plotted against growth CO_2 concentration in Figure 3.3. Each data point is the mean of between 7 to 10 measurements made at the growth CO_2 concentration at midday when irradiance inside the chambers was at its peak of 1250-1370 μmol quanta m^{-2} s^{-1}. The maximum rates were greater as the CO_2 concentration during growth increased. The slope of the response is steeper at the lower CO_2 concentrations. Because the total leaf area of a canopy varied by two-fold over the range of CO_2 concentrations, the canopy photosynthetic rates in Figures 3.2 and 3.3 are a reflection, in part, of the differences in LAI.

Fig. 3.3. Maximum canopy net photosynthetic rate versus growth CO_2 concentration. Photosynthesis is on a land area basis. Each data point is the mean of 7-10 measurements made at midday when the quantum flux density was 1250-1370 μmol quanta m^{-2} s^{-1}. Data are from Fig. 3.2. Vertical lines through data points represent ± SD.


CANOPY NET PHOTOSYNTHESIS
(μmol CO_2 m^{-2} s^{-1})
60
45
30
15
0
0
200
400
600
800
1000
CO_2 CONCENTRATION (μl CO_2 l^{-1})

RuBP Carboxylase Activity and RuBP Levels

The RuBPCase activity was assayed from fully-expanded leaves collected from the upper part of each canopy. The means of triplicate assays are plotted in Figure 3.4 (A). Both initial and total activities decreased significantly as the CO_2 concentration increased, with the highest activities occurring at the lowest CO_2. The initial activity decreased by 28% as CO_2 increased from 160 to 990 μl CO_2 l^{-1}, while the total activity decreased by 23% over the same CO_2 range. The activation of RuBPCase was calculated from data in Figure 3.4 (A) and was found to be quite high, particularly at low CO_2 [Figure 3.4 (B)]. Activation did show a significant but not a great response to CO_2, however, the highest activation (greater than 95%) occurred at the lower CO_2 concentrations. Above atmospheric concentrations of CO_2 there was not much activation response to CO_2. The initial and total RuBPCase activities tended to parallel each other regardless of CO_2 concentration.

The RuBP levels were determined in a subset of the same leaf samples used for RuBPCase assays. The means of triplicate assays are shown in Figure 3.5. The level of RuBP decreased significantly as CO_2 increased, however, at CO_2 concentrations greater than 660 μl CO_2 l^{-1} the measured levels of RuBP did not appear to respond strongly to CO_2. The RuBP at 660 μl CO_2 l^{-1} was only 30% of the level at 160 μl CO_2 l^{-1}. Assuming that RuBP is present only in the chloroplast (Heber, 1974), and that the stromal volume of the chloroplast is 25 μl mg Chl^{-1} (Sicher and Jensen, 1979), chloroplast concentrations of RuBP can be

Fig. 3.4. A. RuBPCase activity versus growth CO_2 concentration. Both initial (●) and total (○) activities are shown. Assays were performed at 30°C at pH 8.0 for 45 s. Data points are the means of triplicate assays. Plant samples were collected October 18 (34 DAP). B. RuBPCase activation versus growth CO_2 concentration. Percent activation calculated from data in A.

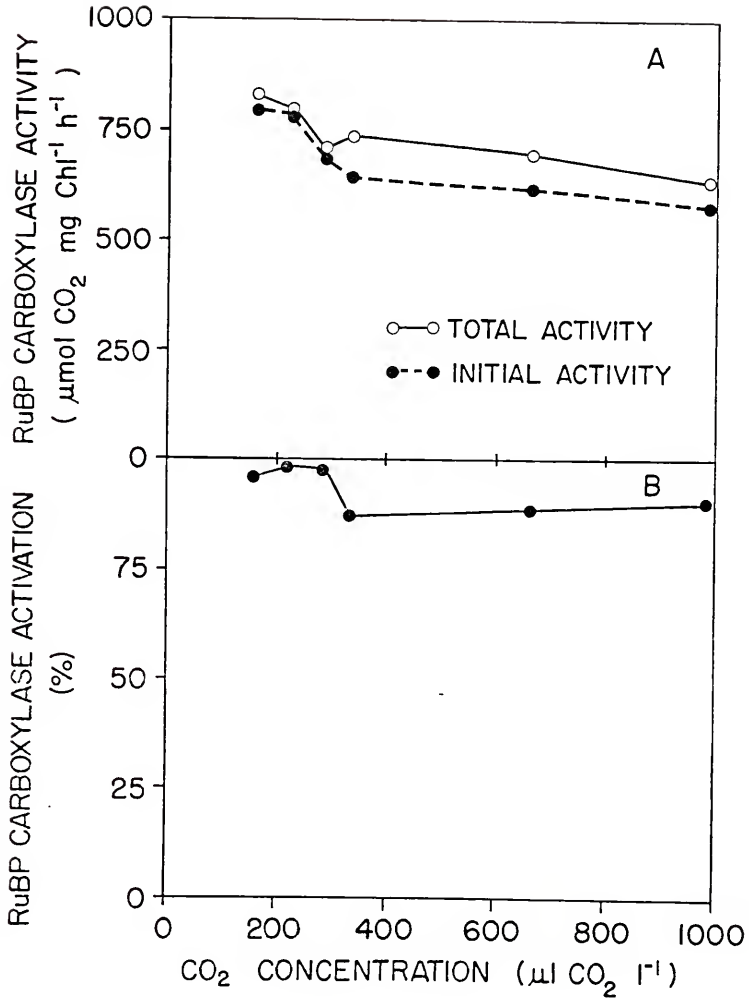
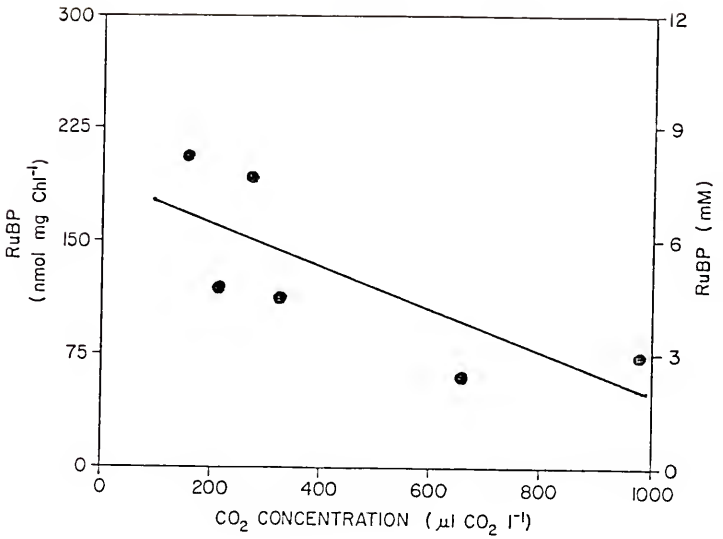

RuBP CARBOXYLASE ACTIVITY
(μmol CO_2 mg Chl^{-1} h^{-1})
1000
750
500
250
0
A
TOTAL ACTIVITY
INITIAL ACTIVITY
RuBP CARBOXYLASE ACTIVATION
(%)
75
50
25
0
B
0
200
400
600
800
1000
CO_2 CONCENTRATION (μl CO_2 l^{-1})

Fig. 3.5. Levels of RuBP versus growth CO_2 concentration. Chloroplast concentration of RuBP (mM) assumes 25 μl stroma volume mg chlorophyll^{-1}. Data points are means of triplicate assays. Leaf samples were subsamples of tissue used for RuBPCase assays in Fig. 3.4.


300
225
150
75
0
RuBP
(nmol mg Chl⁻¹)
12
9
6
3
0
RuBP (mM)
0
200
400
600
800
1000
CO2 CONCENTRATION (μl CO2 l⁻¹)

estimated. These values were determined for each CO_2 concentration and are also presented in Figure 3.5. The RuBP concentration ranged from 2.9 to 8.3 mM as the CO_2 concentration decreased from 990 to 160 $\mu l\ CO_2\ l^{-1}$.

Effects of CO_2 on $K_m(CO_2)$ and V_{max}

Assays of total RuBPCase activity were performed at various HCO_3^- concentrations to determine the Michaelis constant, $K_m(CO_2)$, and V_{max}. Total activity (Mg^{2+}/HCO_3^- activated) was assayed for determination of $K_m(CO_2)$ and V_{max} to separate activation from catalysis kinetics. The mean enzyme activity of triplicate assays are plotted against HCO_3^- concentration, for each growth CO_2 concentration, in Figures 3.6 to 3.10. The HCO_3^- concentrations have been corrected for consumption of the substrate during the assays. The assumption was made that the reaction rate was constant during the 45 s assay. The solid curve in each of the Figures 3.6 to 3.10 represents the predicted response of total RuBPCase activity to HCO_3^- based on the Michaelis-Menten equation,

$$v_c = \frac{V_{max} * [HCO_3^-]}{K_m(HCO_3^-) + [HCO_3^-]}, \qquad [3.2]$$

where v_c is the velocity of the carboxylation reaction (total RuBPCase activity), V_{max} is the theoretical maximum rate of reaction, $[HCO_3^-]$ is the concentration of the substrate, and $K_m(HCO_3^-)$ is the Michaelis constant for HCO_3^-. The kinetic parameters of equation [3.2] [$K_m(HCO_3^-)$ and V_{max}] were calculated from the data in Figures 3.6 to 3.10 by the least squares method of Cleland (1979). The inset in each

Fig. 3.6. RuBPCase activity versus HCO_3^- concentration in leaf tissue grown at 160 μl CO_2 l^{-1}. Assay vials were flushed with N_2 prior to assay. All reagents were prepared CO_2-free. Assays were performed at 30°C and pH 8.0 for 45 s in the presence of CA. The HCO_3^- concentrations are corrected for substrate consumption during assay. Data points are the means of triplicate assays. The solid curve is the predicted response based on Michaelis-Menten kinetics. The Km and Vmax were calculated from the data. Leaf samples were subsamples of tissue used for RuBPCase assays in Fig. 3.4 Inset. Double reciprocal plot of RuBPCase activity versus HCO_3^- concentration.

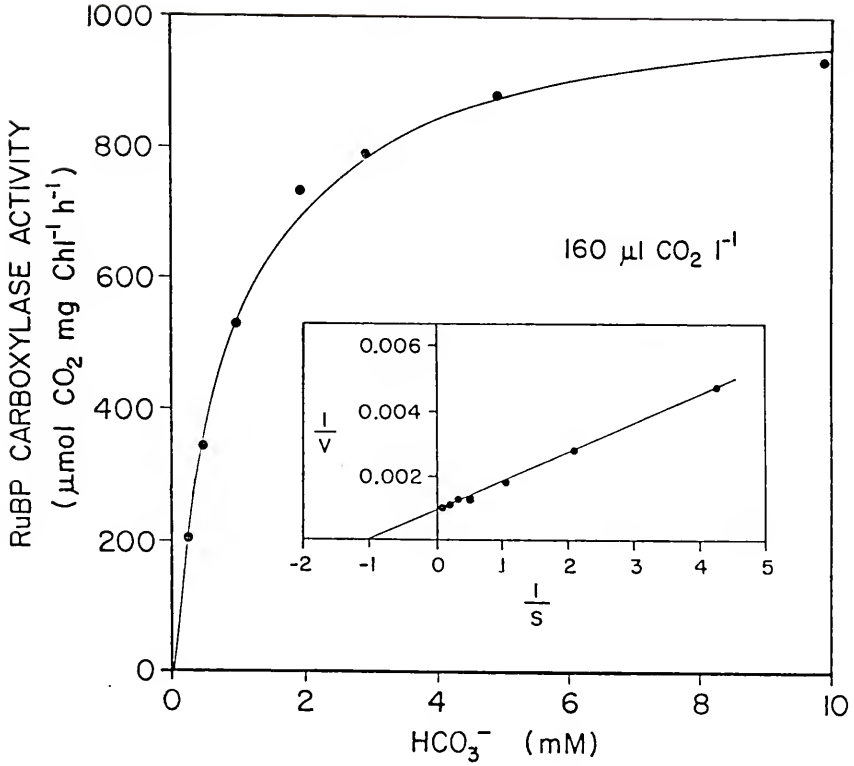

RuBP CARBOXYLASE ACTIVITY
(μmol CO_2 mg Chl^{-1} h^{-1})
1000
800
600
400
200
0
0
2
4
6
8
10
HCO_3^- (mM)
160 μl CO_2 l^{-1}
0.006
0.004
0.002
$\frac{1}{v}$
-2
-1
0
1
2
3
4
5
$\frac{1}{s}$

Fig. 3.7. RuBPCase activity versus HCO_3^- concentration in leaf tissue grown at 280 μl CO_2 l^{-1}. Assay vials were flushed with N_2 prior to assay. All reagents were prepared CO_2-free. Assays were performed at 30°C and pH 8.0 for 45 s in the presence of CA. The HCO_3^- concentrations are corrected for substrate consumption during assay. Data points are the means of triplicate assays. The solid curve is the predicted response based on Michaelis-Menten kinetics. The Km and Vmax were calculated from the data. Leaf samples were subsamples of tissue used for RuBPCase assays in Fig. 3.4 Inset. Double reciprocal plot of RuBPCase activity versus HCO_3^- concentration.

