EFFECTS OF CARBON DIOXIDE ENRICHMENT ON GROWTH, PHOTOSYNTHESIS, AND LEAF SENESECE IN BEAN (PHASEOLUS VULGARIS L.) PLANTS

by

DAVID LLOYD EHRET


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Department of Plant Science

The University of British Columbia
1956 Main Mall
Vancouver, Canada
V6T 1Y3

Date Jan. 18, 1984
Abstract

Incremental increases in absolute growth rate with increasing carbon dioxide concentrations ranging from 340 to 3000 ul l⁻¹ were observed in continuously carbon dioxide enriched bean plants over the course of development. However, relative growth rate of enriched plants was enhanced only early in development. Unit leaf rate was also increased by enrichment primarily in early development, explaining, in part, the trends in relative growth rate. Carbon dioxide enrichment also caused large increases in leaf dry weight, which constituted the major change in carbon partitioning among plant parts.

Leaves of enriched plants showed a decrease in photosynthetic capacity which was not associated with changes in chlorophyll concentration, or photorespiration rate. The decrease in capacity was less evident in older leaves, or in those maintained at low light intensity or with reduced chlorophyll levels, suggesting that the reduced photosynthetic capacity was due to the higher photosynthetic rate of those leaves during growth. Respiration rate in leaves of enriched plants was also increased, but only under conditions which caused a concurrent decrease in photosynthetic capacity. Enriched leaves consistently showed higher starch content with generally a lower photosynthetic capacity that control leaves. Furthermore, an increase in sink demand did not influence the photosynthetic capacity and starch content of enriched leaves to the extent of control leaves.

A high leaf starch content and leaf dry weight correlated
with accelerated senescence of the primary leaves of enriched plants. Conditions such as high light intensity or reduced temperature, which facilitated greater starch accumulation, also resulted in the most rapid and extensive senescence.
# Table of Contents

Abstract ................................................................. ii
List of Tables ............................................................. vi
List of Figures ............................................................ viii
List of Abbreviations .................................................... xi
Acknowledgement ........................................................... xiii

Chapter I
GENERAL INTRODUCTION ................................................ 1

Chapter II
LITERATURE REVIEW .................................................... 7
  2.1 PLANT RESPONSE TO CARBON DIOXIDE ENRICHMENT .......... 7
  2.1.1 Historical Perspective ........................................ 7
  2.1.2 Carbon Dioxide Enrichment And Plant Growth ............. 8
  2.1.3 Duration Of Enrichment ....................................... 11
  2.1.4 Interaction With Plant Age .................................. 12
  2.1.5 Interaction With Light Intensity .......................... 13
  2.1.6 Interaction With Nutrient Regime .......................... 14
  2.1.7 Effects On Partitioning Of Assimilates ................... 15
  2.1.8 Response Of Photosynthetic Capacity ...................... 17
  2.1.9 Leaf Morphology ............................................. 23
  2.1.10 Leaf Carbohydrates ......................................... 23
  2.2 SENESCENCE ...................................................... 24
  2.2.1 General Physiology .......................................... 25
  2.2.2 Growth Regulators .......................................... 27
  2.2.3 Environmental Influences ................................... 29
  2.2.4 Carbon Dioxide And Senescence ............................ 30

Chapter III
EFFECTS ON GROWTH AND PARTITIONING .............................. 32
  3.1 INTRODUCTION .................................................... 32
  3.2 MATERIALS AND METHODS ....................................... 34
  3.2.1 Plant Culture ................................................. 34
  3.2.2 Carbon Dioxide Treatment Chamber Design ................ 34
  3.2.3 Growth Analysis ............................................. 38
  3.3 RESULTS AND DISCUSSION ...................................... 42
  3.3.1 Effects On Growth ........................................... 42
  3.3.2 Effects On Partitioning ..................................... 53
  3.3.3 Summary ..................................................... 62

Chapter IV
EFFECTS ON NET CARBON DIOXIDE EXCHANGE .......................... 65
  4.1 INTRODUCTION ..................................................... 65
  4.2 MATERIALS AND METHODS ....................................... 68
  4.3 RESULTS AND DISCUSSION ...................................... 73
  4.3.1 Generalized Response Of Net Carbon Dioxide Exchange Capacity ................................................... 73
  4.3.2 Response Of Specific Parameters Of Net Carbon Dioxide Exchange Capacity ................................................... 80
  4.3.3 Effect Of Duration ........................................... 84
  4.3.4 Effect Of Photosynthetic Photon Flux Density .......... 85
  4.3.5 Response To Sink Manipulation ............................. 89
## List of Tables

3.1 Analysis of variance of the collective dry weight data of trial 1 and trial 2.......................43

3.2 Total dry weight, pod weight, and Harvest Index of plants grown at 340, 500, 800, 1200, 2000, or 3000 ul l$^{-1}$ carbon dioxide for 44 days.............................................63

4.1a Net carbon dioxide exchange rate of the first and second trifoliates of control (340 ul l$^{-1}$) and enriched (1400 ul l$^{-1}$) plants..................................................74

4.1b Analysis of variance of the data of Table 4.1a........74

4.2 Oxygen sensitivity of the net carbon dioxide exchange rate of the first trifoliate of control (340 ul l$^{-1}$) and enriched (1400 ul l$^{-1}$) plants treated for 13 days.................................78

4.3 Leaf thickness and intercellular air space volume of control (340 ul l$^{-1}$) and enriched (1400 ul l$^{-1}$) plants.................................................................82

4.4 The effect of shading during growth on the net carbon dioxide exchange rate of the first trifoliate of enriched (1400 ul l$^{-1}$) and control (340 ul l$^{-1}$) beans grown at 26 C........................................88

4.5 The effect of shading all but the first trifoliate on starch content and net carbon dioxide exchange rate of the first trifoliate of control (340 ul l$^{-1}$) and enriched (1400 and 3000 ul l$^{-1}$) plants.................................................................92

4.6 Effect of pot size on the net carbon dioxide exchange rate of the first trifoliate of enriched (1400 ul l$^{-1}$) and control (340 ul l$^{-1}$) plants..................................................100

4.7 Stomatal diffusive resistance of the primary leaf and first trifoliate of enriched (1400 ul l$^{-1}$) and control (340 ul l$^{-1}$) plants......................................................105

4.8 Net carbon dioxide exchange rate of control (340 ul l$^{-1}$) and enriched (1000 ul l$^{-1}$) tomato plants at a PI of 5.0.........................................................108
5.1 Net carbon dioxide exchange rate and chlorophyll content of the primary leaves before and after 17 days of carbon dioxide enrichment at 32 or 20°C..........................116

5.2 Specific leaf weight and starch content of the primary leaves of control (340 ul l⁻¹) and enriched (1400 ul l⁻¹) plants at two temperatures and two levels of photosynthetic photon flux density........................................130

5.3 Water and osmotic potentials, and specific leaf weight of enriched (1400 ul l⁻¹) and control (340 ul l⁻¹) plants grown for 7 days at 24°C.................................135
### List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Schematic diagram of one of the eight carbon dioxide treatment chambers</td>
<td>36</td>
</tr>
<tr>
<td>3.2</td>
<td>Leaf area over time of plants grown at 340, 500, 800, 1200, 2000, or 3000 ul l⁻¹ carbon dioxide</td>
<td>44</td>
</tr>
<tr>
<td>3.3</td>
<td>The dry weight over time of plants grown at 340, 500, 800, 1200, 2000, or 3000 ul l⁻¹ carbon dioxide</td>
<td>45</td>
</tr>
<tr>
<td>3.4</td>
<td>Absolute growth rate of dry weight over time of plants grown at 340, 500, 800, 1200, 2000, or 3000 ul l⁻¹ carbon dioxide</td>
<td>46</td>
</tr>
<tr>
<td>3.5</td>
<td>Relative growth rate over time of plants grown at 340, 500, 800, 1200, 2000, or 3000 ul l⁻¹ carbon dioxide</td>
<td>47</td>
</tr>
<tr>
<td>3.6</td>
<td>Unit leaf rate over time of plants grown at 340, 500, 800, 1200, 2000, or 3000 ul l⁻¹ carbon dioxide</td>
<td>49</td>
</tr>
<tr>
<td>3.7</td>
<td>Leaf area ratio over time of plants grown at 340, 500, 800, 1200, 2000, or 3000 ul l⁻¹ carbon dioxide</td>
<td>51</td>
</tr>
<tr>
<td>3.8</td>
<td>Root-shoot ratio over time of plants grown at 340, 500, 800, 1200, and 3000 ul l⁻¹ carbon dioxide</td>
<td>54</td>
</tr>
<tr>
<td>3.9</td>
<td>Leaf weight ratio over time of plants grown at 340, 500, 800, 1200, 2000, or 3000 ul l⁻¹ carbon dioxide</td>
<td>55</td>
</tr>
<tr>
<td>3.10</td>
<td>Leaf weight over time of plants grown at 340, 500, 800, 1200, 2000, or 3000 ul l⁻¹ carbon dioxide</td>
<td>56</td>
</tr>
<tr>
<td>3.11</td>
<td>Ratio of the growth rate of leaf weight to that of the remainder of the plant over time, in plants grown at 340, 500, 800, 1200, 2000, or 3000 ul l⁻¹ carbon dioxide</td>
<td>58</td>
</tr>
<tr>
<td>3.12</td>
<td>Specific leaf weight over time of plants grown at 340, 500, 800, 1200, 2000, or 3000 ul l⁻¹ carbon dioxide</td>
<td>59</td>
</tr>
</tbody>
</table>
3.13 Specific leaf weight over time of the individual leaves of plants grown at 340 (broken line) or 1200 (solid line) ul l\(^{-1}\) carbon dioxide...............................61

4.1 Response of net carbon dioxide exchange rate of the first trifoliate of enriched (1400 ul l\(^{-1}\)) and control (340 ul l\(^{-1}\)) plants (at PI=5.0) to intercellular space carbon dioxide concentration....75

4.2 Response of the net carbon dioxide exchange rate of the first trifoliate of enriched (1400 ul l\(^{-1}\)) and control (340 ul l\(^{-1}\)) plants to photosynthetic photon flux density...............................79

4.3 Response of the net carbon dioxide exchange rate, expressed per unit chlorophyll, of the first trifoliate of enriched (1400 ul l\(^{-1}\)) and control (340 ul l\(^{-1}\)) plants, to photosynthetic photon flux density...............................83

4.4 Response of net carbon dioxide exchange rate of the first trifoliate of enriched (1400 ul l\(^{-1}\)) and control (340 ul l\(^{-1}\)) plants (PI>5.0) to intercellular space carbon dioxide concentration....86

4.5 Relationship between net carbon dioxide exchange rate and starch content of the first trifoliate of enriched (1400 or 3000 ul l\(^{-1}\)) and control (340 ul l\(^{-1}\)) plants...............................97

4.6 Time-course of the response of leaf water vapour and carbon dioxide exchange to a light-dark transition...............................103

5.1 Photograph of the primary leaves of enriched (1400 ul l\(^{-1}\)) and control (340 ul l\(^{-1}\)) plants grown for 20 days at 20 C.........................117

5.2 Time-course of net carbon dioxide exchange rate of primary leaves of enriched (1400 ul l\(^{-1}\)) (1400 ul l\(^{-1}\)) and control (340 ul l\(^{-1}\)) plants grown at 20 C........................................118

5.3 Photograph of the central leaflet of the first trifoliate leaf of enriched (1400 ul l\(^{-1}\)) and control (340 ul l\(^{-1}\)) plants grown for 20 days at 20 C........................................121

5.4 Time-course of stomatal resistance of primary leaves after transfer to treatment chambers at 20 C........................................123
5.5 Time-course of chlorophyll content and net carbon dioxide exchange rate of shaded and unshaded primary leaves of plants grown at 1400 ul l\(^{-1}\) carbon dioxide and at 20 C..............................126

5.6 Chlorophyll content of primary leaves grown at 340 or 1400 ul l\(^{-1}\) carbon dioxide, and 90 or 370 umol m\(^{-2}\) s\(^{-1}\) photosynthetic photon flux density for 17 days.................................127

5.7 Relationship between starch content and specific leaf weight of the primary leaves.........................131
List of Abbreviations

AbA...........abscisic acid
ADP...........adenosine diphosphate
ATP...........adenosine triphosphate
Ci............intercellular carbon dioxide concentration
Co............carbon dioxide concentration external to the leaf
CER...........net carbon dioxide exchange rate
Chl...........chlorophyll
dLw...........change in leaf weight
dRw...........change in the weight of the remainder of the plant
dt...........change in time
E............unit leaf rate
F............leaf area ratio
G............absolute growth rate
J(H2O)........flux of water vapour
J(CO2)........flux of carbon dioxide
kPa...........kilopascals
La............leaf area
LAD...........leaf area duration
Lw...........leaf weight
LWR...........leaf weight ratio
Pi............phosphate
PI...........Plastochron Index
PPFD...........photosynthetic photon flux density
R............relative growth rate
Rm...........mesophyll resistance
RPP cycle.....reductive pentose phosphate cycle
Rs..........stomatal resistance
RuBP........ribulose-1,5-bisphosphate
SLW.........specific leaf weight
UDP-glucose..uridine diphosphate glucose
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I. GENERAL INTRODUCTION

It is well known that air is a fine elastick fluid, with particles of very different natures floating in it, whereby it is admirably fitted by the great author of nature, to be the breath of life, of vegetables, as well as of animals, without which they can no more live, nor thrive than animals can.

Stephen Hales, 1727

Carbon dioxide is, biologically, one of the most important gases in the earth's atmosphere; it is also one of the least abundant. Only carbon dioxide and its dissolved ions can be utilized as a source of carbon by autotrophic organisms, the primary producers in the global ecosystem. The ability of photosynthetic autotrophs to incorporate large quantities of carbon, derived from atmospheric carbon dioxide at ambient concentrations of 340 microlitres per litre is truly a remarkable evolutionary achievement.

Considering the importance of carbon dioxide to plant life, it should not be surprising that carbon dioxide influences a range of physiological processes in higher plants.

Carbon dioxide concentration influences several aspects of the photosynthetic process. Carbon dioxide is a substrate of the reductive pentose phosphate (RPP) cycle of photosynthesis. The concentration of carbon dioxide is therefore a determinant

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1 The notation used to express carbon dioxide concentration in this study is microlitres of carbon dioxide per litre of air (ul l^-1). At 25 C and 1 atmosphere pressure, the density of carbon dioxide is 1.81 ug l^-1. Thus 340 ul l^-1 = 615 ug l^-1.
of the rate of true photosynthesis, which may be defined as photosynthetic carbon assimilation in the absence of processes in the leaf which result in the evolution of carbon dioxide. Photorespiration is one such process which causes release of carbon dioxide in the light, and thereby reduces the rate of net photosynthesis. However, carbon dioxide concentration also influences net photosynthesis by reducing the percentage inhibition of true photosynthesis by photorespiration (Fock et al., 1979). Carbon dioxide not only acts as substrate in photosynthesis, but activates ribulose bisphosphate carboxylase (RuBP carboxylase), an important enzyme in the RPP cycle (Lorimer et al., 1978), and is needed as bicarbonate ion in the operation of Photosystem II and oxygen evolution (Stemler and Govindjee, 1973; Khanna et al., 1980). It may even function in light-induced chloroplast movements (Walczak and Gabrys, 1981).

Carbon dioxide also regulates stomatal aperture (Rashcke, 1975). Carbon dioxide causes stomatal closure and an increase in stomatal diffusive resistance. The intercellular carbon dioxide concentration appears to be more important than the carbon dioxide concentration external to the leaf in regulating stomatal aperture. In many species, an increased carbon dioxide concentration will tend to increase water use efficiency (the ratio of carbon assimilated to water transpired).

Carbon dioxide interacts with ethylene in regulating aspects of plant development, although the nature of the interaction is still controversial. Carbon dioxide is known to competitively inhibit ethylene action in developmental processes.
such as floral senescence, fruit ripening, and seed germination (Abeles, 1973). Carbon dioxide also increases ethylene release from illuminated leaves (Bassi and Spencer, 1982). Recently it has been proposed that photosynthesis is required for ethylene synthesis and its subsequent release from leaves (Grodzinski et al., 1983). Carbon dioxide could influence this process through an effect on photosynthesis rate.

Carbon dioxide also influences developmental processes such as flowering (Bassi et al., 1976; Hicklenton and Jolliffe, 1980a), and fruit ripening (Abeles, 1973). Those effects, along with the inhibition of ethylene action, appear to be non-photosynthetic in nature.

In addition to photosynthetic and developmental effects, there is evidence that high carbon dioxide concentration promotes respiratory gas exchange in non-photosynthetic tissue (Perez-Trejo et al., 1981).

It should be pointed out that the concentrations of carbon dioxide required to influence some of these processes vary substantially. Stomatal aperture and net photosynthesis respond to carbon dioxide concentrations from 0 to approximately 2000 ul l⁻¹, depending on the light intensity; developmental processes, such as inhibition of ethylene action, generally require an order of magnitude greater carbon dioxide concentration (10,000 to 20,000 ul l⁻¹).

In spite of the many and varied actions of carbon dioxide, surprisingly little work, particularly of a physiological nature, has been directed toward studying the effects of long
term exposure of plants to high carbon dioxide concentrations. To date, much of the work has focussed on the commercial applications of increasing growth of horticultural crops through enrichment of greenhouse atmospheres with carbon dioxide. As early as the 1930's, greenhouses in Europe, particularly in Germany, were using carbon dioxide on a routine basis (Wittwer, 1967), with wide-spread use becoming evident in North America and Europe in the early 1960's. In recent years, an increasing number of carbon dioxide enrichment studies have been conducted on field crops (eg. Hesketh et al., 1971; Wong, 1979).

The increased interest in carbon dioxide concentration effects on field crops arises, in part, from the fact that the atmospheric carbon dioxide concentration is increasing. Largely because of the burning of fossil fuels, and to a lesser extent because of deforestation of tropical rainforests, the present carbon dioxide concentration of approximately 340 ul l⁻¹ is expected to double by the early or middle part of the next century (Bach, 1980). Carbon dioxide derived from fossil fuels amounted to 0.1 Gt in 1860 and is now 5 Gt per year (Bach, 1980) (1Gt=10⁹ton). Deforestation influences atmospheric carbon dioxide concentration by reducing the capacity of the biosphere to absorb carbon (through photosynthesis), and also directly contributes carbon dioxide to the atmosphere by the oxidation of soil organic matter in cleared land. A balance sheet of carbon input (via photosynthesis) and carbon output (via respiration) for the biosphere suggests that terrestrial ecosystems, on a global scale, appear to be acting as a net source of carbon.
The oceans, on the other hand, are a major sink for both organic and inorganic forms of carbon (the latter as dissolved carbon dioxide and carbonates). The present day atmospheric carbon dioxide concentration is increasing because source output exceeds the capacity of sinks to absorb the carbon.

In addition to the possible effects of increased atmospheric carbon dioxide concentration on plant growth and physiology, and hence, agricultural productivity, increasing carbon dioxide may also indirectly affect global agriculture. Dramatic shifts in climatic and agricultural zones are expected to occur because of the predicted warming of the earth's atmosphere by 2 to 3.5 C for a doubling of carbon dioxide concentration (see Hansen et al., 1981), the so-called "greenhouse effect".

An increasing atmospheric carbon dioxide concentration is a reality of our time. For this reason alone, it is important to gain an understanding of how living organisms respond to long-term exposure to elevated carbon dioxide concentrations. We are already benefiting from such studies in terms of increased crop production in greenhouses enriched with carbon dioxide. Equally important is the insight to be gained from those studies in understanding how plants function.

The work reported in this thesis deals with the effects of long-term enrichment with carbon dioxide on three separate, but interrelated, aspects of plant function. In Chapter 3, investigations of the growth of plants and the partitioning of
carbon among plant parts as affected by continuous carbon dioxide enrichment will be reported. Those investigations detail the growth response of bush beans over the course of development from a very young vegetative growth stage, to the near-completion of reproductive growth. Growth analysis was used to measure plant growth response to 6 carbon dioxide concentrations ranging from 340 to 3000 ul l⁻¹.

Chapter 4 describes the physiology of carbon dioxide exchange in leaves from bean plants grown at different carbon dioxide concentrations. Since photosynthesis is such a fundamental aspect of plant growth and development, and is known to respond to carbon dioxide concentration, it was pertinent to compare gas exchange characteristics of plants grown in an enriched carbon dioxide atmosphere with those grown in unenriched conditions. The conditions and circumstances of the relative changes in gas exchange characteristics of enriched and control plants were examined.

Lastly, during the course of this study, the observation was made that carbon dioxide enrichment caused extensive chlorosis of specific leaves. Chapter 5 deals with some environmental and physiological aspects of leaf senescence in enriched plants. This aspect of carbon dioxide enrichment has not been previously studied in a rigorous manner. As a first step, it was relevant to determine the environmental conditions under which this effect of high carbon dioxide concentration occurred.
II. LITERATURE REVIEW

2.1 PLANT RESPONSE TO CARBON DIOXIDE ENRICHMENT

2.1.1 Historical Perspective

Over three hundred years ago, Jean-Baptiste van Helmont conducted what would become a milestone experiment in the history of plant science. He found that a willow tree growing for 5 years in 200 pounds of soil increased in weight 164 pounds while soil weight changed negligibly. He concluded that the weight gain derived from water taken up from the soil and that the soil itself contributed very little to plant dry matter. Stephen Hales in 1727 surmised that some of the weight gain derived from the air. This idea was developed by Senebier and Ingenhousz (Rabinowitch and Govindjee, 1969); the component of the air which plants incorporate was described in terms of the new chemistry of Lavousier as carbon dioxide (Rabinowitch and Govindjee, 1969).

Brown and Escombe (1902) were probably the first to explore the effects of higher than ambient concentrations of carbon dioxide on plants. They subjected several horticultural species to 1147 ul l⁻¹ carbon dioxide and found some rather dramatic negative effects. Plants responded to carbon dioxide by shedding flower buds prematurely, and by the shedding and curling of leaves. According to Demoussey (see Wittwer and Robb, 1964) this was perhaps due to impurities in the air. In 1902-04, using a different technique to produce carbon dioxide, Demoussey (see Wittwer and Robb, 1964) found large increases in
plant dry weight varying from 97% for fuchsia to 262% for geranium, at high carbon dioxide concentrations.

By the 1920's and 30's, work was being done with increasing emphasis on the uses of carbon dioxide in glasshouse crop production (White, 1930; Small and White, 1930; Bolas and Melville, 1935).

Work of the 1950's and 60's was spurred by the classic studies by Gaastra (1963) and by Waggoner, Moss and Hesketh (1963) into the effects of carbon dioxide concentration on the net rate of photosynthesis.

To date, a great deal of information has become available describing the effects of carbon dioxide on photosynthesis and the effects of carbon dioxide enrichment on plant productivity. However, relatively little is known about the physiology of plants grown in carbon dioxide enriched atmospheres.

