PHYSIOLOGICAL LIMITATIONS TO THE GROWTH RESPONSE OF BEAN PLANTS (*Phaseolus vulgaris* L.) TO CARBON DIOXIDE ENRICHMENT

by

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THE DEPARTMENT OF PLANT SCIENCE

We accept this thesis as conforming to the required standard

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ABSTRACT

Previous studies on dwarf bean plants have found a very limited growth response to CO$_2$ enrichment (Jolliffe and Ehret, 1985; Ehret and Jolliffe, 1985b). There was no increase in leaf area, and leaf injury was observed after about three weeks of CO$_2$ enrichment (Ehret and Jolliffe, 1985a). Although dry weight was increased, the increase may be limited due to restricted carbon utilization (e.g. no increases in leaf area). In this study, non-photosynthetic limitations, such as the partitioning of dry matter among plant parts, the partitioning of carbon among photosynthetic end products, and the interactive effects of nutrient and carbon supply on growth, that may contribute to the observed growth responses were investigated.

Bean plants responded to CO$_2$ enrichment by increasing their total dry weights. This weight increase was caused by higher growth rate, at least at early growth stages, and higher unit leaf rate. The dry weight increase was mainly in the leaves, and was not evenly distributed among all plant parts. Leaf expansion and branching were not enhanced by CO$_2$ enrichment. The differential effects of CO$_2$ enrichment on growth of different parts caused significant increases in specific leaf weight and shoot root ratio, and a decrease in leaf area ratio. These results indicated that the bean plants used in this study have a limited ability to utilize the extra carbon that was fixed under CO$_2$ enrichment.

There were small increases in glucose, fructose, and sucrose concentrations early in the CO$_2$ treatments. These increases became much larger after three weeks of CO$_2$ enrichment. The timing of the higher increases in leaf soluble sugars coincided with the timing of increases in stem and roots dry weight.

There was also a large increase in starch concentration shortly after plants were transferred to CO$_2$ enriched condition. The higher starch concentration accounted for the majority of the weight increase in CO$_2$ enriched leaves, and this starch level was
maintained for several days after plants were switched back to ambient CO₂ levels. A ¹⁴C study on the partitioning of carbon between leaf pools showed that carbon transfer out of the storage pool under CO₂ enrichment was limited.

CO₂ enrichment had no effects on leaf protein and amino acid concentrations. No difference, or slight increases, were found in inorganic nutrient concentrations per unit leaf area. Plants grown under CO₂ enrichment, however, show a higher loss of nutrients (especially N and K) from older shoot parts (primary leaves and older trifoliates) to younger parts.

High NO₃⁻ supply increased plant dry weight and leaf area under both CO₂ enriched and ambient conditions. The dry weight increases of the stem and roots caused by CO₂ enrichment, however, were much higher and earlier for high NO₃⁻ treated plants. Furthermore, lower leaf starch concentration was also observed for those CO₂ enriched high NO₃⁻ treated plants. High NO₃⁻ supply also increased the leaf nutrient concentrations (N, K, Mg, Ca). Increased uptake of nutrients for high NO₃⁻ treated plants may be partly contributed by the enhanced root growth.

In addition to the growth responses, foliar abnormalities developed gradually in beans under CO₂ enrichment. Chlorosis, assessed by a loss in total chlorophyll concentration, was observed in the primary leaves after about three weeks of CO₂ enrichment. The disorder eventually appeared in the oldest trifoliate leaves after more prolonged CO₂ enrichment. The onset of leaf injury was correlated with the timing of the increases in leaf soluble sugars and the redistribution of nutrient elements from the older shoot parts to the younger parts. High NO₃⁻ supply delayed the development of leaf injury induced by high CO₂.

Results in the present studies indicate that growth responses of dwarf bean plants to CO₂ enrichment were affected by the limited carbon partitioning, and the restriction of starch degradation was indicated to be the probable cause. A higher carbon input under CO₂ enrichment may create a higher demand for inorganic
elements. Effects of nutrient supply (NO$_3^-$) on growth responses and leaf injury of CO$_2$ enriched plants suggested that an imbalance between carbon and nutrient input could be partly related to the limited growth responses of dwarf bean plants to CO$_2$ enrichment.
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<td>A</td>
<td>the initial $^{14}$C activities in Pool 1</td>
</tr>
<tr>
<td>a</td>
<td>the initial $^{14}$C activities in Pool 2</td>
</tr>
<tr>
<td>Ab</td>
<td>the measurement of absorbances of spectrophotometer</td>
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<td>ABA</td>
<td>abscisic acid</td>
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<td>ANOVA</td>
<td>analysis of variance</td>
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<td>B</td>
<td>pool depletion constants for Pool 1</td>
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<tr>
<td>b</td>
<td>pool depletion constants for Pool 2</td>
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<tr>
<td>Bq</td>
<td>bequerel, unit of radioactivity</td>
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<tr>
<td>CER</td>
<td>net carbon dioxide exchange rate</td>
</tr>
<tr>
<td>E</td>
<td>unit leaf rate</td>
</tr>
<tr>
<td>e</td>
<td>the base of natural logarithms</td>
</tr>
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<td>dt</td>
<td>change in time</td>
</tr>
<tr>
<td>dW</td>
<td>change in weight</td>
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<td>$K_{01}$</td>
<td>transfer coefficient determining the rate of carbon export from Pool 1</td>
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<tr>
<td>$K_{21}$</td>
<td>transfer coefficient determining the rate of carbon flow from Pool 1 to Pool 2</td>
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<tr>
<td>$K_{12}$</td>
<td>transfer coefficient determining the rate of carbon flow from Pool 2 to Pool 1</td>
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<td>kPa</td>
<td>kilopascals</td>
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<td>LA</td>
<td>leaf area</td>
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<tr>
<td>LAR</td>
<td>leaf area ratio</td>
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<tr>
<td>loge</td>
<td>Natural logarithm</td>
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<td>NAR</td>
<td>net assimilation rate</td>
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<td>PPFD</td>
<td>Photosynthetic photon flux density</td>
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<td>RGR</td>
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<td>RuBP</td>
<td>ribulose-1,5-bisphosphate</td>
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<tr>
<td>RuBPCase</td>
<td>ribulose-1,5-bisphosphate carboxylase</td>
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<tr>
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<td>sodium dodecyl sulphate</td>
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<td>SE</td>
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<td>shoot root ratio</td>
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CHAPTE R 1 - GENERAL INTRODUCTION

The primary role of CO$_2$ in plants is to serve as the substrate for photosynthesis, thereby being the carbon source for the plant growth. Even though this primary role in photosynthesis has long been recognized, the influences of varying CO$_2$ levels on plant photosynthesis, growth and development remain topics of intensive research, debate and speculation (Lemon, 1983).

For many terrestrial plants, especially those with the C$_3$ pathway of photosynthetic carbon assimilation, an increase in CO$_2$ above present ambient levels produces an increase in net photosynthetic rate (Huber et al., 1984). This is largely attributable to CO$_2$ more completely satisfying the substrate requirement for ribulose-1,5-bisphosphate carboxylase (RuBPCase) and competing more effectively with O$_2$ to inhibit RuBP oxygenase (Enoch, 1978; Ehleringer and Bjorkman, 1977). For C$_4$ plants, the expression of photorespiration is prevented by the additional dicarboxylic acid cycle. Thus, by increasing the CO$_2$ concentration, there is less increase in photosynthesis compared to C$_3$ plants. Higher CO$_2$ concentration also decreases stomatal conductance, and together with additional carbon input this leads to improved the water use efficiency (Rogers et al., 1984b).

There are many examples where short-term increases in photosynthetic CO$_2$ assimilation seem to be directly extrapolated into long-term growth improvements, i.e. higher plant mass, larger leaf area and larger root systems (Lemon, 1983). Many plants, however, exhibit some form of acclimation during extended exposure to high CO$_2$, such that the initial increase in net photosynthesis is moderated or even lost (Wulff and Strain, 1982; Delucia et al., 1985; Peet et al., 1986), and the expected gains in productivity are not fully realized (Peet, 1986). The mechanisms by which plants acclimate to CO$_2$ remain uncertain, however, acclimation has been correlated to increases in leaf starch content (Nafziger and Koller, 1976; Mauney et al., 1979),
decreases in photosynthetic enzyme activity (Porter and Grodzinski, 1984; Sage et al., 1988), decreases in stomatal conductance (Imai and Murata, 1978; Spencer and Bowes, 1986), and indirect growth effects (Poorter et al., 1988).

High CO₂ concentrations may produce effects on growth beyond those caused through photosynthesis. Modification in the partitioning of photosynthate among plant organs has been reported in many studies. Morphological effects of high CO₂ include increased branching (Rogers et al., 1984a), greater stem elongation (Madore and Grodzinski, 1985) and changed root/shoot ratio (Sionit et al., 1981b; Idso et al., 1988). Most studies have found increased leaf area (Ford and Thorne, 1967; Hofstra and Hesketh, 1975; Sionit et al., 1981b) in the seedling stage, which increases subsequent growth (Kriedemann and Wong, 1984). The ability of plants to capitalize on additional photosynthetic carbon input, and the occurrence of non-photosynthetic effects of CO₂, will influence the eventual growth responses to CO₂ enrichment.

The interaction of CO₂ enrichment with inorganic nutrients can play an important role in plant responses to CO₂ enrichment (Lemon, 1983). CO₂ enrichment increased the uptake of inorganic nutrients (N and P) in soybean (Cure, et al., 1988a,b), but no increase of nutrient uptake was observed in cotton (Wong, 1979) and cocklebur (Hocking and Meyer, 1985). The lack of a substantial increase in nutrient uptake may limit the growth stimulation by CO₂ enrichment. Furthermore, in many plants, nutrient concentrations decrease in CO₂-enriched leaves (Porter and Grodzinski, 1984), and decreased leaf nitrogen concentrations were correlated with decreased photosynthetic rate under CO₂ enrichment (Larigauderie, et al., 1988).

In addition, injurious effects of high CO₂ on leaves of tomato (Madsen, 1974), cotton (Hesketh et al., 1971), and bean (Ehret and Jolliffe, 1985a) have been reported. Van Berkel (1984) indicated that the potential for leaf injury induced by high CO₂ may be widespread, with variable symptoms and susceptibilities among plant species. The
cause of CO₂-induced injury effect is not yet clear, but it can also modify the growth effects of CO₂ enrichment.

Previous studies on dwarf bean plants have found a very limited growth response to CO₂ enrichment (Jolliffe and Ehret, 1985). Dry weight was increased, but there was no increase in leaf area, and leaf injury was observed after about three weeks of CO₂ enrichment (Ehret and Jolliffe, 1985a). There was a decline of photosynthetic capacity after CO₂ enrichment (Ehret and Jolliffe, 1985b) when photosynthesis rates were compared at the same CO₂ level. However, photosynthesis rate was still higher in CO₂ acclimated plants at the CO₂ concentrations used during growth. Hence, there was a continuous enhancement of carbon input throughout growth. This additional input could be compounded upon, but the dynamics of growth indicated little evidence of such compounding (Jolliffe and Ehret, 1985).

The current studies were undertaken to investigate non-photosynthetic limitations which influence the ability of bean plants to respond to CO₂ enrichment. Three lines of work were followed:

(i) The dynamics of response of growth and leaf injury to CO₂ enrichment were explored up to the beginning of reproductive development (Chapter 3). A short interval between harvests, and a survey of the distribution of growth among different plant parts, were used to specify the timing and location of growth responses.

(ii) Responses to CO₂ enrichment were mainly in the leaves, and concentrations of leaf carbohydrates, amino acids and proteins, and the partitioning of carbon among leaf pools, were examined to determine their relationship to the growth effects (Chapter 4).
(iii) The final studies (Chapter 5) considered the interaction between CO$_2$ enrichment and inorganic nutrients. Effects of CO$_2$ enrichment on the uptake and partitioning of inorganic nutrients were investigated to determine their coupling with growth and leaf injury responses. Conversely, the effects of NO$_3^-$ supply on CO$_2$ enrichment responses were also explored.
CHAPTER 2 - LITERATURE REVIEW

2.1 JUSTIFICATION FOR CO₂ ENRICHMENT STUDIES ON PLANTS

Three major interests are promoting research on carbon dioxide and plants: (i) the importance of CO₂ in plant function, (ii) the beneficial effects of CO₂ enrichment in greenhouse crop production, and (iii) the impact of increasing atmospheric CO₂ concentration on ecological systems.

CO₂ has many physiological roles in plants. Of primary importance is the role of CO₂ as an essential resource for plant growth as the substrate for photosynthesis, but it also participates in several other important functions. It is a reactant or product of many metabolic processes (e.g. respiration). CO₂ regulates stomatal aperture, ionic forms of CO₂ (e.g. bicarbonate ion) participate in membrane transport systems, and CO₂ opposes the action of ethylene, an important chemical growth regulator. Holistic responses of plants to CO₂ are sometimes difficult to explain simply on the basis of photosynthetic responses, for example the occurrence of leaf injury (Ehret and Jolliffe, 1985a) and altered photoperiodic performance (Hicklenton and Jolliffe, 1980b) at high CO₂ levels. Since CO₂ is involved in so many physiological processes, studies of plant response to CO₂ can contribute significantly to our overall understanding of plant function.

CO₂ enrichment was first used for greenhouse plant production approximately 100 years ago in Northern Europe (Miller, 1938). It was not until the 1960's, when relatively inexpensive and pure sources of CO₂ (e.g. pure CO₂, combustion of propane) became available, that CO₂ enrichment was extensively applied to commercial production in North America. It is difficult to obtain an accurate assessment of the current worldwide use of CO₂ enrichment for greenhouse crops, but there is no doubt that it is extensively used for vegetable, flower, fruit, and forest tree seedling crops. The practice is of greatest value during cool weather when the infiltration of external air
into greenhouses is restricted. In bright sunlight, crops in a closed greenhouse may quickly deplete the available CO\textsubscript{2}, resulting in levels considerably below (150 ppm or less) the normal atmospheric level of about 350 ppm (Wittwer and Robb, 1964). Under such conditions plant growth is restricted because of limited CO\textsubscript{2} availability for photosynthesis. In addition, it is well documented that significant yield increases occur in greenhouse crops when the CO\textsubscript{2} concentration is elevated to double or triple the ambient CO\textsubscript{2} concentration (Enoch and Kimball, 1986a,b). This has led to considerable research on the beneficial effects of CO\textsubscript{2} enrichment on crop yields and on the procedures for the effective application of CO\textsubscript{2} enrichment (Enoch and Kimball, 1986a,b).

Current interest in CO\textsubscript{2} effects is being strongly promoted by concerns relating to the progressive increase in CO\textsubscript{2} concentrations in the earth's atmosphere (Hansen \textit{et al.}, 1981; Keeling \textit{et al.}, 1982). CO\textsubscript{2} is a trace gas in the earth’s atmosphere, with the current concentration being only about 355 parts per million (ppm) by volume. CO\textsubscript{2}, however, is rapidly accumulating in the atmosphere, by more than 1 ppm per year (Bacastow \textit{et al.}, 1985), mainly due to the burning of fossil fuels and deforestation (Woodwell \textit{et al.}, 1983).

A continuation of such rates of accumulation will lead to a doubling of atmospheric CO\textsubscript{2} concentration over the next 50 to 100 years. Atmospheric carbon dioxide intercepts long-wave radiation from the earth’s surface, and this is considered by climatologists to play an important role in atmospheric energy balance. Current models of global climate predict that the mean temperature of the earth’s surface could increase by approximately 2 to 3 C due to a doubling of CO\textsubscript{2} concentration (Mitchell, 1989). This temperature increase could have major effects on biological systems. In addition to effects on organisms of temperature change \textit{per se}, other climate-related effects may occur due to changing precipitation patterns and sea level (Mitchell, 1989). However, beyond these potential impacts of climatic change, increasing atmospheric
CO₂ concentrations should also have direct biological effects. Horticultural studies of CO₂ effects on plants will continue to be an important source of information for forecasts of biological responses to changing atmospheric CO₂ levels.

2.2 PHYSIOLOGICAL EFFECTS OF CO₂ ENRICHMENT

2.2.1 Photosynthesis

CO₂ is the primary substrate of photosynthesis. In short term (minutes to hours) experiments on leaves, increased CO₂ concentration increases photosynthesis rates (Gaastra, 1963; Huber et al., 1984). However, the long term (days to weeks) effect of CO₂ enrichment on photosynthesis is more difficult to define. Hicklenton and Jolliffe (1978) found that, in tomato, under comparable test conditions of CO₂ concentration, the net rate of photosynthesis was greater in leaves developed under CO₂ enrichment. Similar findings were also obtained in other studies (Huber et al., 1984; Poorter et al., 1988; Vu et al., 1989). CO₂ enrichment suppresses photorespiratory activity (Enoch, 1978; Ehleringer and Bjorkman, 1977), and this inhibition of photorespiration may contribute to the enhancement of net photosynthesis under high CO₂.

Many other studies (Aoki and Yabuki, 1977; Imai and Murata, 1978; Clough et al., 1981; Ehret and Jolliffe, 1985b; Delucia et al., 1985; Larigauderie et al., 1986), however, observed inhibitory effects of long term CO₂ enrichment on photosynthesis. The cause of the inhibition is unknown, but, altered photosynthetic enzyme activities (Sage et al., 1988), excessive starch accumulation (Nafziger and Koller, 1976), stomatal responses (Imai and Murata, 1978; Spencer and Bowes, 1986) and indirect growth effects (Poorter et al., 1988) have been implicated.

Growth at elevated CO₂ concentrations has been shown to result in changing the levels of ribulose-1,5-bisphosphate (RuBP) and ribulose-1,5-bisphosphate carboxylase (RuBPCase) activity. In tomato plants, Hicklenton and Jolliffe (1980a) observed an increased RuBPCase activity and a decreased glycolic acid oxidase (an enzyme of
photorespiration) activity under CO₂ enrichment. In contrast, following long term exposure to high CO₂, declines of RuBPCase enzyme activity were observed in several other studies (Vu et al., 1983; Von Caemmerer and Farquhar, 1984; Peet et al., 1986; Sage et al., 1988; Yelle et al., 1989b). Although changes in photosynthetic capacity due to high CO₂ concentration correlate with changes in RuBPCase activity, a cause and effect relationship between this enzyme activity and the photosynthetic capacity is still unproven. Sage et al. (1989) studied the acclimation of photosynthesis to elevated CO₂ in five C₃ species, they found that the content of RuBPCase was lower in only two of the five species, yet its activation state was 19% to 48% lower in all five species following long term exposure to high CO₂. These results indicate that during growth in CO₂-enriched air, leaf RuBPCase content remains in excess of that required to support the observed photosynthetic rates. Similarly, the activity of carbonic anhydrase is also affected by CO₂ enrichment (Porter and Grodzinski, 1984). It was found that carbonic anhydrase activity decreased with the duration of CO₂ treatment and that the low carbonic anhydrase activity increased the resistance to CO₂ assimilation. More work, however, is needed to relate the extent of decreasing activity of carbonic anhydrase to changes in photosynthetic capacity during prolonged CO₂ enrichment.

2.2.2 Carbon Metabolism

Increases in leaf starch content with increasing CO₂ concentration during growth are commonly observed in CO₂ enriched plants (Mauney, et al., 1979; Tolbert, 1984; Madore and Grodzinski, 1985; Poorter, et al., 1988) Delucia et al. (1985) studied the effect of CO₂ enrichment on diurnal responses of starch pool size in cotton plants. They found that degradation and mobilization of starch in the 350 ppm plants at the end of dark period maintained the starch pool in a near equilibrium state. CO₂ enrichment, however, increased leaf starch accumulation, as starch concentration in the high CO₂ plants did not return to the previous morning’s level by the end of the dark
period. The disequilibrium between starch accumulation and loss would presumably lead to a progressive increase in leaf starch concentration in the high CO₂ plants, perhaps until some new equilibrium level was attained.

The decline in photosynthesis at high CO₂ associated with high starch accumulation has been shown in many studies (Nafziger and Koller, 1976; Azcon-Bieto, 1983). Chatterton et al. (1972) found that the reduction in photosynthesis was proportional to the amount of starch in the leaves of pangolagrass plants. Sasek et al. (1985) found that cotton plants grown at 1000 ppm CO₂ contained large starch pools, and photosynthetic rates were lower than expected based on results from brief exposures to high CO₂. The reduction of photosynthesis could be reversed within a few days by transferring plants grown at 1000 ppm CO₂ to 350 ppm CO₂. The recovery of photosynthesis was correlated with the depletion of the starch pool. In contrast, Yelle et al. (1989a) found that carbon exchange rates of young leaves of L. esculentum decrease as plants acclimated to high CO₂. However, no significant accumulation of starch and sugars were found in those CO₂ enriched leaves.