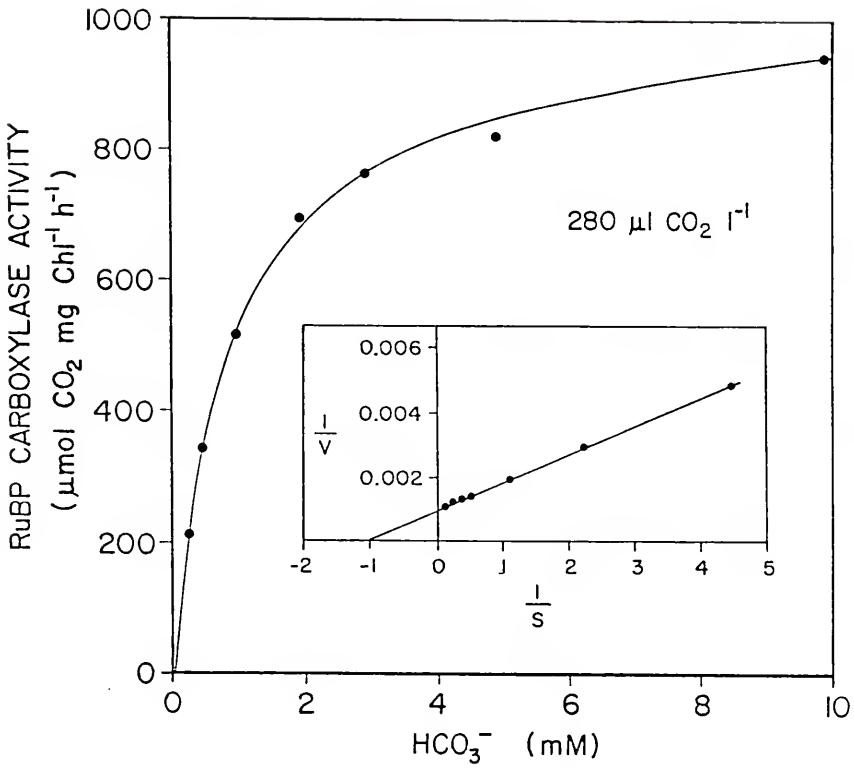

RuBP CARBOXYLASE ACTIVITY
(μmol CO_2 mg Chl^{-1} h^{-1})
1000
800
600
400
200
0
0
2
4
6
8
10
HCO_3^- (mM)
280 μl CO_2 l^{-1}
0.006
0.004
0.002
$\frac{1}{V}$
-2
-1
0
1
2
3
4
5
$\frac{1}{S}$

Fig. 3.8. RuBPCase activity versus HCO_3^- concentration in leaf tissue grown at 330 μl CO_2 l^{-1}. Assay vials were flushed with N_2 prior to assay. All reagents were prepared CO_2-free. Assays were performed at 30°C and pH 8.0 for 45 s in the presence of CA. The HCO_3^- concentrations are corrected for substrate consumption during assay. Data points are the means of triplicate assays. The solid curve is the predicted response based on Michaelis-Menten kinetics. The Km and Vmax were calculated from the data. Leaf samples were subsamples of tissue used for RuBPCase assays in Fig. 3.4 Inset. Double reciprocal plot of RuBPCase activity versus HCO_3^- concentration.

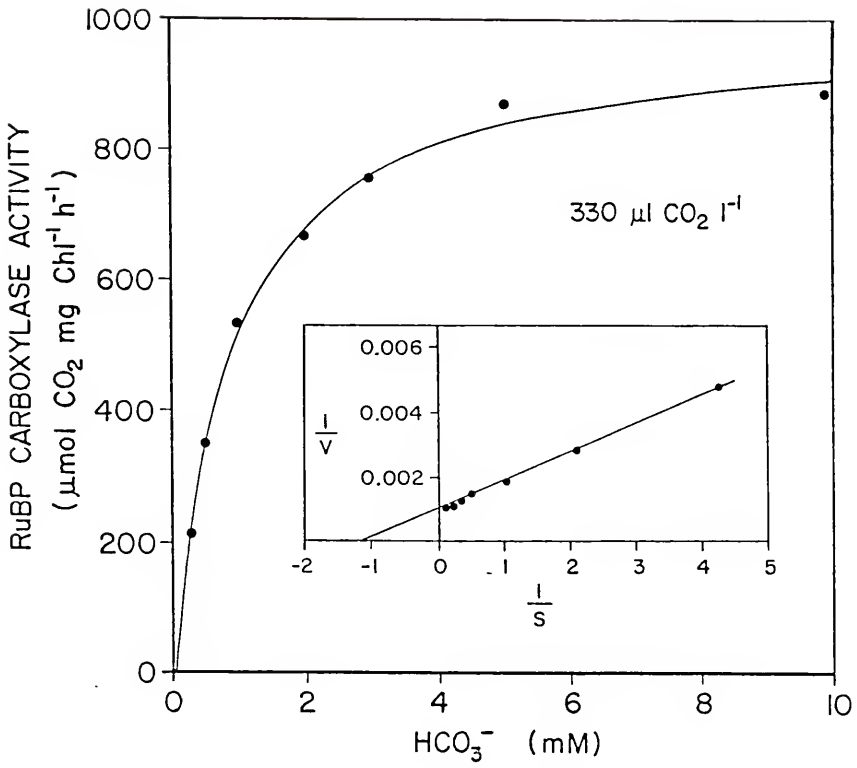

RuBP CARBOXYLASE ACTIVITY
(μmol CO_2 mg Chl^{-1} h^{-1})
1000
800
600
400
200
0
0
2
4
6
8
10
HCO_3^- (mM)
330 μl CO_2 l^{-1}
0.006
0.004
0.002
$\frac{1}{v}$
-2
-1
0
1
2
3
4
5
$\frac{1}{s}$

Fig. 3.9. RuBPCase activity versus HCO_3^- concentration in leaf tissue grown at 660 μl CO_2 l^{-1}. Assay vials were flushed with N_2 prior to assay. All reagents were prepared CO_2-free. Assays were performed at 30°C and pH 8.0 for 45 s in the presence of CA. The HCO_3^- concentrations are corrected for substrate consumption during assay. Data points are the means of triplicate assays. The solid curve is the predicted response based on Michaelis-Menten kinetics. The Km and Vmax were calculated from the data. Leaf samples were subsamples of tissue used for RuBPCase assays in Fig. 3.4 Inset. Double reciprocal plot of RuBPCase activity versus HCO_3^- concentration.

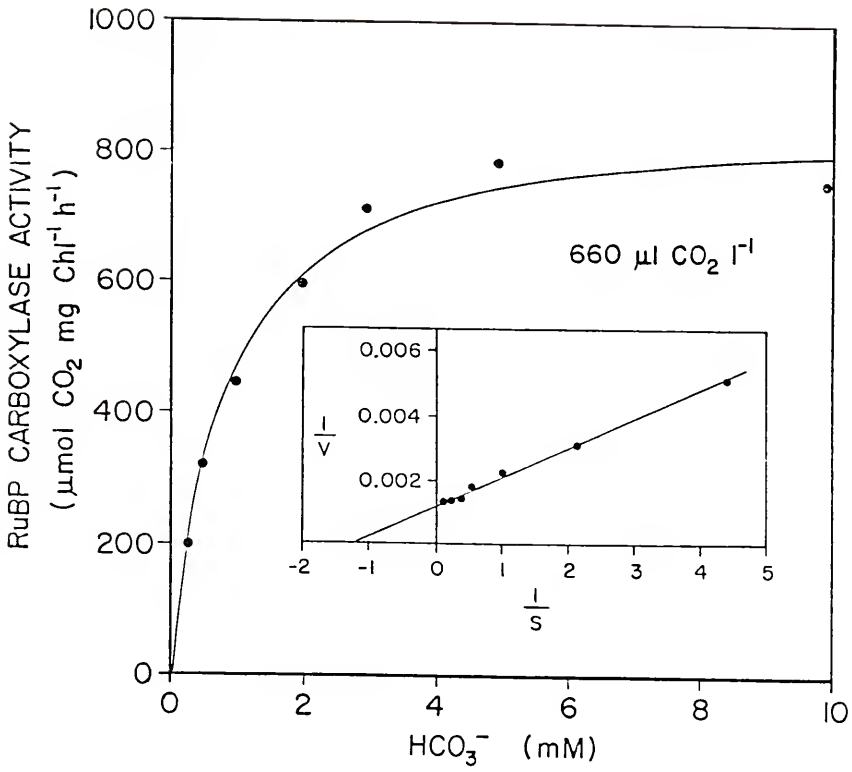

RuBP CARBOXYLASE ACTIVITY
(μmol CO_2 mg Chl^{-1} h^{-1})
1000
800
600
400
200
0
0
2
4
6
8
10
HCO_3^- (mM)
660 μl CO_2 l^{-1}
0.006
0.004
0.002
$\frac{1}{v}$
-2
-1
0
1
2
3
4
5
$\frac{1}{s}$

Fig. 3.10. RuBPCase activity versus HCO_3^- concentration in leaf tissue grown at 990 μl CO_2 l^{-1}. Assay vials were flushed with N_2 prior to assay. All reagents were prepared CO_2-free. Assays were performed at 30°C and pH 8.0 for 45 s in the presence of CA. The HCO_3^- concentrations are corrected for substrate consumption during assay. Data points are the means of triplicate assays. The solid curve is the predicted response based on Michaelis-Menten kinetics. The Km and Vmax were calculated from the data. Leaf samples were subsamples of tissue used for RuBPCase assays in Fig. 3.4 Inset. Double reciprocal plot of RuBPCase activity versus HCO_3^- concentration.

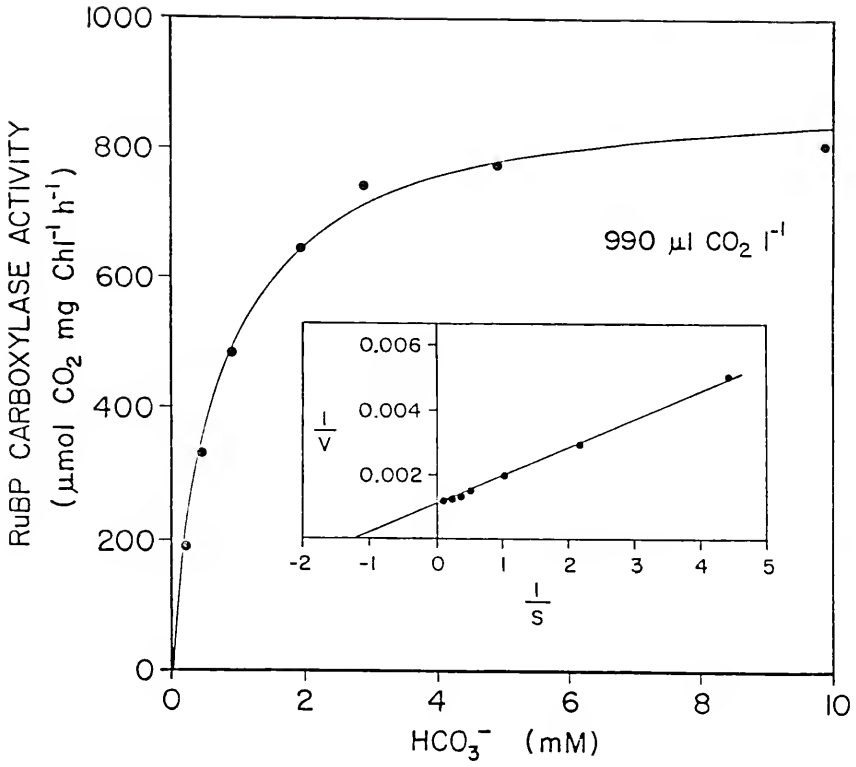

RuBP CARBOXYLASE ACTIVITY
(μmol CO2 mg Chl-1 h-1)
1000
800
600
400
200
0
0
2
4
6
8
10
HCO3- (mM)
990 μl CO2 l-1
0.006
0.004
0.002
1/V
-2
-1
0
1
2
3
4
5
1/S

figure is the double reciprocal plot of total RuBPCase activity versus HCO_3^-. The shape of the response curves are similar for each CO_2 growth treatment. The enzyme activity is generally higher at a given HCO_3^- concentration above 1 mM for leaves grown at lower CO_2 concentrations. The RuBPCase activity was similar in each CO_2 treatment at concentrations of HCO_3^- less than 1 mM. At higher concentrations of substrate the differences between response curves became greater.