2.1.2 Carbon Dioxide Enrichment And Plant Growth

In view of the central role of carbon dioxide in photosynthesis, and hence dry matter production, numerous studies have been undertaken to describe the effects of carbon dioxide enrichment on yield of plants. Much of that work has been done in relation to enrichment of greenhouse crops because of the potential benefits of commercial carbon dioxide applications. Work on tomato (Wittwer and Robb, 1964; Madsen, 1973a, 1973b, 1974; Calvert and Slack, 1976; Hicklenton and Jolliffe, 1978), lettuce (Wittwer and Robb, 1964), and cucumber (Aoki and Yabuki, 1977) has been done on the effect of carbon dioxide enrichment on dry weight accumulation. Similarly, field
crops such as soybean (Hardman and Brun, 1971; Mauney et al., 1978), cotton (Mauney et al., 1978; Wong, 1979), sugar beet (Wyse, 1980), wheat (Gifford, 1977; Sionit et al., 1981a), corn (Wong, 1979), and rice (Imai and Murata, 1976, 1977, 1978b) have been studied.

Both total dry weight and agronomic yield are usually increased by carbon dioxide enrichment. Following are a few examples. Increases in tomato dry weight of 100% occurred after 42 days in 800 to 2000 ul l⁻¹ carbon dioxide (Wittwer and Robb, 1964). Yields were increased 29% to 71% and the quality of fruit was improved. Hicklenton and Jolliffe (1978) also found tomatoes grown at 900 ul l⁻¹ carbon dioxide produced 30% more marketable fruit. In sugar beet, dry weight increased 180% in high carbon dioxide (Wyse, 1980). Enrichment to 500 ul l⁻¹ increased dry weight 32% in wheat; a decrease in carbon dioxide to 150 ul l⁻¹ reduced dry weight 43% (Gifford, 1977). Grain yield increased 43% and decreased 44% in these two treatments respectively. Cotton showed a 2-fold increase in dry weight after 40 days at 640 ul l⁻¹ carbon dioxide while corn showed only a 20% increase at the same level of enrichment (Wong, 1979).

In addition to total yield and reproductive yield, various other features of plant growth have been analysed in relation to atmospheric carbon dioxide enrichment.

Relative growth rate (R) increased in response to carbon dioxide enrichment in cotton (Mauney et al., 1978), wheat (Neales and Nicholls, 1978), and tomato (Hicklenton and Jolliffe, 1978).
Unit leaf rate (E) also increases as a result of carbon dioxide enrichment. Increases have been observed in cucumber (Aoki and Yabuki, 1977), tomato (Hicklenton and Jolliffe, 1978), soybean (Patterson and Flint, 1980, 1982; Gustafson and Breen, 1982), wheat (Neales and Nicholls, 1978), cotton (Wong, 1979), sorghum (Mauney et al., 1978), sunflower (Mauney et al., 1978), and Desmodium (Wulff and Strain, 1982). In one instance, E was found to decrease in enriched plants (Raper and Peedin, 1978). As will be discussed in a later section, this latter case is probably a function of plant age or the duration of treatment. There is generally a good correlation between the effects of carbon dioxide enrichment on unit leaf rate and relative growth rate. (Aoki and Yabuki, 1977; Neales and Nicholls, 1978; Patterson and Flint, 1980, 1982; Gustafson and Breen, 1982; Wulff and Strain, 1982). However, it must be kept in mind that since R is a function of both E and leaf area ratio (F), an increase in E at high carbon dioxide concentration does not necessarily translate into a proportionately greater R; changes in leaf area must also be considered. For example, Hurd (1968) found that enriched tomatoes initially showed increased R and E, but subsequently showed no difference in R with respect to controls, despite a continually greater E. Here, R was being influenced by a decreasing F.

An increase in total leaf area per plant due to enrichment (Ford and Thorne, 1967; Mauney et al., 1978; Wong, 1979; Wyse, 1980; Patterson and Flint, 1982; Wulff and Strain, 1982) and leaf number per plant (Ford and Thorne, 1967; Gifford, 1977)
reflects a change in absolute growth rate, and as such is not a reliable measure of the leaf area in relation to the size of the plant. Leaf area ratio is a better estimate, and is found to decrease in response to enrichment (Ford and Thorne, 1967; Hughes and Freeman, 1967; Tognoni et al., 1967; Neales and Nicholls, 1978; Patterson and Flint, 1982; Wulff and Strain, 1982).

There is much evidence then, that carbon dioxide enrichment results in an increase in total dry weight, R, and E. However, plant responses to enrichment are somewhat more complex than would be anticipated based on this information alone. Some important additional factors will now be considered.

2.1.3 Duration Of Enrichment

There is some evidence that the length of time the plant is exposed to elevated carbon dioxide affects the response. For example, growth of cucumber under carbon dioxide enrichment was 1.5 to 1.8 times greater than that of controls (Aoki and Yabuki, 1977), but as the treatment duration increased, the most effective concentration for growth decreased. Also, rates of net carbon dioxide assimilation at the higher carbon dioxide concentrations (2400 and 5500 ul l⁻¹) decreased to levels below that of controls. Because older leaves experienced a longer period of enrichment than younger leaves, it is reasonable to expect that the older leaves showed the major decrease in net assimilation.

The daily duration of enrichment also affects the growth response. Tomatoes showed increases in yield roughly
proportional to the increase in enrichment time per day (Calvert and Slack, 1976).

2.1.4 Interaction With Plant Age

Plant response to enrichment is influenced by developmental stage. R and E were higher during the juvenile stage (10 to 30 days) in enriched cotton, soybean, sunflower and sorghum (Mauney et al., 1978), but were similar to control plants at the reproductive stage. Sunflower and soybean had a decreased E relative to controls later, during the maturation stage of development, but still maintained greater total dry weight after 100 days of enrichment (due largely to increases in leaf area).

Because the effect of enrichment is influenced by the duration of exposure, it is difficult to separate plant age effects from duration effects in many of the cited studies. In a more definitive study by Neales and Nicholls (1978), wheat plants of various ages were exposed to high carbon dioxide concentrations for the same length of time. R and E of 10 day old plants were increased 35% and 55% respectively, but were reduced 44 and 16% respectively in 24 day old plants relative to controls (Neales and Nicholls, 1978). Although this seems to indicate that young plants responded better to enrichment, Hardman and Brun (1971) found no lasting effects of enrichment administered during the preflowering stage on the mature plant. Only enrichment during the flowering and post-flowering stages had any effect on dry weight or vegetative and reproductive characters. Krizek et al. (1971) however, found that the stimulatory effects of the enrichment of crabapple seedlings for
4 weeks persisted for 2 to 3 months after transfer to ambient carbon dioxide.

2.1.5 Interaction With Light Intensity

Carbon dioxide enrichment increased plant growth and unit leaf rate in greenhouse tomatoes in mid-winter when light intensity was low (Wittwer and Robb, 1964). Hopen and Ries (1962) found that high carbon dioxide could compensate for low light intensity in maintaining growth of cucumber. Similarly, Hurd (1968) showed that a combination of high carbon dioxide/low light produced the same growth as low carbon dioxide/high light.

The magnitude of the response to enrichment is dependent on the light intensity during growth. Hopen and Ries (1962), Ford and Thorne (1967), and Hughes and Cockshull (1971) found that enrichment had a progressively greater effect on growth as light intensity increased. However, others have found a greater stimulation of growth by carbon dioxide under conditions of low light than high light (Macdowall, 1972; Gifford, 1977). Macdowall interpreted this as indicating that carbon dioxide increased the efficiency of net photosynthesis (which would give greater net photosynthesis than controls under limiting light), but had little or no effect on the capacity of photosynthesis (no different than controls at light saturation). However, he did not consider the fact that at elevated carbon dioxide, a normal light response curve for C3 plants would exhibit greater efficiency and greater capacity. Therefore, if plants grown at high carbon dioxide show the same photosynthetic capacity as controls when measured under growth carbon dioxide
concentrations, they probably have depressed photosynthetic capacity if compared to control plants under the same measuring conditions of light intensity and carbon dioxide concentration.

2.1.6 Interaction With Nutrient Regime

Although an increased nutrient level generally increases dry matter production, its influence on plant response to carbon dioxide enrichment is equivocal, and may depend on species. Rice (Imai and Murata, 1978a) and cotton (Wong, 1979) showed proportionately greater dry weight accumulation in response to carbon dioxide at higher nitrogen levels; corn (Wong, 1979), wheat (Sionit et al., 1981a), and soybean (Patterson and Flint, 1982) did not show clear increases in response to nitrogen.

The increase in E due to carbon dioxide enrichment shows no dependence on nitrogen level in rice or Japanese millet (Imai and Murata, 1978a), a negative relationship with nutrient level in soybean (Patterson and Flint, 1982), and a positive relationship with nitrogen level in cotton and corn (Wong, 1979).

There are several other factors which account for a proportionately greater increase in dry weight due to enrichment at higher nutrient levels. Nutrient regime also affects the response of leaf area duration (LAD) to carbon dioxide, increasing it in soybean (Patterson and Flint, 1982) and to some extent in wheat (Sionit et al., 1981a). An increased LAD in soybean at high nutrient and carbon dioxide levels compensated for a decreased E and contributed to greater dry weight accumulation (Patterson and Flint, 1982). In rice, despite no
proportional change in $E$ with nutrient level, tillering was promoted in enriched plants at high nutrient levels, and thereby contributed to proportionally greater dry weight increase (Imai and Murata, 1976).

2.1.7 Effects On Partitioning Of Assimilates

Carbon dioxide enrichment has a variety of effects on plant development, due in part to changing patterns of assimilate partitioning. Many of the changes in the growth of plants or plant parts are a result of an increase in absolute and relative growth rates, and do not necessarily reflect changes in partitioning. However, some growth parameters do respond differentially to carbon dioxide. For example, decreases in $F$ have already been mentioned. LAD is increased by enrichment in soybean (Patterson and Flint, 1982), wheat (Sionit et al., 1981a), and Desmodium (Wulff and Strain, 1982). Specific leaf area (SLA) is decreased at high carbon dioxide concentrations (Ford and Thorne, 1967; Hurd, 1968; Neales and Nicholls, 1978; Wulff and Strain, 1982), indicating that a greater proportion of fixed carbon is retained in the leaves under conditions of high carbon dioxide concentrations. High carbon dioxide concentrations generally result in an increase in tillering in cereals (Ford and Thorne, 1967; Imai and Murata, 1976; Gifford, 1977; Neales and Nicholls, 1978). A greater proportion of assimilate is also directed to root growth as shown by an increased root/shoot ratio (Ford and Thorne, 1967; Kriedemann et al., 1976, Tognoni, et al., 1967; Wittwer, 1967; Wyse, 1980).

In legumes, root nodules are a major sink for
photosynthetic metabolites, and availability of photosynthate is a major factor in determining rates of nitrogen fixation. Carbon dioxide enrichment increased the total nitrogen fixed and the percentage of nitrogen derived from atmospheric nitrogen in soybean and pea (Hardy and Havelka, 1977), which resulted from an increased nodule mass and delayed nodule senescence. In addition, the specific nodule activity was increased. Phillips et al. (1976) found an increase in specific nodule activity only after short term carbon dioxide enrichment (6 hours), but not after long term enrichment (4 weeks). They concluded that a short term increase in current photosynthesis through a short term increase in carbon dioxide concentration increased nitrogen fixation by affecting nodule functioning, and that long term carbon dioxide treatments increased nitrogen fixation by increasing nodule mass. However, Finn and Brun (1982) found no increase in specific nodule activity in short or long term carbon dioxide treatments of soybeans.

The fact that carbon dioxide causes an increase in nitrogen fixation over the long term through an increase in nodule mass does not suggest, however, that partitioning of assimilates to nodules has been affected by enrichment. In this respect, Phillips (1976) found that carbon dioxide did not alter this partitioning.
2.1.8 Response Of Photosynthetic Capacity

Net photosynthesis of plants may increase during exposure to high carbon dioxide concentration, but as mentioned previously, the effect is not consistent through age, developmental stage, or duration of exposure. Growth analysis yields information on E over daily or even weekly periods, but is not well suited for determining specific characteristics of photosynthesis. Rather, its importance lies in its ability to describe growth characteristics (for example, R, E, and F) which apply to the plant over extended periods. However, despite these limitations, Neales and Nicholls (1978) used growth analysis data to calculate that an increase in growth due to carbon dioxide enrichment should lead to much higher increments in dry weight than were actually observed. This would require a sustained increase in R, which in fact did not occur. R diminished with time because of a decrease in F; a concurrent increase in SLW was also suggested to contribute to a failure to maintain full photosynthetic potential by decreasing leaf assimilation rates. The relationship between SLW and net carbon dioxide exchange rate (CER), particularly as affected by starch content, will be discussed more fully in a later section.

Gas exchange analysis, while lacking the integrated approach of growth analysis, allows measurements of photosynthetic efficiency and capacity. It can describe net photosynthesis in terms of its component processes. Although E may increase due to enrichment, simply because of the greater carbon dioxide concentration available as substrate for
photosynthesis, this does not reflect the innate photosynthetic capacity of those plants compared to unenriched plants. To estimate relative capacities, photosynthesis must be measured under the same conditions of carbon dioxide, light, and temperature. Hence, in my study, carbon dioxide exchange capacity is defined as the ability of enriched plants to carry out net carbon dioxide exchange compared to controls when measured under the same conditions of carbon dioxide concentration, photosynthetic photon flux density (PPFD), and temperature.

Gas exchange measurements made at the same concentrations of carbon dioxide show decreased net photosynthesis per unit leaf area in enriched plants (Ho, 1977; Mauney et al., 1979; Clough et al., 1981; Wulff and Strain, 1982). This indicates a reduced photosynthetic capacity in enriched plants. The reason for the decrease has been attributed to stomatal closure in rice (Imai and Murata, 1978c). However, this is not the only possible explanation. Aoki and Yabuki (1977) found a duration-dependent increase in stomatal resistance and a decrease in mesophyll resistance in enriched cucumber. Similarly, Hofstra and Hesketh (1975) showed reduced rates of photosynthesis in soybeans, particularly in older leaves, which could also be a function of duration of exposure. Frydrych (1976) found a reduced photosynthetic capacity in enriched cucumber, but only when measured at high light intensity. However, the measurements made at low light intensity (47.5 W m$^{-2}$) were probably light-limited and differences in photosynthetic
capacity would not be expected. Wulff and Strain (1982), on the other hand, found reduced photosynthesis at all light intensities up to 1300 umol m$^{-2}$ s$^{-1}$. This is probably due to reduced chlorophyll levels in enriched plants. In fact, if photosynthesis was plotted versus chlorophyll content, enriched plants showed higher rather than lower photosynthetic capacity, perhaps due to changes in photosynthetic unit size. Clough et al. (1981) compared photosynthetic rates of podded (high sink) and depodded (low sink) soybeans under two carbon dioxide regimes. Low sink plants had lower photosynthetic rates than high sink plants and low carbon dioxide treatments had higher rates than high carbon dioxide treatments, when measured under the same carbon dioxide concentration. Kriedemann et al. (1976) found that enriched *Vitis* plants had reduced photosynthesis rates at low measurement concentrations of carbon dioxide, relative to controls, but higher rates at high measurement concentrations. This unusual result can perhaps be explained by referring to the leaf anatomy of *Vitis*. Enriched leaves were thicker. Hence, the decreased rate of photosynthesis in enriched plants was probably not due to a reduced internal surface area, but due to a reduced capacity to photosynthesize. How then does one explain greater photosynthesis at higher carbon dioxide concentrations? The authors suggested that because grape leaves were hypostomatous and enriched leaves were thicker, carbon dioxide at low concentrations could be proportionately more limiting in enriched leaves in regions furthest away from the stomata. At
high carbon dioxide concentrations, those regions were no longer limited by carbon dioxide, and contributed proportionately more to net photosynthesis per unit leaf area. Photosynthetic capacity appeared to increase, but was in fact still less than controls.

Most investigations have therefore found that there appears to be a reduction in photosynthetic capacity in enriched plants, the extent of which is dependent on various developmental and environmental factors. Several authors, in fact, have found no effect of high carbon dioxide concentration on photosynthetic capacity (Ford and Thorne, 1967; Gifford, 1977), but this does not preclude the possibility of an effect occurring under different conditions or at a different age.

Somewhat more difficult to reconcile is the work of several authors (Bishop and Whittingham, 1968; Madsen, 1975; Hicklenton and Jolliffe, 1980b) who found that tomato plants grown in enriched atmospheres exhibited an increase in photosynthetic capacity. Hicklenton and Jolliffe (1980b) have provided the most detailed study to date. The carbon dioxide effect on photosynthesis was not caused by stomatal responses. Rather, the photosynthetic machinery per se was being affected. Enrichment caused a decrease in both the carbon dioxide and light compensation points, greater net photosynthesis at a given light intensity and carbon dioxide concentration, and decreased oxygen inhibition of net photosynthesis.

How does one explain an increase in photosynthetic capacity in some studies of tomato, and a decrease in capacity in
virtually all other species studied? It is not because tomato responds differently to enrichment; Ho (1977) found decreased photosynthetic capacity in this species. It may relate to the environmental conditions during growth. Growth temperature, however, does not seem to be a factor. Enriched tomato plants grown at a relatively high temperature of 30 to 35 °C (Hicklenton and Jolliffe, 1980b), or at a lower temperature of 18 °C (Bishop and Whittingham, 1968) both show an increase in photosynthetic capacity. Hence, yet other environmental or developmental conditions may be required to elicit this response.

Changes in photosynthetic capacity due to high carbon dioxide concentration correlate with changes in several photosynthetic enzymes. A decrease in capacity correlates with decreased RuBP carboxylase activity (Kriedemann et al., 1976; Wong 1979), and an increase in capacity coincides with increased activity of this enzyme (Hicklenton and Jolliffe, 1980b). RuBP carboxylase was also reported to increase in enriched barley leaves (Fair et al., 1973) although photosynthesis rates were not measured. Glycolate oxidase activity has been reported to decrease at high carbon dioxide concentration (Fair et al., 1973; Hicklenton and Jolliffe, 1980b), which may account for the reduced photorespiration in enriched plants (Hicklenton and Jolliffe, 1980b). Also, a lower nitrate reductase activity has been shown to occur (Fair et al., 1973; Kriedemann, et al., 1976), which could also contribute to lower photorespiration.

In a study of root zone enrichment of potato (Arteca and Poovaiah, 1982), PEP carboxylase activity of roots increased,
but both PEP carboxylase and RuBP carboxylase activity of leaves remained unchanged.

Carbon dioxide enrichment could also influence photosynthesis rates through changes in chlorophyll content. Madsen (1968) found no effect on chlorophyll content per unit leaf area but showed lower chlorophyll content per unit fresh weight in enriched plants. Cave et al. (1981) found a lower mean chlorophyll content per unit leaf area in both immature and mature leaves of enriched *Trifolium subterraneum*, but this response was not statistically significant. Chlorophyll content per unit dry weight was significantly lower in enriched immature leaves, but not significantly lower in mature leaves. Similarly, chlorophyll content in enriched cotton was not significantly lower than controls (Wong, 1979). In soybean, sicklepod, and showy crotalaria examined at two nutrient levels, chlorophyll content was generally, although not always, significantly lower in enriched plants (Patterson and Flint, 1982).

Hence, enrichment appears to cause a somewhat lower chlorophyll content, although the difference may not always be substantial. A significant decrease in the chlorophyll a:b ratio in enriched plants (Cave et al., 1981) suggests that a change in the composition of the photosynthetic pigment complex occurs.
2.1.9 Leaf Morphology

A number of studies have explored the developmental aspects of carbon dioxide enrichment. In addition to the changes in plant growth and development which have already been discussed, changes in leaf structure are also known to occur.

The number of stomata per unit leaf area has been shown to decrease in tomato (Madsen, 1973b), in response to carbon dioxide enrichment, but is not affected in barley, kale, or maize (Ford and Thorne, 1967).

Generally leaf thickness increases in response to high carbon dioxide concentration (Madsen, 1973b; Hofstra and Hesketh, 1975; Kriedemann et al., 1976), which could have an effect on photosynthetic measurements as previously discussed. The thickness of the palisade layer increases due to increases in cell volume in enriched tomato (Madsen, 1973) and cell number in soybean (Hofstra and Hesketh, 1975).

2.1.10 Leaf Carbohydrates

There are numerous reports of increases in starch content in leaves of enriched plants (Madsen, 1968; Hofstra and Hesketh, 1975; Ho, 1977; Mauney et al., 1979; Cave et al. 1981; Finn and Brun, 1982). These differences are great enough to be seen in light micrographs (Hofstra and Hesketh, 1975; Kriedemann et al., 1976) or in electron micrographs of chloroplasts, where extremely large starch deposits can be seen to disrupt normal grana structure (Cave et al., 1981; Wulff and Strain, 1982). These dramatic changes in starch content presumably are a major
contributor to the increases in SLW that are observed in enriched plants.

Sucrose concentration tended to increase in enriched tomato leaves (Bishop and Whittingham, 1968; Madsen, 1968; Ho, 1977). However, total soluble sugars (including sucrose) decreased in enriched soybean (Hofstra and Hesketh, 1975) and were unaffected in cotton, soybean, sunflower, and sorghum according to Mauney et al. (1979).

2.2 SENESCEENCE

Because of the interesting observation made during the course of my study that high carbon dioxide concentrations appeared to promote leaf senescence, an overview of senescence physiology is provided as background for the reader.

Senescence can be characterized as a collection of deteriorative processes which culminate in death (Nooden and Leopold, 1978). Senescence may occur at virtually any level of organization, from individual cells, to tissues, to the entire organism. Whether responding to environmental or intrinsic signals, senescence is always a highly regulated and directed sequence of events. In many, but not all cases, senescence is related to aging, which may be defined as a gradual accumulation of physiological changes with time (Nooden and Leopold, 1978). Neither aging nor senescence in plants seems to be due to the accumulation of errors in transcription, as suggested in work on animal senescence. For example, leaves may undergo complete senescence in less than 4 days. Conversely, tissue from annual plants can be cultured for years, when, if left on the plant,
would senesce after one flowering season (monocarpic senescence). Aging need not always result in death, or *vice versa*. Monocarpic senescence for example, is not due to aging as defined previously.

### 2.2.1 General Physiology

A number of characteristic physiological changes are associated with leaf senescence. Although most of these are certainly not a cause of senescence, they can be useful indicators of the extent and kinetics of the process.

Chlorophyll loss, one of the most dramatic and visible changes to occur, is frequently used as a measure of senescence. This generally correlates with a decrease in photosynthetic rate. However, a decline in photosynthesis may precede a loss of chlorophyll (Hardwick *et al*., 1968), and in a mutant of fescue, senescence, measured as protein breakdown, occurs without chlorophyll degradation (Thomas and Stoddart, 1975). Loss of chlorophyll need not then be closely associated with the ongoing process of senescence.

Decline in photosynthesis is a major initial event in senescence, although the primary events causing photosynthetic reduction are not known. The decline is accompanied by the loss of RuBP carboxylase (Kannangara and Woolhouse, 1968; Friedrich and Huffaker, 1980) and some other enzymes of photosynthetic carbon assimilation (Batt and Woolhouse, 1975). Wittenbach *et al*. (1980) however, found that the initial decline in photosynthesis was not due to a decrease in RuBP carboxylase activity or level. Rather, the decline was preceded by a
swelling of chloroplasts and disruption of thylakoids, which could result from an observed increase in starch deposition. Large numbers of osmiophilic granules also appear in the chloroplasts, as has also been observed by Barton (1966).