Specific mechanisms of the photosynthetic inhibition correlated with high starch concentrations are still unknown. Some suggestions involve physical mechanisms, such as shading of the chloroplasts by starch grain (Warren-Wilson, 1966) or interference of intracellular CO₂ transport due to large starch grains (Nafziger and Koller, 1976). In addition, the microscopic study done by Vu et al., (1989) showed that the extra starch formed under CO₂ enrichment was distributed as larger starch grains rather than as more starch grains per chloroplast. Large, unusually shaped, starch grains have been postulated to cause damage, either through contortion of the chloroplast lamellae or through actual disruption of chloroplast (Cave et al., 1981; Wulff and Strain, 1982). Visible damage to plants, such as leaf chlorosis, necrosis, and early leaf senescence, occurred after continued and excessive starch accumulation (Cave et al., 1981).
Previous studies have found different effects of CO$_2$ enrichment on soluble sugars. In soybeans, some studies reported increases in sucrose and reducing sugars under CO$_2$ enrichment (Huber et al., 1984; Vu et al., 1989). Other studies (Nafziger and Koller, 1976; Mauney et al., 1979; Finn and Brun, 1982), however, showed no effect of high CO$_2$ on soluble sugar concentrations. Such differences could be attributable to the different plant species used in those studies, but the timing of the measurements could also affect the results. For example, Poorter et al., (1988) found a significant increase in soluble sugar content in the leaf blade of *Plantago major* plants grown under different CO$_2$ conditions. This increase, however, was time dependent; it was large at the beginning of the study and smaller at the end. The regulation of carbon partitioning between starch and sucrose can also help to explain the reason why a higher CO$_2$ effect has been observed on starch concentration than on soluble sugars. Sharkey et al., (1985) found that as CO$_2$ pressure increased, both starch and sucrose synthesis rates increased, but there was always a greater increase in starch than sucrose.

The rate of carbon export from leaves has been shown to be affected by high CO$_2$ concentration. Ho (1977) reported increased rates of photosynthesis and carbon transport in leaves of tomato plants grown under CO$_2$ enrichment, compared to plants grown at ambient CO$_2$. A similar finding was reported by Madore and Grodzinski (1985) in dwarf cucumber plants. In contrast, in bean plants high CO$_2$ concentration increased leaf sucrose concentration slightly, but the rate of assimilate export and the activity of sucrose phosphate synthase were not increased (Huber et al., 1984). Huber et al., (1984) suggested that elevated CO$_2$ apparently affects the distribution of sucrose between transport and 'non-transport' pools in the leaf; only the transport pool would be expected to affect the rate of export. Similarly, Geiger et al. (1983) reported that increased leaf sucrose in sugar beet, produced in response to elevated CO$_2$, was accumulated mainly in the vacuole. Vacuolar sucrose may be less readily available for export compared to sucrose in the cytoplasm and in the minor vein phloem.
2.2.3 Stomatal Responses

Partial stomatal closure at higher than present atmospheric CO\(_2\) concentrations, and opening at lower CO\(_2\) concentrations, is the behavior observed in majority of the reports (Morison, 1985). The sensitivity of stomata to CO\(_2\) varies with the conditions during growth and the conditions during measurement: the leaf age, the light intensity, humidity, and temperature (Morison, 1985). Under CO\(_2\) enrichment, partial closure of stomata will affect photosynthesis, transpiration, water use efficiency, leaf temperature and plant water status (Lemon, 1983). In rice, decreased photosynthesis has been attributed to increasing stomatal resistance (Imai and Murata, 1978), but in most reports the increase in stomatal resistance only contributed a small percentage to the inhibitory effect of high CO\(_2\) on photosynthesis (Spencer and Bowes, 1986).

Based on 46 observations of the effect of high CO\(_2\) on transpiration, Kimball and Idso (in Kimball, 1986) reported a 34% reduction in transpiration for a doubling of CO\(_2\) concentration. Combining the approximate 30% increase in plant growth (Kimball, 1986), it is therefore anticipated that increasing atmospheric CO\(_2\) concentration will, by reducing stomatal resistance and increasing assimilation rate, cause increased water use efficiency. In CO\(_2\) enriched soybean, lower rates of water use delayed or prevented the onset of severe water stress under conditions of low moisture availability (Rogers et al., 1984b). In addition, Sionit et al., (1981b) found that wheat plants grown at increased CO\(_2\) concentration had a lower leaf osmotic potential than those grown at normal CO\(_2\) concentrations. Furthermore, as water stress developed, leaf water potential declined less steeply in the CO\(_2\)-enriched plants. They interpreted these responses as indicative of better adaptation to water and turgor maintenance under high CO\(_2\). An increase in stomatal resistance can be of advantage to the plant not only in conserving water, but also in reducing the uptake of air pollutants (Carlson and Bazzaz, 1982; Allen, 1990).
2.2.4 Interactive Effects with Chemical Growth Regulators

$CO_2$ has been found to interact with plant growth regulators, especially ethylene and abscisic acid (ABA). $CO_2$, a close structural analogue of allene (a compound which substitutes for ethylene in both the pea growth and fruit ripening assays), often acts as a competitive inhibitor of ethylene. Many effects of ethylene have been shown to be blocked by $CO_2$ e.g. growth of etiolated pea stems (Burg and Burg, 1967), abscission (Curtis, 1968), and epinasty (Dhawan et al., 1981). The mechanism of the $CO_2/C_2H_4$ interaction has not been established, but many workers (Beyer, 1979; Gepstein and Thimann, 1981) have observed a simultaneous increase in ethylene release and an inhibition of ethylene action in the presence of high $CO_2$.

The effect of ABA concentration on stomatal sensitivity to $CO_2$ is well documented. ABA application reduces stomatal aperture or conductance, but increases the sensitivity to $CO_2$ (Radin and Ackerson, 1981). Radin et al. (1988) suggested that in a $CO_2$ enriched atmosphere, ABA may function as a messenger between stomatal conductance and assimilation rate because the sensitivity of the system to ABA is enhanced.

2.2.5 Other Effects

In addition, $CO_2$ enrichment can also enhance N$_2$ fixation (Hardy et al., 1978; Finn and Brun, 1982), change the salt tolerance of certain crops (Enoch et al., 1973), elevate the optimum growth temperature (Enoch and Hurd, 1977) and alter the photoperiodic flowering response (Hicklenton and Jolliffe, 1980b). $CO_2$ may regulate the ion transport system in guard cells involving active transport of $H^+$ (Lemon, 1983). $CO_2$ may also directly or indirectly affect other $H^+$ coupled transfer mechanisms, such as phloem loading, sucrose storage, ion uptake by roots, ion transfer causing leaf movement, and solute transport into expanding cells (Lemon, 1983).
2.3 CO₂ ENRICHMENT, YIELD AND PLANT GROWTH

For the past 20 years, with the interest of exploiting CO₂ enrichment in greenhouse crop production, the emphasis of the majority of studies has been on crop yield. Kimball (1986) extracted more than 770 observations from over 140 reports about the effects of CO₂ enrichment on the economic yield or biomass production of 38 agricultural crops and 18 other species. Of these observations, only 66 yielded less than their respective controls. The mean percentage yield increases of C₃ crops were 12, 29, 30, 41, 37, and 52% for flower, fruit, grain, leaf, legume seed, and root crops, respectively. Effects of CO₂ enrichment on yields of flower crops were generally smaller than effects on other crops, because the flower yields were based on the number of blooms per plant, rather than on bloom weight. There were much less data available on the response of C₄ plants to CO₂ enrichment. Immature C₄ crops had an increase of only 11%, while mature C₄ crops responded to CO₂ enrichment with a mean yield increase of 101%.

Fewer reports dealt with the growth and development of plants in response to CO₂ enrichment, and most experiments involved only one harvest at the end of the growing period. In general with C₃ plants, the elevated CO₂ concentrations increased total plant dry weight, total leaf area and plant height (For reviews see Wittwer and Robb, 1964; Hand, 1982).

A dry weight increase, involving larger, more numerous and heavier plant parts, is commonly observed (Kimball, 1986). Madore and Grodzinski (1985) reported that after six weeks of CO₂ enrichment (1150 ppm), there was a 50% increase in shoot dry weight in cucumber plants. They found that this increase was partly due to the presence of at least two more fully expanded leaves on the CO₂ enriched plants. In addition, increased internode length, particularly between the more recently developed nodes,
contributed to the increased dry weight. A similar finding was observed in soybean (Rogers et al., 1984a).

Sasek and Strain (1988) reported that in kudzu, after 14 and 24 days of treatment, plants grown at 675 and 1000 ppm CO₂ accumulated 2.0 and 2.5 times more total dry weight, respectively, than plants grown at 350 ppm CO₂. The dry weights of leaves, stem, and roots were increased at elevated CO₂ concentrations. However, most of the dry weight of the plants was in the leaves, and the largest relative increase in weight due to CO₂ enrichment was in the leaves. Leaf growth was often affected by changing atmospheric CO₂ concentrations. Plants grown under CO₂ enrichment often develop more, heavier, and larger leaves (Mauney et al., 1978; Delucia et al., 1985; Sasek and Strain, 1989). Madore and Grodzinski (1985) found that the increase in leaf dry weight under CO₂ enrichment was largely due to an accumulation of starch in the more mature leaves. Similar findings were reported in bean (Jolliffe and Ehret, 1985), and in cotton (Delucia et al., 1985). Thomas and Harvey (1983) examined the CO₂ enrichment effect on leaf anatomy of four species: corn, soybean, loblolly pine, and sweet gum. They found significant increases in leaf thickness for the three C₃ species under CO₂ enriched conditions, but not for corn, a C₄ plant. An examination of the leaf tissue indicated that in soybean, the differences in thickness were chiefly a result of increased palisade cell initiation and development. A similar finding was reported by Hofstra and Hesketh (1975). In contrast, Ehret (1983) did not find any significant difference in leaf thickness in bean plants. Above results indicate that the anatomic changes in CO₂ enriched leaves may contribute to the weight increases, but different plant species may respond differently. Furthermore, changes in leaf thickness under CO₂ enrichment have been shown to affect the internal cell surface area (Leadly et al., 1987). However, the ramifications of such CO₂-induced changes in leaf anatomy on photosynthesis and water use efficiency are still not clear.
Although few studies (Thomas et al., 1975; Kreidemann and Wong, 1984; Jolliffe and Ehret, 1985; Peet, 1986) have reported no effect of CO$_2$ enrichment on leaf area, for most of the studies, relatively smaller increases in leaf area compared to increases in leaf weight under CO$_2$ enrichment have been frequently reported (Ford and Thorne, 1967; Hofstra and Hesketh, 1975; Sionit et al., 1981b). Elevated CO$_2$ concentrations might increase the total leaf area on a plant in several ways. Sasek and Strain (1989) studied the effects of CO$_2$ enrichment on the expansion of kudzu leaves. They found that at elevated CO$_2$ concentrations, maximum leaf expansion rates were approximately 40% greater. Leaves were fully expanded several days sooner, fully expanded leaves were larger at each leaf position, and leaf production rates were increased 12%. These CO$_2$ enrichment effects on leaf expansion provided the potential for successful seedling establishment.

Leaf area production is very important during early seedling growth because the canopy is not closed and self shading is minimal. During this growth phase, productivity is directly proportional to canopy size and biomass. Therefore, the effects of altered leaf growth may be compounded, with important consequences much later in development. Indeed, in some studies, the dry weight increase under CO$_2$ enrichment was found to be correlated with the leaf area response to CO$_2$ concentration. Kreidemann and Wong (1984) tested four plant species, \textit{(Basella alba} (L), \textit{Raphanus sativus} (L), \textit{Cucumis sativus} (L), and \textit{Brassica pekinensis} (Leur.) Rupr.) grown under CO$_2$ enrichment (1350 ppm) for 40-50 days. For the species \textit{(Basella alba} (L), and \textit{Cucumis sativus} (L)) that had a greater weight increase (6.8 and 5.7 times) there was also a greater leaf area increase (4.23 and 3.7 times respectively). For the other two species, \textit{Raphanus sativus} (L) and \textit{Brassica pekinensis} (Leur.) Rupr, which were not very responsive in dry weight to high CO$_2$ (7% and 38%), there was either no response \textit{(Brassica pekinensis} (Leur.) Rupr) or only a slight response \textit{(Raphanus sativus} (L)) of increase in leaf area.
The responsiveness of different plant parts to CO₂ enrichment can be quite varied, as indicated by a wide range of reported responses of root to shoot ratio. Several studies have suggested that plant roots respond more to atmospheric CO₂ enrichment than plant shoots: e.g., in rice (Imai and Murata, 1976), wheat (Sionit et al., 1981b). Other have suggested that plant shoots are the main beneficiaries of atmospheric CO₂ enrichment: Sionit (1983) in a study of soybean, and Jolliffe and Ehret (1985b) in a study of bean. Lack of effects of CO₂ enrichment on root/shoot ratio have also been reported in maize (Goudriaan and de Ruiter, 1983), soybean (Idso et al., 1988) and cotton (Gifford et al., 1985). Poorter et al., (1988) found there was a time-dependent difference between CO₂ treatments in the shoot/root ratio of Plantago major. This ratio was lower for the CO₂ enriched plants during the first phase of the experiment and higher at the end. In contrast, Gifford et al. (1985) reported that in cotton plants there was more increase in shoot dry weight than root dry weight by high CO₂ at early growing stage (15 days after sowing). However, 22 days after sowing the increase in root dry weight contributed the same as the shoot to the total increase in dry weight. These results indicate that the response of different plant parts to CO₂ enrichment was not just affected by plant species; the other growth conditions and the stage of plant development are also important factors.

Growth in young plants is stimulated more by additional CO₂ than in mature plants. Kimball (1986) reported a mean yield increase of 25% for all mature C₃ agricultural crops compared to a 51% for the immature plants. Studies of the CO₂ enrichment effect on relative growth rate (RGR) often reveal that plants respond the most to CO₂ enrichment at the juvenile stage. For example, Neales and Nicholls (1978) found a strong interaction between time and the stimulation of the RGR of Triticum aestivum plants. During the first week of enrichment the RGR was increased. However, during week 2 a negative correlation between RGR and the applied CO₂ concentration was found. Similar findings were reported in Plantago major (Poorter et
al., 1988), tomato (Yelle et al., 1990), and bean (Jolliffe and Ehret, 1985). The stimulation of RGR by the CO₂ treatment at the beginning of the experiment was due to the increase in net assimilation rate (NAR), however, this stimulation also disappeared at the later stage (Wulff and Strain, 1982). Peet (1986) studied the vegetative and reproductive growth of a monoecious cucumber grown under CO₂ enrichment (1000 ppm). She found that CO₂ enrichment did not increase total fruit weight or number, nor did it increase biomass, leaf area, or RGR beyond the first 16 days after seeding. Further study (Peet et al., 1986) showed that the lack of a sustained growth increase in this cultivar at high CO₂ was caused by an increasing depression of carbon exchange capacity (CER) such that by the time plants were flowering, in situ photosynthetic rates were similar in both 350 and 1000 ppm plants. By the time of fruiting, CER was actually higher in the 350 ppm grown plants than in the 1000 ppm grown plants.

2.4 INTERACTIVE EFFECTS OF CO₂ ENRICHMENT WITH OTHER FACTORS

Environmental factors, such as light, temperature, and nutrient supply, have also been found to modify the effects of CO₂ enrichment on growth. Dons (1988) found that increased CO₂ concentration enhanced growth in *Lemna gibba* under both high and low light intensity; however, the increase was greater under high light intensity than low light intensity. This result was simply due to the fact that maximum photosynthetic enhancement by CO₂ enrichment occurs at light intensities that are saturating for photosynthesis. However, the relative enhancement is usually found to be maximal under light-limiting conditions due to decreases in light compensation point with increases in CO₂ concentrations (Lemon, 1983). Hand and Postlethwaite (1971) reported that CO₂ enrichment advanced the date of first anthesis, promoting earlier cropping and shortened duration of harvest for single-truss tomatoes sown in December and, to a lesser extent, for those sown in July.
Many studies have also shown that the stimulatory effect of atmospheric CO₂ enrichment is strongly temperature dependent (Idso et al., 1987; Nilsen et al., 1983; Sionit et al., 1987a,b; Idso and Kimball, 1989). Idso and Kimball (1989) found that in carrot and radish, cumulative dry matter production was increased by CO₂ enrichment at all temperatures investigated, but with progressively greater effects being registered at higher temperatures. This CO₂ enrichment effect on elevating the optimum growth temperature was also observed by Enoch and Hurd (1977), and this shift in temperature response could have important implications in warm environments. Plants grown under CO₂ enrichment has also been shown to be more tolerant of drought, a response which was related to osmotic adjustment of CO₂ enriched plants (Conroy, 1988b).

Few studies have investigated the effect of nutrient availability on growth responses to CO₂ enrichment. Results from most studies have indicated that the enhancement of dry matter production by CO₂ enrichment is retained even at low nutrient supply (Sionit et al., 1981a; Goudriaan and de Ruiter, 1983; Peet and Willits, 1984). The stimulation by high CO₂ of growth of nutrient-limited plants can be similar to (Wong, 1979; Zangerl and Bazzaz, 1984; Hocking and Meyer, 1985), or lower than, that found for plants grown in optimal conditions (Patterson and Flint, 1982; Sionit et al., 1981a; Goudriaan and de Ruiter, 1983; Peet and Willits, 1982). Cure et al. (1988a,b) reported that in nonnodulated soybean, at all NO₃⁻ and P levels except the lowest, exposure to high CO₂ resulted in increased nutrient (N, P) uptake and nutrient utilization efficiency. The increased nutrient uptake was associated with larger root system, as uptake of per unit root mass was lower than the control. In contrast, Hocking and Meyer (1985) observed a consistent growth response of cocklebur to high CO₂ over a range of NO₃⁻ concentrations, and there was little change in NO₃⁻ uptake relative to control. The above results indicate that plant response to CO₂ enrichment when other nutrients are limiting depends on the nature of those limitations and whether additional CO₂ assimilation will increase access to those limiting nutrients.
Several studies have reported a decrease in leaf nutrient concentrations under CO₂ enrichment (Wong, 1979; Porter and Grodzinski, 1984; Tremblay et al., 1987, 1988), but the reason for this decline remain unclear. Several mechanisms, such as the increased starch content at high CO₂ or reduced nutrient uptake as a result of stomatal closure at high CO₂ (Madsen, 1975), have been proposed. Neyra and Hageman (1976) reported that exposure of the leaf canopy to increasing concentrations of CO₂ decreases the flux of nitrate to the leaf blades. Decreases in nitrate accumulation in the leaf blade with increased CO₂ concentration were only partly accounted for by the difference in transpiration. Yelle et al. (1987) studied the effect of CO₂ enrichment and root-zone temperature on growth of tomato plants. They found the best response to CO₂ enrichment was at 30°C, and appeared to be related to increased NO₃⁻ translocation to the leaves.

Larigauderie et al. (1988) reported a linear photosynthesis versus leaf nitrogen relationship. They found that for the low nitrogen treatment, this resulted in lower photosynthesis rates measured at 650 ppm for the CO₂-enriched plants, compared to the photosynthesis rates measured at 350 ppm for the non-enriched plants. At higher nitrogen availability, photosynthetic rates of plants grown and measured at 650 ppm were greater than photosynthetic rates of the non-enriched plants measured at 350 ppm. These results imply that the declined nutrients (especially nitrogen) may partially explain the adverse long term CO₂ effect on photosynthetic capacity.
2.5 CO₂ ENRICHMENT AND LEAF INJURY

Injurious effects of high CO₂ have been reported in many plant species. In tomato plants, Madsen (1974) found plants grown with additional CO₂ showed rolled and deformed leaves, and such effects increased with CO₂ concentration. In potato, Goudriaan and de Ruiter (1985) observed that leaves of CO₂-enriched plants developed a brownish fringe. Van Berkel (1984) reported that leaf die-off can take place with gerbera, cucumber, tomato and other crops. Similarly, Ehret and Jolliffe (1985a) also reported that leaf chlorosis of CO₂-enriched bean plants can be observed after about three weeks of CO₂ enrichment. These observations indicate that CO₂ induced leaf injury is widespread among different plant species, and the symptoms of injury can be quite varied.

