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Title: EFFECTS OF CO_2 ENRICHMENT DURING FLOWERING AND PODFILL ON NET PHOTOSYNTHESIS, DRY MATTER ACCUMULATION AND YIELD OF BEANS, PHASEOLUS VULGARIS L.

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Patrick J. Breen

Beans were grown in open top chambers outside, either in the field (1980, 1981) or in containers (1982) and were exposed during 12 hr of daylight to air containing 1250 µl CO_2/liter (enriched) for all (continuous, 1980, 1982), part (flowering or podfill, 1980) or none (controls) of the period between first flower and seed maturity. When plants were not exposed to enriched air, they received ambient air (340 µl CO_2/liter). Net photosynthesis of the penultimate mainstem leaf of field (1981) and container grown 'Oregon 1604' was measured by a transient CO_2 depletion method. Enrichment increased net photosynthesis 3-6 fold (field) or 2-5 fold (container) over controls from first flower to seed maturity. Enrichment enhancement of net photosynthesis was maintained despite large accumulations of leaf starch (10.7 g m^{-2}, maximum) and 50% decreases in leaf conductance. In 1980, enrichment during flowering increased shoot dry weight of 'Oregon 1604' and 'Royal Red' by 38% and 30%, respectively, at the end of flowering. By pod maturity, the difference between continuously enriched and controls was no

longer significant. Enrichment increased number of pods per plant at the end of flowering in both cultivars, but these were not maintained at pod maturity. In 1981, continuous enrichment of 'Oregon 1604' increased shoot dry weight by 67%, 42% and 22% at end of flowering, pod and seed maturity harvests, respectively. Greater vegetative (leaf and stem) dry weight accounted for 47%, 59% and 32% of the shoot dry weight increase at the 3 harvests, respectively. Dry weight increases of enriched leaves were associated with approximate 25% greater specific leaf weights. Starch accounted for about 50% of the higher specific leaf weight of enriched plants at pod and seed maturity, respectively. Continuously enriched plants had a higher number of pods than controls at each of the 3 harvests. Higher number of pods did not translate into increased seed dry weight per plant due to fewer seeds per pod. Weight per seed was not affected. The failure of continuous enrichment to increase the difference between shoot dry weight of enriched and control plants after the end of flowering suggests that the enhancement of whole plant net photosynthesis of continuous enrichement during flowering was not maintained during the entire post flowering period. The greater number of pods due to enrichment during flowering strongly supports the hypothesis that available photosynthate is a limiting factor to initial pod set.

EFFECTS OF CO_2 ENRICHMENT DURING FLOWERING AND PODFILL ON NET PHOTOSYNTHESIS, DRY MATTER ACCUMULATION AND YIELD OF BEANS, *PHASEOLUS VULGARIS* L.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

EFFECTS OF CO_2 ENRICHMENT DURING FLOWERING AND PODFILL ON NET PHOTOSYNTHESIS, DRY MATTER ACCUMULATION AND YIELD OF BEANS, PHASEOLUS VULGARIS L.

Chapter 1

INTRODUCTION

Dry beans are a widely consumed food source in developed and developing countries. They serve as a significant source of human dietary protein, especially among less affluent peoples. Snap beans are consumed mainly in developed countries and are a supplementary source of minerals and vitamins in the diet. In 1981, snap bean production for fresh and processed markets in the United States exceeded 8.1×10^5 metric tons with a value of over 200 million dollars (20, 101). Nationally, Oregon ranks second in production of snap beans for processing (101). The farm gate value of the 1982 Oregon crop was 23.8 million dollars.

Increasing yield is a common goal of many in agriculture. Beans and other legumes offer a fascinating challenge to those concerned with yield improvement because their initial yield potential is often radically reduced (up to 80%) by abscission of flowers and young pods (1, 92). Since number of pods per plant is the principal yield component in both snap and dry beans, factors which influence abscission rates play a key role in regulating yield. Many environmental factors have been observed to affect abscission rates in legumes, including drought, extreme

temperatures, irradiance levels and photoperiod (72). However, the mechanism(s) triggering pod abscission in legumes is not well known (98). One hypothesis is that available photosynthate during flowering and early podfill is insufficient, thus resulting in competition among reproductive organs, leading to pod abscission (1). The fact that many of the previously mentioned environmental factors influence both abscission and photosynthesis suggests a probable relationship. A role of available photosynthate in limiting pod set in legumes is further supported by experimental manipulation of source/sink ratios. Procedures which increase the source/sink ratios such as pod removal (35, 103) or CO_2 enrichment (14, 15, 19, 43, 44) resulted in greater number of pods. Conversely, decreasing the source/sink ratios by shading (71) or leaf removal (99) caused a decline in number of pods.