Mitochondria on the other hand, remain structurally intact until the final stages of senescence (Rhodes, 1980). Respiration rate generally does not change until late in senescence, when it decreases rapidly (Rhodes, 1980). A transient rise in respiration, particularly in detached leaves has been reported (Hardwick et al., 1968; Tetley and Thimann, 1974), which may result from an increase in respiration of amino acids, or an uncoupling of respiration (Tetley and Thimann, 1974). The fact that respiration remains relatively high throughout most of senescence suggests that it may be needed in energy production for the synthesis of degradative enzymes (Rhodes, 1980).

Protein composition changes dramatically during senescence. Proteolysis serves the dual function of turning off synthesis of unneeded metabolites, as well as liberating amino acids for export to non-senescent plant parts. Not all proteins decrease however. Degradative enzymes such as RNase and proteolytic enzymes increase during senescence (Matile and Winkenbach, 1971). RNase is responsible for the decrease in RNA and ribosomes observed during senescence (Thimann, 1980).
2.2.2 Growth Regulators

As in virtually every other aspect of plant development, growth regulators are involved in, or at least affect, leaf senescence. The most effective growth regulator to delay senescence is cytokinin and its synthetic analogs. Kinetin effectively delays chlorophyll loss and proteolysis in the dark, and to a lesser extent in the light. If applied to a small area of a leaf, it delays senescence in only that locality, and induces transport of solutes from non-treated areas (Thimann, 1980). Kinetin has its most pronounced effect on detached leaf tissue, with little or no effect on an intact system. The lack of effect in intact systems is perhaps due to high endogenous levels of cytokinin already in the tissue, perhaps transported from the roots. Kinetin also has the ability to induce regreening after senescence has been initiated (Nooden and Leopold, 1978). This is similar to the delaying effects of debudding or decapitation on leaf senescence, suggesting that a redistribution of cytokinin occurs when sinks for the hormone are removed.

Abscisic acid (AbA) is known to induce senescence, or at least promote the various primary or secondary physiological processes associated with it. As is the case with cytokinin, exogenous AbA has its greatest effect on detached tissue. Endogenous levels of AbA increase during senescence. For example, water deficits, which are known to induce senescence, also cause higher AbA levels (see Nooden and Leopold, 1978).

The other major groups of plant hormones do not have the
clearly defined effects of AbA and cytokinin. Auxins have only a small effect in delaying senescence, and gibberellic acids are effective in only a few species, for example, *Taraxacum* and *Rumex*. The relationships between gibberellic acids and cytokinin-induced delay of senescence are not clear.

Although ethylene is clearly involved in promoting flower senescence and leaf abscission (Abeles 1973), its role in leaf senescence is equivocal. Conflicting interpretations may arise if the responsiveness of the tissue to ethylene varies in such a way that it responds at one age or under one set of conditions but not another. For example, ethylene synthesis may increase, but if the tissue is not responsive, the observed increase is of little consequence. One must therefore be cognizant of the distinction between ethylene level and ethylene action. Ethylene has been observed to increase during leaf senescence (see Thimann, 1980). However, levels of ethylene in senescing leaves of beans and *Xanthium* are the same as that of mature, non-senescing leaves (see Osborne, 1978). Work of Aharoni and Lieberman (1979), however, supports the idea of a role of ethylene in senescence. Although ethylene release is actually decreased during the initial stages of senescence in the dark, inhibitors of ethylene synthesis, such as Ag+ and aminoethoxy vinyl glyoxime delay chlorophyll loss.

Carbon dioxide is known to antagonize ethylene action in many higher plants (Abeles, 1973), although the concentrations needed are relatively high (>10,000 ul l⁻¹). High carbon dioxide concentration (5000 ul l⁻¹) is also known to accelerate
ethylene release from leaf tissue, although the effect is only temporary (Bassi and Spencer, 1982). It is now generally believed that carbon dioxide antagonizes ethylene action by displacing ethylene from its binding site.

Very few studies have looked at the interaction of carbon dioxide and ethylene in senescence. Aharoni and Lieberman (1979) found that 50,000 to 10,000 ul l⁻¹ carbon dioxide stimulated ethylene production by tobacco leaf disks senescing in the dark, but in fact delayed senescence.

2.2.3 Environmental Influences

Leaf senescence can be initiated by a number of environmental signals, which in some cases at least, are related to changes in levels of growth regulators. Leaf senescence, for example, is accelerated by low leaf water potentials (see Thimann, 1980), which also cause an increase in leaf ABA content (Wright and Hiron, 1969). Interestingly, Ludlow (1975) found that low water potentials appeared to suspend the aging process in those leaves of Panicum which did not die from the direct effects of water stress. It is also possible that repeated cycles of low water potential may render young leaves less susceptible to the stress-inducing conditions (see Thomas and Stoddart, 1980).

Temperature extremes are also known to initiate leaf chlorosis, presumably as a result of metabolic damage (Thomas and Stoddart, 1980). Cool temperature may interact with light intensity to bring about accelerated senescence (Taylor and Craig, 1971).
Light is generally known to delay senescence of leaf tissue (Goldthwaite and Laetsch, 1967; Thomas and Stoddart, 1980) and is thought to act through effects on photosynthesis.

Mineral deficiency is a major cause of leaf senescence; often low nutrient availability will result in mobile nutrients being transported from the older leaves. This may be one reason why senescence symptoms are first evident in those leaves.

In conclusion, it seems that, unlike monocarpic senescence, which is initiated by a developmental program, senescence induced by the environment is, in large part, a function of tissue susceptibility. Susceptibility of leaves to a stress seems to depend on such factors as leaf age, position on the plant, and previous stress history.

2.2.4 Carbon Dioxide And Senescence

The prospect of carbon dioxide acting as a senescence-inducing agent in plants has received very little attention, even though as early as 1902, negative effects of carbon dioxide enrichment were observed in plants (Brown and Escombe, 1902). This was later ascribed to impurities in the air.

In more recent times, leaves of enriched cucumbers have been observed to have yellow spotting, eventually followed by necrosis at carbon dioxide concentrations above 1000 ul l⁻¹ (Wittwer, 1967). Hesketh et al. (1971) found that leaves of enriched cotton and soybean plants became very chlorotic. This was associated with large starch accumulations in the leaves. Chlorosis occurred at 31°C but not 35°C. In a paper relating
carbon dioxide enrichment to senescence of cotton leaves, Chang (1975) found that 1000 ul l⁻¹ carbon dioxide caused decreased chlorophyll and protein levels after 30 days of enrichment. A decrease in photophosphorylation and Hill reaction activity also occurred when measured in vitro in the presence of bicarbonate. Carbonic anhydrase, an enzyme catalyzing the hydration of carbon dioxide, also decreased in activity. It is not clear, however, whether those changes related to the cause of carbon dioxide-induced senescence.
III. EFFECTS ON GROWTH AND PARTITIONING

3.1 INTRODUCTION

Enhanced plant growth occurs at elevated carbon dioxide concentrations (eg. Wittwer and Robb, 1964). However, there is some indication that the full potential for increased growth, based on a simple model of increased substrate for net assimilation is not achieved (Neales and Nicholls, 1978). Furthermore, the degree of growth enhancement due to carbon dioxide enrichment is highly variable among species (Wong, 1979), over time (Aoki and Yabuki, 1977), developmental stage, (Mauney et al., 1978), and environmental conditions (Ford and Thorne, 1967).

As a first step in elucidating the physiological responses of beans to carbon dioxide enrichment, it was essential to describe the growth trends and relationships of the whole plant under controlled conditions. The primary objectives of the work reported in this chapter, therefore, were to answer the following questions:
1. When and to what degree does carbon dioxide enrichment have an effect on overall plant growth?
2. How is growth partitioned within plants in relation to carbon dioxide enrichment?
3. What factors are responsible for the changes in growth at different carbon dioxide levels? For example, how does net assimilation rate change in response to enrichment and is it correlated with changes in growth?
These questions can, in part, be answered using techniques of plant growth analysis. Various indices of plant performance, such as relative growth rate and unit leaf rate, as well as indices of partitioning such as leaf area ratio, and specific leaf weight, were used to establish the effect of long-term carbon dioxide enrichment on the growth of bean plants from the seedling to the reproductive stage. Note that a study of reproductive development per se was not intended, although the general features of flowering and pod formation were observed during the study.
3.2 MATERIALS AND METHODS

3.2.1 Plant Culture

Unless otherwise indicated, the following pre-treatment cultural procedures were used in all experiments described in this thesis. Three bush bean (Phaseolus vulgaris L. cv., Pure Gold Wax) plants were seeded per 5 inch plastic pot (soil volume was 0.85 l) in sandy loam soil, and were thinned to one plant per pot at 10 to 14 days after planting. During this period, plants were in a Percival (Model PG.78) growth chamber. The temperature regime was 26 C day/20 C night, photoperiod was 16 hours, and light intensity incident on the primary leaves (measured with a Li-Cor model LI-185 quantum meter) was 350 umol m\(^{-2}\) s\(^{-1}\) photosynthetic photon flux density (PPFD). Note that 1 mole m\(^{-2}\) s\(^{-1}\) is equal to 1 Einstein m\(^{-2}\) s\(^{-1}\), or Avogadro's Number of photons m\(^{-2}\) s\(^{-1}\). Plants were watered daily with tap water. At 10 to 14 days, the plants were transferred to specially constructed growth chambers for carbon dioxide treatment.

3.2.2 Carbon Dioxide Treatment Chamber Design

Conventional plant growth chambers do not permit easy control of carbon dioxide levels. Chambers are frequently designed as semi-closed systems, in which a feedback loop controls chamber carbon dioxide levels. In this situation, if the chamber air volume is large, it can be difficult to maintain a constant carbon dioxide concentration because of extended lag times in administering carbon dioxide. Furthermore, the planned
study of carbon dioxide enrichment effects required that many chambers be used simultaneously, and appropriate facilities were not initially available. Eight growth chambers were therefore built which were inexpensive, but provided easy maintenance of carbon dioxide levels.

The chambers were constructed in two sets of four each. In each set, four individual Plexiglas chambers, each 0.45m x 0.45m x 0.80m (L,W,H) and volume of 0.16 m$^3$, shared a common supporting framework, cooling system, and light source. The lighting system for each set consisted of a bank of 25 very high output (VHO) cool-white fluorescent tubes, and 13 60-watt incandescent bulbs, which was suspended 0.10 m above the top of the chambers.

Within each chamber, plants were placed on a movable platform, which also functioned as a heat exchanger to maintain chamber temperature (Fig. 3.1). The platform was suspended by a cable at each corner. The cables exited each chamber through one inch diameter ports in the chamber top and were secured to the chamber framework. Two-inch thick styrofoam sheets, which served as insulation, were positioned adjacent to the outside of the Plexiglas walls of each set of 4 chambers and were held in place with sheets of one quarter-inch plywood.

Chamber temperature was maintained by circulating coolant first through a refrigerated heat exchanger, and then through a secondary copper heat exchanger which comprised the matrix of the chamber platform. Temperature was regulated by adjusting the supply of coolant by means of valves. Chamber heating was
FIGURE 3.1 Schematic diagram of one of the eight carbon dioxide treatment chambers. See text for details.
unnecessary, since daytime temperatures of 38 C could be achieved simply through heat output from the lights when coolant flow was stopped. Chamber temperature was monitored with thermocouples connected to a digital thermometer (Omega, model 199).

The chambers were designed as open systems, with continuous, rapid turnover of air. The turnover time of chamber air was approximately 2 minutes. Fresh ambient air was pulled in from outside the building through galvanized zinc ducting by means of an intake fan (Torin, model TA300) in the bottom of each chamber. Air exited from each chamber through the four ports in the top (through which the platform cables also passed). In addition to the intake fan, four circulating fans (Torin, model TA300) were suspended from each platform and mixed the chamber air. According to the manufacturer's specifications, the rated output of each fan was 1250 l min\(^{-1}\) against zero static pressure. Flow rate through each chamber was 160 l min\(^{-1}\), as measured with a hot wire anemometer (either Datametrics, model 800-VTP, or Weather Measure Corp., model W-141-A1/5). For each measurement, the air velocity through each port was determined in feet per minute, and converted to a volume of air using the following equation:

\[
SCFH = 0.327 \times (FPM)(D^2)
\]

where: SCFH = standard cubic feet per hour

(1 SCFH = 7.8 ml s\(^{-1}\))

\[D = \text{port diameter in inches}\]
FPM = feet per minute (1FPM = 5.08 mm s⁻¹)

Pure carbon dioxide from compressed gas cylinders was bled into each chamber through a Plexiglas tube above the intake fan, which mixed the carbon dioxide with ambient air. The rate of carbon dioxide addition was regulated with flowmeters (Matheson 603 or 610). The carbon dioxide concentration in the air leaving the exit ports was monitored with an infra-red gas analyser (Beckman, model 864). The chamber carbon dioxide concentrations were 340±10, 500±10, 800±20, 1200±20, 2000±40, and 3000±40 ul 1⁻¹.

Over the course of the carbon dioxide treatment period, plants were maintained at 26/20 C day/night temperature, PPFD of 350 umol m⁻² s⁻¹ at the top of the plant canopy, and a photoperiod of 15 hours. Plants were watered twice weekly with half-strength Hoagland's solution and with distilled water when needed. When access to the plants was required, the chamber door was opened for, at most, 2 minutes.

3.2.3 Growth Analysis

For growth analysis, 4 replicate plants in each of the 6 carbon dioxide regimes were harvested 11 days after planting (time zero for enrichment), or at 22, 32, 42, or 55 days of continuous enrichment. Because of space limitations in the treatment chambers, the introduction and removal of plants in the chambers had to be staggered; enough plants for 2 complete harvests (i.e. 8 plants) were placed in the chambers at one
time.

At each harvest date, the area of each leaf was measured with a leaf area meter (Li-Cor model LI-3000), and individually recorded. Dry weights of roots, individual leaves, stems, and pods were determined after oven drying to constant weight at 150°C.

Primary data were log-transformed, and fitted curves describing their time trends were generated using a cubic spline regression procedure. This procedure (Reinsch, 1971) involved the Fortran subroutines DSPLFT and DSPLN available through the University of British Columbia Computing Centre. The procedure also generated fitted curves for the first derivative and the first derivative of the logarithm (i.e. the relative growth rate) of each variate. Smoothing of the spline regressions was determined entirely by the computer program according to the standard deviations of the variates at each harvest. The fitted curves were then used to generate the indices of plant growth defined below:

Relative Growth Rate: the rate of change in dry matter per unit of dry matter already existing. The instantaneous value was calculated as:

\[ R = \frac{1}{W} \frac{dW}{dT} = \frac{d(\ln W)}{dt} \]

Relative growth rate has units of dry weight\(^{-1}\) dry weight\(^{-1}\) time\(^{-1}\).

Leaf Area Ratio: the ratio of total leaf area to total plant dry weight. The instantaneous value was calculated as:
F = La/W

Leaf area ratio has units of leaf area \cdot dry weight\(^{-1}\).

**Unit Leaf Rate**: a measure of the rate of change of dry matter per unit leaf area. The instantaneous value was calculated as:

\[ E = \frac{1}{La} \frac{dW}{dT} \]

The units are dry weight \cdot leaf area\(^{-1}\) \cdot time\(^{-1}\). Unit leaf rate was calculated by dividing R by F.

**Specific Leaf Weight**: the ratio of leaf dry weight to leaf area. It was calculated for the entire plant or for each leaf individually. The instantaneous value was calculated as:

\[ SLW = \frac{Lw}{La} \]

Specific leaf weight has units of dry weight \cdot leaf area\(^{-1}\).

**Leaf weight ratio**: the ratio of leaf dry weight to total plant dry weight. The instantaneous value was calculated as:

\[ LWR = \frac{Lw}{W} \]

The ratio is dimensionless.

**Absolute growth rate**: the rate of change of a variable, such as total plant dry weight. The instantaneous value was calculated as:

\[ G = \frac{dW}{dt} \]

The units in this case are dry weight \cdot time\(^{-1}\).

Absolute growth rate is a function of leaf area times unit leaf rate. Relative growth rate, however, is a function of leaf
area ratio times unit leaf rate.

The experiment was repeated approximately 1 year after completion of the first trial. In the second trial, however, only carbon dioxide concentrations of 340, 500, 1200, and 3000 ul l⁻¹ were used. Analysis of variance was performed on the collective data of those 4 carbon dioxide treatments from both trials. The two trials were treated as two blocks in the analysis. A multifactorial analysis of variance package (MFAV), provided by the University of British Columbia Computing Centre, was used to perform the analysis.
3.3 RESULTS AND DISCUSSION

3.3.1 Effects On Growth

Because of significant block effects in the analysis of variance of total dry weight (Table 3.1), data of the two blocks were not pooled. Henceforth, for simplicity, only the data of the first block are presented; the primary data of the second block showed similar trends in treatment effects (Appendix A).

The primary data and fitted curves for leaf area are given in Figure 3.2. Differences in leaf area were most pronounced at the last harvest date, with enriched plants generally having more total leaf area. The differences among treatments in total dry weight (Fig. 3.3) were greater than the differences in leaf area and were also progressively greater with time. For example, plants grown at 1200 ul l\(^{-1}\) were 1.7 times larger than those at 340 ul l\(^{-1}\) (controls). Note, however, that an additional increase in carbon dioxide concentration to 3000 ul l\(^{-1}\) did not further increase dry weight. The growth response of beans, under the prevailing environmental conditions, appeared to have saturated by 1200 ul l\(^{-1}\).

Absolute growth rate (G) was also higher at elevated carbon dioxide concentrations (Fig. 3.4). Absolute growth rate also increased with time, in part a result of an enlarging total leaf area (Fig. 3.2). Relative growth rate (R), which normalizes growth rate on a unit total plant dry weight basis, decreased in all treatments for the first 19 days after transfer (Fig. 3.5). The relative growth rate of enriched plants, which was initially
TABLE 3.1. Analysis of variance of the collective dry weight data of trial 1 and trial 2.¹

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum Sq.</th>
<th>Mean Sq.</th>
<th>Error F</th>
<th>Required F²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block³</td>
<td>1</td>
<td>140.1</td>
<td>140.1</td>
<td>15.54</td>
<td>3.92</td>
</tr>
<tr>
<td>Harvest</td>
<td>3</td>
<td>9745.2</td>
<td>3248.4</td>
<td>360.37</td>
<td>2.68</td>
</tr>
<tr>
<td>Treat.</td>
<td>3</td>
<td>474.3</td>
<td>158.1</td>
<td>17.54</td>
<td>2.68</td>
</tr>
<tr>
<td>Error</td>
<td>120</td>
<td>1081.7</td>
<td>9.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>127</td>
<td>11441.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ The treatment carbon dioxide concentrations were 340, 500, 1200, and 3000 ul l⁻¹³
² At 5% level of probability
³ Trial 1 and trial 2 were treated as blocks
FIGURE 3.2 Leaf area over time of plants grown at 340, 500, 800, 1200, 2000, or 3000 ul l$^{-1}$ carbon dioxide. Each value is the mean of 4 plants, with standard deviation. Curves are cubic spline functions fitted to data for each carbon dioxide treatment.
FIGURE 3.3 Dry weight over time of plants grown at 340, 500, 800, 1200, 2000, or 3000 ul l⁻¹ carbon dioxide. Each value is the mean of 4 plants, with standard deviation. Curves are cubic spine functions fitted to data for each carbon dioxide treatment.
FIGURE 3.4 Absolute growth rate of dry weight over time of plants grown at 340, 500, 800, 1200, 2000, or 3000 μl l⁻¹ carbon dioxide. Absolute growth rate is expressed in units of g day⁻¹.
FIGURE 3.5 Relative growth rate over time of plants grown at 340, 500, 800, 1200, 2000, or 3000 \text{ul l}^{-1} \text{ carbon dioxide.} 
\text{R is expressed in units of g g}^{-1} \text{ day}^{-1}. 
higher than controls, decreased the most rapidly. By 22 days of age, enriched plants had approximately the same R as controls and this was maintained for the remainder of the experiment.

Relative growth rate is the product of unit leaf rate (E) and leaf area ratio (F), either one of which could change and thereby change R. Unit leaf rate was increased by enrichment during early development, with a maximum at about day 18 (Fig. 3.6). By day 30, it had decreased to nearly control rates in all treatments. The changes in E were similar to those of R after day 25, suggesting that changes in E contributed to the changes in R. This loss of effect of carbon dioxide enrichment on E after prolonged treatments has also been observed in cucumber (Aoki and Yabuki, 1977), sunflower, and soybean (Mauney et al., 1978).

A greater unit leaf rate at high carbon dioxide concentration does not necessarily translate into a greater growth rate. The total leaf surface area is also an important determinant of overall dry matter production. Leaf area is a determinant of both absolute growth rate and relative growth rate. The importance of leaf area in determining growth is pointed out in a study of wheat (Gifford, 1977). On a unit leaf area basis, enriched plants showed 15 to 20% less assimilation than controls at ambient carbon dioxide, but on a unit ground area basis, enriched plants had twice the assimilation rate of controls at high irradiance (Gifford, 1977). This was due to a greater leaf area in enriched plants.

The major influence of enrichment on E was early in
FIGURE 3.6  Unit leaf rate over time of plants grown at 340, 500, 800, 1200, 2000, or 3000 ul l\(^{-1}\) carbon dioxide.

E is expressed in units of g m\(^{-2}\) day\(^{-1}\) x 10\(^{-3}\).
development (Fig. 3.6), while leaf area responded late in development (Fig. 3.2). Hence, early in development, the greater absolute growth rate of enriched plants was determined primarily by increases in E, while later in development, a somewhat greater leaf area also contributed.

Leaf area ratio was progressively lower at higher carbon dioxide concentrations (Fig. 3.7) and this response persisted through time. Thus, enriched plants accumulated greater dry weight per unit of leaf surface area than controls. Note that a change in unit leaf rate can influence the leaf area ratio. The decreased F in enriched plants was in part due to a greater E (assuming leaf area to be constant, an increase in E will cause accumulation of greater dry weight, thereby lowering F; Figure 3.2 showed that leaf area of enriched plants was either equal to or greater than that of controls, therefore a decrease in F in enriched plants must have been due to a greater E).

Since enrichment influenced relative growth rate primarily during early growth, the question arises as to whether sustained enrichment beyond that stage is advantageous. In this regard, it is interesting to note that, irrespective of carbon dioxide treatment, R was similar after 30 days of age, but the absolute growth rate was consistently higher with elevated carbon dioxide concentration during growth. That is, later in development, enriched plants were larger, but did not show differences in R. Therefore, the greater size of enriched plants in later development probably derived from (1) a sustained growth advantage incurred during early development, when R was very
FIGURE 3.7 Leaf area ratio over time of plants grown at 340, 500, 800, 1200, 2000, or 3000 ul l$^{-1}$ carbon dioxide.

F is expressed in units of m$^2$ g$^{-1}$ x10$^{-3}$. 
high, (2) a marginally higher $E$; even though the absolute unit leaf rates were quite low, enriched plants still showed a large percentage increase in $E$ (this would not influence $R$ because of the compensating effect of a lower $F$ value), and (3) a slightly greater leaf area. Hence, a combination of cumulative increases in size, as well as an increase in current growth may have contributed to larger enriched plants during the time when relative growth rate was apparently no greater than in controls. However, whether a growth advantage is maintained if enrichment is discontinued may depend on the species. Krizek et al. (1971) found that the stimulatory effect of 4 weeks of enrichment of crabapple seedlings persisted for 2 to 3 months. Hardman and Brun (1971), however, found no lasting effect of enrichment on soybean at maturity, when carbon dioxide was administered during the first 5 weeks of vegetative growth. Davis and Potter (1983) found that of 5 ornamental species which responded to enrichment, only one (*Peperomia*) retained the size differential after an additional 4 weeks at ambient carbon dioxide. Clearly, more work is needed to determine the long-term effectiveness of short-term enrichment.