The $K_m(CO_2)$ was calculated for each CO_2 treatment from the data in Figures 3.6 through 3.10. The $K_m(CO_2)$ demonstrated little response to CO_2. There was, however, a slight but significant decrease in $K_m(CO_2)$ with increasing CO_2 concentration. The $K_m(CO_2)$ declined by 13% as the growth CO_2 concentration increased six-fold from 160 to 990 μl CO_2 l^{-1} (Table 3.3). The V_{max} was highest in the lowest CO_2 grown leaves. In a response somewhat similar to $K_m(CO_2)$, V_{max} declined significantly by 12% as CO_2 increased from 160 to 990 μl CO_2 l^{-1} (Table 3.3).

CO_2 Concentration at the Cell Wall

The dissolved free CO_2 at the cell wall is an estimate of the CO_2 concentration in equilibrium with the CO_2 in the air in the leaf intercellular spaces for the particular experimental conditions. These values are proportional to the CO_2 concentration ambient to the leaf and are listed in Table 3.3. If the Ci/Ca ratio decreases with increasing CO_2 concentration during growth (as reported in Chapter II), the free CO_2 dissolved at the cell wall would be somewhat higher

Table 3.3. Effect of growth CO_2 concentration on apparent $K_m(CO_2)$, V_{max} and dissolved free CO_2 at the mesophyll cell wall. Apparent $K_m(CO_2)$ and V_{max} were calculated from data in Figs. 3.6-3.10. Cell wall dissolved CO_2 was calculated assuming equilibrium with the CO_2 in the intercellular air spaces in the leaf, a Ci/Ca ratio of 0.72, atmospheric pressure of 760 mm Hg, and 30°C.

Growth CO_2 Concentration	Apparent $K_m(CO_2)$	V_{max}	Dissolved CO_2 at Cell Wall
$\mu l\ CO_2\ l^{-1}$	μM	$\mu mol\ CO_2\ mgChl^{-1}\ h^{-1}$	μM
160	15.9 ± 0.6[a]	1026 ± 10	3.0
220	-	-	4.2
280	15.9 ± 1.2	998 ± 20	5.3
330	15.4 ± 1.2	928 ± 20	6.3
660	14.4 ± 1.2	858 ± 20	12.7
990	13.7 ± 0.8	896 ± 15	19.0

[a]Mean values ± SD.

at CO_2 concentrations below 330 μl CO_2 l^{-1} and somewhat lower at higher CO_2 concentrations.

Discussion

Both specific leaf weight and leaf area index increased with increasing CO_2 during growth. Since all CO_2 treatments had the same number of plants, the increase in LAI was due to a greater leaf area per plant. The increase in SLW with CO_2 may be a result of either an increase in leaf starch, leaf thickness, or in the density of leaf components. Since none of these were measured quantitatively it cannot be determined with certainty which was the causal factor. An increase in SLW with CO_2 in soybean has also been reported by Havelka et al. (1984) and Hofstra and Hesketh (1975). Greater starch content in the leaves exposed to elevated CO_2 was observed by Havelka et al. (1984) and Hofstra and Hesketh (1975). Except at very low CO_2, both soluble protein and chlorophyll decreased (on a dry weight basis) as CO_2 increased. The relative values changed with CO_2 resulting in a decrease in the protein/chlorophyll ratio at higher CO_2 concentrations. This implies that at low CO_2 (160 and 220 μl CO_2 l^{-1}) more protein relative to chlorophyll is produced. Downton et al. (1980) and Wong (1979) also reported lower ratios at elevated CO_2 growth concentrations. Since a large fraction of the total soluble protein of the leaf is dedicated to carbon assimilation and metabolism, while chlorophyll functions in harvesting and transferring light energy, it is not surprising that the concentration of CO_2 during growth affects soluble protein levels more than chlorophyll

levels. Whether the changes in SLW and chlorophyll are of major importance to the photosynthetic rate responses of these plants is not clear, as Bjorkman (1981) has shown poor correlations between light saturated photosynthesis (at air-CO_2 levels) and both SLW and chlorophyll content.

The canopy photosynthetic rate response to increasing solar irradiance (Figure 3.2) indicates that 160 and 220 μl CO_2 l^{-1} grown canopies were light saturated at a relatively low irradiance. The 330 μl CO_2 l^{-1} canopy, and to a greater extent the 660 and 990 μl CO_2 l^{-1} canopies, showed continuously increasing photosynthetic rates as irradiance increased with no light saturation observed. Both Acock et al. (1984) and Jones et al. (1984a) reported results with soybeans that showed canopies grown at elevated CO_2 had greater photosynthetic rates at all light levels. When comparing the photosynthetic rates of the canopies grown at different CO_2 concentrations, it is necessary to consider the differences in LAI (Table 3.1). The photosynthetic rate measurements were made when the plants were 34 days old and the LAI differed two-fold over the range of growth CO_2 concentrations. The lack of complete canopy closure at the lower growth CO_2 concentrations resulted in canopy light saturation at relatively low light levels and also reduced total canopy photosynthetic rates. Increasing the CO_2 concentration leads to less CO_2 limitation to photosynthesis. Thus at high CO_2, light will become even more of a limiting factor. The maximum photosynthetic rates at high light increased with increasing CO_2, however, these rates must be viewed with the respective LAI in mind, as Jeffers and Shibles (1969) reported that canopy

photosynthetic rates were greater and less likely to be light saturated as LAI increased.

The RuBP level, as well as the initial and total activity of RuBPCase, decreased as CO_2 increased. With regard to RuBP this is probably a result of increased consumption of the substrate at high photosynthetic rates associated with high concentrations of CO_2. In experiments where plants were exposed for only short periods of time to a range of CO_2 concentrations, Mott et al. (1984) and Badger et al. (1984) also found the same pattern of response of the RuBP levels. If the concentration of active sites of RuBPCase is 3-4 mM (Jensen and Bahr, 1977), then based on Figure 3.5 RuBP may be nearing limiting concentrations for carboxylation at 660 and 990 μl CO_2 l^{-1}. The reduction in RuBPCase activity is likely due in part to reduced quantities of the enzyme at higher CO_2 concentrations. Since RuBPCase may represent as much as 65% of the total leaf soluble protein (Ellis, 1979), a reduction in total soluble protein may also imply a reduction as well in the quantity of RuBPCase. Except at the lower CO_2 concentrations, the decrease in RuBPCase activity with increasing CO_2 was not great. The difference between the 330 and 990 μl CO_2 l^{-1} initial and total activities were 10 and 14%, respectively. Observations of reduced RuBPCase activity at elevated CO_2 growth conditions have been reported by others (Spencer and Bowes, 1986; Porter and Grodzinski, 1984; von Caemmerer and Farquhar, 1984; Vu et al., 1983; Downton et al., 1980; Wong, 1979). When assayed in vitro, RuBPCase reflected a higher activation status in plants grown at lower CO_2. Other than the drop in activation between 280 and 330 μl CO_2

μl^{-1}, activation was mostly unaffected by growth CO_2 concentration (Figure 3.4-B). These results do not support suggestions that increasing CO_2 concentrations might result in increased RuBPCase activation (Tolbert, 1984) or increased RuBPCase activity (Hesketh et al., 1983).

The growth concentration of CO_2 had a small effect on $K_m(CO_2)$, causing the value to decrease 11% at 990 as compared to 330 μl CO_2 l^{-1}. The $K_m(CO_2)$ values in Table 3.3 are similar to the value of Vu et al. (1986) but only about half of the value of Laing et al. (1974). It has been reported previously that cotton grown at 330 and 640 μl CO_2 l^{-1} had similar $K_m(CO_2)$ values (Yeoh et al., 1981), also implying a minimal effect of growth CO_2 concentration on $K_m(CO_2)$.

The $K_m(CO_2)$ values in Table 3.3 were determined in an atmosphere of nitrogen thus eliminating the competitive oxygenase reaction. Determining $K_m(CO_2)$ in air would raise the value over those determined in nitrogen. This has been demonstrated in a variety of C_3 species (Bird et al., 1982). If $K_m(CO_2)$ values were determined in atmospheres representative of the growth CO_2 concentrations, presumably there would be a greater difference between the high and low CO_2 grown plants due to the reduced oxygen inhibition at high CO_2. In C_4 plants RuBPCase is located in the bundle sheath cells (Hatch, 1976) where the CO_2 concentration is high due to the CO_2 concentrating nature of the pathway. The $K_m(CO_2)$ of RuBPCase of C_4 plants, determined under nitrogen, is higher than for C_3 plants (Yeoh et al., 1980, 1981). Yeoh et al. (1981) have reported mean values of 19 and 31 μM CO_2 for a

variety of C_3 and C_4 plants, respectively. Hence, the data in Table 3.3 and of Yeoh et al. (1980, 1981) indicate that exposure to high CO_2 during growth is in itself not the cause of high $K_m(CO_2)$ values. In addition to higher $K_m(CO_2)$, C_4 plants have RuBPCase that has a higher turnover number (K_{cat}) (Seemann et al., 1984). The K_{cat} is a measure of the maximum number of substrate molecules converted to product per unit time per enzyme active site (Fersht, 1985). The high K_{cat} compensates in part for the high $K_m(CO_2)$. Comparison of the $K_m(CO_2)$ of C_4 plants (Yeoh et al., 1980, 1981) and the estimated CO_2 concentration in the bundle sheath cells of 60 μM (Hatch and Osmond, 1976) shows the CO_2 concentration to be greater than the $K_m(CO_2)$. The situation is reversed in C_3 plants. While it is difficult to measure the free CO_2 concentration in the chloroplast stroma of a C_3 photosynthetic cell, estimates for plants exposed to air of 4 μM (Hesketh et al., 1983), 5-6 μM (Hatch and Osmond, 1976), and 8 μM (Raven and Glidewell, 1981) have been reported. Assuming a value of 5-6 μM CO_2, the $K_m(CO_2)$ of 15.4 μM for 330 μl CO_2 l^{-1} grown plants (Table 3.3) is 2.5 to 3 times higher than the substrate concentrations. The estimates of free CO_2 dissolved at the mesophyll cell walls in Table 3.3 assumes an equilibrium with the intercellular CO_2 concentration which was calculated based on the CO_2 concentration ambient to the leaf. At a CO_2 concentration of 330 μl CO_2 l^{-1}, the estimated dissolved CO_2 concentration at the cell wall is 6.3 μM. This is only slightly greater than the assumed stromal concentration of 5-6 μM. That the CO_2 concentrations are similar in the stroma and the cell wall has been predicted by several researchers (Colman and

Espie, 1985; Farquhar and von Caemmerer, 1982; Werden et al., 1972). It is recognized here that diffusion of CO_2 through an aqueous phase, such as the cytosol, is approximately 10^4 times slower than through air (Nobel, 1970) and this may represent a significant factor in CO_2 flux through the cell. If, in fact, the stromal CO_2 concentration is only marginally lower than the cell wall dissolved CO_2, from data in Table 3.3 it can be shown that only at an ambient CO_2 concentration as high as 990 μl CO_2 l^{-1} will the CO_2 concentration in the vicinity of RuBPCase be greater than the $K_m(CO_2)$. If the $K_m(CO_2)$ values in Table 3.3 had been determined in the presence of atmospheric levels of oxygen, more appropriately comparable to a field situation, the higher $K_m(CO_2)$ values would have required an ambient CO_2 concentration greater than 990 μl CO_2 l^{-1} to supply a stromal CO_2 concentration equivalent to or greater than the $K_m(CO_2)$.

An enzyme with a high K_{cat} will, at a given substrate concentration, have a higher velocity and a higher V_{max} than an enzyme with a lower K_{cat}. The C_3 RuBPCase has a lower $K_m(CO_2)$ (Yeoh et al., 1980, 1981), a lower K_{cat} (Seemann et al., 1984) and thus a lower V_{max} than the C_4 RuBPCase. At atmospheric CO_2 concentrations the stromal CO_2 concentration is less than the $K_m(CO_2)$ in C_3 plants but the bundle sheath CO_2 concentration is higher than the $K_m(CO_2)$ in C_4 plants. It would appear then, that based on the physiology and anatomy of C_3 plants their photosynthetic rate response to increasing CO_2 should be greater than in C_4 plants, and this is what has been observed (Pearcy and Bjorkman, 1983; Downton et al., 1980; Osmond et al., 1980). Seemann et al. (1984) have suggested that the C_4 RuBPCase with its

greater K_{cat} would be more favorably equipped to respond to increasing CO_2. Based on kinetic properties alone this is true. But due to the C_4 pathway, at current atmospheric CO_2 levels bundle sheath CO_2 concentrations are already greater (possibly by a factor of two) than the $K_m(CO_2)$, implying that further CO_2 increases will result in minimal photosynthetic rate increases. Without the anatomy and physiology of the C_4 pathway, the higher K_{cat} of the C_4 RuBPCase would result in larger relative increases in photosynthetic rates with increasing CO_2 compared to the C_3 enzyme with its lower K_{cat}.