The decline in number of pods in response to the hypothetical source limitation during flowering and early podfill can result in the beans being sink limited during seed development (99). Increasing available photosynthate beginning at flowering should lead to greater yield both in terms of number of pods and seed dry weight per plant.

The object of this research was to increase available photosynthate during flowering and/or podfill in order to examine its role as a limiting factor in dry matter accumulation, pod set and seed yield in beans. Photosynthesis and other physiological parameters of a single leaf were monitored during the reproductive period in order to better assess the response to enrichment.

Chapter 2

REVIEW OF THE LITERATURE

I. Introduction

The requirement for carbon dioxide (CO_2) by plants was first reported by Senebier in 1782 (86). The early quantitative studies of the relationship of CO_2 concentrations to photosynthetic rates date back to the late 19th and early 20th centuries. Initial experimentation using elevated CO_2 to enhance plant growth and yield was also conducted in Europe about the same time. The first work in the United States using high CO_2 levels began in 1909 by Cummings and Jones (21). The early work in both of these areas was reviewed by Rabinowitch (86). The use of supplemental CO_2 to enhance plant growth became a common practice in many temperate climate countries (42). Wittwer and Robb (106) reviewed much of the literature through 1962. This review will emphasize the literature which followed.

CO_2 enrichment is a general term and historically has referred to concentrations from 350 to 5000 µl CO_2/liter. Due to increasing atmospheric CO_2 levels (56) and use of controls as high as 400 µl CO_2/liter, CO_2 enrichment in this review will be reserved for concentrations greater than 400 µl CO_2/liter.

The object of this review is to establish a basic foundation of known enrichment effects on photosynthesis, dry matter accumulation, and yield, with emphasis on legumes, in order to assist interpretation of the enrichment effects on these processes in beans.

II. Effects of CO_2 Enrichment on Photosynthesis

A. Chloroplast Level of Organization

1. Ribulose-1,5-bisphosphate Carboxylase-Oxygenase

Photosynthesis in green plants is a process which utilizes light energy to produce carbohydrates from CO_2 and H_2O. This process, which occurs in the chloroplast, is comprised of "light" and "dark" (Calvin cycle) reactions. In the light reactions, light energy, H_2O, adenosine diphosphate (ADP) and oxidized nicotinamide adenine dinucleotide phosphate ($NADP^+$) are converted into O_2, adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide phosphate (NADPH). The ATP and NADPH produced in the light are utilized in the conversion of CO_2 to carbohydrate, which can occur in the dark. The principal and rate limiting dark reaction of photosynthesis is catalyzed by the enzyme ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) and occurs in the stroma of chloroplasts. In this reaction, one molecule of rubulose-1,5-bisphosphate (RUBP) is combined with one molecule of CO_2 to yield 2 molecules of glycerate 3-phosphate. The rate of CO_2 fixation by Rubisco is often referred to as gross photosynthesis. Net photosynthesis is usually defined empirically as the measured rate of net flux of CO_2 into a leaf. The difference between gross and net photosynthesis is the loss of CO_2 by photosynthesizing tissues through respiration. Dark respiration in photosynthesizing tissue of beans is greatly inhibited in the light and generally regarded as negligible (70). Photorespiration occurs simultaneously with photosynthesis in C_3 plants and can account for to 50% of the total CO_2

fixed through photosynthesis (110). Photorespiration (R_L) as defined by Ogren and Chollet (79) is the process comprised of the oxygenation of RUBP and the integrated metabolic pathways followed by the P-glycolate produced in this oxygenation. In this oxygenation reaction, which is also catalyzed by Rubisco, one molecule each of O_2 and RUBP are combined to form one molecule each of phosphoglycolate and glycerate 3-phosphate. Since CO_2 and O_2 compete for the same active sites, CO_2 is a competitive inhibitor of oxygenation, as is O_2 of carboxylation. The $K_i(CO_2)$ of Rubisco for oxygenation is approximately one tenth the $K_i(O_2)$ for carboxylation (53). These kinetic values permit the carboxylase reaction to dominate, even though at normal atmospheric concentrations (i.e., 340 µl CO_2/liter, 210 ml O_2/liter) CO_2 is in much lower supply in the chloroplast than O_2. Still, each O_2 molecule incorporated by Rubisco prevents the fixation of one CO_2 molecule and eventually leads to an additional loss of one half of a CO_2 molecule through photorespiration. Increasing the CO_2/O_2 ratio within the chloroplast will increase the rate of photosynthesis while decreasing photorespiration. Such manipulations have been achieved by elevating CO_2 levels (23, 54,) or reducing O_2 levels (23). Theoretically, complete inhibition of oxygenase alone should result in increased photosynthetic rates of nearly 100%. Four fold increases in rates of net photosynthesis of soybean leaves exposed briefly to high CO_2 levels (1600 µl CO_2/liter) versus rates at normal CO_2 levels (300 µl CO_2/liter) have been obtained (11). Nearly 25% of this increase can be attributed to inhibition of