Although carbon dioxide enrichment increased growth rate, it had no discernable effect on rate of development. Plants in all carbon dioxide treatments flowered at approximately 35 days of age, and began to set pods at approximately 42 days of age. This contrasts to the work of Hicklenton and Jolliffe (1978) who found that flowering in enriched tomato was advanced 3 days. The difference in results between my work and that of Hicklenton
and Jolliffe (1978) may relate to species differences.

3.3.2 Effects On Partitioning

A change in the partitioning of carbon among plant parts may occur as a result of physiological adjustments of plants to different environmental situations.

Although there was some variability in the effect of carbon dioxide concentration on the root-shoot ratio (R/S) early in development (Fig. 3.8), after 30 days of age the R/S was consistently lower in enriched plants. Other workers, however, have reported a greater R/S with enrichment in *Vitis* (Kriedemann *et al*., 1976), tomato (Tognoni *et al*., 1967), barley, kale (Ford and Thorne, 1967), and sugar beet (Ford and Thorne, 1967; Wyse, 1980). It may be significant that all these are non-legumes. *Desmodium*, a legume, showed a decreased R/S (Wulff and Strain, 1982) as did the bean plants in this study (although, in another legume study, Tognoni *et al*., 1967) found an increased R/S in bush beans.

Leaf weight ratio (LWR) was progressively higher with increased carbon dioxide enrichment; this effect was persistent over time (Fig. 3.9). The increase in LWR suggests that high carbon dioxide concentration caused a greater proportion of carbon to be partitioned as leaf weight. This observation is supported by the fact that both leaf weight (Fig. 3.10) and leaf area (Fig. 3.2) increased with enrichment.

There are two aspects of carbon partitioning which are particularly noteworthy. Those concern the partitioning of carbon resources into new leaf area production, and into leaf
FIGURE 3.8. Root-shoot ratio over time of plants grown at 340, 500, 1200, and 3000 ul l⁻¹ carbon dioxide.
FIGURE 3.9 Leaf weight ratio over time of plants grown at 340, 500, 800, 1200, 2000, or 3000 ul l⁻¹ carbon dioxide.
FIGURE 3.10 Leaf weight over time of plants grown at 340, 500, 800, 1200, 2000, or 3000 \( \text{ul l}^{-1} \) carbon dioxide. Each value is the mean of 4 plants, with standard deviation. Curves are cubic spine functions of fitted data for each carbon dioxide treatment.
weight production, respectively.

From the leaf area ratio data (Fig. 3.7), it was apparent that for a given plant size (size being equated with plant weight), enriched plants appeared to partition less carbon into leaf area production than did the controls. Therefore, despite a greater total leaf area in enriched plants, the leaf area relative to plant size was apparently reduced.

Another relevant aspect of partitioning concerns the rate of carbon input into leaf weight. To characterize this partitioning, the absolute growth rate of leaf weight \(\frac{dLw}{dt}\) was compared to that of the remainder of the plant \(\frac{dRw}{dt}\). Carbon dioxide enrichment initially caused a large increase in the ratio of the two growth rates \(\frac{dLw}{dRw}\) (Fig. 3.11). Hence, at least early in growth, a greater proportion of the enhanced dry matter accumulation in enriched plants was partitioned into leaf weight.

This initial increase in the rate of growth of leaf weight could have resulted from either an increase in total leaf area, an increase in leaf weight per unit area, or a combination of the two. For example, the very large increase in specific leaf weight (SLW) during enrichment (Fig. 3.12) suggests that the increase in leaf weight was, in part, due to an increase in the amount of carbon accumulated per unit leaf area. This corroborated studies by Ford and Thorne (1967), Hurd (1968), Neales and Nicholls (1978), and Wulff and Strain (1982). Because total leaf area (Fig. 3.2) did not change significantly in enriched plants compared to controls over the period in which
FIGURE 3.11 Ratio of the growth rate of leaf weight to that of the remainder of the plant over time, in plants grown at 340, 500, 800, 1200, 2000, or 3000 ul l⁻¹ carbon dioxide.
FIGURE 3.12 Specific leaf weight over time of plants grown at 340, 500, 800, 1200, 2000, or 3000 ul l⁻¹ carbon dioxide. SLW is expressed in units of g m⁻² x10⁻³.
a large change in $\frac{dLw}{dRw}$ occurred (Fig. 3.11), it is reasonable to conclude that the change in partitioning was due primarily to an increase in leaf weight per unit leaf area (SLW). An increase in SLW apparently resulted from an increase in the storage function of enriched leaves, and suggests that the partitioning of carbon between the amount transported from leaves, and the amount stored in leaves, was altered due to enrichment. The major decrease in SLW occurred at approximately the time of reproductive growth, which could reflect a mobilization of leaf carbon reserves. However, at the later stages of development where both leaf area and SLW had changed, the relative partitioning of carbon into the production of new leaf area, transport, or storage, could not be evaluated.

When overall plant SLW was sub-divided into component SLW for each leaf, an interesting pattern emerged (Fig. 3.13). The individual leaves of enriched plants initially showed an increasing SLW. The primary leaf had the largest response, which was probably a function of the greater $E$ at that stage of development (11-22 days). The trifoliates did not achieve the high SLW of the primary leaves in either control or enriched plants, perhaps because sink demand, and hence transport rates from source leaves, increased with plant age. The SLW of older leaves (primary and first trifoliates) decreased with a concurrent increase in SLW of younger leaves (fourth trifoliate). This may be due to a progressively reduced $E$ in older leaves (see Chapter 6).

In addition to overall plant growth rates, the partitioning
FIGURE 3.13 Specific leaf weight over time of the individual leaves of plants grown at 340 (broken line) or 1200 (solid line) ul l⁻¹ carbon dioxide.

Data are presented for the primary leaves (P), the first trifoliates (1), and the fourth trifoliates (4).
of dry weight to the pods is an important factor in determining yield. Despite the fact that pod yield in terms of dry weight was greater in enriched plants (Table 3.2), the Harvest Index was the same, or somewhat less than that of controls. Enrichment, therefore, did not cause a greater partitioning of carbon into reproductive growth.

Overall then, it seems that the partitioning of assimilates within the plant was influenced by enrichment mainly to the extent that with greater E, a greater proportion of fixed carbon remained in the leaves.

3.3.3 Summary

Continuous carbon dioxide enrichment of bush beans caused greater growth, that is larger plants, but did not influence rate of development. Although enriched plants were consistently larger than controls, the relative growth rate was greater only during early growth. The trend in relative growth rate was, at least in part, a result of changes in unit leaf rate, which was also higher in enriched plants only during early growth. The data support observations made in other species that continuous carbon dioxide enrichment was effective in increasing relative growth rate and unit leaf rate for only a limited time.

The increased unit leaf rate in enriched plants was probably responsible for the decreased leaf area ratio and increased specific leaf weight compared to controls; those changes have also been observed in other species.

The change in leaf weight partitioning, determined from the ratio of the growth rate of leaf weight to the growth rate of
TABLE 3.2. Total dry weight, pod weight, and Harvest Index of plants grown at 340, 500, 800, 1200, 2000, or 3000 ul l\(^{-1}\) carbon dioxide for 44 days.\(^1\)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pod Weight (g)</th>
<th>Total Weight (g)</th>
<th>Harvest Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>340 ul l(^{-1})</td>
<td>7.54 ±0.28</td>
<td>18.30 ±0.48</td>
<td>0.41±0.01</td>
</tr>
<tr>
<td>500 ul l(^{-1})</td>
<td>9.30 ±0.20</td>
<td>22.64 ±0.87</td>
<td>0.41±0.01</td>
</tr>
<tr>
<td>800 ul l(^{-1})</td>
<td>10.58 ±0.10</td>
<td>26.28 ±0.52</td>
<td>0.39±0.01</td>
</tr>
<tr>
<td>1200 ul l(^{-1})</td>
<td>13.28 ±0.66</td>
<td>31.21 ±0.67</td>
<td>0.43±0.01</td>
</tr>
<tr>
<td>2000 ul l(^{-1})</td>
<td>11.97 ±0.52</td>
<td>29.86 ±1.37</td>
<td>0.40±0.02</td>
</tr>
<tr>
<td>3000 ul l(^{-1})</td>
<td>11.05 ±0.43</td>
<td>31.02 ±0.60</td>
<td>0.36±0.02</td>
</tr>
</tbody>
</table>

\(^1\) Values are the means of 4 plants, with standard error.
the remainder of the plant, is evidence for a change in partitioning of dry matter to the leaves. At least early in development, this partitioning was due entirely to an increase in the storage function of enriched leaves.

To date, the contribution to overall specific leaf weight of that of individual leaves of enriched plants has not been reported. The specific leaf weight of representative leaves of enriched and control plants was tracked through development. This offered a more detailed description of dry matter allocation in representative leaves of enriched and control plants than did a generalized specific leaf weight. The results showed distinctive changes in specific leaf weight for those leaves through time, particularly in enriched plants.
IV. EFFECTS ON NET CARBON DIOXIDE EXCHANGE

4.1 INTRODUCTION

In view of the dramatic changes in E through time and among carbon dioxide treatments, and because of the importance of carbon dioxide assimilation in growth, it was pertinent to determine in a more detailed manner the photosynthetic physiology of enriched plants. The few investigations which have been made of the photosynthetic gas exchange of individual leaves of enriched plants have yielded conflicting results. Some have reported increased photosynthetic capacity (Bishop and Whittingham, 1968; Madsen, 1975; Hicklenton and Jolliffe, 1980b), no effect (Ford and Thorne, 1967; Gifford, 1977), or decreased capacity (for example, Hofstra and Hesketh, 1975; Ho, 1977; Mauney et al., 1979; Clough et al., 1981; Wulff and Strain, 1982).

It is not at all clear why the change in capacity with enrichment occurs; there is evidence that a change in stomatal resistance (Aoki and Yabuki, 1977; Imai and Murata, 1978c) and mesophyll resistance (Aoki and Yabuki, 1977) may be involved, but it is not known why either of those change. However, large accumulations of starch have been observed in enriched leaves (eg. Hofstra and Hesketh, 1975; Wulff and Strain, 1982), and Nafziger and Koller (1976) have shown that leaf starch content was negatively correlated with photosynthesis rate. Therefore, a large starch concentration in leaves of enriched plants may be related to the reduction in photosynthetic capacity of those
leaves. In addition, it is not known if the response to enrichment is influenced by other environmental conditions.

With this in mind, the objectives of this chapter were to answer the following pertinent questions:

1. Does carbon dioxide enrichment change the net carbon dioxide exchange capacity of bean plants?
2. If so, is the apparent change in carbon dioxide exchange capacity due to a change in properties of the leaf which influence CER? For example, changes in stomatal resistance, or changes in leaf thickness or chlorophyll content may influence the measurement of carbon dioxide exchange capacity.
3. Can the change in carbon dioxide exchange capacity of enriched plants be attributed to a change in photorespiration rate?
4. Is dark respiration rate affected by enrichment?
5. Is the change in carbon dioxide exchange capacity of enriched plants modified by other growth conditions, such as light intensity?
6. Is the change in carbon dioxide exchange capacity of an individual leaf consistent through time, and how would this relate to changing environmental conditions (such as shading)?
7. Does starch content of enriched leaves change, and if so, is there a relationship between this change and the carbon dioxide exchange capacity?
8. Is the change in carbon dioxide exchange capacity of enriched plants related to a change in the source/sink relationships of the plant, and can the change in capacity be
alleviated by changing sink demand?

Answers to these questions are pertinent to an understanding of how carbon dioxide enrichment affects the physiology of leaves and ultimately plant growth and productivity.
4.2 MATERIALS AND METHODS

Seeds were germinated, and before transfer to the carbon dioxide treatment chambers, grown according to the conditions previously outlined (section 3.2.1). In one instance, plants were also grown in 3 inch pots (one plant per pot; soil volume was 0.15 l). At 12 days after planting, 4 to 8 bean plants were transferred to each chamber at either 340±10 ul l⁻¹ (control) or 1400±20 ul l⁻¹ (enriched) carbon dioxide, unless otherwise indicated. Chamber temperature was 32/25 C day/night and photosynthetic photon flux density (PPFD) incident on the first trifoliate was approximately 350 umol m⁻² s⁻¹, unless otherwise indicated.

In addition to bean, tomato (Lycopersicon esculentum L., cv. Vendor) plants were used in some experiments, and were transferred at 19 days to 340±10 or 1000±20 ul l⁻¹ carbon dioxide.

Plastochron Index (Erickson, 1957) was used to establish physiological age. The Plastochron Index uses leaf number as an index of development; plants of the same leaf number were assumed to be at the same developmental stage, despite possible differences in growth rate. In effect, this allowed separation of growth from developmental effects of the environment. The Plastochron Index was obtained by counting all leaves over a designated length. Fractional values resulted when the smallest leaf length was a fraction of the reference length. My reference length was 10mm with the first trifoliate being the first plastochron. At the time of transfer, PI=0.5.
Measurements of net carbon dioxide exchange rate (CER, expressed as umoles of carbon dioxide leaf • area•1 time•1), transpiration rates, and dark respiration rates were obtained in the first and second trifoliates. A semi-open gas exchange system was built specifically for this purpose (Ehret and Jolliffe, 1983). The system permitted steady-state measurements of both water vapour and carbon dioxide gas exchanges of a leaf enclosed in a "trap" type cuvette. Conditions of temperature, light intensity, humidity and gas composition in the cuvette varied according to the experiment, and will be outlined where needed. Measurement of water vapour flux (transpiration) permitted calculation of stomatal resistance, which is a measure of the ability of stomata to act as portals for the net diffusion of water vapour. Stomatal resistance was calculated from the following equation derived from Ohm's Law, where net flux is equal to a driving force divided by a resistance:

\[ J(H2O) = \frac{X_i - X_o}{R_s(H2O) + R_a(H2O)} \]

where: \( J(H2O) \) = flux of water vapour (mg m\(^{-2}\) s\(^{-1}\))

\( X_i \) = absolute concentration of water vapour at the site of evaporation in the leaf (mg m\(^{-3}\))

\( X_o \) = absolute concentration of water vapour in the external air (mg m\(^{-3}\))

\( R_s(H2O) \) = stomatal resistance to the diffusion of water vapour (s m\(^{-1}\))

\( R_a(H2O) \) = boundary layer resistance to the diffusion of water vapour (s m\(^{-1}\))
Ra was determined using blotting paper in place of the leaf in the chamber \((Ra(H2O) = 50 \, \text{s m}^{-1})\). Hence, all components except \(Rs\) were known, and \(Rs\) could be determined by solving the above equation.

The same concept applies to the flux of carbon dioxide. The resistance to diffusion of carbon dioxide to the mesophyll cells is again \(Rs + Ra\). However, the diffusivity of carbon dioxide is less than that of water vapour, so in effect, the resistance to transfer of carbon dioxide is greater by a factor of 1.56. The following equation was used to solve for the concentration of carbon dioxide in the intercellular spaces:

\[
J(CO2) = \frac{Co - Ci}{Rs(CO2) + Ra(CO2)}
\]

where: \(J(CO2) = \text{net flux of carbon dioxide (CER, in mg m}^{-2} \, \text{s}^{-1})\)

\(Co = \text{external concentration of carbon dioxide (mg m}^{-3})\)

\(Ci = \text{intercellular space carbon dioxide concentration (mg m}^{-3})\)

\(Rs(CO2) = \text{stomatal resistance to the diffusion of carbon dioxide (s m}^{-1})\)

\(Ra(CO2) = \text{boundary layer resistance to the diffusion of carbon dioxide (s m}^{-1})\)

In some experiments, the starch content of leaf tissue was determined using a modification of the method of Huber and Israel (1982). Samples (200 to 500 mg fresh weight, equivalent to about 4 one-cm leaf disks) were extracted in 80% ethanol at 80 °C until the tissue was pigment-free. This step removed
soluble sugars. Samples were then homogenized in 2 to 5 ml of 0.2N KOH containing 100 mM ethanol and heated for 2 hours at 80 C to solubilize the starch. After cooling, the pH was adjusted to 5.5 with 1M acetic acid. An equal volume of amylglucosidase (Sigma, A-7255, obtained from Rhizopus) solution (400 units ml⁻¹ in 0.1M citrate buffer, pH 5.5) was added and the solution was then incubated for 4 hours at 45 C. This converted starch to glucose. Placing the reaction vessel in boiling water for 1 minute terminated the reaction. After a 20-fold dilution, a 0.5 ml aliquot was taken for glucose determination. Glucose was assayed in a two-enzyme sequence, using Sigma glucose kit 510-A (see also Sigma technical bulletin 510). In this procedure, glucose was first converted to glucuronic acid using glucose oxidase, and this step liberated hydrogen peroxide. Then, in a peroxidase-catalyzed reaction, hydrogen peroxide oxidized o-dianisidine, resulting in colour development. This was measured at 450 nm in a spectrophotometer.

Chlorophyll content was measured in 4 one-cm disks of interveinal tissue taken from the leaves which was used in the gas exchange studies. Samples were stored at -20 C until needed. Chlorophyll was quantified using a modified version of the technique of Bruinsma (1963). Disks were homogenized in 30 ml of cold 80% acetone for one minute in a blender (Osterizer, model Cyclotrol 8). All steps of the extraction were carried out in dim light using cold reagents and vessels. The homogenate was vacuum filtered on Whatman #1 filter paper. The extract was assayed for chlorophyll by measuring absorbance at
645, 652, and 663 nm. Chlorophylls a and b and their sum were then calculated as follows (in mg l⁻¹):

\[
\text{Chi a} = 12.7 \times A_{663} - 2.7 \times A_{645}
\]

\[
\text{Chi b} = 22.9 \times A_{645} - 4.7 \times A_{663}
\]

\[
\text{Chi a + b} = 20.2 \times A_{645} + 8 \times A_{663} = 27.8 \times A_{652}
\]

In some experiments, leaf thickness was determined by dividing leaf area by leaf volume. Leaf area was measured with a leaf area meter (Li-Cor model LI-3000), and leaf volume was determined using a liquid displacement technique (Huxley, 1971). Leaf intercellular air volume was approximated by dividing leaf fresh weight by leaf volume. Assuming water to be the major component of leaf fresh weight, and knowing the density of water to be 1, the difference in measured leaf volume and the expected leaf volume (based on the density of water) was the volume of the intercellular air spaces.

Where required, PPFD was determined with a quantum meter (Li-Cor, model LI-185).

Where appropriate, two regression packages provided by the University of British Columbia Computing Services Centre were used to fit data to a curve. An asymptotic regression package was used to fit carbon dioxide response curves, and a triangular regression package (TRP) was used to fit the data of starch content versus CER to a third-order polynomial regression.
4.3 RESULTS AND DISCUSSION

4.3.1 Generalized Response Of Net Carbon Dioxide Exchange Capacity

Although carbon dioxide enriched beans showed enhanced unit leaf rates compared to controls early in development (see section 3.3.1), the capacity of individual leaves to exchange carbon dioxide was reduced (Table 4.1a and 4.1b). The CER of enriched plants measured at 340 ul l^-1 carbon dioxide after 15 to 19 days of treatment was reduced 19% and 27% in the first and second trifoliates, respectively.

An important distinction in terminology must be reinforced at this point. Measurement of CER of enriched and control plants at their respective carbon dioxide concentrations is not an adequate measure of the comparative ability of those plants to exchange carbon dioxide with the atmosphere. The measurement of CER in this case is obtained at two different carbon dioxide concentrations, and only shows that the rates of carbon dioxide exchange may be different. Therefore, carbon dioxide exchange capacity is defined as the ability of enriched plants to carry out net carbon dioxide exchange (measured as CER) compared to controls under the same measurement conditions. Throughout this chapter, a change in capacity was frequently expressed in terms of a percent of control CER values.

The reduction in CER of enriched leaves was observed at all measurement carbon dioxide concentrations examined (Fig. 4.1). The CER in Figure 4.1 was expressed in terms of intercellular
TABLE 4.1a. Net carbon dioxide exchange rate of the first and second trifoliates of control (340 ul l\textsuperscript{-1}) and enriched (1400 ul l\textsuperscript{-1}) plants.\textsuperscript{1}

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaf</th>
<th>CER\textsuperscript{2} (umol m\textsuperscript{-2} s\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>340 ul l\textsuperscript{-1}</td>
<td>First Trifoliate\textsuperscript{3}</td>
<td>17.8 ±0.9</td>
</tr>
<tr>
<td></td>
<td>Second Trifoliate\textsuperscript{4}</td>
<td>16.7 ±1.2</td>
</tr>
<tr>
<td>1400 ul l\textsuperscript{-1}</td>
<td>First Trifoliate\textsuperscript{3}</td>
<td>14.3 ±1.5</td>
</tr>
<tr>
<td></td>
<td>Second Trifoliate\textsuperscript{4}</td>
<td>12.3 ±0.8</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Values are the means of 3 measurements made on different plants, with standard error

\textsuperscript{2} CER measured at 1000 umol m\textsuperscript{-2} s\textsuperscript{-1} PPFD and 340 ul l\textsuperscript{-1} carbon dioxide

\textsuperscript{3} Measured after 15 days of treatment

\textsuperscript{4} Measured after 19 days of treatment

TABLE 4.1b. Analysis of variance of the data of TABLE 4.1a.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum Sq.</th>
<th>Mean Sq.</th>
<th>Error F</th>
<th>Required F 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>161.29</td>
<td>161.29</td>
<td>11.30</td>
<td>4.67</td>
</tr>
<tr>
<td>Trifoliate</td>
<td>1</td>
<td>21.62</td>
<td>21.62</td>
<td>1.51</td>
<td>4.67</td>
</tr>
<tr>
<td>Error</td>
<td>13</td>
<td>185.59</td>
<td>14.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>368.50</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{1} At 5% level of probability
FIGURE 4.1 Response of net carbon dioxide exchange rate of the first trifoliate of enriched (1400 ul l$^{-1}$) and control (340 ul l$^{-1}$) plants (at PI=5.0) to intercellular space carbon dioxide concentration. Measurement PPFD was 1000 umol m$^{-2}$ s$^{-1}$. Plants were in the treatment chambers for 18 day before CER measurement. PPFD incident on the first trifoliate during growth was 300 to 350 umol m$^{-2}$ s$^{-1}$. Measurements were obtained from 3 plants in each treatment. Enriched leaves, closed circles; control leaves, open circles. Curves were fitted to an asymptotic equation using 13 and 14 values for control and enriched treatments respectively.
carbon dioxide concentration, establishing that the reduction in carbon dioxide exchange capacity was not due to a reduction in stomatal aperture. A knowledge of stomatal aperture was an important consideration, since, for example, a treatment-induced change in stomatal aperture could have changed the carbon dioxide concentration which was available for assimilation in the leaf, and thereby influenced the measured CER value. The substantial influence of stomatal aperture on the measurement of CER is not taken into consideration in many studies of carbon dioxide enrichment effects on plants.