Estimation of the actual CO_2 concentration in the stroma is made difficult by a number of unresolved questions. These include the form of inorganic carbon taken up by both the cell and chloroplast and the location of carbonic anhydrase within the cell. The species of inorganic carbon that crosses the plasmalemma and the chloroplast envelope is generally believed to be CO_2 (Colman and Espie, 1985; Tsuzuki et al., 1985; Espie and Colman, 1982; Werden et al., 1972). These cell membranes are apparently quite permeable to CO_2, the permeability coefficients being in the same range as that of water (Nobel, 1974; Blank and Roughton, 1960). In experiments with isolated C_3 mesophyll cells, Espie and Colman (1982) found the CO_2 supply, without transport of HCO_3^- across the plasmalemma, could support rates of photosynthesis 5 to 8 times higher than the rates observed. However, there are other reports suggesting HCO_3^- crosses the plasmalemma via a transfer mechanism (Volokita et al., 1981) and that HCO_3^- is also the species that crosses the chloroplast envelope

(Poincelot, 1974) possibly with the involvement of ATP (Champigny and Bismuth, 1977).

There is little evidence supporting the presence of a CO_2 concentrating mechanism in C_3 plants and it is usually assumed not to exist (Espie and Colman, 1982; Werden et al., 1972). There have been, however, reports suggesting the mechanism may occur in C_3 species. Recently Lehnherr et al. (1985) have suggested the presence of a CO_2 concentrating mechanism in white clover. Based on discrepancies between gas exchange measurements of high (1000 μl CO_2 l^{-1}) and low (200 μl CO_2 l^{-1}) CO_2 grown plants and enzyme kinetics the concentrating mechanism was predicted. In the plants grown at the lower CO_2 concentration the mechanism was stated to be more efficient. It appears from their data that all plants had approximately the same CO_2 compensation point regardless of growth CO_2 concentration. This seems unlikely if the 200 μl CO_2 l^{-1} grown plants had a more efficient CO_2 concentrating mechanism. A method for determining the presence of an intervening cellular HCO_3^- transport mechanism calls for the evaluation of the ratio of the $K_m(CO_2)$ of RuBPCase and the $K_{1/2}$ (CO_2) for whole cell photosynthesis. If the ratio $K_m(CO_2)/K_{1/2}$ (CO_2) is close to unity the kinetics of RuBPCase will be the major determinant in the establishment of the photosynthetic cell's affinity for CO_2. Whole cell photosynthetic studies were not performed in this study, however, the soybean whole mesophyll cell photosynthetic data of Servaites and Ogren (1977) was collected at 2% oxygen and is applicable here. Correcting the $K_{1/2}$ (CO_2) of Servaites and Ogren from 25°C to 30°C yields a value of 17.2 μM. The $K_m(CO_2)$ of RuBPCase

from soybeans grown at 330 μl CO_2 l^{-1} is 15.4 μM (Table 3.3). The ratio of 0.89 strongly implies the lack of a HCO_3^- transport mechanism in soybean.

The intracellular location of CA is not clear. Carbonic anhydrase catalyses the hydration and dehydration of CO_2 according to equation 3.3 (Umbreit et al., 1972),

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-, \qquad [3.3]$$

the pH determining where equilibrium is established. There appears to be little doubt that CA is located in the chloroplast (Colman and Espie, 1985; Tsuzuki et al., 1985; Werden et al., 1972). Some evidence suggests CA is also located in the cytosol (Colman and Espie, 1985; Reed, 1979). The location of CA is important in calculation of CO_2 movement within photosynthetic cells. In addition to dissolved free CO_2 and HCO_3^- ions, other sources of inorganic carbon in the chloroplast include that bound to the thylakoid membranes (Vermaas and Govindjee, 1982) and to a number of proteins (Colman and Espie, 1985; Yokota et al., 1983). These sources of inorganic carbon contribute to the overall chloroplast pool but they are presumably not free CO_2 available for assimilation by RuBPCase.

Within the range of growth CO_2 concentrations used in this study, it has been shown that increasing CO_2 decreases chlorophyll and soluble protein (both on a dry weight basis), the protein/chlorophyll ratio, and steady state levels of RuBP within the chloroplast. The RuBP levels at high CO_2 concentrations were approximately equal to the active site concentration of RuBPCase. The activation, activity, V_{max}, and $K_m(CO_2)$ of RuBPCase assayed <u>in vitro</u> all exhibited a minor

decrease as CO_2 increased. At high CO_2 concentrations the photosynthetic rate did not appear to be limited by RuBPCase, as increasing CO_2 reduced RuBPCase activity while photosynthetic rates increased.

CHAPTER IV
EFFECTS OF TEMPERATURE ON PHOTOSYNTHESIS AND RuBP CARBOXYLASE AT TWO GROWTH CO_2 CONCENTRATIONS

Introduction

Temperature has the potential for being a critical environmental factor in the regulation of photosynthesis. Temperature effects on photosynthesis in most plants are usually reversible over a range of approximately 10 to 35°C (Berry and Bjorkman, 1980). However, too high a temperature can damage photosystem II while too low a temperature can cause phase separation in cell membranes (Berry and Downton, 1982). Growth of a plant at a particular temperature can lead to photosynthetic acclimation and enhanced performance under that temperature regime (Berry and Bjorkman, 1980).

Increasing temperature can lead to higher photosynthetic rates by allowing some reactions to proceed at greater rates. If the temperature becomes high enough (yet still below the temperature at which heat damage will occur) the increase in temperature will result in a decline in photosynthesis that is not due to heat damage to the plant. This decline is related to the oxygen inhibition of photosynthesis. Oxygen is competitive with respect to CO_2 in C_3 plants (see Chapter I). As temperature increases so do the effects of oxygen inhibition. If the temperature becomes high enough, inhibition will result in a decrease in photosynthesis. Increasing the CO_2 concentration can overcome these oxygen effects (Osmond et al., 1980;

Bjorkman et al., 1978). The major cause of the increased oxygen inhibition at higher temperatures is the change in kinetic parameters of Rubisco (Jordan and Ogren, 1984). The $K_m(CO_2)$ increases with temperature (Jordan and Ogren, 1984; Monson et al., 1982; Badger and Collatz, 1977; Laing et al., 1974) as does V_{max} for both carboxylase and oxygenase activity (Jordan and Ogren, 1984). The $K_m(O_2)$ for oxygenase activity is not strongly affected by temperature (Jordan and Ogren, 1984; Laing et al., 1974). The overall effect of these kinetic changes is an increase in photorespiration relative to photosynthesis at higher temperatures. As a result, the CO_2 compensation point also increases with temperature (Jordan and Ogren, 1984; Azcon-Bieto et al., 1981; Laing et al., 1974). Increasing the CO_2 concentration can compensate for the changes in kinetics, thus increasing the optimal temperature for photosynthesis (Osmond et al., 1980). In addition to the effects on Rubisco kinetics, increasing temperature also increases the solubility of O_2 relative to CO_2 (Ku and Edwards, 1977). Jordan and Ogren (1984) have shown that two-thirds of the temperature dependence of the photorespiration/photosynthesis ratio is due to enzyme kinetic changes and one-third is due to the change in gas solubilities.

The optimal air temperature for soybean canopy photosynthesis is 25 to 30°C (Jeffers and Shibles, 1969). Canopy photosynthetic rates are not strongly dependent on air temperature in this range or at air temperatures up to about 5°C higher (Jones et al., 1985a; Jeffers and Shibles, 1969). Individual leaf photosynthetic rates show a greater response to temperature. As the leaf temperature was increased over

the range of 25 to 35°C leaf photosynthetic rates decreased in a number of C_3 species (Jurik et al., 1984; Sionit et al., 1984; Monson et al., 1982; Enoch and Hurd, 1977). At CO_2 concentrations as high as 700 μl CO_2 l^{-1} leaf photosynthetic rates still declined in the 25 to 35°C range (Jurik et al., 1984; Sionit et al., 1984; Enoch and Hurd, 1977). However, at a CO_2 concentration of 1910 to 1960 μl CO_2 l^{-1} leaf photosynthetic rates of bigtooth aspen increased as leaf temperature was increased to 35°C but then decreased at higher temperatures (Jurik et al., 1984). This implies that leaf temperatures up to 35°C were increasing photorespiration relative to photosynthesis but not causing heat damage to those plants. In studies with soybean, Hofstra and Hesketh (1969) reported an increase in leaf photosynthetic rates as leaf temperature increased from 20 to 40°C. Gourdon and Planchon (1982), working with two cultivars, observed either no effect or a decrease in maximum leaf photosynthetic rates as air temperature was increased from 25 to 30°C.

In addition to the above mentioned response of enzyme kinetic parameters, temperature may have other effects on RuBPCase. Growth at low temperatures (2 to 4°C during cold hardening of rye plants) for a few weeks resulted in an RuBPCase that had an increased activity and a greater specific activity when compared to plants grown at 25°C (Huner and Macdowall, 1979). These changes were found to be related to a conformational change in the enzyme (Huner and Macdowall, 1978). Growth temperature, unlike the assay temperature, did not affect RuBPCase kinetics in Nerium oleander (Bjorkman et al., 1978). It may, however, be responsible for changes in the quantity of RuBPCase in N.

oleander (Osmond et al., 1980). In several experiments where plants were grown at different temperatures ranging from 20 to 45°C (and assayed at a common temperature) the activity of the enzyme was found to be little affected in cotton (Downton and Slayter, 1972) but decreased as the growth temperature of N. oleander and the C_4 Atriplex lentiformis increased (Bjorkman et al., 1978; Pearcy, 1977).

There are little data concerning the effect of growth temperature on levels of RuBP. Berry and Downton (1982) cited work by J. Collatz showing decreased levels of RuBP at temperatures above the photosynthesis temperature optimum. According to Berry and Bjorkman (1980), a substantial decline in the rate of electron transport at higher temperatures could result in a reduction in photophosphorylation or NADPH production leading to the lower levels of RuBP.

The objectives of this study were to determine the effects of air temperature during growth on the activity of RuBPCase and the level of RuBP in soybean grown at atmospheric and twice atmospheric concentrations of CO_2. Air temperature effects on canopy photosynthesis were also investigated.

Materials and Methods

Plant Material and Growth Conditions

Soybeans (Glycine max L. Merr. cv Bragg) were planted in six outdoor environmentally controlled plant growth chambers (described in Chapter II) on 8 Sept. 1985. Three of the chambers had atmospheric CO_2 concentrations controlled (from seed to harvest) to 330 μl CO_2 l^{-1},

the remaining three chambers were controlled to 660 μl CO_2 l^{-1}. Three day/night air temperature regimes were maintained at each CO_2 treatment. The regimes were 26/19°, 31/24°, and 36/29°C and are referred to by the daytime temperatures throughout this chapter. The dew point temperatures maintained in the chambers were 12, 16, and 20°C for the 26, 31, and 36°C air temperature regimes. As in previous experiments (Chapters II and III) the chambers received natural solar irradiation. Leaf tissue was collected at midday, on November 4 (57 DAP), for RuBPCase assays and RuBP determinations. This sampling day was cloud-free. The plant density was 32 plants m^{-2}. Estimates of the canopy LAI's for plants grown at 330 μl CO_2 l^{-1} were 4.13, 4.89, and 5.62 and for 660 μl CO_2 l^{-1} grown plants 5.52, 7.92, and 7.94 for growth temperatures of 26, 31, and 36°C, respectively. The plant sampling procedures described in Chapter II were followed.

Canopy Leaf Temperature and Vapor Pressure Deficit

Leaf temperatures within the upper canopies were measured using an IR temperature transducer (Everest Interscience, series 4000) mounted inside the plant growth chambers. Temperature measurements from three days, November 9, 10, and 12 (62, 63, and 65 DAP), were used to calculate leaf temperature response to air temperature and CO_2 concentration.