The cause of leaf injury remains unclear, but excessive starch accumulation under CO₂ enrichment has been implicated by many authors (Madsen, 1974; Goudriaan and de Ruiter, 1985). Ehret and Jolliffe (1985a) reported that bush bean plants grown in atmospheres enriched to 1400 ppm CO₂ showed accelerated chlorosis of the primary leaves. The degree of injury was regulated by secondary factors, light and temperature. Conditions of relatively high light intensity (340-370 μmole m⁻² s⁻¹ PPFD) or cool temperature (20 °C) promoted leaf injury of the CO₂-enriched plants. Leaf starch accumulation was highest under conditions that caused injury. The microscopic study done by Vu et al., (1989) showed that the extra starch formed under CO₂ enrichment was distributed as larger starch grains rather than as more starch grains per chloroplast. And large, unusually shaped, starch grains have been postulated to cause damage, either through contortion of the chloroplast grana or through actual disruption of chloroplast (Cave et al., 1981; Wulff and Strain, 1982). However, in Gerbera (Van Berkel, 1984), the response was not found to be correlated with starch, indicating that other causes may be involved. For example, due to the similarity of
symptoms, Goudriaan and de Ruiter (1983) suggested that leaf injury of potato plants under CO$_2$ enrichment may be related to potassium deficiency.
CHAPTER 3 - EFFECT OF CO₂ ENRICHMENT ON GROWTH, DRY MATTER PARTITIONING AND LEAF INJURY

3.1 INTRODUCTION

Studies on many plant species have shown that elevated CO₂ concentrations stimulate growth, however, the growth responses vary with plant age, growth pattern (determinate or indeterminate), and other environmental factors (Lemon, 1983; Kimball, 1986). The physiological mechanisms that underlie responses to CO₂ enrichment are complex, and are not fully understood.

Plants acclimate during prolonged exposure to high CO₂ concentration (Hicklenton and Jolliffe, 1980a; Delucia et al., 1985). Some of the acclimation of photosynthesis to CO₂ enrichment is correlated with growth responses (Peet, 1986; Peet et al., 1986; Allen et al., 1988). The lack of a clear compounding effect of increased photosynthesis rate on productivity, however, suggests that the link between photosynthesis and growth responses is complicated (Kramer, 1981). Kriedemann and Wong (1984) reported that the response of dry matter accumulation to CO₂ enrichment was correlated with the leaf expansion responses during the early phases of growth. Their results indicated that the partitioning and utilization of the additional carbon input is critical to the eventual plant responses to CO₂ enrichment.

Previous studies on bean plants showed that atmospheric CO₂ enrichment increased total plant dry weight per plant, however, leaf area development was little affected (Jolliffe and Ehret, 1985). Weight increase appeared to occur more rapidly in leaves than elsewhere in the plant (Jolliffe and Ehret, 1985), and leaf injury was observed after three weeks of CO₂ enrichment (Ehret and Jolliffe, 1985a).
During prolonged CO$_2$ treatment, bean leaves acclimated to enrichment, undergoing a decline of photosynthetic capacity (Ehret and Jolliffe, 1985b). i.e., when compared at the same CO$_2$ concentration, leaves from unenriched plants had higher photosynthesis rates than leaves from CO$_2$-enriched plants. However, leaves of CO$_2$-enriched plants, when tested at high CO$_2$ concentrations, still had higher photosynthesis rates than leaves from unenriched plants tested at ambient CO$_2$ concentration.

Hence, previous research has identified several contributors to the overall reaction of bean plant growth to high CO$_2$: photosynthetic adjustments, leaf expansion, leaf injury, and the ability of plants to compound upon the additional carbon input through efficient dry matter partitioning. Previous growth studies have involved relatively widely spaced times of observation. The following study was done to explore the timing and location of responses to CO$_2$ enrichment in a long-term experiment by studying samples taken with short intervals between harvests. The study examined the effects of CO$_2$ enrichment on the growth of individual plant parts, as well as overall growth, and it was also intended to provide a clear indication of the onset of CO$_2$-induced leaf injury.
3.2 MATERIALS AND METHODS

3.2.1 Plant Culture

Bush bean (*Phaseolus vulgaris* L. cv. 'Gold Crop') seeds were germinated on moistened paper towels in the dark for 72 hours. Germinated seeds having 1 to 1.5 cm radicle length were transplanted to a 12.7 cm diameter pot. The soil medium was a mixture of sand and perlite in 1:1 volume ratio. Plants were grown in a Percival controlled environment chamber (Model PG.78) for 10 days with 26/20 C (day/night) temperature. The light intensity of 250 μE m⁻² s⁻¹ was supplied for 16 hours/day photoperiod. Plants were watered daily with tap water.

After this ten day period the primary leaves were about half expanded; the plants were thinned to one plant per pot and moved to specially constructed CO₂ treatment chambers (Ehret, 1983). Each CO₂ treatment chamber had 0.33 m x 0.33 m x 0.56 m space for plant growth. The CO₂ treatment chambers functioned as open systems through which ambient air, taken from outside the MacMillan Building, University of British Columbia, at 12 m above ground level, was passed. The air turnover rate of each chamber was approximately twice per minute. CO₂ enrichment was achieved by adding gas containing 100% CO₂ to the inlet ambient air stream. Enrichment concentrations were set by regulating the flow rate of pure CO₂ into the chamber, and the concentrations were measured using an infrared gas analyser (Beckman, model 864). The CO₂ concentrations used were 340±30 and 1500±40 μL L⁻¹ (ppm). Four air mixing fans, each rated at 1.22 m³ min⁻¹ at zero static pressure, were present within each chamber to maintain spatial uniformity in gas concentration.

Chamber temperature was controlled by circulating coolant through a copper heat exchanger exposed to the air mixing fans. Temperatures of 26±1/19±1 C (day/night) were maintained during the experiment, as detected by a copper-constantan thermocouple within each chamber. Lighting was provided by banks of 25 cool-white
fluorescent tubes (40 W) and 13 incandescent bulbs (60 W); each bank of lamps illuminated 4 treatment chambers. The light intensity, measured at the pot level, was 150 μE m⁻² s⁻¹, and the photoperiod was 16 hours. Plants were watered daily with half-strength Hoagland's solution (increasing from 100-200 mL every second day as plants grew) and distilled water was supplied on alternate days.

3.2.2 Growth Analysis

A precise definition of what is meant by 'growth' is not at all easy. In this study, 'growth' will be used mainly to describe changes in size (however measured) (Hunt, 1978).

Because space was limited in the CO₂ treatment chambers, the presence of plants in the chambers was staggered. Three plants per chamber were harvested at different times (t): 3, 7, 10, 14, 17, 21, 25, 28, and 35 days after transfer to the CO₂ treatment chambers. At each harvest, the areas (LA) of individual primary and trifoliolate leaves were measured using a leaf area meter (LI-COR model LI-3000). Dry weights (W) were determined after plant parts were dried in an oven at 80 C for 48 hours.

Primary data were log-transformed, and fitted curves describing their time trends were generated using a cubic spline regression procedure (Jolliffe and Courtney, 1984). The fitted curves were then used to generate growth indices (Jolliffe et al., 1982) such as absolute growth rate (dW/dt), relative growth rate (RGR) [d(logₑW)/dt], unit leaf rate (ULR) [(1/LA)(dW/dt)] and leaf area ratio (LAR) (LA/W). Two additional growth indices, not commonly used in plant growth analysis, were: specific leaf weight (SLW), the ratio of leaf dry weight to leaf area, and shoot root ratio (SRR), the ratio of shoot weight to root weight.
3.2.3 Other Determinations: Primary Leaf Water Potential, Osmotic Potential, and Chlorophyll Concentration

At the 14, 21, 24, and 28 day harvests, 1 or 2 leaf disks (1.0 cm in diameter) were punched from the primary leaves of control and CO₂ enriched plants. Leaf disk water potential was determined using a Wescor dew point microvoltmeter (model HR-33T, with sample chamber C-52). To disrupt cell membranes, samples were wrapped in aluminum foil and quickly frozen in liquid nitrogen. The frozen samples were thawed at room temperature and measured again with the dew point microvoltmeter to determine their osmotic potentials.

Chlorophyll concentrations of the primary leaves were determined using the technique of Bruinsma (1963) after extraction from leaf disks in 10 mL of 80% (v/v) cold acetone. The absorbances (Ab) of the extracts were assayed at 645, 652, and 663 nm using a Spectronic 21 model DV spectrophotometer (Bausch & Lomb). Total chlorophyll concentration was calculated from:

Total chlorophyll = 20.2Ab₆₄₅ + 8.0Ab₆₆₃
3.3 RESULTS

3.3.1 Overall Plant Growth under CO₂ Enrichment

The growth data presented in the following sections was the results of first trial. Similar results were obtained from the second trial. The primary data for the both trials was tabulated in the Appendix 1.

Under high CO₂ (1500 ppm), there was a significant increase in total plant dry weight (Fig. 3.1). About seventeen days were required for plants to show a significant response under high CO₂, and the total dry weight increase reached 30% at the final harvest.

Absolute growth rate increased with time for both CO₂ treated and control plants (Fig. 3.2). Minor undulations in the curves for absolute growth rate, and the other growth indices involving \( dW/dt \) (relative growth rate and unit leaf rate), are an artifact of the spline regression procedure, and will not be the subject of interpretation. The increase in absolute growth rate was much higher with plants grown under elevated CO₂ than with the control plants, and the two treatments diverged over time.

Relative growth rate decreased between two and four weeks of treatment in all plants regardless of the CO₂ level (Fig. 3.3). For the first two weeks of treatment, plants grown under high CO₂ concentration had higher relative growth rates. However, relative growth rate of the CO₂ enriched plants later subsided to levels approximately the same as the control plants for the remainder of the experiment.

Unit leaf rate (E) is the rate of change in weight per unit of leaf area (the 'average rate of assimilation'). Plants grown under CO₂ enrichment had higher unit leaf rates throughout the entire experimental period, however, differences diminished toward end of the experiment (Fig. 3.4). The temporal pattern of unit leaf rate was the same at the two CO₂ levels. Unit leaf rate was highest soon after the start of treatment, and then decreased.
Figure 3.1 Effect of CO₂ enrichment on total dry weight per plant.

CO₂ enriched: 1500 ppm (---)
Control: 340 ppm (———)

Each point is the mean ± SE of six plants.
Figure 3.2 Effect of CO₂ enrichment on absolute growth rate.

CO₂ enriched: 1500 ppm  (---)
Control: 340 ppm  (-----)
Figure 3.3 Effect of CO₂ enrichment on relative growth rate.

<table>
<thead>
<tr>
<th></th>
<th>CO₂ enriched:</th>
<th>1500 ppm</th>
<th>Control:</th>
<th>340 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(---)</td>
<td>(-----)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.4 Effect of CO₂ enrichment on unit leaf rate.

CO₂ enriched: 1500 ppm (- - - - -)
Control: 340 ppm (_____)


3.3.2 Effect on Growth of Different Plant Parts

3.3.2.1 Leaf Growth

The increase in total leaf dry weight by CO$_2$ enrichment (Fig. 3.5a) followed a similar pattern to the increase in total plant dry weight just described. The response of total leaf dry weight, however, was earlier and proportionately higher than that of total plant dry weight. The increase of total leaf dry weight ranged from 37% after one week of CO$_2$ treatment to 76% at the final harvest (Fig. 3.5a). It required one week for CO$_2$ enriched plants to show a significant response in total leaf dry weight per plant.

This early increase was mainly due to the increase in primary leaves (Fig. 3.5b). Primary leaves of plants grown under CO$_2$ enrichment were 47% heavier than in the control plants after seven days of CO$_2$ treatment. Primary leaf dry weight increased until about 17 to 21 days of treatment, and under CO$_2$ enrichment became 50 to 70% higher than in control plants (Fig. 3.5b).

Trifoliate leaves began to emerge after 7 days of treatment under both CO$_2$ conditions. The first trifoliates also required 7 to 10 days of CO$_2$ treatment after emergence to show the response in weight (Fig. 3.5c). Dry weight increase of trifoliate leaves by CO$_2$ enrichment persisted until the end of the experiment, and the increase reached 80% at the last harvest.

Contrary to the weight increase, CO$_2$ enrichment did not significantly affect the leaf expansion (Fig. 3.6a, 3.6b, 3.6c). No significant CO$_2$ effects on leaf area were found in either the primary or trifoliate leaves. The number of trifoliate leaves also was unaffected by high CO$_2$ treatment throughout the study (Table 3.1).

Differential effects of CO$_2$ enrichment on leaf attributes are evident when ratios of different variables are compared (Fig. 3.7a, 3.7b, 3.7c). Narrow confidence limits for the ratios, compared with the primary measures from which they are derived (Figs. 3.1 to 3.6), indicate the maintenance of proportionality during growth. i.e. a
Figure 3.5 Effect of CO₂ enrichment on leaf dry weight per plant.

(a) Total leaf dry weight.
(b) Primary leaf dry weight.
(c) Trifoliate leaf dry weight.

CO₂ enriched: 1500 ppm  (--- - - - -)
Control: 340 ppm  (________)

Each point is the mean ± SE of six plants.
Figure 3.6 Effect of CO$_2$ enrichment on leaf area per plant.

(a) Total leaf area.
(b) Primary leaf area.
(c) Trifoliate leaf area.

CO$_2$ enriched: 1500 ppm
Control: 340 ppm

Each point is the mean + SE of six plants.
Table 3.1 Effect of CO₂ enrichment on number of leaves, pods, and pod dry weight per plant

<table>
<thead>
<tr>
<th>Treatment (ppm)</th>
<th>Duration of CO₂ treatment (days)</th>
<th>Number of leaves</th>
<th>Number of pods</th>
<th>Pod dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (350 ppm)</td>
<td>14</td>
<td>8±1</td>
<td>11±2</td>
<td>14±2</td>
</tr>
<tr>
<td>Enriched (1500 ppm)</td>
<td>24</td>
<td>3±1</td>
<td>8±1</td>
<td>11±1</td>
</tr>
</tbody>
</table>

* Values in the table are means±SE of six plants
replicate plant with relatively large leaf area also tended to have large dry weight. Specific leaf weight (SLW) is the ratio of leaf weight (Fig. 3.5) to leaf area (Fig. 3.6). The SLW of CO₂ enriched plants was 20% higher than the control plants after just 3 days of CO₂ treatment (Fig. 3.7a); this difference increased to 50% after 7 to 10 days of CO₂ treatment. This further increase in the difference between the two CO₂ treatments was essentially due to the increase in SLW of CO₂ enriched plants; there was no significant change in SLW of control plants throughout the experimental period.

A larger CO₂ effect was observed on the SLW of primary leaves (Fig. 3.7b). Unlike the results for total leaf SLW, there was an increase in the SLW of primary leaves for the first two weeks of both CO₂ treatments. The primary leaves of the CO₂ enriched plants had an 85% increase in SLW for the first two weeks compared to a 32% increase in the control plants over the same time period. There was a subsequent decrease in SLW after four weeks of treatment. Although the pattern of the SLW changes was similar for both CO₂ treatments, the SLW of primary leaves of CO₂ enriched plants was always approximately 70% higher than the control plants, except in the first week of the experiment.

The effect of CO₂ enrichment on the SLW of trifoliate leaves was less prominent than the effect on primary leaves (Fig. 3.7c). Again, there was little change in the control plants throughout the whole experimental period, except for a decrease in the first three days after trifoliate emergence. The trifoliate SLW of the CO₂ treated plants underwent a 30% increase for the first ten days of treatment and remained at the similar levels until the end of study. Although the difference between the two CO₂ treatments was not as high as observed in primary leaves, the SLW of CO₂ treated trifoliates was still about 50% higher than the controls for the most of the experimental period.
Figure 3.7 Effect of CO₂ enrichment on specific leaf weight (SLW) per plant.

(a) Total specific leaf weight.
(b) Primary leaf SLW.
(c) Trifoliate SLW.

CO₂ enriched: 1500 ppm (---)
Control: 340 ppm (———)

Each point is the mean ± SE of six plants.
3.3.2.2 Stem, Root and Pod Growth

There were no significant differences in stem or root dry weights between CO$_2$ enriched and control plants throughout the experiment except the very last harvest (Fig. 3.8a, 3.8b). The difference of pod dry weight between the two CO$_2$ treatments was not significant at the end of this study (Table 3.1), nor was the number of pods significantly different between the two CO$_2$ treatments (Table 3.1).

3.3.3 Effect on Water Status of Primary Leaves

In order to understand the limitation of leaf expansion under CO$_2$ enrichment, the water status of the primary leaves was investigated. The osmotic and water potentials of the primary leaves were significantly affected by CO$_2$ enrichment (Table 3.2a). Although results were highly variable, the primary leaves of CO$_2$ enriched plants consistently had lower osmotic and water potentials than the primary leaves of the control plants (Table 3.2b). The osmotic potential of the leaves of CO$_2$ treated plants was about 33% lower than the leaves of the control plants, and this difference was maintained until the end of the experiment.

3.3.4 Effect on Partitioning

The more rapid response of leaves to CO$_2$ enrichment, compared with other plant components, indicates the occurrence of CO$_2$-dependent changes in dry matter partitioning. This can be illustrated in several ways. For example, the differential effects of CO$_2$ enrichment on different plant parts were evident when the percentage increases in dry weights of different parts was compared (Fig. 3.9). Throughout the experiment, dry weight gain under higher CO$_2$ was enhanced more for leaves than for
Figure 3.8 Effect of CO₂ enrichment on (a) stem and (b) root dry weight per plant.

- CO₂ enriched: 1500 ppm (---)
- Control: 340 ppm (-----)

Each point is the mean ± SE of six plants.
Table 3.2 Effect of CO₂ enrichment on the osmotic, and water potentials of primary leaves

(a) Summary of ANOVA results

<table>
<thead>
<tr>
<th></th>
<th>Osmotic potential</th>
<th>CO₂ Effect</th>
<th>Harvest Effect</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water potential</td>
<td></td>
<td>*</td>
<td>*</td>
<td>NS</td>
</tr>
</tbody>
</table>

*, **: significant at 5%, 1%. NS: not significant

(b)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration of CO₂ treatment (days)</th>
<th>Osmotic potential (kPa)</th>
<th>Water Potential (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14 21 24 28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (340 ppm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enriched (1500 ppm)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values in the table are means ± SE of four plants
Figure 3.9 Percent dry weight increase of different parts of CO$_2$ enriched plants over control plants.
% Dry Weight Increase

Duration of CO₂ Treatment (days)

- Leaf
- Stem
- Root
stems and roots. It is again evident that the timing of the CO₂ effect was earlier in leaves than in stems and roots.

Similarly, the ratio of shoot to root dry weights (SRR) indicated the differential effects of CO₂ enrichment on shoot and root growth. Similar increases in SRR over the course of experiment were observed in both control and CO₂ enriched plants, particularly near the end of the study (Fig. 3.10). The SRR was higher with CO₂ enriched plants than the control plants throughout the experiment period.

The leaf area ratio (LAR) was significantly reduced by CO₂ enrichment throughout the study. There was no significant change in the LAR of the control plants for the first 24 days of CO₂ enrichment, and there was an approximately 35% decrease by the end of experimental period (Fig. 3.11). For the CO₂ enriched plants, however, there was an initial decrease in LAR during the first few days, and a further decrease near the end of the experimental period.

### 3.3.5 Leaf Injury Induced by CO₂ Enrichment

In addition to the growth and partitioning effects, foliar abnormalities developed gradually in beans under CO₂ enrichment. Chlorosis near the leaf margin between the veins started to appear in the primary leaves about 3 weeks after the onset of enrichment (Fig. 3.12). The disorder eventually appeared in the oldest trifoliate leaves (Fig. 3.13).

The development of injury was accompanied by a decrease of the chlorophyll content (Fig. 3.14). The levels of chlorophyll concentration did not change for the first three weeks of CO₂ enrichment, and there was no difference in chlorophyll concentration between the two CO₂ treatments during this period. After 24 days of treatment, there was a decrease in chlorophyll content in both the CO₂ enriched and control plants until the end of the experiment. For the control plants, the chlorophyll content decreased 50% over four days (day 24 to day 28) followed by a further
Figure 3.10 Effect of CO$_2$ enrichment on shoot root ratio.

<table>
<thead>
<tr>
<th>Condition</th>
<th>ppm</th>
<th>Line Style</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO$_2$ enriched</td>
<td>1500</td>
<td>(---)</td>
</tr>
<tr>
<td>Control</td>
<td>340</td>
<td>(______)</td>
</tr>
</tbody>
</table>

Each point is the mean ± SE of six plants.
Figure 3.11 Effect of CO$_2$ enrichment on leaf area ratio.