Extrapolation of the curves to zero net carbon dioxide exchange suggested that both treatments had similar carbon dioxide compensation points of about 50 ul l⁻¹. The carbon dioxide compensation point is frequently used to characterize the relationship between photosynthesis and respiration in the light, the latter being primarily photorespiration (Canvin, 1979). At the carbon dioxide compensation point, the rate of respiratory carbon dioxide release is equal to the rate of photosynthetic carbon dioxide uptake; a change in the compensation point is indicative of a change in the relative rates of those two processes. Therefore, a similar carbon dioxide compensation point implies that the reduced carbon dioxide exchange capacity of enriched plants was probably not due to a proportionally greater photorespiration rate. The conclusion that there was no difference in photorespiration rate between carbon dioxide treatments when measured under the same conditions was supported by the fact that both treatments showed
the same degree of oxygen inhibition of CER (Table 4.2). Oxygen inhibition of CER has been shown to be related to photorespiration rate (Jolliffe and Tregunna, 1968). Continuous exposure of bean plants to high carbon dioxide concentrations in my study apparently did not change the relative rates of true carbon dioxide exchange (carbon dioxide exchange in the absence of photorespiration) and photorespiratory carbon dioxide exchange when measured under the same conditions. Even though enriched plants presumably had reduced photorespiration rates compared to controls, if measured at their respective growth carbon dioxide concentrations, the ability of enriched plants to photorespire did not differ from controls.

Gifford (1977) found no difference in the carbon dioxide compensation point among plants grown at different carbon dioxide concentrations, which is not surprising since no difference in photosynthetic response was reported. Hicklenton and Jolliffe (1980b), on the other hand, found that together with an enhanced carbon dioxide exchange capacity in plants grown at 1000 ul l⁻¹ carbon dioxide, a decreased carbon dioxide compensation point and reduced oxygen sensitivity also occurred. The significance of those results will be discussed later (sect. 4.3.8).

The response of the first trifoliate leaf of enriched plants to PPFD was determined. At both 340 and 1000 ul l⁻¹ measurement carbon dioxide concentrations and at PPFD approaching saturation, enriched plants showed reduced CER compared to controls (Fig. 4.2). The reduced CER of enriched
TABLE 4.2. Oxygen sensitivity of the net carbon dioxide exchange rate of the first trifoliate of control (340 ul l\(^{-1}\)) and enriched (1400 ul l\(^{-1}\)) plants treated for 13 days.\(^1\)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CER(^2) (1% oxygen)</th>
<th>CER(^2) (21% oxygen)</th>
<th>% Inhibition(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(umol m(^{-2}) s(^{-1}))</td>
<td>(umol m(^{-2}) s(^{-1}))</td>
<td></td>
</tr>
<tr>
<td>340 ul l(^{-1})</td>
<td>24.1±1.3</td>
<td>16.4±0.5</td>
<td>31.9±1.6</td>
</tr>
<tr>
<td>1400 ul l(^{-1})</td>
<td>18.1±1.7</td>
<td>12.2±1.2</td>
<td>32.6±3.2</td>
</tr>
</tbody>
</table>

\(^1\) Values are the means of 3 plants, with standard error
\(^2\) CER measured at 1000 umol m\(^{-2}\) s\(^{-1}\) PPFD
\(^3\) Difference between CER measured at 21% and 1% oxygen within a carbon dioxide treatment, expressed as a percentage of the 1% oxygen value
FIGURE 4.2 Response of the net carbon dioxide exchange rate of the first trifoliate of enriched (1400 ul l⁻¹) and control (340 ul l⁻¹) plants to photosynthetic photon flux density. Leaves measured at 1000 ul l⁻¹ (solid lines) and 340 ul l⁻¹ (broken lines) carbon dioxide concentrations were from different sets of plants. Plants in all cases were treated for 18 days at the carbon dioxide concentrations indicated in the figure. Enriched leaves, closed circles; control leaves, open circles. Each value is the mean of 3 leaves, with standard error.
plants was again not due to a reduction in stomatal aperture since intercellular carbon dioxide concentrations were similar in both treatments at a given level of PPFD (data not presented). At rate-limiting PPFD there was no difference between treatments, indicating an equivalent quantum efficiency and light compensation point. Quantum efficiency decreases with an increase in oxygen sensitivity (Ehleringer and Bjorkman, 1977). The fact that quantum efficiency was not affected by enrichment again points out the lack of enrichment effects on photorespiration rate when measured under the same conditions in both treatments.

In summary, the gas exchange data supported the general trend of reduced net carbon dioxide exchange capacity in enriched plants which has been observed by many others (Hofstra and Hesketh, 1975; Frydrych, 1976; Aoki and Yabuki, 1977; Ho, 1977; Mauney et al., 1979; Wong, 1979; Clough et al., 1981; Wulff and Strain, 1982), and also suggested that the effect was not caused by a change in photorespiration rate.

4.3.2 Response Of Specific Parameters Of Net Carbon Dioxide Exchange Capacity

Many reference points commonly used to express CER may change when plants are grown under different conditions. For example, chlorophyll content, dry or fresh weight, or protein content, all of which are used as a basis for comparing photosynthesis rates, may themselves change in response to carbon dioxide treatment. It was therefore necessary to establish that the measured changes in CER were independent of
changes in those parameters.

Possible effects of carbon dioxide treatments on leaf thickness or total cell volume would diminish the usefulness of leaf area as a basis of expressing CER. Leaf thickness of the first and second trifoliates, however, was not affected by carbon dioxide enrichment (Table 4.3). Hence, expression of photosynthesis on a unit leaf area basis was a reasonable approach in this study. The percentage of leaf volume occupied by air, a parameter which could affect CER through changes in the characteristics of the internal diffusion of carbon dioxide, was also found not to be influenced by enrichment (Table 4.3).

Net carbon dioxide exchange rate is often expressed on a unit chlorophyll basis, even though chlorophyll level does not always correlate with photosynthesis rate (Hesketh et al. 1981). Carbon dioxide enrichment had no effect on chlorophyll levels in mature trifoliates (eg. Fig. 4.3), although chlorophyll levels in young leaves were often reduced (personal observation; Cave et al., 1981). This could represent an influence of carbon dioxide on the rate of greening. Rates of net carbon dioxide exchange given in Figure 4.2 (measured at 1000 ul l⁻¹ carbon dioxide) were converted to a unit chlorophyll basis in Figure 4.3. Carbon dioxide enrichment caused reduced photosynthetic rates not associated with changes in leaf chlorophyll content, an observation in agreement with work on cotton by Wong (1979), but inconsistent with the data of Wulff and Strain (1982) on Desmodium. The latter authors found enriched plants had reduced chlorophyll levels, and that
TABLE 4.3. Leaf thickness and intercellular air space volume of control (340 ul l⁻¹) and enriched (1400 ul l⁻¹) plants.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaf Thickness (mm)</th>
<th>Air Volume (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>340 ul l⁻¹</td>
<td>0.30 ±0.01</td>
<td>16.5 ±2.1</td>
</tr>
<tr>
<td>1400 ul l⁻¹</td>
<td>0.31 ±0.01</td>
<td>16.4 ±0.8</td>
</tr>
</tbody>
</table>

Values are the means of 3 first trifoliates and 3 second trifoliates, with standard error.
FIGURE 4.3 Response of the net carbon dioxide exchange rate, expressed per unit chlorophyll, of the first trifoliate of enriched (1400 ul l\(^{-1}\)) and control (340 ul l\(^{-1}\)) plants, to photosynthetic photon flux density. The data were obtained from the same plants that were used in Figure 2.2 (solid lines). CER was measured at 1000 ul l\(^{-1}\) carbon dioxide concentration. The chlorophyll content was 0.646±0.180 and 0.647±0.240 g m\(^{-2}\) in control and enriched plants respectively. Enriched leaves, closed circles; control leaves, open circles. Each value is the mean of 3 leaves, with standard error.
although carbon dioxide exchange capacity per unit leaf area was reduced by enrichment to 1000 ul l\(^{-1}\) carbon dioxide, the photosynthetic capacity was higher on a unit chlorophyll basis. They found enriched plants to have reduced chlorophyll levels. A number of studies (see Hesketh et al., 1983) have shown that 90% of maximum quantum efficiency was achieved at chlorophyll concentrations of 450 to 600 mg m\(^{-2}\). Measurements of photosynthesis rate per unit chlorophyll in plants not limited by chlorophyll are underestimates and somewhat misleading. Wulff and Strain (1982) report average chlorophyll content values of 466 and 927 mg m\(^{-2}\) in enriched and control plants respectively. They may have been comparing photosynthesis rates at two very different chlorophyll concentrations where one (or both) of the chlorophyll concentrations may not have been limiting photosynthesis. If this was the case, photosynthesis rates, of control plants in particular, when expressed on a per unit chlorophyll basis, may have been underestimated.

4.3.3 Effect Of Duration

The earliest measurements of CER of the first trifoliate leaves were made after a 6 day period in the treatment chambers. At that time, a reduction in carbon dioxide exchange capacity was evident (for example, refer to Table 4.4 in section 4.3.4) Therefore, at least 6 days of high carbon dioxide concentration were required to elicit a reduction in carbon dioxide exchange capacity.

Although a reduction in capacity was evident between 6 and 18 days of enrichment, the reduced net carbon dioxide exchange
capacity exhibited by the first trifoliate of enriched plants was not permanent. If CER was measured after 26 days of treatment, at a PI of greater than 5 (Plastochron Index was not an adequate measure of developmental stage after this point because of extensive branching and no further growth of the main stem), there was no difference in net carbon dioxide exchange capacity between the first trifoliates of control and enriched plants (Fig. 4.4). The response of CER to Ci was more linear than at the earlier stage (Fig. 4.1), and the carbon dioxide compensation point had increased with age in both treatments. These results were similar to those of Hicklenton and Jolliffe (1981b) who found no effect of enrichment on leaves at a PI of 10 which previously had shown an effect at a PI of 5, albeit in a direction opposite to that reported here. Those authors were unable to determine why the effect of carbon dioxide disappeared with time. Hofstra and Hesketh (1975) found that enriched plants recovered full carbon dioxide exchange capacity after 3 days, if transferred to ambient (340 ul 1⁻¹) carbon dioxide concentration. Therefore, the effect of high carbon dioxide concentration in reducing carbon dioxide exchange capacity is not permanent, and may be ameliorated.

4.3.4 Effect Of Photosynthetic Photon Flux Density

One of the most well established effects of elevated carbon dioxide concentration on plants is to increase rates of photosynthesis. The reduced net carbon dioxide exchange capacity of enriched plants may be a function of their greater CER during growth. However, the CER during growth is also a
FIGURE 4.4 Response of net carbon dioxide exchange rate of the first trifoliate of enriched (1400 ul l⁻¹) and control (340 ul l⁻¹) plants (PI>5.0) to intercellular space carbon dioxide concentration. Measurement PPFD was 1000 umol m⁻² s⁻¹. Plants were in the treatment chambers for 26 days. The PPFD incident on the first trifoliate during the latter part of this period was 100 umol m⁻² s⁻¹. Control plants, open circles; enriched plants, closed circles. Measurements were obtained from 3 plants in each treatment. Curves were fitted to an asymptotic equation using 8 and 9 values for control and enriched treatments respectively.
function of PPFD. At low PPFD, the absolute increase in CER because of high carbon dioxide concentration was much less than at higher PPFD (Fig. 4.2), although the percentage increase may be similar. If the reduced carbon dioxide exchange capacity was dependent on a higher CER in enriched plants, then under conditions of PPFD limitation of CER during growth, where carbon dioxide was less effective in increasing CER, there should also be minimal difference in net carbon dioxide exchange capacity between enriched and control plants. Low light conditions in the canopy may in fact explain the disappearance through time of any difference in net carbon dioxide exchange capacity between enriched and control plants. Plants at 26 days of treatment (total of 37 days old) had a well-developed canopy, resulting in extensive shading of lower leaves (<100 umol m$^{-2}$ s$^{-1}$ PPFD).

To test the effect of light intensity on the carbon dioxide-induced reduction of net carbon dioxide exchange capacity, plants were transferred to treatment chambers after the first trifoliate was fully developed. At this point some of the leaflets of the first trifoliate were shaded with tracing paper, while the remainder were maintained at the higher PPFD. As seen in Table 4.4, there was minimal effect of carbon dioxide enrichment on net carbon dioxide exchange capacity at 100 umol m$^{-2}$ s$^{-1}$ PPFD, even though comparable leaflets at 350 umol m$^{-2}$ s$^{-1}$ PPFD showed a 22% reduction. The absolute net carbon dioxide exchange rates of low PPFD leaves were somewhat lower than those at high PPFD, and were probably due to acclimation of those leaves to low PPFD. Such acclimation is typically
TABLE 4.4. The effect of shading during growth on the net carbon dioxide exchange rate of the first trifoliate of enriched (1400 ul l⁻¹) and control (340 ul l⁻¹) beans grown at 26 C.¹

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CER² (umol m⁻² s⁻¹)</th>
<th>%CER³</th>
<th>Ci⁴ (ul l⁻¹)</th>
<th>Respiration⁵ (umol m⁻² s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>340 ul l⁻¹</td>
<td>11.5 ±0.4</td>
<td>-</td>
<td>295 ±8</td>
<td>0.80 ±0.02</td>
</tr>
<tr>
<td>shaded⁶</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1400 ul l⁻¹</td>
<td>11.6 ±0.8 (+)¹</td>
<td>302 ±3</td>
<td></td>
<td>0.80 ±0.02</td>
</tr>
<tr>
<td>shaded⁶</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>340 ul l⁻¹</td>
<td>18.1 ±0.6</td>
<td>-</td>
<td>278 ±7</td>
<td>0.90 ±0.04</td>
</tr>
<tr>
<td>unshaded⁷</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1400 ul l⁻¹</td>
<td>14.1 ±0.6</td>
<td>22</td>
<td>289 ±3</td>
<td>1.30 ±0.02</td>
</tr>
<tr>
<td>unshaded⁷</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Values are means of 3 leaves, with standard error; all measurements were taken after 6 days of treatment.
² CER measured at 1000 umol m⁻² s⁻¹ PPFD.
³ Difference in CER between control and enriched plants, measured at the same PPFD, and expressed as a percentage of the control value.
⁴ Ci refers to the CER measurements only.
⁵ Respiration measured at 21% oxygen and 340 ul l⁻¹ carbon dioxide concentration.
⁶ 100 umol m⁻² s⁻¹ PPFD during growth.
⁷ 350 umol m⁻² s⁻¹ PPFD during growth.
characterized by a greater efficiency of photosynthesis measured under low light conditions, but with reduced rates under high light (Boardman, 1977).

When PPFD limited CER during growth, the effect of carbon dioxide enrichment was negligible, suggesting that the reduction in net carbon dioxide exchange capacity was related to the enhanced CER of enriched plants. Furthermore, the disappearance of any effect of enrichment on the carbon dioxide exchange capacity in the first trifoliate of older leaves was, at least in part, explained by shading of the trifoliate by the leaves above. It was also possible that since CER is known to decrease with age (Davis and McCree, 1978), the lower CER in the older leaves may again limit any effect of enrichment. However, this possibility was not investigated.

4.3.5 Response To Sink Manipulation

Reduced net carbon dioxide exchange capacity in enriched leaves was not due to differences in chlorophyll content nor to altered rates of photorespiration, both of which are intrinsic properties of leaves which influence CER in a direct and proportional manner.

Carbon dioxide enrichment could influence net carbon dioxide exchange capacity by the somewhat more indirect means of altering the source-sink relationships of the plant, in which case, enrichment would not simply be affecting the leaves as isolated units separate from each other, or from the rest of the plant.

A sink is defined as a plant part which undergoes a net
input of carbon from the rest of the plant (Zeevaart, 1979). Conversely, a source is a plant part showing a net export of carbon to the remainder of the plant. Source or sink strength is defined as the product of the size and the activity of the source or sink. The source or sink strength may change in response to a change in the demand for, or supply of, assimilates.

There is now a substantial body of evidence suggesting that sink strength influences source leaf net photosynthesis rates (Zeevaart, 1979; Gifford and Evans, 1981). However, the evidence for a mechanism by which the regulation occurs remains controversial (Neales and Incoll, 1968). High starch levels, which are related to reduced photosynthesis (Nafziger and Koller, 1976), have been proposed as a means by which sink demand influences source leaf photosynthesis. Starch synthesis in source leaves appears to be responsive to sink demand. Sucrose phosphate synthetase, an enzyme involved in sucrose metabolism, may be an important control point (Huber and Isreal, 1982). For example, when sink demand for sucrose was limited by excision of the source leaf, starch accumulation increased and sucrose phosphate synthetase activity fell in the source leaf; conversely when sink demand was increased by defoliation of other source leaves, starch accumulation decreased with a concurrent increase in sucrose phosphate synthetase activity (Rufty and Huber, 1983).

Starch and sucrose levels have been shown to be inversely related and responsive to sink demand in soybean (Thorne and
Koller, 1974), where a direct correlation was observed between starch concentration and mesophyll resistance. Interestingly, a reduced CER associated with high starch content has been shown to occur in widely diverse physiological situations, such as a result of flooding of the roots of sunflower (Wample and Davis, 1983), and in non-tillering Pangola at cool night temperatures (Chatterton et al., 1972). Azcon-Bieto (1983) also found a decline in photosynthesis rate which correlated with carbohydrate accumulation in wheat leaves. Either increasing the carbon dioxide concentration, or reducing the rate of translocation by chilling the base of the source leaf, increased the rate of decline in photosynthesis when measured over a 6 to 8 hour period.

In view of the apparent relationship between carbohydrate level and net carbon dioxide exchange capacity, both starch concentration and CER were determined in enriched and unenriched plants. Enrichment to 1400 ul l⁻¹ or 3000 ul l⁻¹ carbon dioxide resulted in a 21% and 18% decrease in net carbon dioxide exchange capacity and a 4.4-fold and 3-fold increase in starch concentration, respectively, compared to control plants (Table 4.5, pre-shaded values). This observation agreed with previous studies showing an increase in starch content of enriched soybeans (Hofstra and Hesketh, 1975; Nafziger and Koller, 1976) and cotton (Mauney et al., 1979) which correlated with reduced photosynthesis rates. However, Fondy and Geiger (1982) found that although starch increased from 20 to 140 ug cm⁻² over the course of a 14 hour photoperiod, net carbon dioxide exchange was
TABLE 4.5. The effect of shading all but the first trifoliate on starch content and net carbon dioxide exchange rate of the first trifoliate of control (340 ul l$^{-1}$) and enriched (1400 and 3000 ul l$^{-1}$) plants.  

<table>
<thead>
<tr>
<th>Treatment</th>
<th>[Starch] (g m$^{-2}$)</th>
<th>CER$^1$ (umol m$^{-2}$ s$^{-1}$)</th>
<th>%CER$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before shading$^3$:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>340 ul l$^{-1}$</td>
<td>7.26±0.68</td>
<td>31.1±1.3</td>
<td>-</td>
</tr>
<tr>
<td>1400 ul l$^{-1}$</td>
<td>31.92±3.88</td>
<td>24.6±0.5</td>
<td>-</td>
</tr>
<tr>
<td>3000 ul l$^{-1}$</td>
<td>21.60±2.21</td>
<td>25.6±1.6</td>
<td>-</td>
</tr>
</tbody>
</table>

After selective shading$^4$:  

<table>
<thead>
<tr>
<th>Treatment</th>
<th>[Starch] (g m$^{-2}$)</th>
<th>CER$^1$ (umol m$^{-2}$ s$^{-1}$)</th>
<th>%CER$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>340 ul l$^{-1}$ shaded$^5$</td>
<td>3.77±1.03</td>
<td>33.2±0.9</td>
<td>-</td>
</tr>
<tr>
<td>340 ul l$^{-1}$ unshaded$^6$</td>
<td>8.10±1.46</td>
<td>23.7±1.5</td>
<td>29</td>
</tr>
<tr>
<td>1400 ul l$^{-1}$ shaded</td>
<td>26.88±1.97</td>
<td>24.1±2.1</td>
<td>-</td>
</tr>
<tr>
<td>1400 ul l$^{-1}$ unshaded</td>
<td>27.96±2.20</td>
<td>19.3±0.3</td>
<td>20</td>
</tr>
<tr>
<td>3000 ul l$^{-1}$ shaded</td>
<td>28.56±2.71</td>
<td>23.5±1.3</td>
<td>-</td>
</tr>
<tr>
<td>3000 ul l$^{-1}$ unshaded</td>
<td>31.12±3.01</td>
<td>20.5±1.3</td>
<td>13</td>
</tr>
</tbody>
</table>

$^1$ CER measured at 1000 umol m$^{-2}$ s$^{-1}$ PPFD and 1000 ul l$^{-1}$ carbon dioxide  
$^2$ Difference in CER between shaded and unshaded treatments within each carbon dioxide treatment, expressed as a percentage of control values  
$^3$ Pre-shading values were taken after 8 days of carbon dioxide treatment; values are the means of 8 plants, with standard error  
$^4$ Measurements were made after 5 days of selective shading; values are the means of 4 plants, with standard error  
$^5$ shaded leaves maintained at 90±10 umol m$^{-2}$ s$^{-1}$ PPFD  
$^6$ unshaded leaves maintained at 350±20 umol m$^{-2}$ s$^{-1}$ PPFD
not altered. It can be argued, however, that starch reduces net carbon dioxide exchange capacity only if accumulation reaches some critical level, achieved in enriched plants but not in control plants in my study. This is supported by the fact that cotton showed a negative correlation between photosynthesis and starch content, but soybean, sunflower, and sorghum, which did not accumulate as much starch, did not show any correlation (Mauney et al., 1979).

The reduction in carbon dioxide exchange capacity in enriched plants in my study, may have been associated with the carbohydrate status of the leaves. The fact that the difference in capacity occurred at high, but not low PPFD, coupled with the established influence of sink demand on carbohydrate content and photosynthesis rate of source leaves, would suggest that the reduction in net carbon dioxide exchange capacity of enriched plants was influenced by the relative rates of carbon assimilation and carbon export. The rate of translocation (Ho, 1977; Potter, 1980), as well as the efficiency of translocation (Ho, 1977) have been shown to increase in enriched plants. However, if sink demand was influencing, and perhaps limiting, the rate of translocation from enriched source leaves in my study, the greater CER at high carbon dioxide concentrations may have contributed to reduced net carbon dioxide exchange capacity.

To test the influence of sink demand on the carbon dioxide exchange capacity of enriched and control plants, plants were first grown for 9 days in one of three carbon dioxide
concentrations to establish differences in starch content and net carbon dioxide exchange capacity in the first trifoliate leaves (Table 4.5, before shading). All leaves but the first trifoliate were then shaded with opaque paper to increase sink demand on the unshaded leaf; other plants were left unshaded.

Low sink demand (unshaded plants) resulted in decreased net carbon dioxide exchange capacity relative to high sink demand in all carbon dioxide treatments, with a reduction of 29, 20, and 13% in 340, 1400, and 3000 ul l$^{-1}$ treated plants respectively (Table 4.5, after selective shading). Carbon dioxide enrichment also resulted in lower carbon dioxide exchange capacity. Those trends were also found by Clough et al. (1981) who manipulated fruit number in soybeans to create high or low sink plants, at two carbon dioxide concentrations. Their experiment differed from that reported here in that carbon dioxide and sink demand treatments were initiated simultaneously, and were not intended to evaluate the response of enriched plants to a change in sink strength. Furthermore, their measurements of photosynthesis were based on the whole plant.