The vapor pressure deficit (VPD) was calculated using the dry bulb and dew point temperatures of the air inside the growth chambers, according to Murray (1967). The VPD calculations were performed using data collected during the same time periods in which leaf temperature

measurements were made. The plants were non-water-stressed during these measurements.

Canopy Photosynthesis Measurements

Canopy net photosynthesis was measured as described in Chapter III. Measurements were made on November 4 (57 DAP), between the hours of 1100 and 1230 EST when quantum flux density (400 to 700 nm) at the upper canopy level was 1250 to 1350 μmol quanta m^{-2} s^{-1}. The reported photosynthetic rates are the means of six measurements at each treatment. Canopy net photosynthesis is expressed on a land area basis.

RuBP Carboxylase Assay

The assay procedure for RuBPCase activity was similar to the previously described methods with some modifications. A quantity of frozen leaf powder (70 to 150 mg dry weight) was removed from liquid N_2 storage and placed in a pre-chilled Ten Broeck tissue homogenizer. Added to the leaf powder was 5 ml of extraction buffer consisting of 100 mM Tris-HCl (pH 8.0), 5 mM DTT, 10 mM isoascorbate, and 1.5% (w/v) PVP-40. The leaf tissue was homogenized for approximately 60 s at 0°C. An aliquot of the homogenate was reserved for chlorophyll determination and the remainder was centrifuged at 12,000 g for 3 min. The supernatant of the crude extract was either used immediately to initiate the initial RuBPCase assays or was activated (as described later) and then used to initiate the total RuBPCase assays. Assays were performed in triplicate at 26, 31, or 36°C in a waterbath with

continuous shaking (125 strokes min^{-1}). The assay buffer consisted of 50 mM Tris-HCl (pH 8.0), 5 mM DTT, 5 mM $MgCl_2$, and 10 mM isoascorbate. To the assay buffer was added 0.5 mM RuBP. The 5-ml glass vials were sealed with screw-on septum caps and through the septum was added 20 mM $NaH^{14}CO_2$ (7.54 GBq/mol). Initial RuBPCase assays were initiated with the injection of 50 µl of crude extract. For total RuBPCase activity assays 50 µl of crude extract was injected into the assay vials minus RuBP. The enzyme was allowed to activate for 5 min at the assay temperature. Following activation the assay was initiated with the injection of RuBP. For all assays, the total assay volume was 5 ml. Assays were terminated after 45 s with the injection of 0.1 ml of 6 N formic acid in methanol. Determination of acid-stable ^{14}C products was as described in Chapter III.

RuBP Determination

The assay procedure for RuBP was similar to that described in Chapter II with some minor modifications. A quantity of frozen leaf powder (70 to 150 mg dry weight) was removed from liquid N_2 storage and placed in a pre-chilled Ten Broeck tissue homogenizer. Added to the leaf powder was 5 ml of 0.5 N HCl at 0°C. The leaf tissue was homogenized for approximately 60 s at 0°C and an aliquot was reserved for chlorophyll determination. The remaining homogenate was centrifuged at 12,000 g for 5 min. To 2.5 ml of the supernatant was added 0.37 ml 2M Tris base and 0.22 ml 4 N KOH. The neutralized supernatant (pH 8.3) was then stored on ice. The assay buffer consisted of 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, and 5 mM DTT. The

assay buffer was added to 5-ml glass assay vials which were capped with screw-on septum caps. Through the septum was injected 20 mM $NaH^{14}CO_2$ (7.54 GBq/mol) and 0.25 ml neutralized leaf extract supernatant. The assay was initiated by the injection of 50 μl of activated RuBPCase from tobacco (as described in Chapter II). Assays were performed in triplicate at 26°C in a waterbath with continuous shaking (125 strokes min^{-1}). Total assay volume was 0.5 ml. After 60 min the assay was terminated with the injection of 0.1 ml of 6 N formic acid in methanol. Determination of acid-stable ^{14}C products was as described in Chapter III.

Chlorophyll Determination

Chlorophyll was determined in sample extracts used for RuBPCase activity and RuBP assays by the methods described in Chapter II.

Analysis of Statistical Significance

Simple linear and quadratic regression analyses were performed to determine the statistical significance (at the 5% level) of experimental results. In this chapter, air temperature during growth was used as regressor. The methods used are described further in Chapter II. Regression parameters are tabulated in Appendix C.

Results

Canopy Leaf Temperature

The temperature of leaves in the upper canopies was always several degrees below the air temperature, regardless of the CO_2 or

air temperature treatment. Increases in air temperature were accompanied by increases of a lesser magnitude in leaf temperature.

The measured difference in dry bulb temperature between chambers at the two lowest control temperatures (26 and 31°C) were actually 4.9 and 4.6°C for the 330 and 660 μl CO_2 l^{-1} treatments, whereas the difference in leaf temperatures were only 2.2 and 1.9°C, respectively. The measured difference in dry bulb temperatures between the lowest and highest control temperatures (26 and 36°C) were actually 9.6 and 9.0°C for the 330 and 660 μl CO_2 l^{-1} treatments, whereas the difference in leaf temperatures were only 3.5 and 4.4°C, respectively. The lower temperature of the leaves, with respect to the air, was due to evaporative cooling. The mean leaf to air temperature differential (T_L-T_A) and VPD were calculated and were found to be closely related. A linear regression of T_L-T_A with VPD as regressor yielded a correlation coefficient of -0.993. At 330 μl CO_2 l^{-1} T_L-T_A and VPD were, for the 26°C treatment, -3.1°C and 1.78 kPa, for the 31°C treatment, -5.8°C and 2.53 kPa, and for the 36°C treatment, -9.2°C and 3.87 kPa. At 660 μl Co_2 l^{-1} T_L-T_A and VPD were, for the 26°C treatment -3.4°C and 1.96 kPa, for the 31°C treatment, -6.1°C and 2.77 kPa, and for the 36°C treatment, -8.0°C and 3.56 kPa.

Canopy Net Photosynthesis

Within the range of temperatures used in the experiments reported here, air temperature during growth of soybeans had no effect on the maximum midday rates of canopy net photosynthesis (Table 4.1). At each CO_2 concentration the differences in canopy net photosynthesis at

Table 4.1. Effect of air temperature on maximum canopy net photosynthetic rates. Plants were grown at 330 or 660 $\mu l\ CO_2\ l^{-1}$. Measurements were made between 1100 and 1230 EST on November 4 (57 DAP). The quantum flux density was 1250 to 1350 μmol quanta $m^{-2}\ s^{-1}$ at the upper canopy level. The plant density at all treatments was 32 plants m^{-2}.

Growth temperature	Canopy Net Photosynthesis	
	330 $\mu l\ CO_2\ l^{-1}$	660 $\mu l\ CO_2\ l^{-1}$
°C	μmol $CO_2\ m^{-2}\ s^{-1}$	
26	29.3 ± 1.8 [a]	55.4 ± 1.2
31	28.1 ± 1.7	55.0 ± 1.0
36	30.6 ± 1.7	54.5 ± 2.3

[a] Mean ± SD.

growth air temperatures of 26, 31, and 36°C were not significant. The canopy net photosynthetic rates averaged 87% greater in the 660 compared to the 330 $\mu l\ CO_2\ l^{-1}$ grown canopies.

RuBP Carboxylase Activity

Initial and total RuBPCase activities were assayed at both 26°C and at the growth temperature for plants grown at 330 and 660 $\mu l\ CO_2\ l^{-1}$. Initial RuBPCase activity (Figure 4.1) showed no significant response to growth air temperature or CO_2 treatment when assayed at 26°C. When assayed at the growth temperature initial activity increased with temperature. Initial activity increased 76% when assay temperature was raised from 26 to 36°C in the 330 $\mu l\ CO_2\ l^{-1}$ grown plants and by 53% in the 660 $\mu l\ CO_2\ l^{-1}$ grown plants. Total RuBPCase activity (Figure 4.2) also showed no significant response to growth air temperature or CO_2 treatment when assayed at 26°C. Total activity increased with temperature when assayed at the plant growth temperature. Total activity increased 37% when assay temperature was raised from 26 to 36°C in the 330 $\mu l\ CO_2\ l^{-1}$ grown plants and by 23% in the 660 $\mu l\ CO_2\ l^{-1}$ grown plants. These increases correspond to Q_{10} values (between 26 and 36°C) of 1.7 and 1.4 for 330 $\mu l\ CO_2\ l^{-1}$ grown plants and 1.5 and 1.2 for 660 $\mu l\ CO_2\ l^{-1}$ grown plants for initial and total RuBPCase activity, respectively. The percent activation of RuBPCase was calculated from data in Figures 4.1 and 4.2 and is shown in Figure 4.3. When assayed at 26°C (Figure 4.3 A) activation was in the range of 73 to 81% for all growth temperatures in both CO_2 treatments. The response of RuBPCase activation to growth air

Fig. 4.1. Initial RuBPCase activity versus growth air temperature for 330 (●) and 660 (○) μl CO_2 l^{-1} grown plants. RuBPCase was assayed at either 26°C (broken line) or at the growth temperature (solid line). Assays were performed at pH 8.0 for 45 s. Data points are the means of triplicate assays. Plant samples were collected November 4 (57 DAP).

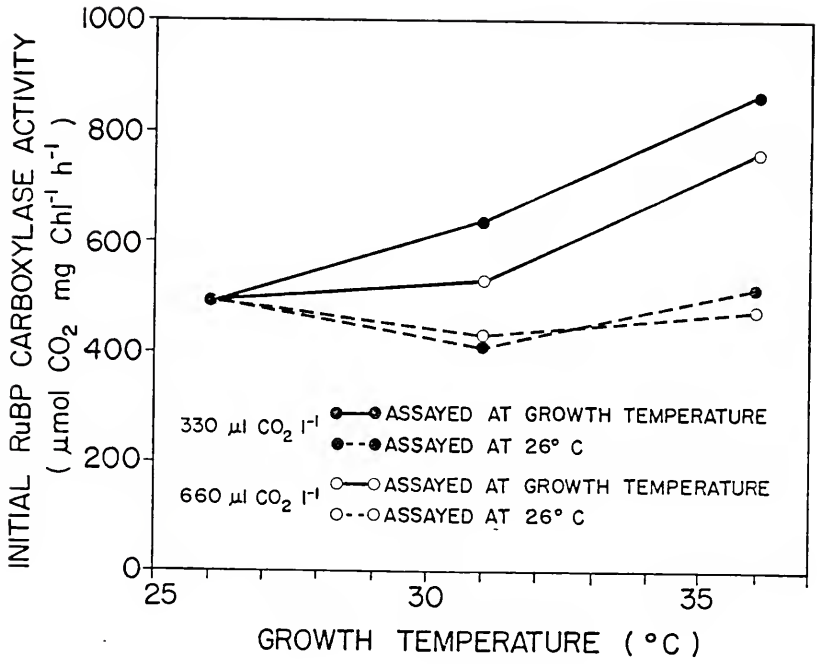

INITIAL RuBP CARBOXYLASE ACTIVITY
(μmol CO_2 mg Chl^{-1} h^{-1})
1000
800
600
400
200
0
25
30
35
GROWTH TEMPERATURE (°C)
330 μl CO_2 l^{-1}
ASSAYED AT GROWTH TEMPERATURE
ASSAYED AT 26° C
660 μl CO_2 l^{-1}
ASSAYED AT GROWTH TEMPERATURE
ASSAYED AT 26° C

Fig. 4.2. Total RuBPCase activity versus growth air temperature for 330 (●) and 660 (o) μl CO_2 l^{-1} grown plants. RuBPCase was assayed at either 26°C (broken line) or at the growth temperature (solid line). Assays were performed at pH 8.0 for 45 s. Data points are the means of triplicate assays. Plant samples were collected November 4 (57 DAP).