<table>
<thead>
<tr>
<th></th>
<th>CO$_2$ enriched:</th>
<th>Control:</th>
</tr>
</thead>
<tbody>
<tr>
<td>ppm</td>
<td>1500</td>
<td>340</td>
</tr>
<tr>
<td></td>
<td>(---)</td>
<td>(-------)</td>
</tr>
</tbody>
</table>

Each point is the mean ± SE of six plants.
Figure 3.12 Photograph of CO$_2$-induced leaf chlorosis of a primary leaf.

(a) Control
(b) CO$_2$ enriched
Figure 3.13 Photograph of CO₂-induced leaf chlorosis of the first trifoliate leaf.
Figure 3.14 Effect of CO₂ enrichment on total chlorophyll concentration of primary leaves.

Each point is the mean ± SE of six plants.
Total Chlorophyll Concentration (g/m²)

Duration of CO₂ Treatment, days

- Control
- CO₂ Enriched
decrease of 39% over the next ten days (days 28 to 38). In contrast, the decrease in CO$_2$ enriched plants was much faster than the control plants. Over the corresponding periods, there was a 82% decrease followed by another 68% decrease. Thus, the chlorophyll concentration became significantly lower in CO$_2$ enriched plants than in the control plants during the development of leaf injury induced by CO$_2$ enrichment.
3.4 DISCUSSION

3.4.1 Plant Growth

The sustained enhancement of growth under high CO₂ was evident from the increases in total plant dry weight and absolute growth rate. This growth stimulation by CO₂ enrichment has been reported by many authors (for review see Kramer 1981). Kimball (1986) reported that, in his analysis of 430 studies of plant response to CO₂ enrichment, an average stimulation in yield of 30-35% is obtained with a doubling of the CO₂ concentration. That value is comparable to the increase found in this study.

The extended beneficial effect of CO₂ enrichment can't be directly explained by the response of relative growth rate; in this study the enhancement of RGR was only observed during the first two weeks of the experiment. Jolliffe and Ehret (1985) explained the discrepancy between the eventual depression in RGR and the steady increase in dry weight caused by elevated CO₂. It is due to the cumulative increase in plant dry weight by CO₂ enrichment. RGR is equal to absolute growth rate divided by plant dry weight; after sufficient time under CO₂ enrichment, the cumulative increase in plant dry weight necessarily causes a reduction in relative growth rate. They also showed that when plants of equal size are compared RGR was higher in CO₂-enriched plants. Similarly, Rogers et al. (1984a) also found an increase in relative growth rate of Glycine max only during the first two weeks of enrichment. Thereafter, high CO₂ concentrations no longer increased RGR. They concluded that the equalization of RGR occurred through adjustment of both ULR and LAR (RGR equals ULR times LAR). They suggested that as soybean plants develop, two mechanisms serve to modify or buffer the initial response of RGR to elevated CO₂: 1) a dampening of the stimulation of ULR through self shading or through a more direct effect on photosynthesis and 2) a downshift in LAR resulting from reduced specific leaf area and altered leaf anatomy. Poorter et al. (1988) corrected the ontogenetic drift by plotting
the RGR against a measure of plant size, i.e. leaf area. High CO₂ concentrations stimulated the RGR of *P. major* throughout the entire experiment.

Reduced LAR at high CO₂ was observed in this study (Fig. 3.11). Increased selfshading, however, seems unlikely to explain the present results because leaf area was unchanged (Fig. 3.6a). In this study the lack of promotion of RGR by CO₂ enrichment at the later stage can be explained by the accumulative increase in dry weight and the diminished response of both ULR and LAR.

### 3.4.2 The Effect of CO₂ Enrichment on Leaf Growth

Since leaves perform most of photosynthetic carbon fixation, it was not surprising that leaf dry weight was directly affected by CO₂ enrichment. In this study, there was a rapid CO₂-induced increase in leaf dry weight, and the effect was more prominent in the oldest leaves (primary leaves) than in the younger leaves (trifoliates). This increase in weight, however, was not matched by effects of CO₂ enrichment on leaf area, leaf number or leaf emergence.

In the majority of plant species, an increase in leaf area has usually been obtained using CO₂ enrichment (Ford and Thorne, 1967; Imai and Murata, 1976; Delucia *et al.*, 1985). However, as in this study, studies using bean (Jolliffe and Ehret, 1985), and tobacco (Thomas *et al.*, 1975) found that leaf area was virtually unaffected by CO₂ enrichment. This greater effect of CO₂ enrichment on leaf weight increase compared with area increase caused a considerable increase in specific leaf weight. Madore and Grodzinski (1985) reported that leaves from successive positions along the stem of CO₂ enriched plants had higher SLW than leaves at the similar positions on the control plants. When compared on an age basis, increases in SLW were most apparent in the oldest leaves. This effect of CO₂ enrichment in leaf growth has been noted in other studies (Jolliffe and Ehret, 1985; Delucia *et al.*, 1985).
Kriedemann and Wong (1984) tested four plant species, (*Basella alba* (L), *Raphanus sativus* (L), *Cucumis sativus* (L), and *Brassica pekinesis* (Leur.) Rupr.) grown under CO$_2$ enrichment (1350 ppm) for 40 to 50 days. They found that the magnitude of the CO$_2$ effect seemed to correlate with the response of the leaf area of the test species to CO$_2$ enrichment. This finding can be partially explained by the results that in some plant species leaves grown in high CO$_2$ have the same photosynthetic rate per unit area as leaves grown in low CO$_2$ (Ford and Thorne, 1967; Mauney *et al.*, 1979). These studies imply that expansion of the leaf area may be one key to achieving the full benefit from CO$_2$ enrichment. The lack of response of leaf area under high CO$_2$ found in this study would certainly limite the relative response to CO$_2$ enrichment of dwarf beans compared to most other plant species.

Water status of a leaf may affect the rate of leaf expansion and ultimately the rate of canopy development. In this study, leaf osmotic and water potentials were consistently lowered by CO$_2$ enrichment. The pressure potentials (data not shown), estimated by taking the differences between water and osmotic potentials, were consistently higher in CO$_2$ enriched leaves than control. Although the differences in pressure potentials were relatively small (due to the high variability of results), the trend was consistently observed throughout the experiment. These results suggest that leaf area expansion of CO$_2$ enriched bean plants was not restricted in terms of plant water status.

In addition to the plant water status, there are several other related factors that can affect the leaf expansion; such as genetic control of the growth form, environmental factors (light, temperature *etc.*), supply of growth substrates and inorganic nutrients. The effects of environmental factors were not investigated in this study, however, other studies indicate that light conditions can indeed alter the CO$_2$ effect on leaf expansion. For example, Gifford (1977) reported that leaf area index was not altered by CO$_2$ enrichment when wheat was grown under high light, but was enhanced under low light.
Low light levels were used in this study, but bean leaf expansion was not increased by high CO2. The fact that CO2 enrichment increased the leaf dry weight indicates that there was no shortage of carbon resource for leaf growth. The allocation of this carbon to additional leaf expansion was not realized implying some limitation in carbon partitioning.

3.4.3 Dry Matter Partitioning

In dwarf bean, it seems that not only is the expansion of individual leaf area restricted, but the number of leaves, and the stem and root dry weight are also not significantly promoted by CO2 enrichment. These selective effects on partitioning were obvious when the percent increase in dry weight was compared between different plant parts (Fig. 3.9). This uneven dry matter partitioning consequently was the cause of the increase in shoot root ratio and the decrease in leaf area ratio by high CO2. Contrary to this finding, there are reports of decreased shoot root ratios by CO2 enrichment for barley and kale (Ford and Thorne, 1967), sugar beet and radish (Sionit et al., 1982). There was also a lack of responsiveness in chrysanthemum (Hughes and Cockshull, 1971). Many factors can account for the different observations among various reports, such as different plant species used, the other environmental factors, etc. For example, Sionit et al. (1982) found that the effect of CO2 on shoot root ratio was larger with greater light flux density and increased plant age. In the case of bean plants, again, the accumulation of dry matter in the leaves under high CO2 indicates there was no shortage of carbon supply; the lack of growth of non-leaf parts (increasing in dry weight) should be attributed to limited ability of source leaves to translocate carbon to the other plant parts.
3.4.4 Effect on Leaf Injury

In addition to restricted leaf expansion, the leaf injury observed in this study may also cause some limitation of growth to CO$_2$ enrichment. High CO$_2$ induced leaf chlorosis has been reported in other species, such as cucumber (Witter, 1967), cotton (Hesketh et al., 1971), basil (Wallick and Zinnen, 1990), soybean (Chang, 1975; Hesketh et al., 1971) and bean (Ehret and Jolliffe, 1985a). Van Berkel (1984) indicated that high CO$_2$ induced leaf injury may be widespread among plant species with different symptoms and susceptibility. The injury has been correlated with higher leaf starch content in some studies (Cave et al., 1981; Ehret and Jolliffe, 1985a). Cave et al. (1981) further proposed that the disruption of normal chloroplast structure by irregularly shaped starch grains caused the CO$_2$ induced injury. This may not apply to all plant species. For example, CO$_2$ enriched sunflower, which also had high starch levels due to enrichment, did not show a similar relationship (Hesketh et al., 1971). An alternative explanation, implicating inorganic nutrients in leaf injury, has been investigated in the present study, and the results will be considered in later chapters.
CHAPTER 4 - EFFECT OF CO₂ ENRICHMENT ON LEAF METABOLITES AND CARBON PARTITIONING

4.1 INTRODUCTION

Beyond the CO₂ response of photosynthesis itself, the manner and the extent of carbon use by CO₂-enriched plants may be critical to their whole plant response (Lemon, 1983). Partitioning of assimilated carbon occurs within the leaf, between immobile products such as starch and transport intermediates such as sugars and amino acids, and between leaves and sinks elsewhere in the plant. Many studies have found that leaf starch concentrations increase under CO₂ enrichment (Delucia et al., 1985; Poorter et al., 1988). A relatively small increase in leaf sugar concentrations has also been observed in some plant species (Havelka et al., 1984; Huber et al., 1984; Allen et al., 1988; Vu et al., 1989), but not in soybean, cotton, sunflower and sorghum (Mauney et al., 1979). Sharkey et al. (1985) studied the effect of CO₂ concentration on leaf sucrose:starch ratio in bean plants. They found that below about 150 ppm CO₂, more carbon was found in sucrose than in starch. As CO₂ concentrations increased, there was always more starch made than sucrose. This switch from sucrose to starch synthesis implied an inadequate use or transport of sucrose at elevated CO₂ concentrations, and an improved ability to utilize sucrose might increase plant responses to CO₂ enrichment.

The changed partitioning of dry matter in response to CO₂ enrichment (Chapter 3) implies that there is a limitation in carbon distribution from leaves: leaves of bean plants grown under CO₂ enrichment had early and large dry matter increases, while stem and roots had later and smaller responses. The utilization of the extra dry weight of the CO₂-enriched bean leaves was also limited since there was no increase in
number of leaves or leaf area. This part of the study was done to investigate the effect of CO$_2$ enrichment on the deposition of carbon into different end products (starch, soluble sugars, protein and amino acids). Furthermore, the effects of CO$_2$ enrichment on the flow of carbon into storage and export pools in leaves were studied using compartmental analysis.
4.2 MATERIALS AND METHODS

Plants were grown as described earlier and harvested at 10, 14, 17, 21, 24, 28, and 38 days after CO$_2$ treatments. Three plants were harvested from each CO$_2$ treatment and discs (1.0 cm diameter) were punched from primary leaves of each plant and stored in the liquid nitrogen until ready for analysis.

4.2.1. Determination of Carbohydrates

Five leaf discs from each plant were extracted in 80% ethanol at 80°C until the tissue was pigment-free. The supernatant was used to determine the concentrations of soluble sugars (sucrose, glucose, and fructose) and the insoluble fraction was used to determined the starch concentration. Three subsamples were measured for the sample of each plant.

4.2.1.1 Starch Concentration

Starch concentration was determined using a modification of the method of Huber et al. (1984). The pigment-free discs obtained after leaf disc extraction was suspended in 2 mL of 0.2 N KOH and placed in the boiling water for 30 min. After cooling, the pH of the mixture was adjusted to about 5.5 with 1M acetic acid. An equal volume of amyloglucosidase (from *Rhizopus* genus mold) solution (400 units/mL in 0.1 M citrate buffer, pH 5.5) was then added and the mixture was incubated at 45°C for 4 to 6 hours. After digestion, the mixture was placed in boiling water for 1 min. to terminate the reaction. A 25 μL aliquot of the supernatant was analyzed for glucose using glucose kit 510-A (see 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 color development. This was measured at 450 nm in a spectrophotometer.

4.2.1.2. Sucrose, Fructose, and Glucose Concentrations

The ethanol extracts (supernatant) were dried in a bath of boiling water. Four mL of distilled water were added to the residue. To 2 mL of the aqueous solution was added 2 mL of a 0.5% invertase solution in pH 4.5 acetate buffer, followed by incubation at 37 C for 2 hours. Levels of glucose and fructose in the initial extract, and the total glucose and fructose following invertase digestion of the sucrose, were then measured spectrophotometrically at 340 nm with Boehringer Mannheim D-glucose/D-fructose assay kit. In this procedure, D-glucose and D-fructose are phosphorylated by the enzyme hexokinase and adenosine-5'-triphosphate (ATP) to glucose-6-phosphate (G-6-P) and fructose-6-phosphate (F-6-P). In the presence of enzyme glucose-6-phosphate dehydrogenase (G6P-DH), G-6-P is oxidized by nicotinamide-adenine dinucleotide phosphate (NADP) to gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH). The amount of NADPH formed in this reaction is stoichiometric with the amount of D-glucose. It is NADPH which is measured by the increase in absorbance at 340 nm.

4.2.1.3 Determination of Amino Acid, and Protein Concentrations.

4.2.1.3.1 Protein and Amino Acid Extraction

Freeze dried leaf sample (0.05 g) from each plant were ground with 1 mL of 0.01 M phosphate buffer (pH 7.2) in an ice bath. The extract was washed out with 19 mL of buffer and centrifuged (2000g) at 4 C for 20 min. The supernatant was used for quantification of protein and amino acids. Three subsamples were taken from sample of each plant for protein and amino acids assay.
4.2.1.3.2 Protein Determination

Total soluble protein was determined by a modification (Bensadoun and Weinstein, 1976) of the procedure of Lowry et al. (1951). This procedure used 1% (w/v) sodium deoxycholate and 24% (w/v) trichloroacetic acid to quantitatively precipitate proteins thus removing substances which interfered with Lowry protein determination procedure. The precipitated protein then was measured at 660 nm after reacting with the Lowry agents.

The proteins in the crude extract were separated by the SDS gel electrophoresis (Laemmli, 1970). Five μL of sample (50 mg/mL) was applied to a 12% running gel.

4.2.1.3.3 Amino Acid Determination

Total amino acids were determined spectrophotometrically at 570 nm by the ninhydrin assay (Moore and Stein, 1948).

4.2.2 Compartmental Analysis of Carbon Efflux and Storage

4.2.2.1 The Concept

A two-compartment model (Fig. 4.1a) (Dale et al., 1980; Hoddinott and Jolliffe, 1988) was used to study leaf carbon partitioning between pools. Carbon fixed by the leaf from atmospheric CO₂ is deposited in Pool 1. The carbon in Pool 1 can be transferred either to storage in Pool 2 or exported from the leaf. Carbon can also enter Pool 1 by transfer back from storage Pool 2.

Following a brief ¹⁴CO₂ feeding, the count rate detected by the Geiger-Muller tube is related to the total ¹⁴C contents (Q) of Pools 1 and 2. An exponential decline in ¹⁴C activity in each pool is expected as ¹⁴C is exported from the leaf. The mathematical model is as follows:

\[ Q = Q_1 + Q_2 = Ae^{-Bt} + ae^{-bt} \]
where:
Q is the measure of total $^{14}$C activity (counts per min)
t is time (min)
e is the base of natural logarithms
A and a are the initial $^{14}$C activities in Pools 1 and 2 respectively
B and b are pool depletion constants for Pools 1 and 2 respectively

Observations were made of counts per minute (Q) at different times (t) after pulse $^{14}$CO$_2$ feeding. The observation was standardized as a percentage of estimated initial counts per minute at the end of feeding. A regression was then established using the BMDPAR program package (Dixon, 1985), providing estimates for A, a, B and b.

Transfer coefficients (rate constants) reflecting the rates of carbon flow through the system can then be calculated as follows:

$$K_{12} = (AB + ab)/(A + a)$$
$$K_{21} = Bb(A + a)/(AB + ab)$$
$$K_{01} = AB(B - b)^2/((A + a)(AB + ab))$$

During this study, it was found that negative values for the remobilization rate constant ($K_{12}$, associated with carbon flow from Pool 2 to Pool 1) were obtained, especially for CO$_2$ enriched plants. The results indicate that for CO$_2$ enriched plants, the carbon transfer from Pool 2 to Pool 1 did not occur. A modified model (Fig. 4.1b, Appendix 2) was developed as follows:

$$Q = Q_1 + Q_2 = Ae^{-P_3t} + AP_2(1 - e^{-P_3t})$$
where $P_2$ equals $K_{21}/(K_{21} + K_{01})$, and $P_3$ equals to $K_{21} + K_{01}$.

4.2.2.2 Experimental Design

Plants were grown in CO$_2$ chambers as described earlier. On each day for two weeks, beginning after 15 days of CO$_2$ treatment, one plant from either a control or CO$_2$ enriched chamber was transferred to a Plexiglas chamber in a fume hood (Fig. 4.2). One primary leaf of the tested plants was then enclosed in a secondary chamber at the side of the main chamber enclosing the rest of the plant. The same CO$_2$ concentration under which plants were grown was maintained about the plant. Light intensity at the primary leaf level was 380  $\mu$mole m$^{-2}$ s$^{-1}$ PPFD provided by incandescent lamps. The primary leaf was labelled by exposing it to a closed loop flow of air containing a pulse of $14.8 \times 10^5$ Bq $^{14}$CO$_2$ for five minutes followed by chasing with unlabelled air with the same CO$_2$ concentration as the rest of the plant.

A Geiger-Muller tube, attached to a linear ratemeter and a chart recorder, was mounted in the base of the leaf chamber close to the adaxial surface of the leaf. Following labelling, the activity of a fed leaf was monitored for at least the next six hours. Data were analysed as described in section 4.2.2.1.
Figure 4.1 The two compartment model used to describe partitioning.

(a) The original model.
(b) The modified model

Carbon enters the system by photosynthesis (Pn) and is exported to the rest of the plant according to the transfer coefficient $K_{01}$, or stored in Pool 2 according to $K_{21}$. Some of the stored carbon is refluxed back to the export Pool 1 according to $K_{12}$. 
Figure 4.2 The system used for $^{14}$C compartmental analysis experiment.
4.3 RESULTS

4.3.1 Effects on Carbohydrates Concentrations

4.3.1.1 Sucrose, Glucose and Fructose Concentrations

Plants grown under CO$_2$ enrichment had higher sucrose concentration in the primary leaves than control plants (Fig. 4.3), however, the difference between the two treatments was greatest following 21 days of CO$_2$ treatment. At that time there was a large increase in sucrose concentration for the CO$_2$ enriched plants while there was no change of sucrose concentration of the control leaves. The increase in sucrose concentration by CO$_2$ enrichment varied from 23-94% at the earlier stages (before 21 days of CO$_2$ treatment) to 260 to 360% at the later stages.

Glucose and fructose concentrations followed the similar patterns to that observed for sucrose concentrations (Fig. 4.4, 4.5). There was an initial decrease in both glucose and fructose concentrations after 10 days of CO$_2$ treatment followed by a large increase following 21 days of CO$_2$ treatment. In contrast to the sucrose results, changes in glucose and fructose concentrations after 21 days were observed for both the control and CO$_2$ enriched plants. However, the glucose and fructose concentrations of CO$_2$ enriched plants were consistently 2 to 4 times higher than the control plants.

4.3.1.2 Starch Accumulation

CO$_2$ enrichment increased the starch concentrations in the leaves. In fact, among all the carbohydrates studied, primary leaf starch concentration showed the most prominent and immediate effects. Starch concentration was about seven times higher in CO$_2$ enriched leaves than in the controls after ten days of CO$_2$ enrichment (Fig. 4.6). Similar levels of starch concentrations were maintained for plants within each treatment for the remainder of the experiment.
Figure 4.3 Effect of CO$_2$ enrichment on the sucrose concentration of primary leaves.

Each point represents mean $\pm$ SE of 3 samples
Sucrose concentration (g/m²)

Duration of CO₂ treatment (days)

- Control (340 ppm)
- Enriched (1500 ppm)
Figure 4.4 Effect of CO$_2$ enrichment on the glucose concentration of primary leaves.