In my study, the rate of change in net carbon dioxide exchange capacity over time in unshaded plants was similar in all carbon dioxide treatments (decreases of 24, 22, and 20% in 340, 1400, and 3000 ul l$^{-1}$ treatments respectively) (Table 4.5), and therefore the results were not confounded by age-related differences in net carbon dioxide exchange capacity among treatments.

Increasing sink demand decreased the age-related decline in
carbon dioxide exchange capacity of the first trifoliate, and in this respect, enriched plants did not respond to shading as well as controls. Comparing carbon dioxide exchange capacity within shaded (high demand) treatments, the percent reduction in enriched plants compared to controls was even greater than that before shading (for example, in comparing 3000 with 340 ul 1⁻¹ plants, there was an 18% reduction in carbon dioxide exchange capacity before shading, but a 29% reduction after shading). Similar results were obtained when comparing the effect of shading on the capacity within carbon dioxide treatments; there was progressively less difference in carbon dioxide exchange capacity between shaded and unshaded plants with increasing carbon dioxide concentration treatments (Table 4.5). Therefore, an increase in sink demand did not alleviate the reduction in net carbon dioxide exchange capacity of enriched compared to control plants.

Starch content of the first trifoliate was also determined in relation to carbon dioxide exchange capacity. Unshaded control plants had virtually the same net carbon dioxide exchange capacity as shaded 3000 ul 1⁻¹ plants, despite a 3-fold difference in starch content, and also showed a 24% reduction in carbon dioxide exchange capacity compared to initial rates, even though starch content was unaltered (Table 4.5). Therefore, changes in net carbon dioxide exchange capacity because of shading (sink demand) were independent of starch concentration. This observation supports the work of Potter and Breen (1980) who found that although continuous light for 52 hours resulted
in starch accumulation to 3.3 mg cm\(^{-2}\) and reduced photosynthetic rates in rapidly expanding sunflower leaves, other leaves which were mature and slowly expanding accumulated less starch but showed greater photosynthetic reduction. Note that because the leaves were of different ages, their sensitivity to starch may have been different, as suggested by Nafziger and Koller (1976).

However, as shown in Figure 4.5, when starch content was plotted versus CER for all treatments, enriched plants generally had higher starch content and lower net carbon dioxide exchange capacity, although the data were quite scattered. It is possible that enrichment affected net carbon dioxide exchange capacity through some aspect of starch accumulation which set a lower maximum carbon dioxide exchange potential. Other factors (e.g. shading or aging) may have then modified the capacity within that limit, independently of starch concentration.

It may be that starch accumulation per se was not causing the decline in net carbon dioxide exchange capacity but was rather a reflection of a complex metabolic adjustment, some other aspect of which could have caused the reduced carbon dioxide exchange capacity. For example, Thorne and Koller (1974) found that at high starch levels, RuBP carboxylase activity was reduced. In addition, there is currently a great deal of interest in the possibility that regulation of net photosynthesis rate may reside at the level of carbohydrate transport from chloroplasts. This possibility has been discussed with particular reference to sink control of source leaf photosynthesis rates (Herold, 1980; Fondy and Geiger,
FIGURE 4.5 Relationship between net carbon dioxide exchange rate and starch content of the first trifoliate of enriched (1400 or 3000 ul l$^{-1}$) and control (340 ul l$^{-1}$) plants.

The data are based on the measurements given in Table 4.5. The curve was fitted to a third-order polynomial equation, using 40 values. $R^2=0.46$
Sucrose is the major metabolite transported through the plant, and the proposed regulation of photosynthesis involves, at the outset, sucrose metabolism. Sucrose is first synthesized in the cytoplasm of mesophyll cells before it is loaded into the phloem for export. The synthesis of sucrose involves first the conversion of triose phosphates, which are exported from the chloroplast, to fructose-1,6-bisphosphate. After dephosphorylation to fructose-6-phosphate, and some conversion to UDP-glucose, the products are then converted by sucrose phosphate synthetase to sucrose phosphate, which is then converted to sucrose by sucrose phosphate phosphatase. If high cytoplasmic sucrose concentrations occurred, for example, because of decreased loading due to low sink demand, the accumulation of sucrose would limit further sucrose synthesis by feedback inhibition of sucrose phosphate synthetase (Herold, 1980; Fondy and Geiger, 1981). Alternatively, high sucrose levels could limit sucrose phosphate phosphatase activity (Herold, 1980). In either case, phosphorylated compounds would increase in the cytoplasm, and phosphate (Pi) concentration would decrease. This would favour retention of triose phosphates in the chloroplast since Pi is required for exchange of triose phosphates across the chloroplast inner membrane. ADP-glucose pyrophosphorylase, an enzyme of starch synthesis, is stimulated by a high triose phosphate/Pi ratio (see Fondy and Geiger, 1981), and triose phosphates would thus be redirected into starch synthesis.

Triose phosphates could conceivably inhibit photosynthesis
through a mass action effect. Alternatively, low Pi concentrations in the chloroplast could reduce net photosynthesis, possibly through a reduced ATP/ADP ratio (Robinson and Walker, 1979) or through a direct effect on RuBP carboxylase activity (Heldt et al., 1978). Low levels of Pi in leaves have, in fact, been correlated with reduced rates of net photosynthesis (Terry and Ulrich, 1973) and high levels of leaf starch (Jensen, 1980). Low levels of Pi would nicely explain a decrease in carbon dioxide exchange capacity with increased leaf carbohydrate accumulation.

As a corollary to increasing sink demand, an additional experiment was designed to investigate the effect of a decrease in sink demand on the carbon dioxide-induced reduction in carbon dioxide exchange capacity. Demand for assimilates was manipulated by restricting plant size. Plants were grown in pots of two sizes, with those in the smaller pots growing less rapidly because of restricted root development, and presumably having reduced sink demand.

The difference in net carbon dioxide exchange capacity between enriched and control plants in 3 inch pots, measured on an area basis after both 11 and 18 days, was smaller than in 5 inch pots (Table 4.6). Hence, enriched and control plants approached the same net carbon dioxide exchange capacity when growth was severely restricted. The similarity of exchange capacity was a result of either there being no carbon dioxide enrichment effect, or because control plants were suppressed to
TABLE 4.6. Effect of pot size on the net carbon dioxide exchange rate of the first trifoliate of enriched (1400 ul l\(^{-1}\)) and control (340 ul l\(^{-1}\)) plants.\(^1\)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CER(^2)</th>
<th>%CER(^3)</th>
<th>Chlorophyll • CER(^4)</th>
<th>%CER(^5)</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(umol m(^{-2}) s(^{-1}))</td>
<td>(g m(^{-2}))</td>
<td>(umol g(^{-1}) chl. s(^{-1}))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>340 ul l(^{-1}), 5&quot; Pot</td>
<td>18.4±1.6</td>
<td>-0.710±0.025</td>
<td>2.59</td>
<td>-</td>
<td>4.5</td>
</tr>
<tr>
<td>1400 ul l(^{-1}), 5&quot; Pot</td>
<td>12.1±1.1</td>
<td>0.710±0.060</td>
<td>1.70</td>
<td>39</td>
<td>4.5</td>
</tr>
<tr>
<td>340 ul l(^{-1}), 3&quot; Pot</td>
<td>11.7±0.6</td>
<td>0.382±0.043</td>
<td>3.06</td>
<td>-</td>
<td>2.5</td>
</tr>
<tr>
<td>1400 ul l(^{-1}), 3&quot; Pot</td>
<td>10.2±1.2</td>
<td>0.490±0.041</td>
<td>2.09</td>
<td>32</td>
<td>2.5</td>
</tr>
<tr>
<td>340 ul l(^{-1}), 3&quot; Pot</td>
<td>17.7±1.3</td>
<td>0.582±0.018</td>
<td>3.05</td>
<td>-</td>
<td>4.5</td>
</tr>
<tr>
<td>1400 ul l(^{-1}), 3&quot; Pot</td>
<td>15.3±1.1</td>
<td>0.588±0.056</td>
<td>2.61</td>
<td>14</td>
<td>4.5</td>
</tr>
</tbody>
</table>

\(^1\) Values are the means of 3 plants, with standard error; all measurements were taken after 11 days of treatment, except for the last two rows, which were after 18 days.

\(^2\) CER measured at 1000 umol m\(^{-2}\) s\(^{-1}\) PPFD and 340 ul l\(^{-1}\) carbon dioxide; CER expressed on a unit area basis.

\(^3\) Difference in CER between control and enriched treatments at the same pot size and Plastochron Index, and on a per unit area basis, expressed as a percentage of control values.

\(^4\) CER measured at 1000 umol m\(^{-2}\) s\(^{-1}\) PPFD and 340 ul l\(^{-1}\) carbon dioxide; CER expressed on a unit chlorophyll basis.

\(^5\) Difference in CER between control and enriched treatments at the same pot size and Plastochron Index, and on a per unit chlorophyll basis, expressed as a percentage of control values.
the same extent as enriched plants. Judging from the similarity of capacity of control plants in 5 and 3 inch pots at the same Plastochron Index, when expressed on a unit leaf area basis, it seems that pot size did not serve to reduce capacity, at least in the first trifoliate.

At 11 days, chlorophyll levels of plants in the smaller pots were reduced compared to those in large pots (Table 4.6), and the net carbon dioxide exchange capacity per unit chlorophyll was somewhat higher. Referring to the argument presented earlier, it may be that comparing two quite different chlorophyll levels, one or more of which may be non-limiting, gives an underestimate of CER, particularly at the higher chlorophyll level (control, 5 inch pot). Alternatively, it may be that net carbon dioxide exchange capacity on a per unit chlorophyll basis was indeed somewhat increased in small pots precisely because of the decrease in chlorophyll level. When plant growth and sink demand were restricted, higher chlorophyll levels would possibly reduce net carbon dioxide exchange capacity. At low chlorophyll levels, absolute rates of carbon dioxide exchange were correspondingly lower and so a reduction in net carbon dioxide exchange capacity was less likely to occur. This possibility was supported by the fact that enriched and control plants after 18 days in small pots, and having reduced chlorophyll levels (relative to those in large pots), showed a higher, but similar, carbon dioxide exchange capacity when measured per unit leaf area, or per unit chlorophyll. Hence, it seems that reducing sink demand by size restriction
need not result in a reduction of net carbon dioxide exchange capacity if chlorophyll levels were reduced in the process.

4.3.6 Effects On Dark Respiration

Leaves which showed reduced net carbon dioxide exchange capacity due to enrichment also showed greater respiration rates (Table 4.4, unshaded leaves).

Differences in stomatal resistance among treatments were observed, but the measurements of respiratory carbon efflux were not complicated by differences in stomatal resistance, unlike measurements of photosynthetic carbon flux. As stomata closed in the dark, stomatal resistance increased. Intercellular carbon dioxide concentration correspondingly increased because of respiratory production, which increased the gradient in concentration from the inside of the leaf to the outside. An increased driving force thereby maintained constant carbon dioxide efflux in spite of an increase in stomatal resistance. As illustrated in Figure 4.6, respiration rate was unaffected by stomatal closure.

Conditions of low PPFD, which did not elicit a decrease in carbon dioxide exchange capacity in enriched plants, also did not cause an increase in respiration rate (Table 4.4, shaded leaves). This effect of low PPFD on respiration rate may have related to a reduced CER of both enriched and control plants. In my study, the CER of plants in their respective treatment chambers was not determined. However, it was apparent from the unit leaf rate data (Chapter 3), and from Figure 4.2 (the CER of the first trifoliate under growth conditions can be approximated
FIGURE 4.6 Time-course of the response of leaf water vapour and carbon dioxide exchange to a light-dark transition.

The leaf was darkened at time zero. Solid lines are chamber outlet measurements and broken lines are reference inlet measurements. The absolute magnitude of the difference between the inlet and outlet is unimportant here, since it is a function of the degree of loop recirculation in the gas exchange measuring system. However, the relative changes in the difference between inlet and outlet of the two gas exchanges should be noted.
at a PPFD of 350 umol m\(^{-2}\) s\(^{-1}\) and at a measurement carbon dioxide concentration of 340 ul l\(^{-1}\) for controls and 1000 ul l\(^{-1}\) for enriched plants), that enriched plants had enhanced CER compared to controls during growth.

A linear relationship between net photosynthesis and the subsequent dark respiration rate has been reported (Penning de Vries, 1972; Ludwig et al., 1975). When photosynthesis was varied by changing either light intensity or carbon dioxide concentration (Ludwig et al., 1975), both treatments showed a linear positive relationship between net photosynthesis rate and dark respiration rate, although the responsiveness to carbon dioxide was somewhat less.

Enrichment may have caused both reduced net carbon dioxide exchange capacity and enhanced respiration because of greater carbon dioxide exchange rates. Further, the greater carbohydrate content of enriched plants may have been related to the increase in respiration. There is some evidence of a positive relationship between carbohydrate content and respiration rate. Azcon-Bieto and Osmond (1983) have found that a high carbon dioxide efflux in the dark, immediately following a period of photosynthesis was correlated with a greater concentration of carbohydrates in wheat leaves.

4.3.7 Effects On Stomata

The stomata of both enriched and control plants closed in response to increased carbon dioxide concentration when measured at 1000 umol m\(^{-2}\) s\(^{-1}\) PPFD (Table 4.7). However, stomatal resistance of enriched plants was generally lower than, or equal
TABLE 4.7. Stomatal diffusive resistance of the primary leaf and first trifoliate of enriched (1400 ul l\(^{-1}\)) and control (340 ul l\(^{-1}\)) plants.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rs(^2) at 340 ul l(^{-1}) (s mm(^{-1}))</th>
<th>Rs(^3) at 1000 ul l(^{-1}) (s mm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Leaf(^a)</td>
<td>340 ul l(^{-1})</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1400 ul l(^{-1})</td>
<td>-</td>
</tr>
<tr>
<td>First Trifoliate(^b)</td>
<td>340 ul l(^{-1})</td>
<td>12.4 ±0.7</td>
</tr>
<tr>
<td></td>
<td>1400 ul l(^{-1})</td>
<td>9.6 ±0.7</td>
</tr>
</tbody>
</table>

1 Values are the means of measurements of 4 leaves, with standard error
2 Rs was measured at an external carbon dioxide concentration of 340 ul l\(^{-1}\)
3 Rs was measured at an external carbon dioxide concentration of 1000 ul l\(^{-1}\)
4 Measured 6 days after transfer to the treatment chamber
5 Measured 2 days after transfer to the treatment chamber, and from a different set of plants than the primary leaves
to, that of controls when measured at the same carbon dioxide concentration. The data were quite variable, and further work is needed to determine the circumstances of the somewhat reduced stomatal resistance of leaves of enriched plants. The reduction of stomatal resistance in enriched plants implies that stomata have acclimated to high carbon dioxide concentrations, at least under some conditions. This has not been previously reported.

4.3.8 Effect On Tomato

Several authors (Bishop and Whittingham, 1968; Madsen, 1975) have found increased photosynthetic rates in enriched tomato plants compared to controls, when measured at the same external carbon dioxide concentration. Increased photosynthetic rates could, in part, have been due to differences in stomatal aperture between treatments. Hicklenton and Jolliffe (1980b), however, showed that enriched tomato plants had increased photosynthetic ability even when measurements were based on comparable intercellular carbon dioxide concentrations. They attributed the increased capacity to a decrease in photorespiration rate. Interestingly, the effect occurred only in enriched plants at 1000 ul l⁻¹ carbon dioxide and not in those at 5000 ul l⁻¹ carbon dioxide, and only at a particular age. The data were inconclusive as to why the effect eventually diminished.

It is possible that species or environmental differences were responsible for the discrepancy between my data and those of Hicklenton and Jolliffe (1980b). In an effort to resolve the contradiction, the same cultivar of tomato which was used in
their study was grown under conditions as similar as possible to that described in their work; light intensity was 150 umol m$^{-2}$ s$^{-1}$ PPFD, temperature was 35 C, and photoperiod was 15 hours. Measurements of CER which were obtained from leaves at the same node and at the same developmental stage as that reported by Hicklenton and Jolliffe (1980b) are given in Table 4.8. A concentration of 1000 ul l$^{-1}$ carbon dioxide caused reduced net carbon dioxide exchange capacity, just as in bean plants.

There are, however, several points of difference between my experiments and those of Hicklenton and Jolliffe (1980b). Although the growth chambers used in both cases were open and had continuous flow of fresh air, my chambers had a turnover time of 1 minute, whereas the chambers used by Hicklenton and Jolliffe had a 10 minute turnover time. This would lead to higher chamber humidity and a 10-fold increase in concentration of any volatiles, such as ethylene, produced by the plant. Ethylene is a known plant growth regulator and could conceivably have affected CER. Ethylene concentration in my chambers was less than 0.2 ul l$^{-1}$, but was not measured by Hicklenton and Jolliffe (1980b).

4.3.9 Summary

Bush beans grown in atmospheres enriched with carbon dioxide showed a reduced carbon dioxide exchange capacity, thus supporting the majority of reports dealing with gas exchange in enriched plants of other species. The reduction in capacity was not associated with changes in leaf thickness, nor was it due to increased stomatal resistance, which has been previously
### TABLE 4.8 Net carbon dioxide exchange rate of control (340 ul l⁻¹) and enriched (1000 ul l⁻¹) tomato plants at a PI of 5.0

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CER² at 340 ul l⁻¹ (umol m⁻² s⁻¹)</th>
<th>%CER³</th>
<th>CER⁴ at 1000 ul l⁻¹ (umol m⁻² s⁻¹)</th>
<th>%CER⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>340 ul l⁻¹</td>
<td>13.4 ±1.4</td>
<td>-</td>
<td>20.7 ±1.8</td>
<td>-</td>
</tr>
<tr>
<td>1000 ul l⁻¹</td>
<td>9.4 ±1.3</td>
<td>30</td>
<td>14.7 ±1.6</td>
<td>29</td>
</tr>
</tbody>
</table>

¹ Values are the means of 3 plants, with standard error
² CER measured at 650 umol m⁻² s⁻¹ PPFD and 340 ul l⁻¹ carbon dioxide
³ Difference in CER between control and enriched plants measured at 340 ul l⁻¹ carbon dioxide, and expressed as a percentage of control values
⁴ CER measured at 650 umol m⁻² s⁻¹ PPFD and 1000 ul l⁻¹ carbon dioxide
⁵ Difference in CER between control and enriched plants measured at 1000 ul l⁻¹ carbon dioxide, and expressed as a percentage of control values
associated with the apparent reduction in capacity (Imai and Murata, 1978c). The reduction in carbon dioxide exchange capacity with enrichment was also not due to reduced chlorophyll levels. A reduced chlorophyll concentration has been observed in enriched plants of other species, and could conceivably have accounted for the difference in carbon dioxide exchange capacity in other studies.

Enriched beans showed the same degree of oxygen sensitivity as unenriched beans, suggesting that photorespiration rate was unaltered by enrichment and was therefore not associated with the reduced carbon dioxide exchange capacity. This contrasts with a decrease in oxygen sensitivity in enriched tomato plants which showed an increased carbon dioxide exchange capacity (Hicklenton and Jolliffe, 1980b).

Enriched and unenriched leaves maintained at a low PPFD, and hence at low and similar carbon dioxide exchange rates, did not show a difference in carbon dioxide exchange capacity. This observation suggests that the reduction in capacity of enriched plants was a function of the higher carbon dioxide exchange rates at the higher PPFD. This relationship has not been previously demonstrated.

If chlorophyll levels were reduced in control and enriched plants, there again was no difference in carbon dioxide exchange capacity. Assuming that reduced chlorophyll levels were limiting CER in both control and enriched plants, the evidence would support the idea of a dependence of gas exchange capacity on overall CER.
As noted in previous studies, enriched plants showed increased starch content in the leaves. However, the relationship between starch content and carbon dioxide exchange capacity, in response to a change in sink demand in enriched compared to unenriched plants, has not been studied previously. Enriched bean plants did not show the large changes in gas exchange capacity found in unenriched plants when sink demand was altered. The relatively small changes in capacity possibly related to a consistently higher starch content in enriched plants. The higher starch levels may have reduced the upper limit of carbon dioxide exchange capacity of enriched plants.

Respiration rate increased in enriched leaves but only if there was a concurrent decrease in carbon dioxide exchange capacity. This correlation is a new and interesting finding in that it suggests that the two processes were being influenced by some common factor, perhaps an increased carbohydrate status.
V. EFFECTS ON LEAF SENESCENCE

5.1 INTRODUCTION

During the course of this study, it was observed that specific leaves of enriched beans became prematurely chlorotic. There have been only a few observations of this effect reported in the literature (Wittwer, 1967; Hesketh et al., 1971; Chang, 1975). Carbon dioxide-induced senescence correlated with a higher leaf starch content in cotton and soybean, although enriched sunflower, which also had high starch levels due to enrichment, did not show a similar relationship (Hesketh et al., 1971). The senescence was also associated with reduced photophosphorylation and Hill reaction activity (Chang, 1975).

Those observations constitute the extent of knowledge about this potentially interesting physiological problem. I therefore was interested in examining some of the environmental and physiological parameters associated with accelerated chlorosis and senescence of enriched bean leaves.

The following questions were addressed:
1. Which leaves are induced to senesce by carbon dioxide enrichment, and how rapid is the response?
2. How is high carbon dioxide-induced senescence modified by the environment? For example, how does temperature influence the induction or subsequent rate of carbon dioxide-induced senescence? Secondly, is carbon dioxide-induced senescence influenced by light intensity?
3. Since an increase in starch content figures so prominently
as a consequence of enrichment (see Chapter 4), is increased starch content related to the accelerated senescence at high carbon dioxide concentrations?

4. Is high carbon dioxide-induced senescence related to changes in solute content of enriched leaves? Conceivably, an increase in solute concentration may parallel the increase in starch, which could result in an osmotically-induced senescence.

5. Since carbon dioxide causes stomatal closure, is the senescence caused by carbon dioxide enrichment related to stomatal closure?

Answers to these questions will contribute significantly to an understanding of how carbon dioxide enrichment influences leaf senescence, and more generally, how this response relates to other aspects of carbon dioxide enrichment and plant function.
5.2 MATERIALS AND METHODS

Pre-treatment plant culture conditions were as described previously (Chapter 3). At 10 days, when the primary leaves were fully expanded, 4 to 8 plants were transferred to each treatment chamber and exposed to either 340±10 ul l⁻¹ (control) or 1400±20 ul l⁻¹ (enriched) carbon dioxide. Depending on the experiment, growth temperature was either 32 C day/25 C night (warm), or 20 C day/15 C night (cool). Subsequent reference to the growth temperature will mention the day temperature only. A PPFD of 350 umol m⁻² s⁻¹ was generally used, but in some experiments, two levels of PPFD were used. In this instance, each plant was positioned in the chamber such that one primary leaf received 350 umol m⁻² s⁻¹ PPFD and the other 100 umol m⁻² s⁻¹ PPFD. Light intensity was also manipulated in similar experiments by shading one primary leaf with a single layer of opaque paper. Photoperiod was 16 hours unless otherwise indicated.