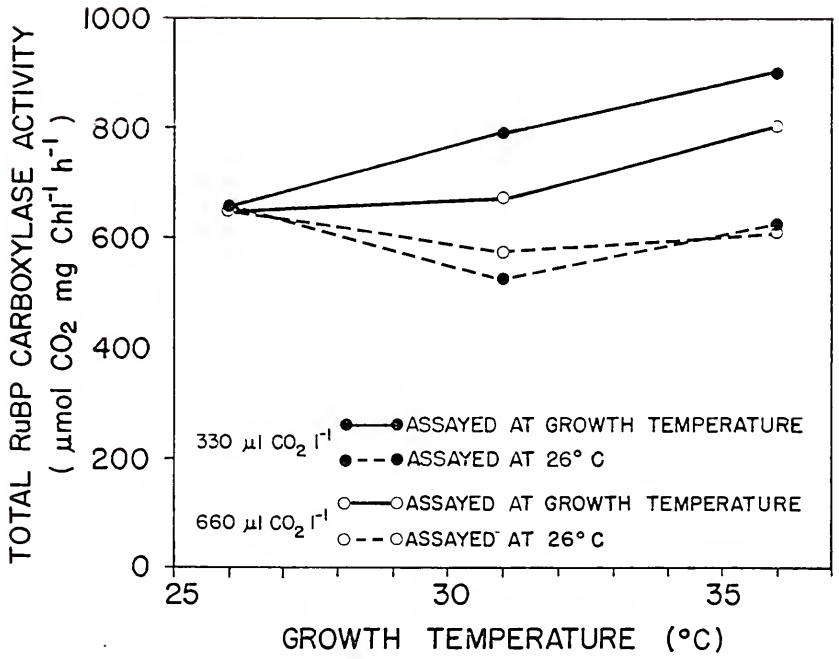
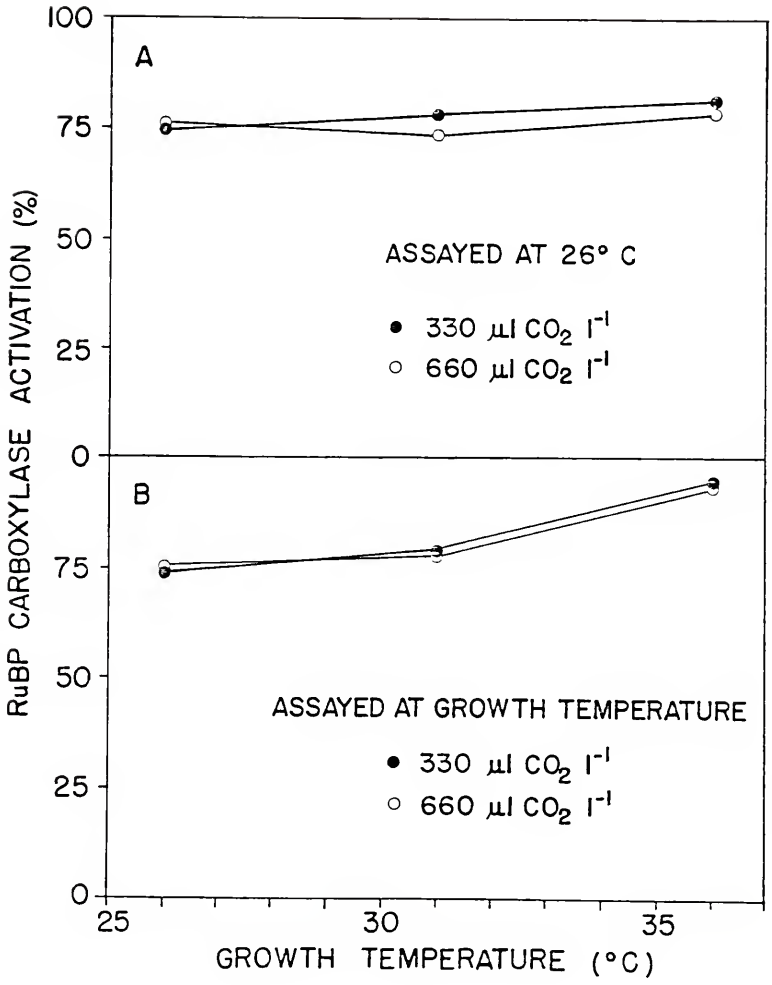

TOTAL RuBP CARBOXYLASE ACTIVITY
(μmol CO_2 mg Chl^{-1} h^{-1})
1000
800
600
400
200
0
25
30
35
GROWTH TEMPERATURE (°C)
330 μl CO_2 l^{-1}
ASSAYED AT GROWTH TEMPERATURE
ASSAYED AT 26° C
660 μl CO_2 l^{-1}
ASSAYED AT GROWTH TEMPERATURE
ASSAYED AT 26° C

Fig. 4.3. A, B. RuBPCase activation (%) versus growth air temperature for plants grown at 330 (•) or 660 (○) μl CO_2 l^{-1}. Activation was calculated from data in Figs. 4.1 and 4.2. Assays were performed at A) 26°C, and B) the respective growth temperatures.


A
B
100
75
50
25
0
RuBP CARBOXYLASE ACTIVATION (%)
ASSAYED AT 26° C
● 330 µl CO2 l-1
○ 660 µl CO2 l-1
ASSAYED AT GROWTH TEMPERATURE
● 330 µl CO2 l-1
○ 660 µl CO2 l-1
25
30
35
GROWTH TEMPERATURE (°C)

temperature was minimal, however, it was found to be statistically significant in the 330 μl CO_2 l^{-1}, but not the 660 μl CO_2 l^{-1}, treatment. When assayed at growth temperatures (Figure 4.3 B) activation was between 74 and 80% for assay temperatures of 26 and 31°C. When assayed at 36°C the activation climbed to greater than 93%.

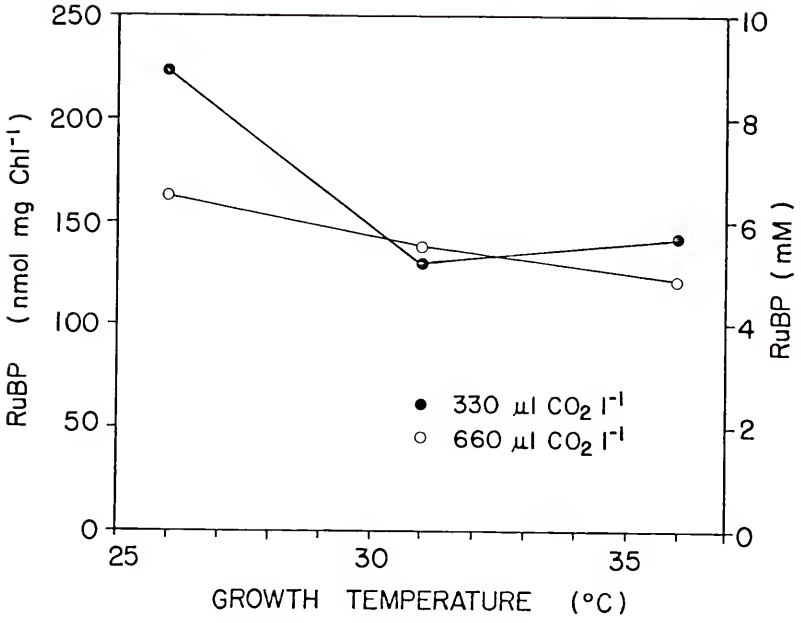
RuBP Levels

The levels of RuBP are plotted against growth air temperature for both CO_2 treatments in Figure 4.4. The highest levels of RuBP were found in plants grown at 26°C. In both CO_2 treatments the RuBP level decreased significantly as growth air temperature increased above 26°C. There was not a significant difference in the response of RuBP to growth air temperature between the two growth CO_2 concentrations. Based on assumptions made in Chapter II (concerning cellular location of RuBP and stromal volume) the chloroplast concentration of RuBP (mM) in leaves grown at air temperatures below 31°C was quite a bit greater than the 3 to 4 mM active site concentration of RuBPCase (Jensen and Bahr, 1977). At 31 and 36°C, RuBP levels were approaching, but still greater than, the RuPBCase active site concentration.

Discussion

The lack of response of maximum canopy photosynthetic rates, within a CO_2 treatment, to growth air temperature is due, in part, to the fact that leaf temperatures remained several degrees cooler than air temperatures. There was a smaller differential in leaf

Fig. 4.4. Levels of RuBP versus growth air temperature for plants grown at 330 (●) or 660 (○) μl CO_2 l^{-1}. Chloroplast concentration of RuBP (mM) assumes 25 μl stroma volume mg $chlorophyll^{-1}$. Data points are the means of triplicate assays. Leaf samples were subsamples of tissue used for RuBPCase assays in Figs. 4.1 and 4.2.


250
200
150
100
50
0
RuBP (nmol mg Chl^{-1})
10
8
6
4
2
0
RuBP (mM)
25
30
35
GROWTH TEMPERATURE (°C)
330 µl CO_2 l^{-1}
660 µl CO_2 l^{-1}

temperatures, between treatments, than in air temperatures. At 330 μl CO_2 l^{-1}, when air temperature was increased by 4.9 and 9.6°C, leaf temperature only increased by 2.2 and 3.5°C, and at 660 μl CO_2 l^{-1}, when air temperature was increased by 4.6 and 9.0°C, leaf temperature only increased by 1.9 and 4.4°C. The leaf temperature remained below the air temperature due to evaporative cooling of the leaves. As the dry bulb temperatures were increased the magnitude of T_L-T_A became greater. This was due to an increasing VPD, as a result of greater increases in dry bulb temperatures relative to dew point temperatures. There was a strong correlation between increasing VPD and the increasing magnitude of T_L-T_A, due to evaporative cooling. Generally speaking, T_L-T_A becomes more negative as VPD increases (Ehrler, 1973; Idso, 1982). The value of T_L-T_A is typically negative when plants are well-watered (Idso, 1982), as was the case with the plants in this study. Thus, as the dry bulb temperatures were increased (both the absolute temperature and relative to the dew point temperature) VPD increased, as did evaporative cooling, resulting in a greater differential between leaf and air temperature. The effect of this relationship was that leaf temperatures were not as high, nor spanned as wide a range, as did air temperatures.

Jones et al. (1985a) also reported a lack of response of canopy photosynthetic rates when soybeans were exposed to different air temperatures. In the experiments of Jones et al. (1985a), plants were grown at 31°C and canopy photosynthesis was measured at air temperatures of 28 and 33°C (and constant dew point temperature) with no apparent difference in rates.

Higher photosynthetic rates in the high CO_2 canopies (Table 4.1) is a widely observed response and is similar to results in Figure 3.3 (Chapter III).

Both initial and total RuBPCase activities, when assayed at 26°C, were independent of growth air temperature and CO_2 concentration. These results (with regard to temperature) agree with the results expressed on a leaf area basis of Downton and Slayter (1972) working with cotton. However, when Downton and Slayter (1972) expressed their results on a chlorophyll basis (as are the data in Figures 4.1 and 4.2), increases in growth temperature from from 25 to 40°C caused a decrease in RuBPCase activity. This was the result of an increase in chlorophyll per unit leaf area as growth temperature increased. Phillips and McWilliam (1971) found a small decrease in the specific activity of RuBPCase (assayed at 25°C) in wheat as the growth temperature increased over the range of approximately 25 to 35°C.

When RuBPCase was assayed at the respective growth temperature (Figures 4.1 and 4.2) the activities were greater at higher temperatures. This is not surprising as higher temperatures typically increase reaction rates. The greater RuBPCase activity at increasing temperatures is not contradictory with observed decreases in leaf photosynthesis as temperature increases (Jurik et al., 1984; Monson et al., 1982; Enoch and Hurd, 1977). As Jordan and Ogren (1984) have shown, photosynthesis decreases relative to photorespiration at higher temperatures, but increasing CO_2 can overcome this decline (Osmond et al., 1980). When RuBPCase was assayed in the experiments reported here, inorganic carbon was present at saturating levels and thus the

oxygenase reaction was essentially eliminated. Hence, the higher temperatures during the enzyme assays increased carboxylase reaction rates but probably did not increase oxygenase reaction rates. While RuBPCase activity assayed at growth temperatures indicates the potential CO_2 assimilation rates, they should not be regarded as accurate reflections of *in situ* leaf photosynthetic rates. The activation of RuBPCase was not greatly affected by CO_2 or air temperature during growth.

The significant decrease in RuBP levels with increasing growth air temperature was observed in both CO_2 treatments. Berry and Bjorkman (1980) have suggested that reduced rates of photophosphorylation and NADPH production could cause decreased RuBP levels at higher temperatures. The increased rates of RuBP consumption as both the RuBP carboxylase and RuBP oxygenase activities increase *in vivo* with temperature is probably also a factor.

The results presented here indicate that air temperature during growth had no significant effect on RuBPCase activity when assayed at a common temperature. The RuBP levels decreased significantly as temperature increased, possibly as a result of greater consumption due to higher rates of both carboxylation and oxygenation occurring *in vivo*. At a given CO_2 concentration, air temperature had no effect on maximum canopy photosynthetic rates. The lack of effect of air temperature on RuBPCase activity and canopy photosynthesis is due, partially, to the fact that evaporative cooling kept the leaves from reaching temperatures as high as the air. The fact that RuBP levels did respond to the increases in temperature suggests that RuBP may be more sensitive to moderate changes in the environment.

CHAPTER V
GENERAL SUMMARY AND CONCLUSIONS

The studies described in the previous chapters were initiated to examine the effects of CO_2 concentration on photosynthesis in soybean. The focus of these studies was placed on the enzyme (RuBPCase) and the substrate (RuBP) responsible for the CO_2 fixation reaction. In addition, leaf characteristics such as chlorophyll and protein content were also examined for response to the CO_2 treatments.