Each point represents mean ± SE of 3 samples
Glucose concentration (g/m²)

Duration of CO₂ treatment, days

- Control (340 ppm) - Enriched (1500 ppm)
Figure 4.5 Effect of CO$_2$ enrichment on the fructose concentration of primary leaves.

Each point represents mean $\pm$ SE of 3 samples
Fructose concentration (g/m²)

Duration of CO₂ treatment, days

- Control (340 ppm)
- Enriched (1500 ppm)
Figure 4.6 Effect of CO₂ enrichment on the starch concentration of primary leaves.

Each point represents mean ± SE of 3 samples.
The effects of CO₂ treatments on starch accumulation were further studied by switching plants to different CO₂ conditions after eight days of treatment at an initial CO₂ level. SLW and starch concentration of the primary leaves were measured both before and after switching. As before (Fig. 3.7), the SLW of CO₂ enriched plants increased with time, while the SLW of control plants remained at a lower level (Fig. 4.7). It took about 12 to 14 days of CO₂ enrichment for SLW of the primary leaves to reach its maximum. When the CO₂ enriched plants were switched to the control CO₂ level after 8 days of treatment, the SLW remained at about the same level for another 5 days before significant decrease was observed. Plants switched from control CO₂ condition to high CO₂, however, underwent a rapid increase in SLW, reaching the similar SLW as plants kept at the high CO₂ concentration after 9 days of switching.

Changes in primary leaf starch concentration corresponded to the changes in SLW (Table 4.1). There was no difference in starch concentration after one day of CO₂ enrichment. There was a 50% increase in starch concentration after 8 days of CO₂ treatment followed by a further 60% increase after an additional 4 days of CO₂ enrichment. The control plants maintained a similar level of the starch concentration over the same period of time. For plants switched to the higher CO₂ concentrations, there was a 90% increase in primary leaf starch concentration during this 4 day period. For plants switched from high to ambient CO₂ condition, however, the starch concentration was not significantly changed 4 days after switching.

4.3.1.3 The Partitioning of Carbon between Carbohydrates and Carbon Pools

In beans, sucrose is the major transport carbohydrate, while starch is the storage carbohydrate. Comparing the carbon partitioning between these two carbohydrates can provide an indication of how carbon was utilized under different CO₂ conditions. The partitioning of carbon between starch and sucrose can be expressed by taking the ratio between these two variables (Fig. 4.8). Although the ratio of
Figure 4.7 The specific leaf weights of primary leaves of control, CO₂ enriched, and plants switched from initial CO₂ concentration to the other CO₂ treatment at day 8 of CO₂ enrichment.

Each point represents mean ± SE of 3 plants.
Table 4.1 Effect of different CO\textsubscript{2} conditions over two periods of time on specific leaf weight and starch concentrations of primary leaves

<table>
<thead>
<tr>
<th>Treatment CO\textsubscript{2} (ppm)</th>
<th>Duration of CO\textsubscript{2} Treatment (days)</th>
<th>Specific leaf weight (g/m\textsuperscript{2})</th>
<th>Starch concentration (g/m\textsuperscript{2})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>340/340</td>
<td>32±2.2</td>
<td>34±4</td>
<td>10.4±.2</td>
</tr>
<tr>
<td>340/1500</td>
<td></td>
<td></td>
<td>37±3</td>
</tr>
<tr>
<td>1500/1500</td>
<td>30±2</td>
<td>57±8</td>
<td>10.8±.2</td>
</tr>
<tr>
<td>1500/340</td>
<td></td>
<td></td>
<td>45±4</td>
</tr>
</tbody>
</table>

* Plants were transferred to the other CO\textsubscript{2} condition at the eighth day of CO\textsubscript{2} treatment

** Values in the table are means ± SE of three plants
Figure 4.8 Effect of CO$_2$ concentrations on the starch/sucrose concentration ratio of control and CO$_2$ enriched plants.
Ratio of starch/sucrose concentration

Duration of CO₂ treatment, days

- Control (340 ppm)  - Enriched (1500 ppm)
starch to sucrose fluctuated over time for both control and CO₂ enriched plants, two distinctive stages were observed. Up to 21 days of treatment, the ratio of starch/sucrose concentration was much higher in the leaves of CO₂ treated plants than the leaves of control plants. After 21 days of CO₂ enrichment, however, the difference in the ratio of starch to sucrose in leaves between two CO₂ treatments diminished. Eventually, the starch to sucrose ratio became about the same for both CO₂ treated and control plants.

In addition to the observations on carbon partitioning between the transport and storage carbohydrates, a two compartment model was used to study the partitioning of newly fixed carbon (Fig. 4.1a,b). Photosynthesis and remobilized carbon from storage Pool 2 are the sources of the carbon in storage Pool 1, while carbon in Pool 1 can be transported to the storage Pool 2 or exported from the leaves to the other parts of the plant. The depletion of carbon from both pools was monitored by measuring ¹⁴C loss in pulse chase studies.

The export rate constant, $K_{01}$, associated with carbon export from Pool 1 to other parts of the plant, decreased for CO₂ enriched plants after 15 days of CO₂ treatment (Fig. 4.9a). A similar trend was found for the export rate constant of control plants except for two brief increases at 18 and 24 days of CO₂ treatment.

The storage rate constant ($K_{21}$, associated with carbon flow from Pool 1 to Pool 2) of CO₂ enriched plants was maintained at a steady level throughout the experiment, except for a drop at the end of the experiment (Fig. 4.9b). The storage rate constant of control plants, however, was higher than the CO₂ enriched plants before 20 days of treatment and later decreased to similar levels as the CO₂ enriched plants after 20 days of CO₂ treatment.

Similarly, the rate constant for carbon release from the storage Pool 2 ($K_{12}$) was higher for control plants than CO₂ enriched plants until the end of the experimental period (Fig. 4.9c). After 17 days of CO₂ treatment, there was no
Figure 4.9 Effect of CO_2 enrichment on the transfer rate constants between carbon storage pools.

(a) The export rate constant (K_{01}).
(b) The storage rate constant (K_{21}).
(c) The remobilization rate constant (K_{12}).
Transfer Coefficient $K_{01}$ (export) ($x10^{-4}$ min)

Transfer Coefficient $K_{21}$ (storage) ($x10^{-4}$ min)

Transfer coefficient $K_{12}$ (remobilization) ($x10^{-4}$ min)

Duration of CO$_2$ treatment (days)

- Control (340 ppm)
- Enriched (1500 ppm)
detectable remobilization of carbon from the storage pool 2 for CO2 enriched plants. For control plants, the reflux rate constant also decreased over time, but remobilization of carbon from storage pool 2 did not stop until the end of the experiment.

The ratio of the rate constants for export and storage continuously decreased for the CO2 enriched plants (Fig. 4.10). At 15 days of CO2 treatment, the ratio of export to storage was larger than one, thereafter, the ratio decreased below one for CO2 treated plants. For control plants, the ratio of export to storage rate constants was always below one, except at the end of the experiment.

4.3.2 Effects on Protein and Amino Acid Concentrations

Total leaf protein concentration per unit area decreased with time for both control and CO2 treated plants (Fig. 4.11). Decreases were most notable following 14 and 24 days of CO2 treatment. There was no significant difference in protein concentration between the two CO2 treatments. A similar decreasing trend was observed when the protein profile was investigated (Fig. 4.12). However, the rate of decrease varied among different bands between the two CO2 treatments, especially in a band corresponding in molecular weight to the carbonic anhydrase standard (31,000 Daltons). After 21 days of CO2 treatment this band was evidently lighter for CO2 enriched plants than control plants. After 24 days of CO2 treatment this band had almost completely disappeared for CO2 enriched plants, while it was still visible for control plants.

Total amino acid concentration was highly variable (Fig. 4.13), however, a decreasing trend can be observed for both CO2 treatments. There were no CO2 enrichment effects observed throughout the experiment.
Figure 4.10 Ratio of the export and storage rate constant of control and CO₂ enriched plants.
The diagram shows the ratio of $K_{01}/K_{21}$ (export/storage) over the duration of CO$_2$ treatment in days. The duration is measured from 10 to 30 days.

A control group with 340 ppm CO$_2$ and an enriched group with 1500 ppm CO$_2$ are compared.

- **Control (340 ppm)**: The ratio starts at approximately 3.5 and decreases to around 0.5 by the 30th day.
- **Enriched (1500 ppm)**: The ratio starts at approximately 3.5 and decreases to around 0.5 by the 22nd day, showing a steeper decrease compared to the control group.

The graph illustrates how the ratio changes with the duration of CO$_2$ treatment, with the enriched group showing a faster decline.
Figure 4.11 Effect of CO₂ enrichment on the protein concentration of primary leaves.

Each point represents mean ± SE of 3 samples
Leaf protein concentration (g/m²)

Duration of CO₂ treatment, days

- Control (340 ppm)  - Enriched (1500 ppm)
Figure 4.12 Photograph of polyacrylamide gel (12%) electrophoresis of soluble protein extracts of control and CO2 enriched leaves.

Lane 1 and 10 are the low molecular weight standards:
(A) Phosphorylase b (97,400 KD),
(B) Bovine serum albumin (66,200 KD),
(C) Ovalbumin (42,699 KD),
(D) Carbonic anhydrase (31,000 KD),
(E) Soybean trypsin inhibitor (21,500 KD),
(F) Lysozyme (14,400 KD).

Lane 2, 3 : samples from day 14
Lane 4, 5 : samples from day 21
Lane 6, 7 : samples from day 24
Lane 8, 9 : samples from day 28

Even number Lanes: CO2 enriched
Odd number Lanes: Control
Figure 4.13 Effect of CO$_2$ enrichment on the amino acid concentration of primary leaves.

Each point represents mean ± SE of 3 samples
Leaf amino acid concentration (g/m²)

Duration of CO₂ treatment, days

- Control (340 ppm)
- Enriched (1500 ppm)
4.4 DISCUSSION

4.4.1 Effects on Carbohydrates

4.4.1.1 Levels of Soluble Sugars

Higher ambient CO$_2$ will increase net photosynthesis, but it is impossible to predict the CO$_2$ enrichment effect on growth if the extent and manner in which the plant uses the extra assimilated carbon is not fully understood. To date, results of CO$_2$ enrichment on soluble sugars have been varied. Mauney et al. (1978) studied four different plant species (cotton, soybean, sunflower, sorghum) under high CO$_2$. They found that high CO$_2$ increased starch concentration in all species, but no significant changes in soluble sugars was observed. Similar results (Finn and Brun, 1982; Nafziger and Koller, 1976) were found for soybean in studies of short term effects of CO$_2$ enrichment. In contrast, Huber et al. (1984) and Hrubec et al. (1985) found that CO$_2$ enrichment of soybean plants usually resulted in a slight increase in leaf sucrose concentration, whereas Hofstra and Hesketh (1975) reported that soybean plants in normal air had consistently higher sugar concentration than at high CO$_2$. These conflicting results could partly be due to the use of different species or varieties by various researchers. Most of these reports only examined the sugar concentrations at one specific time. Other studies, however, have indicated that the timing of sampling can also affect the results. Poorter et al. (1988) found there was a marked difference in starch and soluble sugar concentrations of leaf blades of Plantago major under different CO$_2$ conditions, although the differences in soluble sugars were quantitatively less. In addition, they found that the difference in concentration of starch and soluble sugar was time dependent: large at the beginning of the experiment, smaller at the end. Similarly in this study, significant differences in both starch and soluble sugars concentrations were observed, but the timing of the CO$_2$ enrichment effect was very different. The significant difference in the starch concentration between the two CO$_2$ treatments was
observed after ten days of CO₂ treatment. However, prominent differences in soluble sugars were not observed until the later stages of the experiment (after 21 days of CO₂ treatment).

4.4.1.2 Starch Accumulation

For most plant species grown under elevated CO₂ an increase in the starch concentration in the leaves has been observed (Dons, 1988; Spencer and Bowes, 1986; Sasek et al., 1985). Increased starch accumulation in CO₂ enriched leaves can be caused by either an increase in carbon partitioning to the starch pool or a decrease in mobilization of carbon out of this pool. Delucia et al. (1985) examined the diurnal pattern of starch accumulation for control and CO₂ enriched cotton plants. Degradation and mobilization of starch in 340 ppm plants at the end of the dark period maintained the starch pool in a near equilibrium state. CO₂ enrichment produced a step-function in leaf starch accumulation as starch concentration in the high CO₂ plants did not return to the previous morning’s level by the end of dark period. This disequilibrium in pool size would presumably lead to a daily increase in leaf starch concentration in the high CO₂ plants until some equilibrium level was reestablished. Although the diurnal pattern of the starch accumulation was not examined in present study, the compartmental partitioning of newly fixed carbon was investigated. Results indicate that the capability for carbon accumulation into starch from newly fixed carbon was not any faster in CO₂ enriched leaves than the controls, since the storage rate constant of CO₂ enriched plants was not higher than the controls. However, remobilization of this newly fixed carbon from the storage pool was either very low or lacking in the CO₂ enriched plants, while for the control plants a significantly higher capability for remobilization was observed throughout the experiment. These results indicate that the high accumulation of starch under high CO₂ was partly caused by depressed starch degradation. This effect of CO₂ enrichment on starch degradation
was also observed when CO\textsubscript{2} enriched plants were switched back to the ambient CO\textsubscript{2} condition: the starch concentration was still maintained at a high level four days after switching. For those plants switched from ambient to CO\textsubscript{2} enriched condition, however, an immediate increase of starch concentration was observed.

4.4.2 Effects of Carbon Partitioning on Growth under CO\textsubscript{2} Enrichment

4.4.2.1 Leaf Growth

Studies of CO\textsubscript{2} effects on growth reported earlier (Chapter 3) showed that the early increase in growth was mainly found in leaves. Furthermore, the leaf dry weight increase was accompanied by large increases in both SLW and leaf starch concentrations. The increase in leaf starch concentrations has been correlated with the dry weight increase found in leaves of CO\textsubscript{2} enriched plants in many other studies. For example, Madore and Grodzinski (1985) concluded that the increase in leaf dry weight under CO\textsubscript{2} enrichment was largely due to an accumulation of starch in the more mature leaves. Similarly, Ehret and Jolliffe (1985b) found a linear relationship between leaf starch concentration and SLW. They noted that increases in SLW to about 30 g m\textsuperscript{-2} were not accompanied by noticeable increases in leaf starch concentration, but beyond that value, increases in SLW were attributable solely to increases in leaf starch concentration. The correlation between starch concentration and SLW was confirmed in this study by the results of acclimation experiment (Table 4.1). The changes in SLW and starch concentration were found to be correlated both before and after plants were switched to different CO\textsubscript{2} conditions. These results indicate that the leaf dry weight increase found in CO\textsubscript{2} enriched bean plants is due mainly to starch accumulation. Other studies, however, have shown that the structural adaptation of leaves under CO\textsubscript{2} enrichment can also partly account for the dry weight increase found under CO\textsubscript{2} enrichment. In melon plants, Acock and Pasternak (1986) found that differences in structural dry matter per unit leaf area were at least as important as stored
carbohydrates in determining SLW. In addition, some researchers (Hofstra and Hesketh, 1975; Thomas and Harvey, 1983) have published micrographs showing that CO\textsubscript{2}-enriched soybean leaves are thicker, have more densely packed palisade mesophyll cells, and can contain additional layers of palisade cells. In addition to the highly correlated relationship between SLW and starch concentration, there was no significant difference in leaf thickness between CO\textsubscript{2} enriched and control bean plants (Ehret, 1983). Therefore, it is unlikely that major contributions to SLW were made through anatomical changes of leaves under high CO\textsubscript{2} in the present study.

**4.4.2.2 Growth of Stem and Root**

The growth of stem and root rely on the carbon transported from the leaves. Therefore, the availability of the transport sugar will be a potential limitation to the CO\textsubscript{2} enrichment effect on the growth of these plant parts. The availability of the transport sugar can be regulated by the partitioning of carbon between starch and sucrose. In this study, the starch/sucrose ratio of CO\textsubscript{2} enriched plants decreased at the later stage of the experiment. At the same time, there were large increases of soluble sugars in the leaves of CO\textsubscript{2} enriched plants. The timing of these changes of carbon partitioning and the increase in soluble sugars levels coincided with the initial dry weight increase in stem and root. These results suggest that the increase in dry weight of non-photosynthetic plant parts of CO\textsubscript{2} enriched plants is dependent on the level of the transport sugars from the source leaves.

In addition to the availability of the transport sugars, the rate of transport may also limit the growth effect of CO\textsubscript{2} enrichment. Increased carbon transport in CO\textsubscript{2} enriched plants has been reported in some studies (Ho, 1977; Madore and Grodzinski, 1985), but considerable differences may exist among species in both the short-term and long-term effects of CO\textsubscript{2} enrichment. This study has shown that the export rate constant for newly fixed carbon for CO\textsubscript{2} treated plants was lower or similar to the
control plants. Similar results have been reported by Hoddinott and Jolliffe (1988). They reported that in bean plants, the rate constant for export was lower in CO₂ enriched plants than control plants in the light period; however, in the dark period CO₂ enriched plants had a higher rate constant for export than the control plants. Since the newly fixed carbon is only part of the potential carbon for export, this information is insufficient to conclude if the CO₂ enrichment had any effect on the carbon transport in beans. More studies are needed to verify the correlation between increased sucrose concentrations and the carbon transport rate in bean plants.

4.4.3 Effects on Protein and Amino Acid Composition

Protein and amino acids are secondary products following photosynthetic carbon fixation. The effects of CO₂ enrichment on the composition of proteins and amino acids varies among plant species. Wong (1979) reported that cotton grown in 330 ppm CO₂ had higher extractable protein concentration per unit leaf area than in plants grown in CO₂ enriched air, but there was no difference in maize. Similarly, no CO₂ effect on protein concentration was found in soybean (Havelka, et al., 1984). In bean, Porter and Grodzinski (1984) reported that soluble protein was not altered by CO₂ treatment after 7 days. My study showed that the protein concentration in bean plants decreased at two developmental stages (flowering and pod formation) for both CO₂ enriched and control plants but the levels of protein concentration per unit leaf area were not significantly changed by CO₂ treatment. The lack of change in total protein concentration does not eliminate the possibility of a change in protein composition induced by CO₂ enrichment. In fact, the preliminary study of the protein profile suggests there could be differential changes in specific proteins under different CO₂ conditions. Although there were no quantitative results, the qualitative difference of the protein profiles between the two CO₂ treatments suggests there was a faster rate of decrease of a protein. Although I did not demonstrate that this protein has carbonic
anhydrase activity, its molecular weight correspond to this enzyme which has a well established role in \( \text{CO}_2 \) biology. Porter and Grodzinski (1984) also found that in the mature leaves of bean plants (cv Seafarer) the levels of carbonic anhydrase declined markedly over the time course of experiment (\textit{i.e.} 7 days), and the rate of loss of this enzyme was greater in high \( \text{CO}_2 \) treated tissue. In C\(_3\) plants, carbonic anhydrase is a chloroplast enzyme which catalyzes the interconversion of \( \text{CO}_2 \) and \( \text{HCO}_3^- \). The significance of a reduced carbonic anhydrase activity during \( \text{CO}_2 \) enrichment is unclear, because the precise role of carbonic anhydrase in the stroma has not been defined (Reed and Graham, 1981). Carbon fixation in algae grown in high \( \text{CO}_2 \) is depressed when assayed in ambient \( \text{CO}_2 \), an effect attributed to suppression of carbonic anhydrase activity (Reed and Graham, 1981). Reed and Graham (1981) concluded that perhaps in higher plants less carbonic anhydrase is required because the \( \text{CO}_2 \) gradient between atmosphere and the stroma is sufficient to ensure high rates of photosynthesis.
CHAPTER 5 - EFFECTS OF CO₂ ENRICHMENT AND NITRATE SUPPLY ON GROWTH, INORGANIC NUTRIENT UPTAKE AND LEAF INJURY

5.1 INTRODUCTION

As growth and photosynthesis increase in response to higher CO₂, a correspondingly greater amount of mineral nutrients must be absorbed through the roots. Therefore, in keeping with the principle of limiting factors, any restriction in nutrient availability will limit the response of growth to additional CO₂. Indeed, it has been found in many studies that the effects of CO₂ on growth were increased by higher nutrient supply (Sionit et al., 1981a; Patterson and Flint, 1982; Cure et al., 1988a,b). This greater growth stimulation by CO₂ enrichment under conditions of optimal nutrient supply was associated with higher nutrient uptake under these conditions (Cure et al., 1988a,b). In contrast, Hocking and Meyer (1985) observed a consistent growth response of cocklebur to high CO₂ over a range of NO₃⁻ concentrations with little change in NO₃⁻ uptake relative to control. The above studies indicated that plant responses to CO₂ enrichment when other nutrients are limiting depends on the nature of those limitations and whether additional CO₂ assimilation will increase the availability of potentially limiting nutrients.