CER was measured at various times during senescence using a semi-open gas exchange system (see section 4.2). In time-course studies, each leaf could only be used once, because use of the gas exchange cuvette left a residual ring of grease, deposited when the leaf was sealed in the gas exchange chamber. Hence, time-course studies of CER during senescence used leaves of different plants at each sampling date.

In all experiments, CER was determined at 1000 umol m⁻² s⁻¹ PPFD, and at the prevailing growth temperature; CER was based on an intercellular carbon dioxide concentration of 340 ul l⁻¹.
(interpolated from carbon dioxide response curves) in the time-course studies, and on an external carbon dioxide concentration of 340 ul l$^{-1}$ in all other experiments.

Chlorophyll determinations were made as previously described (see section 4.2).

Specific leaf weight was determined by taking the oven dry weight of a known area of interveinal leaf tissue.

Measurements of stomatal resistance were obtained in the treatment chambers and under growth conditions. A diffusive resistance porometer was used (Li-Cor, model LI 60).

Plastochron Index was determined as in Section 4.2.

In one experiment, leaves were excised from the plant after 10 days in the pre-treatment growth chamber, placed with their petioles in small beakers of distilled water, and transferred to the treatment chambers. Chamber temperature was maintained at 23 C day/17 C night, and light intensity was 370 umol m$^{-2}$ s$^{-1}$ PPFD. Carbon dioxide concentration was either 340 or 3000 ul l$^{-1}$.

Water potentials of leaf disks of control and enriched plants were determined with a Wescor dew point microvoltmeter (model HR-33T, with sample chamber C-52). The samples were then frozen on dry ice in aluminum foil and subsequently measured again with the dew point microvoltmeter to determine osmotic potentials. Specific leaf weight was also determined using disks obtained from the same leaves.
5.3 RESULTS AND DISCUSSION

As leaves develop, they exhibit a characteristic pattern in photosynthetic capacity. Photosynthesis per unit leaf area increases steadily during the time of net importation of assimilates and reaches a maximum as the rate of import reaches zero. At that time the leaf is a net exporter (Giaquinta, 1978). Photosynthesis rate then declines in the latter stages of leaf development (Davis and McCree, 1978). The decline in CER in mature primary leaves is evident in Table 5.1. Within 17 days of transfer to carbon dioxide treatment chambers, CER decreased to zero in enriched plants, irrespective of the growth temperature. The decline in CER of unenriched leaves was much less evident. Chlorophyll levels also decreased dramatically in enriched primary leaves at both temperatures (Table 5.1). Primary leaves of enriched plants at 20 C had lost virtually all chlorophyll by 20 days after transfer (Figure 5.1).

Changes in CER were first evident in plants at 20 C (Fig. 5.2) 6 days after transfer.

5.3.1 Effects Of Temperature

Accelerated senescence may be due simply to the normal advancement of aging or it may involve deterioration not associated with normal aging, for example, due to environmental stress. Plants at the lower temperature (20 C) did not grow as rapidly as those at the higher temperature (32 C), as shown by their lower Plastochron Index (Table 5.1). The initiation of senescence, as estimated by the first signs of chlorophyll loss
TABLE 5.1. Net carbon dioxide exchange rate and chlorophyll content of the primary leaves before and after 17 days of carbon dioxide enrichment at 32 or 20 °C.1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CER(^2) ((\text{umol m}^{-2} \text{ s}^{-1}))</th>
<th>Chlorophyll PI (g m(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Enrichment(^3):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>340 ul l(^{-1})/20</td>
<td>15.3±2.6</td>
<td>0.67±0.03</td>
</tr>
<tr>
<td>340 ul l(^{-1})/32</td>
<td>15.9±1.8</td>
<td>0.67±0.03</td>
</tr>
<tr>
<td>After Enrichment:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>340 ul l(^{-1})/20</td>
<td>10.5±1.3</td>
<td>0.60±0.02</td>
</tr>
<tr>
<td>1400 ul l(^{-1})/20</td>
<td>0.0±0.0</td>
<td>0.25±0.08</td>
</tr>
<tr>
<td>340 ul l(^{-1})/32</td>
<td>8.5±0.5</td>
<td>0.54±0.04</td>
</tr>
<tr>
<td>1400 ul l(^{-1})/32</td>
<td>0.0±0.0</td>
<td>0.13±0.07</td>
</tr>
</tbody>
</table>

1 Values are the means of 3 measurements, with standard error
2 CER was measured at 1000 umol m\(^{-2}\) s\(^{-1}\) PPFD and an external carbon dioxide concentration sufficient to give a Ci of 340 ul l\(^{-1}\)
3 The same plants were used for all initial values, hence only one initial chlorophyll value is given
FIGURE 5.1 Photograph of the primary leaves of enriched (1400 ul l\(^{-1}\)) and control (340 ul l\(^{-1}\)) plants grown for 20 days at 20 C.
FIGURE 5.2  Time-course of net carbon dioxide exchange rate of primary leaves of enriched (1400 ul l⁻¹) and control (340 ul l⁻¹) plants grown at 20 C. CER was measured at 1000 umol m⁻² s⁻¹ PPFD and an intercellular space carbon dioxide concentration of 340 ul l⁻¹. Each value is a measurement of a different leaf. Enriched plants, closed circles; control plants, open circles.
occurred somewhat sooner in physiologically younger leaves at cool temperatures than in physiologically older leaves at warm temperature (data not presented). Furthermore, both enriched and control primary leaves at 32°C had a CER of 13 umol m⁻² s⁻¹ at day 8, similar to initial rates (Table 5.1), but the CER of primary leaves of enriched plants at the cool temperature had decreased to 5 umol m⁻² s⁻¹ by that time (Fig. 5.2). Therefore, the induction of senescence was probably not due to accelerated aging. This conclusion requires that the Plastochron Index be an effective measure of physiological age.

One must be careful in comparing rates of senescence at two different temperatures. Once initiated, senescence proceeded at a rate determined by the prevailing temperature, even though the actual induction of senescence may have been earlier at the cooler temperature. After induction, the rate of senescence may have been slower because of a temperature-related slowing of metabolism. As a case in point, if enriched plants were transferred to a higher temperature at the onset of the first sign of chlorosis, further yellowing proceeded at an accelerated rate compared to enriched plants which were not transferred (data not shown). Also, control plants at both temperatures (Table 5.1) showed an equivalent decline in photosynthesis and chlorophyll content, which may be misleading for the reasons just discussed.

However, there did seem to be a temperature-related effect on senescence. Trifoliates of enriched plants also showed chlorosis, but only at the cool temperature. The chlorosis
appeared in small patches of interveinal tissue (Fig. 5.3), which eventually progressed to complete yellowing. Those leaves did not expand to normal size, and the chlorotic lesions developed when the trifoliates were still quite small (about 30% of normal maximum leaf size).

Senescence is known to be accelerated by extremes of temperature (Thomas and Stoddart, 1980). Two days of chilling stress at 10 C and a light intensity of 215 W m⁻² reduced photosynthesis to zero in *Sorghum*, although chlorophyll levels were not affected (Taylor and Rowley, 1971). The effects in *Sorghum* were attributed to solarization, which is a light-dependent destruction of the photosynthetic apparatus, or as initially defined, a reduced ability of chloroplasts to form starch (Holman, 1930). The temperatures that accelerated senescence in my study were quite moderate by comparison, and the PPFD was a maximum of only 370 umol m⁻² s⁻¹ (130 W m⁻²), quite low compared to that generally needed for solarization.

5.3.2 Effects On Stomata

Stomatal closure is associated with leaf senescence, although the nature of the relationship is not clear. Stomata are known to close with decreasing temperature, (Hofstra and Hesketh, 1969) decreasing light intensity, or increasing carbon dioxide concentration. It was therefore conceivable that in this study, any one or all of those parameters may have affected senescence through a change in stomatal aperture. Stomatal resistance was therefore measured in situ, that is, in leaves in the treatment chambers under their respective growth
FIGURE 5.3 Photograph of the central leaflet of the first trifoliate leaf of enriched (1400 ul l$^{-1}$) and control (340 ul l$^{-1}$) plants grown for 20 days at 20 C.
conditions.

For the first three days of treatment with two carbon dioxide concentrations and two levels of PPFD at 20 C, there were no significant differences in stomatal resistance (Fig. 5.4). However, plants which senesced the most rapidly, those at high carbon dioxide (1400 ul l⁻¹) and high light (370 umol m⁻² s⁻¹), also had the most rapid and extensive increase in stomatal resistance. By 17 days, plants at high carbon dioxide and low PPFD also began to show stomatal closure concurrently with yellowing.

Temperature also had no initial effect on stomatal resistance. After 7 days at 32 C, stomatal resistance was 0.91 ±0.05 s mm⁻¹ in controls and 0.78 ±0.06 s mm⁻¹ in enriched plants, values similar to those at 20 C (Fig. 5.4). The lack of immediate temperature or carbon dioxide effects on stomatal resistance was probably due to a limitation of stomatal opening by the relatively low PPFD. Furthermore, the two levels of PPFD used were not sufficiently different to significantly affect stomatal aperture (Fig. 5.4).

It has been suggested by Thimann and Satler (1979) that stomatal closure is not simply a result of the senescence process, but is the cause. Thimann (1980) supported this with several lines of evidence: (1) peeling of the epidermis of tobacco leaf discs greatly decreased the effects of kinetin on senescence, (2) coating with Vaseline promoted senescence, (3) fusicoccin, which promoted stomatal opening in the dark, delayed the onset of senescence in the dark, (4) phenyl mercuric
FIGURE 5.4 Time-course of stomatal resistance of primary leaves after transfer to treatment chambers at 20 C.

Measurements were made in the treatment chambers. Primary leaves were maintained at either 370 (solid lines) or 100 (broken lines) umol m$^{-2}$ s$^{-1}$ PPFD, and 340 (open circles) or 1400 (closed circles) ul l$^{-1}$ carbon dioxide concentration. Each data point is the mean of measurements of 4 to 6 plants, with standard error.
nitrate, which promoted stomatal closure in the light, also promoted senescence in the light, and (5) kinetin was found to cause stomatal opening in the dark.

Leaf gas exchange is altered because of stomatal closure, and it may be that the concentration of ethylene or carbon dioxide in the leaf could be related to the onset of senescence. The evidence for and against a role for ethylene in senescence has been previously discussed (see Chapter 2). The possibility of low carbon dioxide concentrations causing senescence is supported by recent work by Satler and Thimann (1983a). They found that oat leaves in air minus carbon dioxide senesced as rapidly in light as in the dark. I believe there are two possible explanations for this effect. First, in the absence of carbon dioxide, electron transport in the photoreactions of photosynthesis would not continue to reduce NADP, but would instead reduce oxygen to hydrogen peroxide in the Mehler reaction (Elstner, 1982). Over the long term, this could have damaging effects on the photosynthetic apparatus. Secondly, if carbon dioxide displaces ethylene from its binding sites in the leaf, the absence of carbon dioxide would cause a greater effective concentration of ethylene in the leaf, which could influence senescence as previously discussed.

Even if low carbon dioxide concentration causes senescence, the possibility of carbon dioxide concentrations becoming low enough to cause senescence as a result of stomatal closure was not likely in my study. Senescence was observed at high carbon dioxide concentrations. Even when stomata closed, the
intercellular carbon dioxide concentration would be higher than ambient in those treatments. Clearly, stomatal closure, if related to senescence, was not acting through a low carbon dioxide concentration.

5.3.3 Effects Of PPFD

Since carbon dioxide enrichment seemed to influence carbon dioxide exchange capacity through its effect on increasing CER (Chapter 4), senescence may have been induced for the same reason. In this context, light also caused chlorophyll loss in sugarcane (Edelman and Schoolar, 1969), and it may be significant that sugarcane is a C4 species, and as such was capable of very high photosynthesis rates. If so, decreasing PPFD should decrease CER, and hence senescence. Figure 5.5 shows that carbon dioxide enrichment had a greater effect on loss of carbon dioxide exchange capacity in primary leaves maintained at 375 umol m^{-2} s^{-1} than in those at 90 umol m^{-2} s^{-1}. Similarly, loss of chlorophyll was more rapid at the higher PPFD (Fig. 5.6) At low PPFD, carbon dioxide enrichment had no clear effect on chlorophyll levels. It should be kept in mind that the levels of PPFD differed by only 285 umol m^{-2} s^{-1}, and differences in senescence were still observed.

The ability of light to accelerate senescence was not consistent with previous studies showing that light delayed senescence compared to dark treatments in oats (Thimann et al., 1977) and Phaseolus vulgaris (Goldthwaite and Laetsch, 1967). Light was postulated to exert this effect through photosynthesis, and more specifically in the case of oats,
FIGURE 5.5 Time-course of chlorophyll content and net carbon dioxide exchange rate of shaded and unshaded primary leaves of plants grown at 1400 umol l⁻¹ carbon dioxide and at 20°C. Shaded leaves (broken lines) were grown at 90 umol m⁻² s⁻¹ PPFD, and unshaded leaves (solid lines) were grown at 375 umol m⁻² s⁻¹ PPFD. Each data point is a measurement of a different leaf.
FIGURE 5.6 Chlorophyll content of primary leaves grown at 340 or 1400 \textmu l l^{-1} carbon dioxide, and 90 or 370 \textmu mol m^{-2} s^{-1} photosynthetic photon flux density for 17 days. The growth temperature was 20°C. Each treatment is the mean of 4 leaves, with standard error.
through cyclic photophosphorylation. This was based on the observation that 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) completely blocked sugar formation in photosynthesis, yet did not prevent the delay of senescence by light (Thimann et al., 1977; Thimann, 1980). Light may possibly also act through phytochrome (see Thomas and Stoddart, 1980) although in Phaseolus vulgaris the response to light did not have the characteristics of a phytochrome response (Goldthwaite and Laetsch, 1967).

Experiments designed to study the effect of carbon dioxide on senescence in the dark compared to in the light were unsuccessful because of the technical difficulties in placing entire plants in absolute darkness while maintaining adequate ventilation and control of carbon dioxide concentration.

The conflicting data pertaining to the role of light in senescence may be resolved if one assumes that light influences two different processes, depending on intensity. Low levels of PPFD may be required to maintain a certain degree of stomatal opening, or level of energy charge in mesophyll cells through photosynthesis, in order to delay senescence. However, PPFD at higher levels may have contributed to senescence through yet other consequences of photosynthetic metabolism. In my study, light was probably not acting through a solarization process, since the highest level of PPFD was still relatively low in those experiments compared to the level needed for solarization.
5.3.4 Effects On Leaf Dry Weight

Treatments which caused a greater rate of senescence (e.g. 1400 ul l\(^{-1}\) carbon dioxide/370 umol m\(^{-2}\) s\(^{-1}\) PPFD) also caused a greater SLW (Table 5.2). Specific leaf weight was determined 6 days after the initiation of each treatment, a time when senescence symptoms were not yet apparent. By sampling at this time, changes in leaf dry weight which were a result of senescence were avoided. In samples taken at 10 days, when senescence symptoms were evident in some treatments, the trend in SLW was unaltered (data not shown). As shown in Figure 5.7, a higher SLW correlated with a greater starch content in leaves.

The SLW of young expanding first trifoliates of enriched plants at cool temperature (60 ±1 g m\(^{-2}\)) was also higher than that of controls also at cool temperature (38 ±2 g m\(^{-2}\)). Again, there was a positive relationship between leaf dry weight and senescence. Since those leaves were presumably still functioning as sinks, the higher specific leaf weights may result, in part, from a greater transport of assimilate from source leaves, which was then being accumulated, rather than being used in leaf area expansion.

5.3.5 Preliminary Experiments With Detached Leaves

Prevention of assimilate movement out of source leaves should increase specific leaf weight if net assimilation continues. For this reason it was of interest to observe how prevention of assimilate export from illuminated primary leaves would affect the rate of senescence with respect to carbon
TABLE 5.2. Specific leaf weight and starch content of the primary leaves of control (340 ul l\(^{-1}\)) and enriched (1400 ul l\(^{-1}\)) plants at two temperatures and two levels of photosynthetic photon flux density.\(^1\)

<table>
<thead>
<tr>
<th>T (C)</th>
<th>PPFD (umol m(^{-2}) s(^{-1}))</th>
<th>SLW (g m(^{-2}))</th>
<th>Starch (g m(^{-2}))</th>
<th>SLW (g m(^{-2}))</th>
<th>Starch (g m(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>340</td>
<td>33 ±2</td>
<td>1.4 ±0.9</td>
<td>65 ±3</td>
<td>25.9 ±3.5</td>
</tr>
<tr>
<td>32</td>
<td>100</td>
<td>23 ±1</td>
<td>0.2 ±0.0</td>
<td>27 ±0</td>
<td>0.8 ±0.5</td>
</tr>
<tr>
<td>22</td>
<td>340</td>
<td>48 ±2</td>
<td>15.1 ±0.6</td>
<td>78 ±0</td>
<td>46.8 ±1.9</td>
</tr>
<tr>
<td>22</td>
<td>100</td>
<td>36 ±0</td>
<td>3.0 ±0.8</td>
<td>45 ±3</td>
<td>10.9 ±0.8</td>
</tr>
</tbody>
</table>

\(^1\) Values are the means of 3 measurements, with standard error
FIGURE 5.7 Relationship between starch content and specific leaf weight of the primary leaves.
Plants were grown at either 340 or 1400 ml l⁻¹ carbon dioxide, and at either 32 or 20 C. Primary leaves were maintained at either 90 or 370 umol m⁻² s⁻¹ PPFD.
Leaves were sampled after 6 days of treatment. The curve was computer-fitted to a third-order polynomial equation, using 13 data points. \( R^2 = 0.98 \)
dioxide concentration.

In one set of experiments, the petioles of mature primary leaves were heat-girdled to kill the phloem. Girdling caused accelerated abscission of leaves in both carbon dioxide treatments and so this result in relating export to senescence was inconclusive.

In a second experiment, mature primary leaves were detached from the plant and treated with either 340 or 3000 ul l⁻¹ carbon dioxide at 23 C. Preliminary results showed a more rapid senescence and greater SLW in the high carbon dioxide leaves (data not presented). Most studies of senescence employ detached leaves or leaf disks, but the senescence-related biochemistry of excised material differs from that of attached leaves (Lewington et. al., 1967). Such differences may be due to the disruption of normal transport processes, to and from the leaf, which alters hormonal and metabolite status. This influences both the onset of senescence and the export of amino acids and other products during senescence (Spencer and Titus, 1973). Nevertheless, it is interesting to note that carbon dioxide accelerated senescence in both attached and detached leaves. Apparently, any changes incurred by removal from the plant did not undermine the relationship between carbon dioxide and senescence. Carbon dioxide therefore had a fundamental effect on senescence per se, separate from any possible effect on transport processes which could indirectly influence senescence. The possible effect of high carbon dioxide on the production or transport of growth regulators from other plant
parts is apparently not involved in the carbon dioxide-induced senescence process.

The possibility that carbon dioxide induced senescence through a deleterious accumulation of some product of photosynthesis is supported by other studies relating carbohydrate levels to senescence. For example, leaf disks of *Xanthium* floated on 0.01M sucrose solutions at 3000 Lux lost 90% of their chlorophyll compared to 60% loss in controls floated on water (Khudairi, 1970). The sucrose effect was light and temperature dependent, and was not due to an osmotic effect of the sucrose solutions, which had a solute potential of only -3.0 kilopascals (kPa). Similarly, Moore *et al.*, (1974) found that excised *Sinapis* cotyledons floated on sucrose solutions had greater chlorophyll loss, as well as a greater carbohydrate content, than controls. Starch content also correlates with senescence. Wittenbach *et al.*, (1980) described an increase in starch content coincident with the onset of senescence in mature soybean leaves, which was associated with a disorientation of chloroplast lamellae preceding a decline in photosynthesis, chlorophyll content, and total leaf nitrogen content, and an increase in proteolytic activity. Schoolar and Edelman (1970) found that if starch synthesis was prevented by application of iodoacetate, the light dependent decrease in chlorophyll content was slower.

It is interesting to speculate on how accumulated carbon may induce senescence of leaves. With enhanced photosynthesis and accumulation of fixed carbon, one might expect a lower
solute potential. This could induce senescence in a way similar to that induced by a water deficit. However, carbon dioxide-induced senescence did not seem to be osmotic in nature, since the water potential and solute potential of leaves sampled just prior to the onset of senescence symptoms were not significantly different between carbon dioxide treatments (Table 5.3).

At high carbon dioxide concentrations, the chloroplasts presumably assumed more of a storage function, with progressively greater accumulations of starch. The distortion of chloroplasts which results (Cave et al., 1981; Wulff and Strain, 1982) could influence the interception of light, the diffusion of carbon dioxide, or could disrupt the grana. Alternatively, a metabolic change could be involved. For example, Edelman and Hanson (1971) found that sucrose suppresses chloroplast development and chlorophyll synthesis in a carrot tissue culture with low invertase activity.

It should be kept in mind, however, that carbon dioxide may be influencing senescence by means other than (or in addition to) carbohydrate accumulation. Carbon dioxide may cause changes in the sensitivity to growth regulators, such as ethylene, or influence their levels or action. Possibly carbon dioxide as bicarbonate influences cell pH, the extent of which would depend on the buffering capacity of the cell (see Smith and Raven, 1979).
TABLE 5.3. Water and osmotic potentials, and specific leaf
leaf weight of enriched (1400 ul l⁻¹) and
control (340 ul l⁻¹) plants grown for 7 days
at 24 C.¹

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Water potential (kPa)</th>
<th>Osmotic potential (kPa)</th>
<th>SLW (g m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>340 ul l⁻¹</td>
<td>-63 ±6</td>
<td>-100 ±17</td>
<td>59 ±4</td>
</tr>
<tr>
<td>1400 ul l⁻¹</td>
<td>-89 ±14</td>
<td>-121 ±13</td>
<td>113 ±6</td>
</tr>
</tbody>
</table>

¹ Values are the means of 3 leaves, with standard error.
5.3.6 Summary

The induction of leaf senescence by high carbon dioxide concentrations has not been previously characterized. Carbon dioxide-induced senescence of primary bean leaves was positively correlated with light intensity (PPFD). Trifoliates also showed accelerated chlorosis, but only at cool temperatures; senescence of primary leaves may also be influenced by temperature but the data were equivocal on this point.

The fact that higher PPFD caused a more rapid senescence would suggest that carbon dioxide-induced senescence was related to the higher carbon dioxide exchange rates of enriched leaves. Furthermore, those treatments which caused the most rapid senescence also caused the greatest SLW and leaf starch content.
VI. GENERAL DISCUSSION

By way of introducing a general discussion of the findings of this study, I would like to review the major points which were described.