photorespiration, while the remainder was probably due to higher carboxylation rates. Robinson and Walker (90) found that kinetic values of Rubisco support 2-3 fold increases in carboxylation rates under high CO_2 levels over those at normal levels. Therefore, the major physiological effects of high CO_2 levels (enrichment) on net photosynthesis are inhibition of the oxygenase reaction and simultaneous enhancement of the carboxylation reaction of Rubisco resulting in potentially large increases in net photosynthesis.

The carboxylase-oxygenation reactions discussed occur in all C_3 mesophyll cells which contain chloroplasts. In C_4 plants, CO_2 is first fixed by phosphoenol pyruvate (PEP) carboxylase in mesophyll cells to form C_4 organic acids (malate or aspartate) which are then transported to bundle sheath cells where they are decarboxylated. In the bundle sheath cell chloroplasts, the CO_2 is then fixed by Rubisco and further metabolized via the Calvin cycle as in C_3 plants. A major result of this process is to elevate the partial pressure of CO_2 to near saturating levels for Rubisco in the bundle sheath chloroplasts, thereby virtually eliminating oxygenation and glycolate production (5). Therefore, CO_2 enrichment has a much smaller impact on rates of carboxylation and oxygenation in C_4 than in C_3 plants.

CO_2 enrichment can apparently affect the levels of photosynthetic and photorespiratory enzymes within leaves. The level of Rubisco in enriched leaves was lower in grape but higher in Lea brunoniana relative to controls (62). Rates of net photosynthesis of enriched leaves followed a similar pattern when measured at 300

μl CO_2/liter, but not at CO_2 levels higher than 500 μl CO_2/liter. Hicklenton and Jolliffe (48) reported that in tomato plants grown at 1000 μl CO_2/liter, RUBP carboxylase activity was higher in younger, but lower in older leaves relative to comparable leaves of plants grown at 300 μl CO_2/liter. They also found that CO_2 enrichment reduced the activity of the photorespiratory enzyme, glycolic acid oxidase. Fair et al. (28) also reported decreased glycolic acid oxidase activity and corresponding reduction of photorespiration in barley leaves enriched with CO_2 at levels higher than 1%.

2. Starch Accumulation in the Chloroplasts

Leaves of CO_2 enriched plants often have much higher starch levels than those grown in normal atmospheric air (17, 33, 49, 62, 68, 74, 75, 82, 87, 108). Starch is synthesized and accumulated in the chloroplasts of leaves. Large accumulations of starch can result in end-product inhibition of photosynthesis (40). Confirmation of end-product inhibition requires not only the demonstration of negative correlations between starch levels and net photosynthesis, but also of a plausible mechanism (40). Proposed mechanisms for starch inhibition of photosynthesis includes binding of magnesium ions, interference with light transmission, increased diffusion pathway for CO_2 and physical disruption of the chloroplasts (40). These are mainly based on physical versus biochemical effects and are not mutually exclusive (40).

Supportive evidence for starch end-product inhibition in CO_2 enriched plants is supplied by several experiments which show negative correlations between net photosynthesis and high leaf starch