Lower nutrient concentrations in CO₂ enriched leaves have been observed in some studies (Wong, 1979; Porter and Grodzinski, 1984; Tremblay et al., 1987, 1988). Several mechanisms for this have been suggested, such as increased carbon gain associated with elevated atmospheric CO₂ which increases the relative amount of carbon to other elements in plant tissue (Lemon, 1983), and reduced nutrient influx to leaves due to stomatal closure (Neyra and Hageman, 1976). Larigauderie et al. (1988) reported a linear photosynthesis versus leaf nitrogen relationship under both ambient
and CO₂ enriched conditions. Thus, lower leaf nitrogen concentrations observed under CO₂ enrichment may partly limit plant growth responses to CO₂ enrichment.

As observed earlier (Chapter 3), leaf injury occurred after three weeks of CO₂ enrichment. The similarity of the symptoms of injury and nutrient deficiency effects (especially N, K) suggested that there may be some connection. A similar suggestion was made by Goudriaan and de Ruiter (1983) who suggested that leaf injury of potato plants under CO₂ enrichment may be related to potassium deficiency.

The studies in this chapter were done to examine the relationship between nutrients and CO₂ enrichment effects. The concentrations and the partitioning of the inorganic elements among different plant parts were examined to study their responses to CO₂ enrichment and to identify elements which may become limiting under CO₂ enrichment. Furthermore, the effect of nutrient availability on growth responses of CO₂ enrichment was investigated by studying growth and leaf injury under two levels of NO₃⁻ supply.
5.2 MATERIALS AND METHODS

5.2.1. Effect of CO₂ Enrichment on Inorganic Nutrients

Plants were grown under control (340 ppm CO₂) and enriched (1500 ppm CO₂) conditions as described in Chapter 3. The experiment was designed as a randomized block, with four chambers per block. Twelve plants were assigned to each chamber and four plants were harvested after 14, 21, and 28 days of CO₂ treatment. The concentrations and contents of various inorganic elements were determined for different plant parts. The elements assessed were: N, P, K, Ca, Mg, Fe, Mn, Zn, and Cu. The plant components examined were: primary leaves, successive trifoliate leaves formed during the course of the study, stem, and pods. At each harvest, different plant components were bagged and dried in the oven at 80 C for 48 hours. The dried plant samples were pooled for each chamber and ground with a Phillips coffee grinder to a coarse powder and stored in a desiccator before further analysis.

5.2.1.1 Nutrient Analysis

Dry samples (0.5 to 1.0 g) were weighed and digested (Parkinson and Allen, 1975) with sulfuric acid and hydrogen peroxide as oxidants under high heat for 30 minutes. Liquid samples were diluted to 100 mL with distilled water and transferred to plastic bottles for the further analysis. The sample solutions were then analyzed using the following procedures, with further dilution as appropriate. For nitrogen and phosphorus, an AutoAnalyzer II (Technicon Autoanalyzer II Methodology, 1976) was used. The determination of nitrogen is based on a colorimetric method in which an emerald-green color is formed by the reaction of ammonia, sodium salicylate, sodium nitroprusside and sodium hypochlorite in a buffered alkaline medium at a pH of 12.8-13.0. The ammonia-salicylate complex is read at 660 nm. The determination of phosphorus is based on the colorimetric method in which a blue color is formed by the
reaction of ortho phosphate, molybdate ion and antimony ion followed by reduction with ascorbic acid at an acidic pH. The phosphomolybdenum complex is read at 660 nm. For the remainder of the elements (K, Mg, Fe, Ca, Cu, Mn, Zn, Na), the concentrations were determined by a atomic absorption spectrophotometer (Perkin-Elmer 806).

5.2.2 Interactive Effects of CO2 Enrichment and NO3⁻ Supply

5.2.2.1 Experimental Design

The experiment was designed as a 2 x 2 factorial, with two CO2 concentrations (340 ppm, 1500 ppm) and two levels of nutrient supply (full NO3⁻, 6 mmole/L; and 1/2 NO3⁻, 3 mmole/L). Plants were grown in chambers under the conditions as described earlier (Chapter 3), except for their nutrient supply. Full strength Hoagland's solution was supplied to high NO3⁻ treated plants while low NO3⁻ treated plants received the same nutrient solution except that it contained only half of the controls' NO3⁻ concentration. The reduced NO3⁻ solution was prepared by lowering the concentrations of KNO3 and Ca(NO3)2 to half the levels found in Hoagland's solution and replacing Ca^2+ and K⁺ with appropriate concentrations of CaCl2.6H2O and KCl. The pH of nutrient solution was adjusted to 6.0 by NaOH (0.1 M) for each nutrient treatment. Nutrient solutions were supplied every other day starting at the beginning of the CO2 treatments, while distilled water was supplied when needed.

The experiment consisted two blocks with four chambers in each block. Twelve plants were assigned to each chamber and three plants were harvested from each chamber at 17, 21, 25, and 29 days after CO2 treatment. At each harvest, two samples (primary leaf of two individual plants) from each chamber were taken for analysis of leaf constituents (protein, amino acids, starch, and chlorophyll) while the remainder of the plant parts were bagged and dried at 80 C for 48 hours. After drying, each plant component was weighed and the dried primary leaves were used for the determination
of inorganic nutrient elements (N, P, K, Mg, Ca, Na). The methods of determination of leaf constituents were the same as described in Chapters 3 and 4.
5.3 RESULTS

5.3.1 Effect on Inorganic Nutrients

5.3.1.1 Levels of Inorganic Nutrients

Although nine elements were studied in this experiment, only results of two elements (N, K) will be presented in the results section. The remainder of the results will be presented in the appendix 3. Out of the nine elements studied in this experiment, there were two distinctive groups according to the distribution of the elements among different leaf positions. For nitrogen, phosphorus, potassium and zinc the concentrations decreased with the age of the leaves, whereas for magnesium, calcium, iron, copper and manganese the concentration generally increased with the age of leaf. No consistent CO₂ effects on the concentrations of nutrient elements were found (Table 5.1), however, the significant interaction term (CO₂ effect x harvest) reflected the changing trend of CO₂ effects over time.

The concentrations of all the tested elements in all leaf positions were higher or the same in control plants than in CO₂ enriched plants, when the concentration was expressed per unit dry weight (Fig. 5.1). On the other hand, when the concentration of the nutrient elements were expressed per unit leaf area, the opposite trend was observed: that plants grown under CO₂ enrichment had higher concentrations per unit leaf area than the control plants (Fig. 5.2). Since leaf area was unaffected by CO₂ level, while dry weight was strongly influenced (Chapter 3), the former is the better basis for interpretation. Absolute amounts of most elements contained in different leaf components tended to increase during the study (Fig. 5.3). CO₂ enrichment caused greater accumulation in most cases, but some exceptions to this occurred at the final harvest (N, Mg, Cu, Mn).
Table 5.1 Summary of ANOVA results:  
Effect of CO$_2$ enrichment on concentrations of nutrients of various leaves

<table>
<thead>
<tr>
<th>Element</th>
<th>CO$_2$ Effect</th>
<th>Harvest Effect</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td>Primary Leaves</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>5.6</td>
<td>.25</td>
<td>1.1</td>
</tr>
<tr>
<td>P</td>
<td>5.9</td>
<td>.25</td>
<td>.26</td>
</tr>
<tr>
<td>K</td>
<td>12.3</td>
<td>.18</td>
<td>2.3</td>
</tr>
<tr>
<td>Mg</td>
<td>79.9</td>
<td>.07</td>
<td>1.3</td>
</tr>
<tr>
<td>Ca</td>
<td>168.9</td>
<td>.05*</td>
<td>.14</td>
</tr>
<tr>
<td>Fe</td>
<td>8.9</td>
<td>.21</td>
<td>1.1</td>
</tr>
<tr>
<td>Cu</td>
<td>17.3</td>
<td>.15</td>
<td>.9</td>
</tr>
<tr>
<td>Mn</td>
<td>19986</td>
<td>.005**</td>
<td>.03</td>
</tr>
<tr>
<td>Zn</td>
<td>11.8</td>
<td>.18</td>
<td>.58</td>
</tr>
<tr>
<td>1st&amp;2nd Trifoliates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>3.0</td>
<td>.33</td>
<td>2.6</td>
</tr>
<tr>
<td>P</td>
<td>9.4</td>
<td>.20</td>
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</tr>
<tr>
<td>K</td>
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<td>.02**</td>
<td>6.7</td>
</tr>
<tr>
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<td>Ca</td>
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<tr>
<td>Zn</td>
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<td>.23</td>
<td>7.6</td>
</tr>
<tr>
<td>3&amp;4th Trifoliates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>1.8</td>
<td>.40</td>
<td>8.4</td>
</tr>
<tr>
<td>P</td>
<td>25281</td>
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</tr>
<tr>
<td>K</td>
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<td>Mg</td>
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<tr>
<td>Zn</td>
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<td>.37</td>
<td>2.8</td>
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<td>Remainder of the Leaves</td>
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<td></td>
</tr>
<tr>
<td>N</td>
<td>1.4</td>
<td>.45</td>
<td>.28</td>
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<tr>
<td>P</td>
<td>4.6</td>
<td>.28</td>
<td>.02</td>
</tr>
<tr>
<td>K</td>
<td>3678</td>
<td>.01**</td>
<td>.19</td>
</tr>
<tr>
<td>Mg</td>
<td>28561</td>
<td>.004**</td>
<td>4.1</td>
</tr>
<tr>
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<td>.04*</td>
<td>8.9</td>
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<td>Fe</td>
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<td>.20</td>
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<tr>
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<tr>
<td>Mn</td>
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<td>.22</td>
<td>.79</td>
</tr>
<tr>
<td>Zn</td>
<td>.8</td>
<td>.53</td>
<td>.73</td>
</tr>
</tbody>
</table>

* significant at 5% level  ** significant at 1% level  
F = F value, P = probability.
Figure 5.1 Effect of CO$_2$ enrichment on nutrient concentrations (\%, g.g$^{-1}$ x10$^{-2}$) of different plant parts.

Harvest I: 14 days of CO$_2$ treatment  
Harvest II: 21 days of CO$_2$ treatment  
Harvest III: 28 days of CO$_2$ treatment  

Plant parts:  
1. Primary leaves  
2. 1st and 2nd trifoliate leaves  
3. 3rd and 4th trifoliate leaves  
4. Remainder of the leaves  
5. Stem  
6. Pods
LEGEND
- 340 ppm CO₂
- 1500 ppm CO₂

HARVEST I

HARVEST II

HARVEST III

N CONCENTRATION, %

PLANT PARTS
LEGEND

\[
\begin{align*}
340 \text{ppm } CO_2 & \quad \text{light blue} \\
1500 \text{ppm } CO_2 & \quad \text{dark blue}
\end{align*}
\]

HARVEST I

PLANT PARTS

0.0 - 0.2 - 0.4 - 0.6 - 0.8 - 1.0

0.2 - 0.3 - 0.3 - 0.3 - 0.3 - 0.2

HARVEST II

PLANT PARTS

0.0 - 0.2 - 0.4 - 0.6 - 0.8 - 1.0

0.2 - 0.3 - 0.2 - 0.5 - 0.5 - 0.4

HARVEST III

PLANT PARTS

0.0 - 0.2 - 0.4 - 0.6 - 0.8 - 1.0

0.2 - 0.3 - 0.2 - 0.5 - 0.4 - 0.6 - 0.7
Figure 5.2 Effect of CO$_2$ enrichment on nutrient concentrations (mg per unit of leaf area) of various leaves.

Harvest 1: 14 days of CO$_2$ treatment
Harvest 2: 21 days of CO$_2$ treatment
Harvest 3: 28 days of CO$_2$ treatment

Plant parts
1: Primary leaves
2: 1st and 2nd trifoliate leaves
3: 3rd and 4th trifoliate leaves
4: Remainder of the leaves
Harvest 1

Harvest 2

Harvest 3

Plant parts

Control (340 ppm)  Enriched (1500 ppm)
Figure 5.3 Effect of CO₂ enrichment on nutrient contents of different plant parts per plant.

Harvest 1: 14 days of CO₂ treatment
Harvest 2: 21 days of CO₂ treatment
Harvest 3: 28 days of CO₂ treatment

Plant parts:

- a: Primary leaves
- b: 1st and 2nd trifoliate leaves
- c: 3rd and 4th trifoliate leaves
- d: Remainder of the leaves
- e: Stem
- f: Pods

Control: first bar of each pair
Enriched: second bar of each pair
N CONTENT, g (x 10^-2)

1.2 1.4 0.9 1.1 4.4 3.4 4.1 3.2 3.5 8.4 3.0 4.9 8.2 5.9 6.0 1.7

HARVEST NUMBER
5.3.1.2 Partitioning of Nutrients Among Plant Parts

These results allowed the calculation of the change in nutrient contents during two periods of growth: 14 to 21 days of CO₂ treatment (period 1) and 21 to 28 days of CO₂ treatment (period 2). For most elements and parts, absolute content increased during both periods (Fig. 5.4). Increases were greatest for those parts undergoing most rapid growth. In a few cases, however, there was a decrease in absolute elemental content in the older plant parts during period 2. These losses appeared to be greater for CO₂ enriched plants. For example, during period 2, for nitrogen there was a loss of nutrient content for the primary leaves and majority of the trifoliate leaves (1st, 2nd, 3rd and 4th) for CO₂ enriched plants. However, in the control plants, this loss was only found in the primary leaves and oldest trifoliate leaves (1st and 2nd). The amount of nutrient loss was also higher in the CO₂ enriched plants than the control plants (Fig. 5.4a-i). Presuming that the losses involve redistribution to other plant parts, these losses can be expressed as net contributions of older plant parts to nutrient accumulation in the younger parts (Fig. 5.5). The results show that in the the first period between harvests, older parts made little contribution except for copper. During the period 1, 20 to 30% of copper accumulated in the younger plant parts was contributed by the older plant parts. However, in the second period, the contributions of nutrient elements from older plant parts was greater in CO₂ enriched plants for all the nutrients tested except phosphorus. There were no losses of phosphorus from any plant part regardless of CO₂ treatment. Among those nutrients that had high loss in the CO₂ enriched plants, nitrogen and potassium had the greatest differences. There was a 2% loss of potassium in the older parts of control plants, while the loss in the CO₂ enriched plants was 22%. For nitrogen, there was 10% loss from older parts in the control plants compared to more than 50% found for CO₂ enriched plants. The timing
Figure 5.4 Effect of CO$_2$ enrichment on nutrient gain or loss of different plant parts over two periods.

(a) Period 1: between 14 and 21 days
(b) Period 2: between 21 and 28 days

Plant parts
1: Primary leaves
2: 1st and 2nd trifoliate leaves
3: 3rd and 4th trifoliate leaves
4: Remainder of the leaves
5: Stem
6: Pods
PLANT PARTS

(a) 340 ppm CO₂
(b) 1500 ppm CO₂

CHANGE IN N CONTENT (g x 10⁻²)

1 2 3 4 5 6

1.1 0.8 4.4 1.1 6.1 1.9 2.6

0.5 0.3 0.7 1.1 0.5 1.3 1.7 1.0
Figure 5.5 The percentage of nutrient contribution from older plant parts to younger plant parts

(a) Period 1: between 14 and 21 days
(b) Period 2: between 21 and 28 days
of redistribution of nutrients from older to younger plant parts (period 2) corresponded with the period of development of leaf injury.

5.3.2 Interactive Effects of CO$_2$ Enrichment and NO$_3^-$ Supply

5.3.2.1 Plant Growth

High CO$_2$ concentration increased total plant dry weight under both levels of NO$_3^-$ supply (Table 5.2, Fig. 5.6). The magnitude of the CO$_2$ effect on total plant dry weight was about 30 to 40% for both NO$_3^-$ treatments. The total plant dry weight was also increased by increasing NO$_3^-$ supply at each CO$_2$ concentration by 20 to 30%, but the effect of NO$_3^-$ treatment only commenced after second harvest (21 days of treatment). At the first harvest (17 days after CO$_2$ treatment), dry weight was only affected by CO$_2$ treatment. Except for the first harvest, there was no difference in total plant dry weight between high CO$_2$ and low NO$_3^-$ treated plants and low CO$_2$ and high NO$_3^-$ treated plants. Plants treated with high CO$_2$ and high NO$_3^-$ had the highest total plant dry weights, and low CO$_2$ and low NO$_3^-$ plants had the lowest plant dry weights for the entire experiment.

The effects of CO$_2$ and NO$_3^-$ concentrations on total leaf dry weight followed a similar pattern (Fig. 5.7). Again, both CO$_2$ enrichment and higher NO$_3^-$ supply increased the leaf dry weight after 17 and 21 days of treatment respectively (Table 5.2). However, the magnitude of effects of CO$_2$ and NO$_3^-$ treatments were different. Total leaf dry weight was increased about 40 to 80% by high CO$_2$ treatment, and there was only a 20 to 50% increase by higher NO$_3^-$ supply. Leaf dry weight was found significantly higher in high CO$_2$ low NO$_3^-$ treated plants than low CO$_2$ and high NO$_3^-$ treated plants throughout the experiment, except the last harvest. The effect of CO$_2$ enrichment on leaf dry weight was 15 to 20% higher in those plants treated with low NO$_3^-$ than those plants treated with high NO$_3^-$ in all harvests except the second harvest.
Table 5.2 Summary of ANOVA results: Interactive effects of CO$_2$ enrichment and NO$_3$ supply on plant growth

(a) Total plant dry weight

<table>
<thead>
<tr>
<th></th>
<th>Harvest 1</th>
<th>Harvest 2</th>
<th>Harvest 3</th>
<th>Harvest 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO$_2$ Effect</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>NO$_3$ Effect</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Interaction</td>
<td></td>
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<td></td>
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</tbody>
</table>

(b) Total leaf dry weight

<table>
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<th>Harvest 2</th>
<th>Harvest 3</th>
<th>Harvest 4</th>
</tr>
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<tbody>
<tr>
<td>CO$_2$ Effect</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>NO$_3$ Effect</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Interaction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(c) Total leaf area

<table>
<thead>
<tr>
<th></th>
<th>Harvest 1</th>
<th>Harvest 2</th>
<th>Harvest 3</th>
<th>Harvest 4</th>
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<tbody>
<tr>
<td>CO$_2$ Effect</td>
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<td>*</td>
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<tr>
<td>NO$_3$ Effect</td>
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<td></td>
<td>*</td>
</tr>
<tr>
<td>Interaction</td>
<td></td>
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</table>

(d) Specific leaf area

<table>
<thead>
<tr>
<th></th>
<th>Harvest 1</th>
<th>Harvest 2</th>
<th>Harvest 3</th>
<th>Harvest 4</th>
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</tr>
<tr>
<td>Interaction</td>
<td></td>
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(e) Root dry weight

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<tr>
<td>NO$_3$ Effect</td>
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<td>Interaction</td>
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(f) Stem dry weight

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-- not significant
*, ** significant at 5%, 1% levels, respectively.
Figure 5.6 Effect of CO₂ enrichment and NO₃⁻ supply on total dry weight per plant.
Low CO$_2$, Low NO$_3$  
High CO$_2$, Low NO$_3$  
Low CO$_2$, High NO$_3$  
High CO$_2$, High NO$_3$
Figure 5.7 Effect of CO₂ enrichment and NO₃⁻ supply on total leaf dry weight per plant.
Total Leaf Dry Weight, g/plant

Treatment Duration, days

- Low $\text{CO}_2$, Low $\text{NO}_3$
- Low $\text{CO}_2$, High $\text{NO}_3$
- High $\text{CO}_2$, Low $\text{NO}_3$
- High $\text{CO}_2$, High $\text{NO}_3$
Contrary to the dry weight response, the growth of leaf area responded much more to NO$_3^-$ treatment than CO$_2$ treatment (Table 5.2, Fig. 5.8). Plants treated with high NO$_3^-$ had a 35 to 55% increase in leaf area over low NO$_3^-$ treated plants regardless of the CO$_2$ treatment. There was no CO$_2$ effect on leaf area of the low NO$_3^-$ treated plants, but for those plants treated with high NO$_3^-$, there was a decrease in leaf area by high CO$_2$ in the first half of the experiment, although this difference diminished later in the experiment.