Continuous long-term carbon dioxide enrichment caused large increases in plant dry weight, which were reflected in increases in growth rate compared to unenriched plants. The increases in growth rate were dependent on increases in unit leaf rate early in development, and on increases in leaf area and to a lesser extent increases in unit leaf rate, later in development. The observed increases in unit leaf rate of whole plants were also reflected in increases in the net carbon dioxide exchange rate of individual leaves of those plants. In addition, the increase in carbon dioxide exchange rate of enriched leaves correlated with a reduced carbon dioxide exchange capacity and an increased respiration rate in those leaves. An increase in carbon accumulation (measured as an increase in specific leaf weight and an increase in starch content), which resulted, in part, from the increase in carbon dioxide exchange rate of enriched leaves, correlated with a reduction in carbon dioxide exchange capacity of the first trifoliates, and accelerated senescence of the primary leaves. Furthermore, the carbon dioxide-induced changes in relative growth rate and unit leaf rate of whole plants, and the changes in carbon dioxide exchange capacity, respiration rate, and senescence of individual leaves, were most evident early in plant development.
In a number of cases, the magnitude of a response to carbon dioxide enrichment changed by increments with an increasing carbon dioxide concentration. The best examples of incremental change were the increases in absolute growth rate, relative growth rate, and unit leaf rate of beans at progressively greater carbon dioxide concentrations (Chapter 3). The incremental response of those parameters appeared to decrease between 800 and 1200 ul l⁻¹ carbon dioxide, which suggests an approach to saturation in that concentration range. Commercial greenhouses frequently have enriched atmospheres of 1000 to 1200 ul l⁻¹ carbon dioxide (Wittwer and Robb, 1964). According to my data, a 1000 to 1200 ul l⁻¹ concentration range would optimize increases in growth with a minimal carbon dioxide addition to the greenhouse atmosphere. Bear in mind that environmental conditions may modify the growth response/carbon dioxide concentration relationship. For example, because of the severity of carbon dioxide-induced senescence at cool temperatures (Chapter 5), the growth response at 1000 to 1200 ul l⁻¹ may be reduced when compared to that at lower concentrations. Accelerated leaf senescence is an important consideration in determining optimum carbon dioxide concentrations for greenhouse enrichment, particularly at cool temperatures. However, the dose-response relationship of carbon dioxide concentration and senescence was not determined in my study.

The net carbon dioxide assimilation rate of leaves
generally increases with increasing carbon dioxide concentration if other environmental factors are not limiting (Gaastra, 1963). The importance of carbon dioxide as a determinant of net carbon dioxide assimilation rate cannot be underestimated in any study of the effects of long-term carbon dioxide enrichment on plant growth or function. The increase in unit leaf rate (Chapter 3) and CER (Chapter 4) of enriched leaves shows that high carbon dioxide concentration resulted in an increase in net carbon dioxide assimilation rate in those plants.

A basic conclusion of my study is that a higher net carbon dioxide exchange rate in enriched plants was responsible for many of the observed physiological effects of enrichment. For example, an increase in relative growth rate correlated with an increase in unit leaf rate (Chapter 3). Furthermore, the higher CER of enriched leaves correlated with a reduction in carbon dioxide exchange capacity and an increase in respiration rate compared to controls (Chapter 4). If the difference in CER between control and enriched plants was reduced by shading, or by low chlorophyll levels (Chapter 4), the effectiveness of enrichment in altering carbon dioxide exchange capacity and dark respiration rate was diminished.

The effect of carbon dioxide enrichment on senescence was also dependent on the level of PPFD, once again showing a probable relationship between enhanced CER and a carbon dioxide enrichment response.

Similarly, a limitation of unit leaf rate by low light intensity during the latter stages of development probably
reduced the effectiveness of carbon dioxide enrichment in increasing unit leaf rate of whole plants (Chapter 3). Attenuation of light intensity through a shoot canopy is logarithmic in nature, and hence the unit leaf rate of much of a well-developed shoot canopy may have been limited by light intensity. In my study, the relative growth rate of both enriched and unenriched plants decreased with maturity, presumably because an increasing proportion of the leaves were functioning at reduced light levels. At those intensities, the effectiveness of high carbon dioxide concentration in increasing unit leaf rate was diminished. This interpretation is supported by data on the response of individual leaves to increasing light intensity reported in Chapter 4. At low PPFD, the absolute increase in CER with increased carbon dioxide concentration was much less than the absolute increase in CER at high PPFD. A light-carbon dioxide interaction has been observed previously, in that the effect of carbon dioxide was progressively greater with increasing light intensity (Hopen and Ries, 1962; Ford and Thorne, 1967; Hughes and Cockshull, 1971). However, the possibility that the shading of leaves during development was responsible for the diminishing effect of enrichment on relative growth rate has not been discussed in the literature.

My study therefore suggests a number of reasons why the growth of whole plants did not respond to enrichment as would be predicted from short-term carbon dioxide-response curves of net photosynthesis rate. First, when CER was not limited by light intensity, the net carbon dioxide exchange capacity of enriched
leaves was reduced compared to the CER of control leaves. Second, when light intensity in the canopy was low, the absolute increase in unit leaf rate at high carbon dioxide concentration was diminished, thereby contributing to a lower relative growth rate. Third, accelerated senescence of leaves of enriched plants probably reduced the leaf surface area available for net carbon dioxide exchange.

An often overlooked factor in determining the response of unit leaf rate to carbon dioxide concentration is the degree of air turbulence within the plant canopy. In all experimental situations outlined in my study, chamber air was well mixed. However, ventilation can be a confounding influence when extrapolating the response of CER, obtained from gas exchange measurements in well-ventilated chambers or cuvettes, to a whole plant or crop situation. Profiles of carbon dioxide concentration within an enriched shoot canopy show gradients in concentration which are dependent on the position in the canopy, and on plant density (Duncan and Barfield, 1968). Hence, the carbon dioxide concentration at the leaf surface may vary substantially with leaf position or plant density. As a result, carbon dioxide response curves generated in well-ventilated measurement chambers may be quite different than those in a field situation. In a poorly ventilated crop, I suspect that unit leaf rate would approach saturation at a higher carbon dioxide concentration than one would expect from laboratory measurements.

The effectiveness of high carbon dioxide concentration in
increasing plant growth, which is concurrently limited by other environmental factors, such as light intensity (Chapters 3 and 4), has relevance agronomically. Water and nutrient availability may also limit the growth potential of vegetation in many areas, but little information is available on the effects of enrichment on growth when those conditions are limiting.

In describing the effect of carbon dioxide enrichment on growth which is limited by other factors, the effects of carbon dioxide on unit leaf rate must be separated from effects on leaf area. For example, growth of cotton was increased by carbon dioxide enrichment at low nitrate levels only because of an increase in leaf area; unit leaf rate was not increased by enrichment (Wong, 1979).

Carbon dioxide enrichment also appears to be of use in improving plant growth under conditions of low water availability. Growth of wheat under water stress conditions was better in enriched than unenriched plants (Gifford, 1979). When stressed, enriched plants showed greater relative increases in growth than unenriched plants, compared to an unstress situation, although the absolute increases in growth were smaller. Sionit et al. (1980) found that the better growth of enriched compared to unenriched wheat under water stress, appeared to be due to better turgor maintenance, as a result of lower osmotic potentials in enriched plants. This also allowed better stomatal opening at low water potentials (Sionit et al., 1981b).
In summary, carbon dioxide enrichment appears to result in a growth increment even when other environmental factors may be limiting. However, it is important to recognize that the greatest absolute growth increments will result when other environmental factors do not constrain growth. If growth is restricted by a stress, the relative increase in growth due to enrichment may be much larger than the absolute increase in growth. This increase in growth potential under rate-limiting conditions is a result of the nature of the involvement of carbon dioxide in photosynthesis. High carbon dioxide concentration decreases the percentage inhibition of true photosynthesis caused by photorespiration (Fock et al., 1979), and thereby increases the net photosynthetic rate, even when other environmental factors may be limiting.

It is interesting to note that the increments in growth due to enrichment may be tempered, not only by other environmental constraints, but also by the effect of high carbon dioxide concentrations on stomatal aperture. Stomatal closure at high carbon dioxide concentrations would increase water use efficiency, but may concurrently limit the growth response. Stomatal closure in response to carbon dioxide results in one of two situations with respect to net photosynthesis (van Keulen et al., 1980). In the first instance, stomatal closure is sufficient to offset an increase in intercellular carbon dioxide concentration, resulting in no increase in net photosynthesis, but a decrease in transpiration. Hence, water use efficiency increases. In this case, stomatal aperture would appear to
regulate Ci. The maintenance of a constant Ci has also been observed when extracellular carbon dioxide is held constant but the photosynthetic rate is varied; stomatal aperture adjusts with changes in photosynthesis rate (Wong et al., 1979). In the second case, stomatal aperture does not regulate Ci and an increase in extracellular carbon dioxide will decrease transpiration rate, but increase net photosynthesis rate. Again water use efficiency increases, but both transpiration rate and net assimilation rate have changed.

In my study, beans showed stomatal closure (i.e. increased stomatal resistance) in response to high carbon dioxide concentrations, when measured at light intensities approaching saturation (1000 umol m\(^{-2}\) s\(^{-1}\) PPFD). However, Ci appeared not to be regulated by stomatal aperture, and increases in Ci resulted in a greater CER. Also, stomatal resistance measured in plants under growth conditions (350 umol m\(^{-2}\) s\(^{-1}\) PPFD) was not affected by carbon dioxide concentration (Chapter 5). There also appeared to be some acclimation of stomata to continuous carbon dioxide enrichment (Chapter 4), which would serve to maintain somewhat greater stomatal opening at high carbon dioxide concentrations. Therefore, stomatal closure in beans was not an important factor in limiting the growth response to enrichment.

As previously outlined, many of the carbon dioxide enrichment effects described in this study were associated with a relatively higher CER in enriched than control plants.
One possible way a higher CER could influence carbon dioxide exchange capacity, respiration rate, or senescence of enriched plants, is through an increase in the carbon assimilation/carbon export ratio of source leaves, resulting in an increase in the leaf carbohydrate status. A high SLW and starch content correlated with all those responses to enrichment.

The relationship between starch content and photosynthetic capacity has been debated extensively in the literature. A negative relationship between starch content and photosynthetic capacity was observed by Nafziger and Koller (1976) with short-term (16 hour) carbon dioxide enrichment of soybean. Mauney et al. (1979) and Hofstra and Hesketh (1975) have observed the same correlation in long-term enrichment studies. In fact, the negative correlation between starch content and photosynthetic capacity is evident in all long-term carbon dioxide enrichment studies to date.

The relationship between starch content and photosynthetic capacity has also been postulated as an integrator of sink and source activity in plants. It has been suggested that sink demand controls source leaf photosynthesis through an effect on starch content of source leaves. For example, if sink strength decreases relative to source strength, starch would presumably accumulate in source leaves, and inhibit photosynthesis in those leaves. Although the relationship has been documented in many studies (Herold, 1980), others do not find a correlation, particularly in short-term studies (Geiger, 1976; Potter and
Breen, 1980; Fondy and Geiger, 1982).

It has been suggested (Mauney et al., 1979) that where no apparent relationship between starch content and photosynthetic capacity is apparent, the starch levels were not high enough. However, in a study of soybeans exposed to continuous light for 54 hours, there was no decline in photosynthesis, despite a 14-fold increase in starch content, which suggests that there was no relationship (Potter and Breen, 1980). Data of Chapter 4 may be relevant in this discussion. There appears to be a negative relationship between starch content and the maximum carbon dioxide exchange capacity. However, changes in carbon dioxide exchange capacity within the limits determined by starch content were evidently independent of starch levels.

With this discussion in mind, I would like to review several effects of carbon dioxide enrichment on leaf physiology which were observed in this study.

First, carbon dioxide enrichment was less effective in decreasing the carbon dioxide exchange capacity of leaves if chlorophyll content of the leaves was low, or the level of PPFD was limiting. This reduced the CER of enriched, as well as control plants, but also decreased the difference in CER between those treatments. The relationship between photosynthetic rate and photosynthetic capacity in enriched plants has not received much attention in the literature. At low CER, a more favorable leaf carbohydrate balance would presumably be achieved, and high carbon dioxide would therefore be less effective in reducing carbon dioxide exchange capacity.
In this context, I consider the relationship between chlorophyll content and carbon dioxide exchange capacity to be particularly noteworthy. When growth, and hence sink demand, was severely restricted in plants growing in small containers (Chapter 4), the lower chlorophyll levels found in source leaves would likely reduce carbon accumulation in those leaves. A reduction in carbon dioxide exchange capacity, at least per unit chlorophyll, would therefore be less likely to occur. To the best of my knowledge, this relationship has not been discussed in the literature.

An increase in sink demand did not alleviate the difference in carbon dioxide exchange capacity among enriched and control treatments, suggesting that the leaf carbon balance was not sufficiently affected, perhaps because of a constant supply of new assimilate. In fact, enriched plants did not respond as readily as controls to a change in sink demand in terms of an increased carbon dioxide exchange capacity or reduced starch content. Enriched plants were, therefore, not as flexible in adjusting source leaf carbon dioxide exchange capacity to a new demand situation. This has not been observed before.

Reproductive growth, acting through an increased sink demand, is known to increase CER in source leaves (Gifford and Evans, 1981). Although reproductive growth may contribute to a lower SLW in enriched plants (see Chapter 3), it is not known whether the onset of reproductive growth influences the carbon dioxide exchange capacity of those plants.

Carbon dioxide-induced senescence was also associated with
high carbon retention in the leaves; SLW and starch content were extremely high in those leaves which were the most rapidly senescent. Increasing SLW, either by lowering the temperature or by increasing net assimilation at a higher light intensity, resulted in a more rapid senescence of enriched plants. Here again, as in the relationship between starch content and carbon dioxide exchange capacity, one must be cautious in suggesting that a high starch content causes senescence. Even so, I feel it is physiologically significant that the correlation occurred irrespective of the manner in which senescence was induced.

Carbon dioxide was very effective in inducing senescence in primary leaves, which probably related to the fact that those leaves attained the highest SLW (Chapter 3). Carbon dioxide induced senescence at 1400 ul l⁻¹, which is unusually low when compared to the high concentrations (>10000 ul l⁻¹) required in other developmental processes, such as interaction with ethylene (Abeles, 1973) or in flower induction in Pharbitis nil under a non-inductive long-day photoperiod (Hicklenton and Jolliffe, 1980a). This large difference in concentration suggests that the mode of action of carbon dioxide in the induction of senescence may be unique, compared to its role in other developmental processes. An increase in SLW may be an indication that the plant was unable to use carbon at a rate sufficient to keep pace with its assimilation. Consequently, senescence of those leaves was a means of reducing total leaf area and thereby achieving a more equitable balance of supply and demand. Hence, senescence in this case was not as much a
function of age as it was of an altered carbon balance, a relationship not widely recognized in the literature. Evidently, those factors which induced senescence were also responsible for a shift in leaf starch metabolism, either because of a change in carbon partitioning, or simply because of greater leaf carbon accumulation under those conditions (here, accumulation/export remains the same).

In this respect, source/sink relationships may be quite important in determining the induction or subsequent rate of senescence. Using a technique of surgically manipulating transport from the flag leaf of wheat, Lazan et al., (1983) showed that restricted export of carbon and nitrogen promoted senescence. The onset of senescence was associated with the attainment of a threshold level of ethanol-soluble carbohydrate.

Similarly, the effect of temperature on source/sink relationships may be important in carbon dioxide-induced senescence. Plant growth was much slower at 20 C than at 32 C, and this was presumably associated with a lower demand for assimilates by developing sinks, and hence, reduced translocation rates. The reduced demand may have resulted in a greater carbohydrate content of enriched primary leaves (see Table 5.2), and contributed to an earlier onset of senescence and a more extensive senescence, than in enriched plants at a higher temperature. There is evidence that the translocation/photosynthesis ratio increases with the measurement temperature, from 10 to 30 C (bean) and from 10 to 40 C (soybean), in plants previously grown at 20 C (Marowitch et
Hence, a lower translocation/photosynthesis ratio at cooler temperatures may have contributed to an accumulation of carbohydrates in source leaves in my study. Alternatively, a change in temperature may have influenced carbohydrate status independently of an effect on plant source/sink relationships.

The majority of studies which explore source-sink relationships and senescence do so specifically in relation to monocarpic senescence. Seed removal in legume species generally delays senescence. However, carbohydrate levels do not seem to be involved since pod removal delayed chlorosis of soybean, despite large increases in leaf starch and soluble carbohydrate content (Mondal et al., 1978). Similarly, male-sterile soybeans set 85% fewer pods than non-sterile soybeans, an effect which was associated with delayed senescence and a higher nitrogen content (Wilson et al., 1978). Yet male-sterile soybeans accumulated more carbohydrates in leaves.

Seed removal in some non-legumes, on the other hand, accelerates senescence. Ear removal resulted in accelerated senescence and carbohydrate accumulation in leaves of corn (Allison and Weinmann, 1970; Christensen et al., 1981), and barley (Mandahar and Garg, 1975). Removal of flowers in pepper accelerated the age-related loss of RuBP carboxylase activity (Hall and Brady, 1977).

In an effort to resolve those conflicting data, Thomas and Stoddart (1980) proposed that sink demand for nitrogen was reduced when seeds of legumes (which have a relatively high N:C ratio) were removed, thereby delaying senescence through a
greater retention of nitrogen in the leaves. Conversely, removal of sinks with a high demand for fixed carbon but only a limited demand for mobile nutrients (for example in non-legumes), causes carbohydrate accumulation and eventual senescence. This view is based on the nutrient diversion hypothesis of senescence, and as such, fits nicely with the self-destruct concept of Sinclair and de Wit (1975), who have suggested that the nitrogen requirement of developing seeds of legumes is so great that it results in extensive nitrogen loss from leaves, and subsequent senescence of those leaves. In my study, carbon dioxide-induced senescence of *Phaseolus* was unrelated to nutrient removal. First, senescence occurred well before pod development, so the only organs requiring mobile nutrients were new leaves and roots, both of which presumably had relatively low demands. Second, detached leaves, which could not transport mobile nutrients (other than leakage from the petiole), showed the same trends as attached leaves (Chapter 5).

If carbon dioxide-induced senescence is unrelated to monocarpic death, one could suggest that it may be acting as a stress. A high carbon dioxide concentration is not usually considered an environmental stress. On the contrary, high carbon dioxide concentrations are generally associated with enhanced plant growth; deleterious affects of high concentrations have rarely been considered or even observed.

Instances of stress, in which deficiency of resources cause senescence are well known. For example, mineral, light, or
water deficiencies all can induce senescence. Conversely, excessive conditions of temperature or light (solarization) may also lead to photosynthetic dysfunction and eventual death. In this respect, carbon dioxide-induced senescence is unlike solarization. In an early study of solarization, Holman (1930) found that beans which had large starch deposits in the leaves were less prone to solarization than those with less starch. Further, the tendency toward solarization was offset by carbon dioxide enrichment.

It is now apparent that excessive concentrations of carbon dioxide will also induce senescence, the relationships of which are unique compared to other environmental extremes.

Further carbon dioxide enrichment studies are needed to follow up several points arising from the present work.

Better information is needed on the relative rates of translocation and carbon storage in leaves, as influenced by enrichment. This has bearing on the effect on carbon dioxide enrichment on dry matter partitioning in plants.

Future work should also include studies of the growth response of enriched plants when growth is restricted by other environmental factors. My work suggests that the growth response of enriched plants was reduced during the latter stages of development by a combination of light limitation as well as by a reduced carbon dioxide exchange capacity of leaves. Future work might include studies of growth and carbon partitioning in enriched plants when temperature limits growth. A study of the
growth response of enriched plants in relation to temperature is particularly relevant in view of the effect of carbon dioxide on senescence at cool temperatures.

The observations recorded in this study are relevant in widely diverse areas of physiological investigation. Because of the applicability of carbon dioxide enrichment to such areas of study as plant senescence and source-sink relationships, it is conceivable that future work may incorporate carbon dioxide enrichment as a tool of investigation of those processes. Three such areas of investigation come to mind. Carbon dioxide enrichment effectively changes the source strength of leaves, and this may be useful in defining how net photosynthesis rate is controlled by integration of plant source-sink activity. Second, the increases in dark respiration rate in enriched plants may be a function of the higher CER of those plants. Hence, carbon dioxide enrichment studies may be useful in exploring the nature of the relationship between net photosynthesis rate and respiration rate. Third, carbon dioxide-induced senescence of leaves seems to be related to carbohydrate metabolism of those leaves. This may have applicability in more general studies of senescence. For example, it would be worthwhile to determine the influence of growth regulators on the carbohydrate metabolism of leaves induced to senesce at high carbon dioxide concentrations.

It is clear that an understanding of carbon dioxide enrichment effects of plants must encompass developmental, as well as photosynthetic responses. Much more work is required to
determine the inter-relationships between those two broad sets of responses to carbon dioxide enrichment.
VII. CONCLUSIONS

Continuous long-term carbon dioxide enrichment of bean plants increased plant growth rate. The primary influence of carbon dioxide enrichment on relative growth rate occurred earlier in development than did the major influence on absolute growth rate. The increase in relative growth rate of enriched plants early in development was paralleled by an increase in unit leaf rate at that time. Therefore, the increase in relative growth rate was probably a reflection of an increased unit leaf rate. Similarly, the major increase in carbon dioxide exchange rate of individual leaves of enriched plants occurred early in leaf development. Accompanying the increase in unit leaf rate or carbon dioxide exchange rate were a decrease in carbon dioxide exchange capacity and an increase in respiration rate of trifoliate leaves, and accelerated senescence of primary leaves. It was therefore concluded that the effects of carbon dioxide enrichment on carbon dioxide exchange capacity, respiration rate, and leaf senescence resulted from the large increases in unit leaf rate or carbon dioxide exchange rate which occurred early in plant or leaf development respectively.

Carbon dioxide enrichment caused large increases in leaf starch content which accompanied the reduction in carbon dioxide exchange capacity of enriched leaves. A consistently higher leaf starch content may explain why the carbon dioxide exchange capacity of enriched source leaves did not respond as strongly as unenriched leaves to an increase in sink demand. The increase in leaf starch content was even more pronounced in
enriched primary leaves which showed accelerated senescence. Therefore, the effect of an increased carbon dioxide exchange rate on carbon dioxide exchange capacity, respiration rate, and senescence in enriched plants may have been through a greater leaf carbohydrate status.

Changes in the growth temperature or light intensity influenced leaf carbohydrate status and the rate of primary leaf senescence. Light intensity also modified the affect of carbon dioxide enrichment on leaf carbon dioxide exchange capacity of trifoliates. Therefore, it was concluded that the effectiveness of carbon dioxide enrichment in eliciting those plant responses was influenced by the environment, possibly through an effect on leaf carbohydrate status.
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The primary data of trials 1 and 2 were not pooled because of significant block error (see Table 3.1). Hence, the primary data for both trials were analysed separately.

The primary data of leaf area over time (Fig. A1) and plant dry weight over time (Fig. A2) are given for the 4 carbon dioxide treatments of trial 2. As in trial 1, the leaf area and plant dry weight of enriched plants increased in comparison to controls, with the changes in plant dry weight being more pronounced than the changes in leaf area. Generally, the trends in leaf area and plant dry weight over time among carbon dioxide treatments were similar to the changes in those parameters in trial 1, although the data were more variable in trial 2.
FIGURE A1 Leaf area over time of plants grown at 340, 500, 1200, or 3000 ul l⁻¹ carbon dioxide. The data are from Trial 2. Each value is the mean of 4 plants, with standard deviation. Curves are cubic spline functions fitted to data for each carbon dioxide treatment.
FIGURE A2 The dry weight over time of plants grown at 340, 500, 1200, or 3000 ul l\(^{-1}\) carbon dioxide.

The data are from Trial 2. Each value is the mean of 4 plants, with standard deviation. Curves are cubic spline functions fitted to data for each carbon dioxide treatment.