The photosynthetic rate response of soybean leaflets to a wide range of CO_2 concentrations indicated plants grown at twice atmospheric concentrations of CO_2 had higher rates than plants grown at atmospheric CO_2 concentrations. These measurements, made under conditions of high solar irradiance and during the pod filling stage, indicate an enhanced leaf photosynthetic capacity for plants grown at an elevated CO_2 concentration. This supports the hypothesis stated in Chapter I. There was no significant effect of the high CO_2 treatment on chlorophyll or protein content nor on the activity of RuBPCase (all expressed on a leaf area basis). Furthermore, RuBP was present in what appeared to be saturating concentrations for photosynthesis and therefore may not have been limiting photosynthetic rates in either CO_2 treatment. These results apparently preclude these characteristics from playing a significant role in the enhanced photosynthetic capacity. Previously published and non-published results of other researchers have shown growth at high CO_2

concentrations to result in changes in the leaf mesophyll of soybean. These changes can lead to increased photosynthetic rates, and may have occurred in the studies reported here. It is concluded that the enhanced photosynthetic capacity of leaflets grown at elevated CO_2 may be a result of an increase in the mesophyll cell surface area/external leaf surface area ratio, or, as a result of the increased assimilate demand of plants grown at elevated CO_2 with their greater pod weights. Alternatively, a combination of these two factors or possibly an as yet to be described factor may also be involved.

Long-term growth of soybeans at CO_2 concentrations ranging from subatmospheric to superatmospheric provided information on the response to CO_2 of RuBPCase activity and kinetics and RuBP, as well as on canopy photosynthesis. The level of RuBP in leaves (on a chlorophyll basis) decreased at the higher CO_2 concentrations. It appeared that RuBP was probably at saturating concentrations for photosynthesis, however, at high CO_2 the concentration of RuBP was approaching the concentration of RuBPCase active sites. The initial and total activities of RuBPCase (on a chlorophyll basis) decreased following growth at increasing concentrations of CO_2. The apparent $K_m(CO_2)$ and V_{max} of RuBPCase also demonstrated small decreases with increasing CO_2 concentrations. Since initial RuBPCase activity is an estimate of <u>in vivo</u> enzyme activity, these data suggest that RuBPCase activity in the leaf may be reduced following exposure to high concentrations of CO_2. Whether this reduction in activity is a result of a lowered specific activity or a decrease in the quantity of RuBPCase protein was not determined. Rates of canopy photosynthesis

increased with CO_2. Since the LAI also increased two-fold over the CO_2 range, at least part of the canopy photosynthetic response was due to a greater photosynthetic surface area. At high CO_2, light becomes more important as a limiting factor for maximum canopy photosynthesis.

The effects of temperature on RuBPCase, RuBP, and canopy photosynthesis were investigated in soybeans grown at atmospheric and twice atmospheric concentrations of CO_2. Canopy photosynthetic rates were independent of air temperature but were greater at the higher CO_2 concentration. The activity of RuBPCase (on a chlorophyll basis) was also independent of air temperature, although the level of RuBP (on a chlorophyll basis) did decrease with increasing temperature. Due to the increased VPD at higher air temperatures, and as a result of evaporative cooling, leaf temperatures were not as high as air temperatures. The actual temperature treatments were apparently not great enough to cause significant effects with respect to RuBPCase activity or canopy photosynthesis.

It is concluded that in addition to the previously mentioned enhanced leaflet photosynthetic capacity, growth of soybeans at elevated CO_2 concentrations may result in reduced RuBPCase activity (expressed on a chlorophyll basis). The physiological significance (if any) of a small decrease in $K_m(CO_2)$ is not known. It is also concluded that the response of RuBP to short-term changes in the CO_2 concentration, increases in temperature, and its rapid turnover rate, suggest that the leaf RuBP concentration may be more sensitive to moderate changes in the environment than RuBPCase activity or photosynthetic rates.

Because of the importance of the process of photosynthesis, and to better understand the response of plants to CO_2, future work on these problems could proceed into a number of areas. Several questions remain unanswered following the studies presented here. These questions can be used to define areas for future research. Specifically, four such areas are described.

1. Determine if the decrease in RuBPCase activity with increasing CO_2 concentration represents a reduction in the quantity of RuBPCase protein.

2. Once RuBPCase is quantified, examine the relationship between the concentration of RuBPCase active sites and the concentration of RuBP in the leaf at elevated CO_2 concentrations.

3. Pursue an investigation of the effects of CO_2 on internal leaf anatomy and how any changes may affect photosynthetic rates.

4. Continue efforts to describe the relationship between carbon fixation rate, translocation, and assimilate demand. This is an area that has received the attention of researchers in many areas of plant physiology. Because of the effects of CO_2 on photosynthesis and yield, this relationship should be examined with respect to the concentration of CO_2.

These areas of research should yield information helpful in better understanding photosynthesis and potentially useful in increasing agricultural productivity.

APPENDIX A
LEAF AND CANOPY PHOTOSYNTHETIC RATE RESPONSES TO LIGHT AT TWO CO_2 CONCENTRATIONS

Introduction

Increasing irradiation typically results in higher photosynthetic rates. Carbon dioxide interacts with the photosynthetic rate response to light via the quantum yield. Quantum yield is essentially the efficiency of utilization of absorbed light (Radmer and Kok, 1977). At high CO_2 the quantum yield increases due to the reduction in RuBP oxygenase activity (Ehleringer and Bjorkman, 1977). Increasing the supply of CO_2, therefore, not only provides more substrate for assimilation, it also improves the efficiency of use of light energy.

Materials and Methods

Plant Material and Growth Conditions

The plants and growth conditions used in this study were the same as described in Chapter II, the only difference being that this study was conducted later in the same season. Estimations of the canopy LAI were made prior to and following the photosynthesis measurements and indicated the LAI was 25 to 30% greater in the 660 compared to the 330 $\mu l\ CO_2\ L^{-1}$ grown canopy.

Leaf and Canopy Photosynthesis Measurements

Both leaf and canopy photosynthesis measurements were made on 14 Nov. 1983 (76 DAP). This was a cloud-free day with a maximum quantum flux density, as measured outside of the growth chambers, of 1350 μmol quanta m^{-2} s^{-1}. The growth and leaf chambers each transmitted 88% of the light incident to their upper surfaces. The appropriate corrections for transmission of solar irradiance were made in Figures A.1 and A.2. Leaf photosynthetic rates were measured as described in Chapter II. At each CO_2 concentration they are the combined responses of two leaflets. Canopy photosynthetic rates were measured as described in Chapter III. Both leaf and canopy measurements were made at the respective growth CO_2 concentrations. The different quantum flux densities represent the natural daily variation in solar irradiance. Leaf photosynthetic rates are expressed on a leaf area basis while canopy rates are on a land area basis.

Results and Discussion

The leaf photosynthetic rate increased with light in both CO_2 treatments (Figure A.1). At all light intensities, the high CO_2 grown leaves had greater photosynthetic rates. The absolute difference in rates was greatest at high light intensity. Leaves from both CO_2 treatments appeared to light saturate at 900 to 1000 μmol quanta m^{-2} s^{-1}. Canopy photosynthetic rates also increased with light intensity at both CO_2 concentrations (Figure A.2). As was the case with leaves, the absolute difference in rates was greatest at high light intensity.

Fig. A.1. Leaf net photosynthesis versus quantum flux density for plants grown and measured at 330 (●) and 660 (○) μl CO_2 l^{-1}. Measurements were made on November 14 (76 DAP). Data points represent mean values of two leaflets at each CO_2 concentration. Vertical lines through points are ± SD for photosynthetic rates and horizontal lines are ± SD for quantum flux density. Photosynthesis is expressed on a leaf area basis.

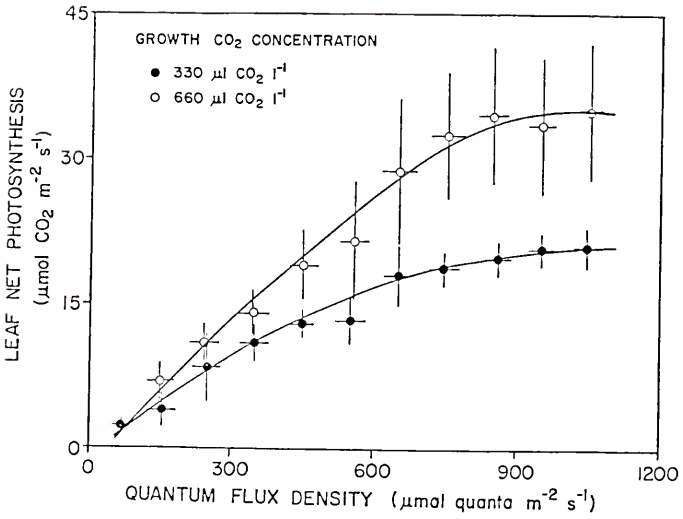
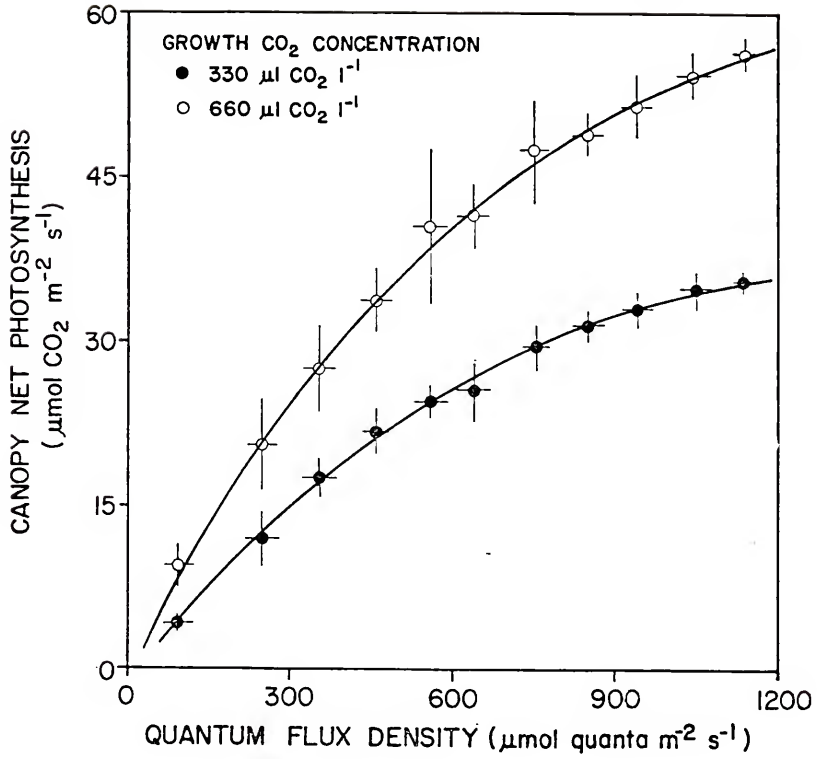

GROWTH CO_2 CONCENTRATION
● 330 µl CO_2 l^{-1}
○ 660 µl CO_2 l^{-1}
LEAF NET PHOTOSYNTHESIS
(µmol CO_2 m^{-2} s^{-1})
45
30
15
0
0
300
600
900
1200
QUANTUM FLUX DENSITY (µmol quanta m^{-2} s^{-1})

Fig. A.2. Canopy net photosynthesis versus quantum flux density for canopies grown and measured at 330 (•) and 660 (○) $\mu l\ CO_2\ l^{-1}$. Measurements were made on November 14 (76 DAP). Data points represent mean values. Vertical lines through points are ± SD for photosynthetic rates and horizontal lines are ± SD for quantum flux density. Photosynthesis is expressed on a land area basis.


GROWTH CO_2 CONCENTRATION
● 330 µl CO_2 l^{-1}
○ 660 µl CO_2 l^{-1}
CANOPY NET PHOTOSYNTHESIS
(µmol CO_2 m^{-2} s^{-1})
60
45
30
15
0
0
300
600
900
1200
QUANTUM FLUX DENSITY (µmol quanta m^{-2} s^{-1})

Light saturation of canopy photosynthesis was not observed in either canopy.

The responses to light of leaf and canopy photosynthesis are similar except for light saturation in the leaves at high light intensity. This is not surprising considering the leaf rates were from leaves in the upper unshaded layer of the canopy. The leaves in the upper layer of a canopy contribute, by far, the majority of the photosynthetic response (Acock et al., 1978; Hatfield and Carlson, 1977). Hatfield and Carlson (1977) reported that 80% of the CO_2 uptake in a soybean canopy occurred in the upper 20% of the canopy.