Due to the increase in leaf dry weight by CO$_2$ enrichment, high CO$_2$ concentration significantly increased specific leaf weight regardless of NO$_3^-$ treatment (Table 5.2, Fig. 5.9). With each CO$_2$ treatment, plants treated with low NO$_3^-$ had higher specific leaf weight than plants treated with high NO$_3^-$, but this difference also diminished later in the experiment.

Root dry weight was mainly affected by CO$_2$ treatment (Table 5.2, Fig. 5.10). For high NO$_3^-$ treated plants there was a 30 to 55% increase in root dry weight by CO$_2$ enrichment, but there was no CO$_2$ effect on plants treated with low NO$_3^-$ concentration until the final harvest. There was also no NO$_3^-$ effect on the root dry weight until the final harvest regardless of the CO$_2$ treatment. In contrast, NO$_3^-$ treatment had the predominant effect on stem dry weight (Table 5.2, Fig. 5.11). High NO$_3^-$ supply significantly increased stem dry weight by 20 to 50% in plants grown under both CO$_2$ concentrations. There was a 14 to 38% increase in stem dry weight in high CO$_2$ high NO$_3^-$ treated plants, but there was no CO$_2$ effect on stem dry weight of plants treated with low NO$_3^-$.

The different responses of plant components (leaf, stem and root) to different treatments described above was evident when percent dry weight increase by CO$_2$ enrichment was compared between high and low NO$_3^-$ treated plants (Fig. 5.12). For leaves, CO$_2$ effects were observed throughout the experiment for both high and low NO$_3^-$ treated plants. For stem and roots, higher and earlier CO$_2$ effects were
Figure 5.8  Effect of CO$_2$ enrichment and NO$_3^-$ supply on total leaf area per plant.
Total Leaf Area, $m^2$/plant

Treatment Duration, days

- Low CO$_2$, Low NO$_3$
- High CO$_2$, Low NO$_3$
- Low CO$_2$, High NO$_3$
- High CO$_2$, High NO$_3$
Figure 5.9 Effect of CO$_2$ enrichment and NO$_3$" supply on specific leaf weight per plant.
Specific leaf weight (g/m²)

Treatment Duration, days

- Low CO₂, Low NO₃
- Low CO₂, High NO₃
- High CO₂, Low NO₃
- High CO₂, High NO₃
Figure 5.10 Effect of CO$_2$ enrichment and NO$_3^-$ supply on root dry weight per plant.
Total Root Dry Weight, g/plant

Treatment Duration, days

- Low CO₂, Low NO₃
- Low CO₂, High NO₃
- High CO₂, Low NO₃
- High CO₂, High NO₃
Figure 5.11 Effect of CO₂ enrichment and NO₃⁻ supply on stem dry weight per plant.
Total Stem Dry Weight, g/plant

Treatment Duration, days

- Low CO₂, Low NO₃
- Low CO₂, High NO₃
- High CO₂, Low NO₃
- High CO₂, High NO₃
Figure 5.12 Percent dry weight increase over control by CO$_2$ enrichment of high and low NO$_3^-$ treated plants.
observed for high NO$_3^-$ treated plants. For low NO$_3^-$ treated plants, CO$_2$ enrichment did not affect stem and root dry weights until the last harvest.

Under ambient CO$_2$, higher NO$_3^-$ supply increased the shoot/root dry weight ratio, but no consistent NO$_3^-$ effect was observed with CO$_2$ enriched plants (Table 5.3). Similarly, CO$_2$ enrichment increased the shoot/root dry weight ratio only with low NO$_3^-$ treated plants; this increase ranged from 10 to 40%.

Although plants were not inoculated during the experiment, root nodules were found in all the plants at later stages of the experiment. The source of inoculum for this nodulation could be the non-sterilized soil medium used in this study. Plants treated with low NO$_3^-$ concentration had significantly higher nodule fresh weight than plants treated with high NO$_3^-$ (Table 5.4). Although CO$_2$ enrichment did not significantly increase nodule fresh weight until later in the experiment, nodule fresh weight was consistently higher in plants treated with high CO$_2$ compared with ambient CO$_2$ plants, regardless of NO$_3^-$ treatment.

**5.3.2.2 Starch Accumulation**

Starch concentrations observed in this study (Fig. 5.13) were considerably higher than expected: the starch content accounted for 70-90% of leaf dry weight while earlier results (Fig. 4.6) showed a 10-30% starch/leaf weight ratio. The cause of this high levels of starch is not clear, but contamination of the assay enzyme (amyloglucosidase) was suspected. Although absolute values of starch concentration were questionably high, a similar trend on the effect of CO$_2$ enrichment on leaf starch concentration was observed. CO$_2$ enrichment significantly increased the starch concentration in the leaves of both high and low NO$_3^-$ treated plants (Table 5.5). Although there was a higher percent increase in starch concentration by CO$_2$ enrichment with high NO$_3^-$ treated plants, higher absolute starch concentrations were observed with the
Table 5.3 Interactive effect of CO$_2$ enrichment and NO$_3$ supply on Shoot/Root dry weight ratio

<table>
<thead>
<tr>
<th>Treatment CO$_2$ NO$_3$</th>
<th>Shoot/Root (g/g)</th>
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<th>Harvest 2</th>
<th>Harvest 3</th>
<th>Harvest 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 1</td>
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<td>3.0</td>
<td>3.5</td>
<td>3.3</td>
<td>4.6</td>
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<tr>
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<td>4.4</td>
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<tr>
<td>2 1</td>
<td></td>
<td>4.1</td>
<td>3.8</td>
<td>4.6</td>
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<td>3.9</td>
<td>4.7</td>
<td>5.2</td>
<td>5.1</td>
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</table>

CO$_2$ - 1: 340 ppm  2: 1500 ppm  
NO$_3$ - 1: 1/2 NO$_3$  2: full NO$_3$
Table 5.4 Interactive effect of CO$_2$ enrichment and NO$_3$ supply on root nodule fresh weight

(a)

<table>
<thead>
<tr>
<th>Treatment CO$_2$ NO$_3$</th>
<th>Nodule Fresh Weight (g)</th>
<th>Harvest 1 (day 17)</th>
<th>Harvest 2 (day 21)</th>
<th>Harvest 3 (day 25)</th>
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<tr>
<td>1 1</td>
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<td>2 2</td>
<td>0.42</td>
<td>0.79</td>
<td>1.27</td>
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CO$_2$ - 1: 340 ppm  2: 1500 ppm
NO$_3$ - 1: 1/2 NO$_3$  2: full NO$_3$

(b) ANOVA

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<tr>
<th></th>
<th>Harvest 1</th>
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<tbody>
<tr>
<td>CO$_2$ Effect</td>
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<tr>
<td>NO$_3$ Effect</td>
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<tr>
<td>Interaction</td>
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-- not significant
*, ** significant at 5%, 1% levels, respectively.
Table 5.5 Summary of ANOVA results:
Interactive effect of CO$_2$ enrichment and NO$_3$ supply on starch, protein, amino acid, and chlorophyll concentrations of primary leaves.

(a) Starch concentration

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<th>Harvest2</th>
<th>Harvest3</th>
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<td>NO$_3$ Effect</td>
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(b) Protein concentration

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<td>NO$_3$ Effect</td>
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<td>Interaction</td>
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(c) Amino acid concentration

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<td>NO$_3$ Effect</td>
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(d) Chlorophyll concentration

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-- not significant
*, ** significant at 5%, 1% levels, respectively.
Figure 5.13 Effect of CO₂ enrichment and NO₃⁻ supply on starch concentration of primary leaves.
Starch Concentration (g/m²)

Treatment Duration, days

- Low CO₂, Low NO₃
- Low CO₂, High NO₃
- High CO₂, Low NO₃
- High CO₂, High NO₃
low NO$_3^-$ treated plants regardless of CO$_2$ treatment throughout the experiment except the last harvest.

5.3.2.3 Inorganic Nutrient Composition

There was no CO$_2$ enrichment effect on nutrient concentrations in the leaves (expressed on an area basis) for all the nutrients tested except at the last harvest for magnesium and nitrogen, and at the first harvest for calcium (Table 5.6). However, there were significant NO$_3^-$ effects on the leaf concentrations of nitrogen, potassium, magnesium, and calcium at most harvests. High NO$_3^-$ supply increased the potassium concentration (Fig. 5.14) in the leaves of CO$_2$ enriched plants by 100% at the first harvest and to more than three fold at the last harvest. The increase was lower for plants grown under ambient CO$_2$ concentration, and it ranged from 70% at the early harvest to 130% at last harvest. Relatively lower NO$_3^-$ effects on the leaf concentrations were found for nitrogen (Fig. 5.15), magnesium (Fig. 5.16) and calcium (Fig. 5.17). In addition, the increases were characteristic of the CO$_2$ enriched plants. The increases associated with higher NO$_3^-$ supply ranged from 10 to 60% for nitrogen and calcium, and 10 to 40% for magnesium. The concentrations of phosphorus and sodium (Figs. 5.18, 5.19) were not affected by either CO$_2$ or NO$_3^-$ treatment at any harvest.

5.3.2.4 Protein and Amino Acid Concentrations

Results of protein concentrations (Fig. 5.20) were lower than expected when compared with the result of total nitrogen concentrations (Fig. 5.15). There was no significant difference in protein concentration per unit leaf area among treatments at the first harvest (after 17 days of treatment) (Table 5.5). The protein concentration for all treatments also decreased with time after the first harvest, but this decrease was partly reversed for plants treated with high NO$_3^-$ due to the protein increase between second and third harvests. Plants treated with high CO$_2$ and NO$_3^-$ always had the
Table 5.6 Summary of ANOVA results: Interactive effect of CO$_2$ enrichment and NO$_3$ supply on nutrients concentrations of primary leaves.

(a) Nitrogen concentration (g/m$^2$)

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<th>Harvest1</th>
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<td>NO$_3$ Effect</td>
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(b) Potassium concentration (g/m$^2$)

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(c) Magnesium concentration (g/m$^2$)

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(d) Calcium concentration (g/m$^2$)

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(e) Phosphorus concentration (g/m$^2$)

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(f) Sodium concentration (g/m$^2$)

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--, not significant
•, • • significant at 5%, 1% levels, respectively.
Figure 5.14 Effect of CO₂ enrichment and NO₃⁻ supply on potassium concentration of primary leaves.
Potassium Concentration (g/m²)

Treatment Duration, days

- Low CO₂, Low NO₃
- Low CO₂, High NO₃
- High CO₂, Low NO₃
- High CO₂, High NO₃
Figure 5.15 Effect of CO$_2$ enrichment and NO$_3^-$ supply on nitrogen concentration of primary leaves.
Total Nitrogen Concentration (g/m²)

Treatment Duration, days

- Low CO₂, Low NO₃
- Low CO₂, High NO₃
- High CO₂, Low NO₃
- High CO₂, High NO₃
Figure 5.16 Effect of CO₂ enrichment and NO₃⁻ supply on magnesium concentration of primary leaves.
Figure 5.17 Effect of CO₂ enrichment and NO₃⁻ supply on calcium concentration of primary leaves.
Figure 5.18 Effect of CO₂ enrichment and NO₃⁻ supply on phosphorus concentration of primary leaves.
Phosphorus concentration (g/m²)

Treatment Duration, days

- Low CO₂, Low NO₃
- High CO₂, Low NO₃
- Low CO₂, High NO₃
- High CO₂, High NO₃
Figure 5.19 Effect of CO$_2$ enrichment and NO$_3^-$ supply on sodium concentration of primary leaves.
Sodium concentration (g/m²)

Treatment Duration, days

- Low CO₂, Low NO₃
- Low CO₂, High NO₃
- High CO₂, Low NO₃
- High CO₂, High NO₃
Figure 5.20 Effect of CO$_2$ enrichment and NO$_3^-$ supply on protein concentration of primary leaves.
Protein concentration (g/m²)

Treatment Duration, days

- Low CO₂, Low NO₃
- Low CO₂, High NO₃
- High CO₂, Low NO₃
- High CO₂, High NO₃
highest protein concentrations while low CO₂ and NO₃⁻ treated plants were consistently the lowest. CO₂ enrichment and high NO₃⁻ supply significantly increased the protein concentration after the first harvest. The increase ranged from 40 to 130% for CO₂ enriched plants, and for high NO₃⁻ treated plants the increase ranged from 70 to 200%. There was no interaction between the effect of CO₂ and NO₃⁻ treatments. There were no significant differences in the concentration of amino acids from different treatments at the early harvests (Table 5.5, Fig. 5.21). However, there was a significant CO₂ and NO₃⁻ interaction at the last two harvests. CO₂ enrichment significantly increased the amino acid concentration after the second harvest. This increase by CO₂ enrichment was sustained to the end of the experiment in the high NO₃⁻ treated plants, but for plants treated with low NO₃⁻ concentration the level of amino acids decreased to a level similar to the CO₂ control plants.

5.3.2.5 Leaf Injury

High NO₃⁻ supply significantly delayed the leaf injury under CO₂ enrichment (Fig. 5.22), and this delay of leaf injury was also evident when total chlorophyll concentrations were compared (Table 5.5, Fig. 5.23). The chlorophyll concentration decreased during the course of the experiment for plants of all treatments. The greatest decrease (60%) was found in the high CO₂ and low NO₃⁻ treated plants, while the plants treated with high CO₂ and high NO₃⁻ only had about a 20% decrease over the course of experiment. In addition, the decrease in the high CO₂ and high NO₃⁻ treated plants was delayed by about four days compared to plants from all other treatments. Higher NO₃⁻ supply increased the chlorophyll concentration for CO₂ enriched plants, but no significant difference was found with CO₂ control plants. In contrast, high CO₂ decreased the chlorophyll concentration in low NO₃⁻ treated plants, but not in high NO₃⁻ treated plants.
Figure 5.21 Effect of CO₂ enrichment and NO₃⁻ supply on amino acid concentration of primary leaves.
Amino acid concentration (g/m²)

Treatment Duration, days

- Low CO₂, Low NO₃
- Low CO₂, High NO₃
- High CO₂, Low NO₃
- High CO₂, High NO₃
Figure 5.22  Photograph of CO$_2$ enriched primary leaves treated with high (left) and low (right) NO$_3^-$.
Figure 5.23 Effect of CO$_2$ enrichment and NO$_3^-$ supply on chlorophyll concentration of primary leaves.
Total Chlorophyll Concentration, g/m²

Treatment Duration, days

- Low CO₂, Low NO₃
- High CO₂, Low NO₃
- Low CO₂, High NO₃
- High CO₂, High NO₃
5.4 DISCUSSION

5.4.1 Effect of CO₂ Enrichment on the Composition and Partitioning of Inorganic Elements

5.4.1.1 Element Levels

The increased carbon input by CO₂ enrichment should tend to increase the amount of carbon relative to other essential elements in plant tissue. This is clearly shown in the present study where nutrient concentrations per unit dry weight were lower in leaves of CO₂ enriched plants than the control plants. Porter and Grodzinski (1984) also showed that leaves from high CO₂ grown bean plants contained approximately 75 and 65% of the control levels of N, P, K, Ca, and Mg after 7 and 14 days of CO₂ treatment, respectively. In Wong's study (1979), the reduction of total nitrogen on a dry weight basis when plants were grown under high CO₂ was observed in cotton but not in maize. This different response in total nitrogen was found to correlate with the effect of CO₂ enrichment on plant dry weight. Wong (1979) found that in 40 day old cotton plants grown in high CO₂, there was a 2-fold increase in dry weight compared with plants in ambient CO₂. In 30 day old maize plants there was only a 20% increase in dry weight in plants grown in 640 ppm CO₂ compared with plants grown in 330 ppm. These results indicate that the reduction in nutrient level per unit dry weight in the tissue of high CO₂ plants was mainly due to the dry weight increase by CO₂ enrichment. In the bean plants used for the present study, leaf area did not respond to CO₂ enrichment. Thus, the compounding effects of CO₂ enrichment can be avoided by using leaf area as the basis for expressing the levels of the metabolites. Contrary to the findings on a dry weight basis mentioned above, the opposite trend was observed when nutrient levels were expressed per unit leaf area. For the majority of the elements tested, CO₂ enrichment had no significant effect on the level of nutrients in various
leaves. The significant CO₂ and harvest interactive effect, however, indicates the effect on nutrient levels by CO₂ enrichment can vary among different plant stages.

5.4.1.2 Partitioning of Nutrients and Leaf Injury

Restricted utilization of the extra carbon entering the leaves limited the growth effects of CO₂ enrichment in beans. Root growth was promoted less under CO₂ enrichment than was leaf growth (Chapter 3). With higher carbon input under high CO₂, higher nutrient demand would be expected, especially at the later stages of plant development. When this demand could not be met by more inorganic nutrient uptake, the redistribution of some inorganic nutrients from older to younger parts of the plant was promoted. Similar finding was reported by Koch et al. (1988). They found that in wild radish, older leaves lost NO₃⁻ and NH₂-N, and roots and young leaves gained NH₂-N in response to N stress.

Although there were greater losses of nitrogen from older parts of CO₂ enriched plants than the control plants, no significant differences in protein and amino acid concentrations of the primary leaves were observed between two CO₂ treatments (Figs. 4.11, 4.13). The discrepancy could be due to the high variability in measurements of leaf protein and amino acid concentrations.

In addition, the timing of this redistribution of inorganic nutrients corresponded with the development of leaf injury. The symptoms of leaf injury were also quite similar to the symptoms of nitrogen and potassium deficiencies, which were the elements that had the highest losses from older to younger plant parts. The temporal pattern of leaf injury development (starting from primary leaves and progressing to the trifoliates) also corresponded with the pattern of nutrient loss. All these correlations suggest that leaf injury and nutrient balance are related.
5.4.2 Interactive Effects of CO₂ Enrichment and Nitrate Supply

5.4.2.1 Plant Growth and Development

In keeping with the principle of limiting factors, if any nutrient is in short supply, its lack will limit growth and the supply of additional CO₂ will not produce greater growth. In this study, total plant dry weight was increased by both CO₂ enrichment and by higher NO₃⁻ supply indicating that the dry weight production of bean plants was limited by both ambient CO₂ and low NO₃⁻ supply. However, the results have also shown that the proportionate dry weight increases caused by CO₂ enrichment were similar when the supply of the NO₃⁻ was reduced by half (from 6 mmole/l to 3 mmole/l). The present results are in agreement with Wong (1979) who found no effect of the level of N supply on the proportional increase in dry-matter production of CO₂ enriched cotton. Hocking and Meyer (1985) found there was no consistent effect of N supply on the magnitude of the growth response to CO₂ enrichment; in fact, the most N-stressed plants had the biggest percentage increase in dry matter in response to CO₂ enrichment. Similar results were also reported in a study of tomato (Peet and Willits, 1984). This contrasts with other studies in which CO₂ enrichment had the least effect on dry-matter production when plants were stressed for nutrients (Patterson and Flint, 1982; Goudriaan and de Ruiter, 1983; Sionit, 1983). This diversity of results suggests that plant responses to increased CO₂ when other nutrients are limiting depends on the nature of those limitations and whether additional CO₂ assimilation will increase the availability or the efficiency of those limiting nutrients.

In beans, the nitrogen concentration per unit leaf area was not increased by CO₂ enrichment when N was limiting. The increased N-use efficiency under CO₂ enrichment has been thought to be related to the more efficient carbon fixation by the enzyme RuBPCase. Wong (1979) found decreased RuBPCase content with high CO₂, and this reaction may represent an acclimation to a lowered demand for RuBPCase at high CO₂. This notion was supported by his finding that the relative increase in dry
weight per shoot due to CO₂ enrichment was almost invariant with nitrogen treatment, while the enhancement of the production capacity (the product of assimilation rate of a leaf and total leaf area per plant) was smaller with lower level of nitrogen.

Although the same proportional increase in total plant dry weight by CO₂ enrichment was observed under both NO₃⁻ conditions, detailed studies of dry matter partitioning among different plant parts showed that the effects of CO₂ enrichment were quite different under each NO₃⁻ treatment. For low NO₃⁻ treated plants, increasing CO₂ concentration increased the dry weight only in the leaves and there were no CO₂ effects on stem and root dry weights until the last harvest. In contrast, for high NO₃⁻ treated plants, CO₂ enrichment not only increased the leaf dry weight but also increased the stem and root. These differences explain why the increase in shoot root dry weight ratio by CO₂ enrichment was only evident in the low NO₃⁻ treated plants. With low NO₃⁻ supply, the CO₂ effect was mainly confined to the leaves, and the weight increase was basically contributed by the increase in the starch accumulation in those leaves. By increasing the level of NO₃⁻ supply, the increased carbohydrates generated under CO₂ enrichment were able to be transported to other plant parts (stem and root). In other words, the partitioning of carbohydrates under CO₂ enrichment was altered according to the NO₃⁻ supply. This different pattern of dry weight partitioning had a long term implication, since the pod dry weight was increased by CO₂ enrichment only under high NO₃⁻ supply.

NO₃⁻ supply not only had an effect on dry matter partitioning, it also affected plant form by producing larger leaves, which in turn increased the potential for carbon fixation. It is not known, however, if the larger leaves had the same capability for CO₂ fixation per unit leaf area. Koch et al. (1988) reported that in wild radish, NO₃⁻ deprivation resulted in a rapid cessation of leaf area expansion while total leaf weight continued to increase. This increase in specific leaf weight under N stress condition was also found in this study.
5.4.2.2 Starch Accumulation and Levels of Inorganic Nutrients

Starch accumulation correlated with the growth responses under different CO₂ and NO₃⁻ conditions. Low NO₃⁻ treated plants with limited leaf area growth response, consistently accumulated higher starch. Other data relating N availability to plant growth and carbohydrate content also indicate that under N-limiting conditions, stored carbohydrates accumulate (Wilson, 1975., Williams et al., 1981). It is doubtful, therefore, that stimulating even more carbohydrates to accumulate by increasing the CO₂ assimilation rate will increase crop production in the long term unless other limiting nutrients become available.

In beans, there were no CO₂ effects on the concentrations of inorganic nutrients regardless of NO₃⁻ supply. Few studies have been done to investigate the effect of NO₃⁻ supply on nitrogen uptake under different CO₂ conditions. Results from those studies have varied with the plant species studied and the levels of N concentration supplied. Hocking and Meyer (1985) observed a consistent growth response of cocklebur to high CO₂ over a range of NO₃⁻ concentrations with little change in NO₃⁻ uptake relative to the control. Similarly, in an experiment with vegetative cotton plants (Wong, 1979), carbon dioxide enrichment increased dry matter production at moderate NO₃⁻ treatment levels (0.6, 4.0, and 12.0 mM), even though no increases occurred in NO₃⁻ uptake. At the highest nitrate level (24.0 mM), uptake increased 28% while dry matter increased 118% in response to CO₂ enrichment. Those results suggest that nutrient uptake under CO₂ enrichment can not be increased simply by increasing the external N supply. Contrary to the above findings, Cure et al., (1988a) found in soybean that at all NO₃⁻ levels except the lowest (0.05, 1.0, 2.5, 5.0, or 10.0 mM), exposure to high CO₂ resulted in increased NO₃⁻ uptake and N utilization efficiency. Increased NO₃⁻ uptake, however, was associated with a larger root system, as uptake per unit of root mass was lower than the control. Hence, the increased nitrogen concentration was
mainly due to a stimulation of root growth. As reported in the results, although there was no effect of NO$_3^-$ supply on root growth at ambient CO$_2$, the stimulation of root growth by CO$_2$ enrichment was enhanced by high NO$_3^-$ supply. Thus, providing more NO$_3^-$ could benefit nutrient uptake both by a growth effect and by increased the availability of the nutrient itself.

Higher NO$_3^-$ supply not only increased nitrogen uptake, it also increased the concentrations of other nutrients (K, Mg, Ca) examined in this study. Among those nutrients affected, potassium had the most significant response. It is well documented that potassium is a key ion involved in the process of sugar transport. Thus, this increase in potassium concentration may help to explain the changes in carbon partitioning to the stem and root between high and low NO$_3^-$ treated plants under CO$_2$ enrichment.

5.4.2.3 Leaf Injury

Although a resemblance between symptoms of nutrient deficiency and CO$_2$-induced leaf injury was noted in an earlier study (Goudriaan and de Ruiter, 1983), no studies have previously correlated the interactive effects of CO$_2$ enrichment and nutrient supply on leaf injury. As noted earlier, the corresponding timing of the redistribution of nutrients and leaf injury development indicated that the CO$_2$ induced leaf injury may be nutrient related. The increased leaf nutrient concentrations (especially N, and K) and the delay of the injury observed under higher NO$_3^-$ supply further confirmed the link between leaf injury and nutrient supply under CO$_2$ enrichment. Thus, the results are consistent with the idea that the CO$_2$-induced leaf injury can be caused by a higher demand for nutrients (N, K) under CO$_2$ enrichment. Further studies are needed however, to define directly the cause and effect relationships between the redistribution of nutrients from older to younger plant parts and leaf injury under CO$_2$ enrichment.
CHAPTER 6 - GENERAL DISCUSSION

Earlier research showed that photosynthesis rate in bean is increased by CO₂ enrichment, although photosynthetic capability, when assessed at the same CO₂ level, is depressed in leaves acclimated to high CO₂ (Ehret and Jolliffe, 1985b). Despite the continuously added input of carbon, there was little compounding effect on growth of increased photosynthesis under CO₂ enrichment in beans (Ehret and Jolliffe, 1985b; Jolliffe and Ehret, 1985) and many other species (Kramer, 1981). The present results indicate that the effect of CO₂ enrichment on growth is influenced by more than just photosynthetic responses alone. In this study, non-photosynthetic responses which may contribute to this lack of compounding growth effect were investigated.

In beans, although total dry weight was increased by CO₂ enrichment, the majority of the increase was confined to the leaves. There were no increases in leaf area expansion or leaf formation. In addition, the growth enhancement of the stem and roots was relatively small and did not occur until the later stages of the experiment.

The lack of increase in leaf area under CO₂ enrichment indicates that the added carbon input is not exploited as a resource to increase the capacity of foliage to assimilate carbon via added leaf area. This restriction is unusual among species studied so far; it has only been found in dwarf bean, corn (Ford and Thorne, 1967), Chinese cabbage (Kriedemann and Wong, 1984) and *Sorghum* (Mauney et al., 1978), it may be connected to the dwarf growth habit, or other developmental control.

Growth depends on the export of photosynthetic products from source leaves to growing regions of plants. There are several processes that can control the allocation of photosynthetic products within the leaf, thus influencing export from the leaf. Partitioning of carbon between starch and sucrose appears to be a particularly effective means of controlling carbon export from leaves. Results in the present studies show
that CO₂ enrichment significantly increased both starch and sucrose concentrations in the leaves. The additional carbon, however, was largely directed into starch formation since the increases in starch concentrations were about 10 to 30 times higher than the increases in sucrose concentrations. This starch accumulation accounted for most of the SLW increase, and it reflects the extent to which production of assimilate exceeds utilization; as a storage product, starch is not directly participating in the construction of new cells to support additional growth.

Prolonged CO₂ enrichment seemed to cause a dysfunction in starch metabolism in the bean leaves. In the transfer experiment, levels of starch concentration were maintained for several days even after plants were transferred from the CO₂ enriched atmosphere back to the ambient CO₂ condition. In contrast, increases in starch concentrations were observed as soon as plants were subjected to CO₂ enrichment. The ¹⁴C compartmental analysis studies showed that the remobilization of carbon from the storage pool could not be detected in CO₂ enriched plants. These results indicate that the degradation of starch was impaired under CO₂ enrichment. Further studies on the effect of CO₂ enrichment on biochemical processes of starch degradation should be conducted in order to pinpoint the cause of this limitation. In contrast, earlier work done by Hoddinott and Jolliffe (1988) found that increasing in CO₂ concentrations had no effect on the rate of carbon remobilization in the light. Their measurements were based on only one specific plant age, while in the present study the measurements were made continuously over a period of two weeks. In this study the disappearance of carbon remobilization was not observed until 17 days after CO₂ treatment, therefore, the discrepancy found between studies could be due to the ages of the plants that were used.
The lag between the dry weight increases in the leaves and the other plant parts demonstrates that there was a limited net carbon efflux from the leaves. Although there were small increases in soluble sugars in leaves of CO₂ enriched plants at the beginning of the experiment, these sugars were not effectively mobilized to support growth of other plant parts. As reported for soybean (Huber et al., 1984), the increased concentrations of soluble sugars found at the early stages of this experiment may not be available for export. At later stages of growth, the decreased starch/sucrose ratio in the CO₂ enriched leaves indicates that relatively more carbon was partitioned to the soluble sugars. This coincided with increases in stem and root dry weights. These results suggest that the limitation in carbon export from CO₂ enriched leaves could be partly due to starch formation which traps carbon before it can be exported. The partitioning and utilization of carbon can also be affected by the availability of other nutrients. For example, carbon export from the leaves can be affected by the level of potassium due to the physiological roles of potassium in phloem loading and carbohydrate metabolism.

The effect of CO₂ enrichment on partitioning of carbon among plant parts is especially important when economic yield is considered. So far, most studies have found that there was no effect of CO₂ enrichment on harvest index (the ratio of economic yield to the total yield of the plant). My observation of early pod growth conform to this pattern. The increases in economic yield occur simply because of greater overall size; harvest index is not strongly affected. For example, with wheat, enhanced yield occurred through greater tillering (branching), each extra tiller eventually bearing an ear, such that harvest index is scarcely changed (Gifford, 1977). Further understanding of the control of carbon partitioning within the leaves and among plant parts is needed to establish whether carbon transport to economic components of the plant can be facilitated.
The alteration of nutrient relations under CO₂ enrichment has been clearly shown by this study. At early stages of growth, inorganic nutrients required for the new growth were supplied mainly by nutrient uptake from the roots. At later stages, a large portion of the supply required for new growth under CO₂ enrichment was from nutrients redistributed from older plant parts. For control plants, however, only a small percentage was redistributed from older plant parts, while the majority of the nutrients were still provided by root uptake. Among the nutrients studied, the relocations of nitrogen and potassium were shown to be most strongly affected by CO₂ enrichment. Limited nutrient uptake also could be connected to the lack of stimulation of root growth under CO₂ enrichment.

The involvement of nutrient availability in growth responses to CO₂ enrichment was further illustrated by the interactive effects of CO₂ enrichment and NO₃⁻ supply. By increasing NO₃⁻ supply, there were increases in both plant dry weight and leaf area. The increase in total leaf area by high NO₃⁻ supply would increase the potential for further growth enhancement. With high NO₃⁻ supply, the CO₂ enrichment effects on the growth of the stem and roots were much higher and earlier. This indicates that for high NO₃⁻ treated plants, the extra carbon assimilated under CO₂ enrichment was utilized more effectively for growth of the non-photosynthetic plant parts. This is correlated with the finding that higher NO₃⁻ supply also decreased leaf starch accumulation. The underlying mechanisms which promoted the carbon translocation are not clear, but increased inorganic nutrient uptake could be involved. In this study, it was found that higher NO₃⁻ supply not only increased the uptake of nitrogen, but also increased the uptake of other elements such as potassium, magnesium, and calcium. Therefore, higher NO₃⁻ supply could promote the carbon transport by simply increasing the availability of potassium to meet the higher demand for that inorganic nutrient under CO₂ enrichment. Relatively few studies have been done to investigate the interactive effects of CO₂ enrichment and nutrient supply on plant growth. More
studies are needed to define the optimum levels of nutrient supply under CO₂ enrichment in greenhouse crop production. Similarly, caution should be taken when predicting plant responses to future atmospheric CO₂ concentrations due to the low level of nutrients that are often available under natural conditions.

Leaf injury induced by high CO₂ concentrations has been found in many plant species (Van Berkel, 1984). It is a concern both in relation to plant quality in greenhouse production systems and to vegetation damage if harmful levels of CO₂ accumulate in the atmosphere. The leaf injury has been associated previously with photosynthetic decline (Ehret and Jolliffe, 1985b). The cause of leaf injury under CO₂ enrichment is not clear, however, it has been correlated with starch accumulation (Ehret and Jolliffe, 1985a). In addition, environmental conditions that promote the starch accumulation such as low temperatures and high light intensities also promote leaf injury under CO₂ enrichment (Ehret and Jolliffe, 1985a). In the present study, the development of injury was correlated with timing of the chlorophyll loss, increased leaf sucrose concentration, changed starch turnover and changed partitioning of nutrient elements (particularly N and K). The possibility that the injury could be due to nutrient imbalances is supported by the ability of added NO₃⁻ to delay it. More direct evidence is needed to prove that the nutritional correlations are causally related to the injury, and not consequences of injury.
CONCLUSIONS

1. The effects of CO₂ enrichment on plant growth depend upon more than just the photosynthetic CO₂ response or photosynthetic acclimation to CO₂. Except at high NO₃⁻ supply, leaf expansion was little affected by CO₂ enrichment, and this may have limited the ability of the dwarf bean plants to compound their growth in response to high CO₂.

2. Restricted distribution of extra dry matter formed under CO₂ enrichment among plant parts is a major factor in determining the ultimate growth responses. In dwarf bean plants, much of the extra carbon input under CO₂ enrichment was confined to the leaves. During later stages of growth, increases in sucrose concentrations under CO₂ enrichment correlated with the growth enhancement of stem and roots.

3. A limitation in starch degradation in CO₂ enriched plants was indicated by two observations: (i) there was a delay in the decrease in starch concentration after plants were switched back to ambient air from CO₂ enrichment, (ii) The disappearance of the remobilization of newly fixed carbon from the carbon storage pool (presumably starch) after extended CO₂ enrichment. Limited starch degradation may contribute to the restriction of carbon relocation and utilization.

4. CO₂ enrichment did not significantly affect total leaf protein and amino acid concentrations, but the level of individual proteins (such as carbonic anhydrase) may be affected.
5. CO₂ enrichment did not significantly increase nutrient uptake, which may partly be due to a lack of enhancement of root growth. Higher carbon input may create higher demand for inorganic nutrients to balance the growth. Greater nutrient losses (N and K) from older shoot parts to younger parts suggest that nutrient uptake for CO₂ enriched bean plants is insufficient to meet the demand.

6. CO₂ enrichment effects on growth are also influenced by the availability of inorganic nutrients. High NO₃⁻ supply enhanced the overall growth of CO₂ enriched plants. This was achieved by increases in leaf area and an improved ability to relocate carbon for stem and root growth. The enhanced growth of the stem and roots correlated with decreased leaf starch concentrations under CO₂ enrichment.

7. High NO₃⁻ supply increased the nutrient uptake under CO₂ enrichment, especially K uptake. This may have contributed to the improved ability for carbon relocation.

8. Prolonged exposure to high CO₂ concentrations induces leaf injury in dwarf bean plants. The injury may partly contribute to the limited growth responses under CO₂ enrichment. The injury, marked by the decrease of leaf chlorophyll concentration, can be delayed by high NO₃⁻ supply.
LITERATURE CITED


Technicon Autoanalyzer II Methodology. 1976. Individual/simultaneous determination of nitrogen and/or phosphorus in BD acid digests. Industrial Method NO. 334-74A.


### Mean Values of Growth Data from Trials 1 and 2 of Growth Experiment (Chapter 3)

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<th>Variable</th>
<th>Duration of CO₂ Treatment (days)</th>
<th>Trial 1</th>
<th>Trial 2</th>
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<td>Leaf dry weight</td>
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<td>0.19±.01</td>
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<tr>
<td>Enriched</td>
<td>0.26±.03</td>
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<td>Stem dry weight</td>
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<td>Pod dry weight</td>
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<td>Total Leaf area</td>
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<td>Control</td>
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<td>232±19</td>
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<tr>
<td>Enriched</td>
<td>59±8</td>
<td>208±19</td>
<td>554±34</td>
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* 38 days for trial 2.
** Dry weight unit (g plant⁻¹), leaf area unit (m²x10⁻⁴ plant⁻¹).
*** Data is mean ± SE.

Flowering started around day 21 and pod formation started around day 28.
APPENDIX 2

The Derivation of a Modified Mathematical Model for Compartmental Analysis.

A modified model, as showed in figure 4.1b, was developed as follows:

\[ \frac{dQ_1}{dt} = -K_{21}Q_1 - K_{01}Q_1 \]
\[ \frac{dQ_2}{dt} = K_{21}Q_1 \]
\[ Q_1 = Q_1(0)e^{-(K_{21}+K_{01})t} \]
\[ Q_2 = Q_1(0)\left(\frac{K_{21}}{K_{21}+K_{01}}\right)(1-e^{-(K_{21}+K_{01})t}) \]

Set

\[ P_1 = Q_1(0) = A \]
\[ P_2 = \frac{K_{21}}{K_{21}+K_{01}} \]
\[ P_3 = K_{21}+K_{01} \]

Hence:

\[ Q = Q_1 + Q_2 = P_1e^{-P_3t} + P_1P_2(1-e^{-P_3t}) \]

and transfer coefficients can be calculated as follows:

\[ K_{21} = P_2P_3 \]
\[ K_{01} = (1 - P_2)P_3 \]
APPENDIX 3

The Effect of CO₂ Enrichment on the Levels of Inorganic Nutrients.
APPENDIX 3.1 Effect of CO$_2$ Enrichment on Nutrient Concentrations

(\%, g g$^{-1}$ x 10$^{-2}$) of Different Plant Parts.

Harvest I: 14 days of CO$_2$ treatment
Harvest II: 21 days of CO$_2$ treatment
Harvest III: 28 days of CO$_2$ treatment

Plant parts:
1. Primary leaves
2. 1st and 2nd trifoliate leaves
3. 3rd and 4th trifoliate leaves
4. Remainder of the leaves
5. Stem
6. Pods
PLANT PARTS
LEGEND
- 340ppm CO₂
- 1500ppm CO₂

HARVEST I

HARVEST II

HARVEST III

Ca CONCENTRATION, %

PLANT PARTS
APPENDIX 3.2 Effect of CO₂ Enrichment on Nutrient Concentrations

(mg per unit of leaf area) of Various Leaves.

Harvest 1: 14 days of CO₂ treatment
Harvest 2: 21 days of CO₂ treatment
Harvest 3: 28 days of CO₂ treatment

Plant parts:
1. Primary leaves
2. 1st and 2nd trifoliate leaves
3. 3rd and 4th trifoliate leaves
4. Remainder of the leaves
Ca concentration, mg/m²

Harvest 1

Harvest 2

Harvest 3

Plant parts

Control (340 ppm)  Enriched (1500 ppm)
Fe concentration, mg/m²

Harvest 1

Harvest 2

Harvest 3

Plant parts

Control (340 ppm)    Enriched (1500 ppm)
Cu concentration, mg/m²

Harvest 1

Harvest 2

Harvest 3

Plant parts

Control (340 ppm) Enriched (1500 ppm)
Mn concentration, mg/m²

Harvest 1

Harvest 2

Harvest 3

Plant parts

- Control (340 ppm)
- Enriched (1500 ppm)
Zn concentration, mg/m

Harvest 1

Harvest 2

Harvest 3

Plant parts

Control (340 ppm)    Enriched (1500 ppm)
APPENDIX 3.3 Effect of CO₂ Enrichment on Nutrient Content of Different Plant Parts.

Harvest 1: 14 days of CO₂ treatment  
Harvest 2: 21 days of CO₂ treatment  
Harvest 3: 28 days of CO₂ treatment

Plant parts:  
a. Primary leaves  
b. 1st and 2nd trifoliate leaves  
c. 3rd and 4th trifoliate leaves  
d. Remainder of the leaves  
e. Stem  
f. Pods

Control: first bar of each pair  
Enriched: second bar of each pair
APPENDIX 3.4 Effect of CO₂ enrichment on Nutrient Gain or Loss of Different Plant Parts Over Two Periods.

(a) Period 1: between 14 and 21 days  
(b) Period 2: between 21 and 28 days

Plant parts:  
1. Primary leaves  
2. 1st and 2nd trifoliate leaves  
3. 3rd and 4th trifoliate leaves  
4. Remainder of the leaves  
5. Stem  
6. Pods
PLANT PARTS

(a) 340 ppm CO₂  
(b) 1500 ppm CO₂

CHANGE IN K CONTENT (g x 10⁻²)

1 2 3 4 5 6

-0.5 -0.5 -0.5 -0.5

0.9 1.2 3.2 3.0 2.2 4.8

0.8 0.6 3.6 3.6 2.3 4.1

0.8 0.6 3.6 3.6 2.3 4.1
(a) 340ppm CO₂
(b) 1500ppm CO₂

CHANGE IN Ca CONTENT (g x 10⁻²)

PLANT PARTS

1.7
2.2
1.8
1.6
1.8
1.3
2.3
2.7
1.3
1.3
0.3
0.2
0.3
0.2
(a) 340 ppm CO$_2$
(b) 1500 ppm CO$_2$

CHANGES IN Fe CONTENT (ug)

PLANT PARTS