The higher photosynthetic rates in leaves grown and measured at 660 μl CO_2 l^{-1} are due to the greater supply of substrate for assimilation, increased quantum yield, and the inherent capacity for increased photosynthesis in soybean leaves grown in elevated CO_2 (discussed in Chapter II). Canopy photosynthetic rates are increased by the same factors described above for leaves. Additionally, increased light intensity and CO_2 concentration improves the supply (to the lower levels of the canopy) of energy and substrate that normally become attenuated with depth in the canopy.

APPENDIX B
EFFECT OF LEAF SAMPLE SIZE ON IN VITRO RuBP CARBOXYLASE ACTIVITY

Introduction

The degree of activation as well as the quantity of RuBPCase are key factors in the regulation of CO_2 assimilation in vivo (Jensen and Bahr, 1977). It has been shown that activation of RuBPCase is dependent on Mg^{2+} and CO_2 (Laing and Christeller, 1976; Lorimer et al., 1976). Assays of RuBPCase following incubation of the enzyme with added Mg^{2+} and HCO_3^- yield "total" activity, while assays without added Mg^{2+} and HCO_3^- yield "initial" activity (Perchorowicz et al., 1981). The initial activity is often used as an estimate of the in vivo RuBPCase activity (Perchorowicz et al., 1982). The percent activation is calculated by the ratio of initial to total activity times 100%. It thus provides an idea of the activation status of RuBPCase in vivo.

Materials and Methods

Plant Material and Growth Conditions

Field grown soybeans (Glycine max L. Merr.) were used to supply leaf tissue in two successive years for this study. On 15 Aug. 1984 (75 DAP), leaves of soybean (cv Braxton) were collected and on 15 May 1985 (55 DAP), leaves of soybean (cv Biloxi) were collected. On both

dates leaves were sampled rapidly and were immediately plunged into liquid N_2, ground to a powder, and stored in liquid N_2 as described in Chapter II.

RuBP Carboxylase Assay

Assays of RuBPCase were performed as described in Chapter II with the exception that 10 mM $MgCl_2$ was added to the extraction buffer where noted.

Results and Discussion

Initial and total RuBPCase activities are shown in Figure B.1 as a function of leaf sample size used in the assay. Total activity was not affected by sample size. Below a leaf sample size of approximately 150 mg (dry weight), initial activity decreased with decreasing sample size. The percent activation (Figure B.2) shows the same dependence on sample size below 150 mg (dry weight). All samples were homogenized in 10 ml of extraction buffer, hence, the smaller sample sizes were effectively diluted to a greater degree. Apparently with small amounts of leaf material the endogenous Mg^{2+} present in the tissue becomes too dilute and RuBPCase deactivation occurs prior to the assay. Incubation of RuBPCase with Mg^{2+} during the activation process in the total activity assay prevents this deactivation. To determine if Mg^{2+} added during RuBPCase extraction would prevent deactivation in the tissue samples, 10 mM $MgCl_2$ was added to the extraction buffer of some assays. The addition of $MgCl_2$ to small tissue samples increased the percent activation over two-fold. With

Fig. B.1. Initial (---) and total (——) RuBPCase activity versus leaf sample size used in assay. RuBPCase was extracted without added Mg^{2+}. Assays were performed at 30°C at pH 8.5 for 45 s. Leaves were collected from field grown plants at 75 DAP.

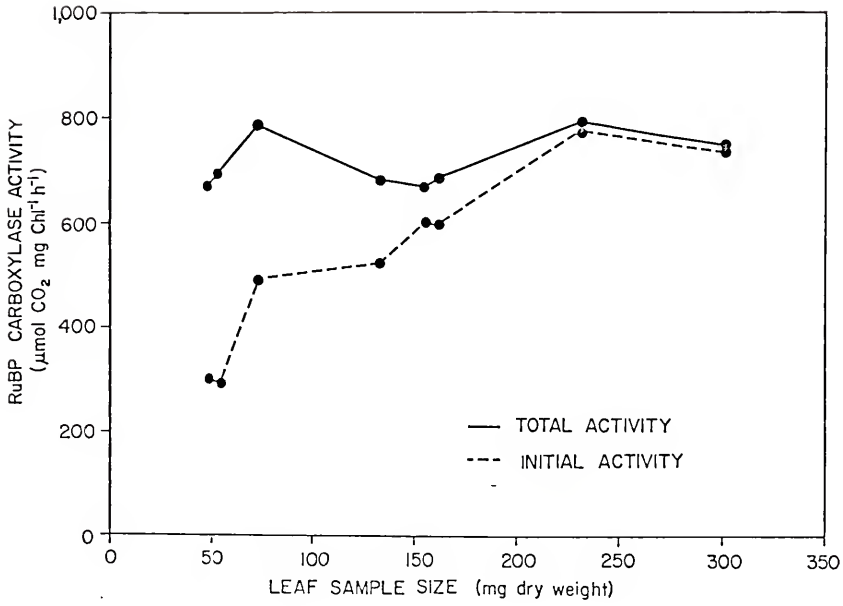
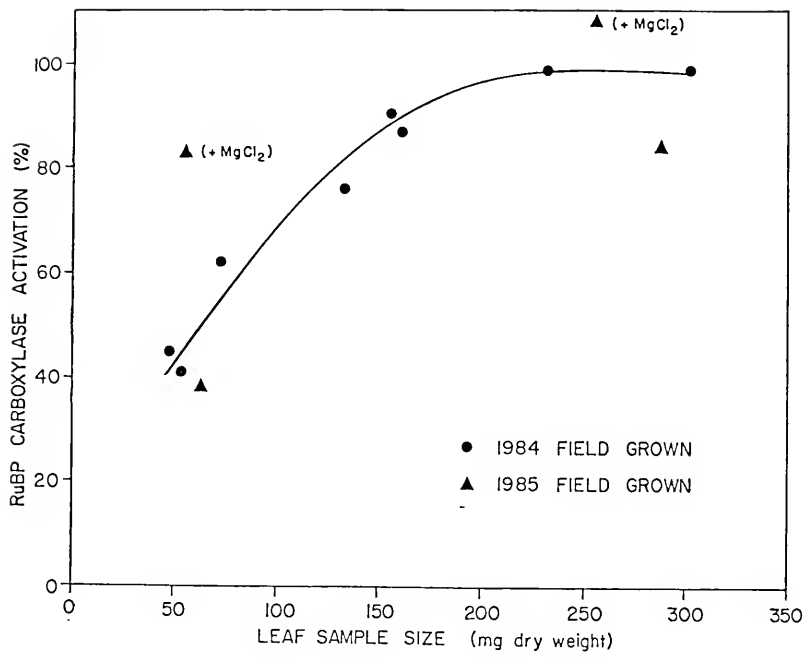

1,000
800
600
400
200
0
RuBP CARBOXYLASE ACTIVITY
(μmol CO_2 mg Chl^{-1} h^{-1})
TOTAL ACTIVITY
INITIAL ACTIVITY
0
50
100
150
200
250
300
350
LEAF SAMPLE SIZE (mg dry weight)

Fig. B.2. Percent activation of RuBPCase versus leaf sample size used in assay. The 1984 results (●) were calculated from activities in Fig. B.1 where no $MgCl_2$ was added to the extraction buffer. The 1985 results (▲) were calculated from activities with and without 10 mM $MgCl_2$ added to the extraction buffer. Except for added $MgCl_2$ all assays followed the same procedure. The 1985 results are from leaves collected from field grown plants at 55 DAP.


▲ (+ MgCl2)
▲ (+ MgCl2)
100
80
60
40
20
0
RuBP CARBOXYLASE ACTIVATION (%)
● 1984 FIELD GROWN
▲ 1985 FIELD GROWN
0
50
100
150
200
250
300
350
LEAF SAMPLE SIZE (mg dry weight)

the added Mg^{2+} the activation level was similar to that measured in the larger tissue samples without added $MgCl_2$. With added $MgCl_2$ activation also increased in samples weighing more than 250 mg (dry weight) although not as dramatically as with the smaller samples. Servaites (1984) showed a similar dependence of the initial activity on the ratio of leaf tissue weight to extraction buffer volume. While there may be factors other than Mg^{2+} concentration involved in the loss of initial activity, use of an appropriate leaf sample size is recommended to avoid problems of excess dilution of Mg^{2+} and possibly other endogenous leaf effectors.

APPENDIX C
LINEAR REGRESSION PARAMETERS

Table C.1. Linear regression parameters (for short-term CO_2 concentrations) for data in Chapter II.

Figure/Table	Dependent variable	Regression parameters		Model
		β_0	β_{CO_2}	R^2
Fig. 2.1	Ci (330)	-17.37**[1]	0.721**	0.971
Fig. 2.1	Ci (660)	- 9.01NS	0.546**	0.932
Fig. 2.4A	initial RuBPCase (330)	100.79**	-0.010NS	0.143
Fig. 2.4A	initial RuBPCase (660)	104.36**	-0.010NS	0.056
Fig. 2.4B	total RuBPCase (330)	107.90**	-0.001NS	0.003
Fig. 2.4B	total RuBPCase (660)	113.61**	-0.002NS	0.004
Fig. 2.5	RuBPCase activation (330)	93.69**	-0.009*	0.315
Fig. 2.5	RuBPCase activation (660)	91.72**	-0.008NS	0.142
Fig. 2.6	RuBP (330)	84.29**	-0.030**	0.267
Fig. 2.6	RuBP (660)	63.07**	-0.014**	0.469
Table 2.3	initial RuBPCase (330)	763.67**	-0.077NS	0.142
Table 2.3	initial RuBPCase (660)	722.52**	-0.077NS	0.065
Table 2.3	total RuBPCase (330)	817.58**	-0.014**	0.003
Table 2.3	total RuBPCase (660)	786.49**	-0.016NS	0.004
Table 2.4	RuBP (330)	132.66**	-0.031**	0.479
Table 2.4	RuBP (660)	161.40**	-0.057**	0.262

[1]Regression parameter = 0 rejected at a probability level of 0.05 (**) or 0.01 (*), or not significant (NS).

Table C.2. Linear regression parameters (for growth CO_2 concentration) for data in Chapter III.

Figure/Table	Dependent variable	Regression parameters		Model
		β_0	β_{CO_2}	R^2
Table 3.1	SLW	1.78**[1]	0.001**	0.559
Table 3.1	LAI	1.58**	0.001**	0.565
Table 3.1	chlorophyll	0.02**	-7*10-7NS	0.009
Table 3.1	protein	0.28**	-6*10-5NS	0.310
Table 3.2	chlorophyll	0.01**	-5*10-6**	0.674
Table 3.2	protein	0.14**	-7*10-5**	0.748
Fig. 3.1	protein/chlorophyll ratio	12.18**	-0.002**	0.287
Fig. 3.4A	initial RuBPCase	789.42**	-0.243**	0.692
Fig. 3.4A	total RuBPCase	812.35**	-0.189	0.712
Fig. 3.4B	RuBPCase activation	97.10**	-0.009**	0.226
Fig. 3.5	RuBP	191.89**	-0.144**	0.584
Table 3.3	$K_m(CO_2)$	16.51**	-0.002**	0.423
Table 3.3	V_{max}	1040.44**	-0.181**	0.645

[1]Regression parameter = 0 rejected at a probability level of 0.05 (**) or not significant (NS).

Table C.3. Linear regression parameters (for growth air temperature) for data in Chapter IV.

Figure/Table	Dependent variable	Regression parameters		Model
		β_0	β_{CO_2}	R^2
Table 4.1	canopy photosynthesis (330)	25.58**[1]	0.121NS	0.069
Table 4.1	canopy photosynthesis (660)	57.62**	-0.085NS	0.051
Fig. 4.1	initial RuBPCase 26° (330)	399.87**	2.233NS	0.043
Fig. 4.1	initial RuBPCase 26° (660)	503.07**	-1.300NS	0.033
Fig. 4.2	total RuBPCase 26° (330)	694.41**	-2.966NS	0.041
Fig. 4.2	total RuBPCase 26° (660)	733.12**	-3.900NS	0.246
Fig. 4.3A	RuBPCase activation 26°(330)	55.96**	0.700**	0.633
Fig. 4.3A	RuBPCase activation 26°(660)	68.10**	0.233NS	0.102
Fig. 4.4	RuBP (330)	414.73**	-8.066**	0.643
Fig. 4.4	RuBP (660)	267.17**	-4.066**	0.980

[1]Regression parameter = 0 rejected at a probability level of 0.05 (**) or not significant (NS).

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BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

J. W. Jones
Professor of Agricultural Engineering

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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