levels. Net photosynthesis of control and enriched soybean leaves was negatively correlated with starch, mesophyll resistance and specific leaf weight (49). Nafziger and Koller (75), using CO_2 enrichment to elevate starch levels in soybean leaves, found decreases in net photosynthesis in leaves whose starch levels exceeded 10.0 g/m^2 leaf area. Net photosynthesis of leaves of cotton plants grown in ambient air initially increased when they were exposed to high CO_2 levels, but then gradually declined as starch accumulated (74). In all three experiments, negative correlations were only found between starch levels and net photosynthesis measured under normal atmospheric CO_2 levels and not when photosynthesis was measured at elevated CO_2 levels. Those findings are consistent with a mechanism of increased distance for CO_2 diffusion (74, 75). Inhibition by this mechanism would be expected to be less when photosynthesis was measured at high CO_2 levels since the CO_2 concentration levels at the chloroplasts should still be higher than at normal atmospheric CO_2 levels. Further evidence of starch inhibition of photosynthesis in leaves of enriched plants was presented by Wulff and Strain (108). They found that net photosynthesis was lower in leaves of enriched Desmodium paniculatum than of controls. Chloroplasts from enriched plants showed large starch accumulation, decreased chlorophyll concentration and reduced grana formation, indicating chloroplast disruption as a possible mechanism for reduced photosynthesis. Similarly, Cave et al. (13) reported that enrichment of Trifolium subterraneum at 1000 µl CO_2/liter resulted in leaf starch content of 5% which resulted in disorder of

chloroplast structure and reduction in chlorophyll relative to controls.

B. Leaf Level of Organization

1. Anatomical and Morphological Changes

A number of anatomical changes in leaf structure in response to CO_2 enrichment have been reported. Hofstra and Hesketh (49) observed greater cell density and a higher number of palisade layers in leaves of enriched soybeans. Rogers et al. (91) also found an extra layer of palisade cells in leaves of enriched soybeans. CO_2 enrichment resulted in longer palisade cells and more layers of spongy mesophyll layers in leaves of Lea brunoniana and grape (62). In both species these changes were associated with increases in net photosynthesis when measured at higher CO_2 levels, but only L. brunoniana showed the increase at atmospheric CO_2 levels. The higher rates of net photosynthesis per unit area were linked to greater chloroplast density as evidenced by increased chlorophyll concentration, which resulted in more efficient light interception (62). These anatomical changes due to enrichment resulted in higher specific leaf weight (SLW) (49, 62).

Large increases in SLW in response to enrichment have often been reported in several C_3 species including soybean (18, 49, 82), velvet leaf (82) and sugar beets (109) (Table 2.1). SLW reflects leaf thickness and thicker leaves in response to enrichment have been reported in tomatoes (50, 68) and in soybeans (91). Higher SLW can also result from increases in structural or reserve (starch) materials. Whether increased SLW due to environment results in

Table 2.1. CO_2 enrichment effects on specific leaf weight (SLW)

Plant	Treatment CO_2 (µl/liter)	Treatment Duration (Days)	SLW Enriched/Control	SLW Day Measured	Ref.
C4 Species					
Corn	1000	45	0.94	45	82
Corn	600	45	NS	45	82
Itchgrass	1000	45	NS	45	82
Itchgrass	600	45	NS	45	82
C3 Species					
Desmodium paniculatum	1000	33	1.30	10	108
Kale	1000	24	1.15	24	31
Soybean	1000	24	1.37	25	18
Soybean	1000	14	1.37	14	49
Soybean	1000	45	1.47	45	82
Soybean	600	45	1.10	45	82
Sugarbeet	1000	24	NS	24	31
Sugarbeet	1000	10	1.51	10	109
Tomato	1000	44	1.09	23	50
Velvet leaf	1000	45	1.25	45	82
Velvet leaf	600	45	1.25	45	82
Grape	850-1000	14	1.37	14	62

enhanced photosynthesis is dependent on the partitioning of dry matter increase in photosynthetic structural, non-photosynthetic structural and/or reserve materials.

2. Stomatal Conductance

This discussion will briefly examine some general relations between stomatal conductance and elevated CO_2 levels and how these may affect net photosynthesis of leaves in CO_2 enriched environments. For more detailed reviews of stomatal functions, the reader is referred to reviews by Raschke (88) and more recently by Farquhar and Sharkey (29). Stomata have a dual function, regulating transpiration while permitting CO_2 to diffuse into the leaf to maintain photosynthesis at sufficient rates to support growth (88, 29). To facilitate this function, stomata respond both to vapor pressure deficits and to the intercellular CO_2 concentration (29). It is well documented that stomata close (as measured by decreased conductance) in response to increasing CO_2 levels and that stomata of C_4 species close at lower external CO_2 levels than stomata of C_3 species (29). Stomata of C_3 species, cotton, soybean and tomato, did not completely close even at 5000 µl CO_2/liter (81). Stomatal closure in response to high CO_2 levels has been shown to last several hours, but is not permanent (55).

The impact of decreased stomatal conductance in response to increased CO_2 concentrations on rates of net photosynthesis is uncertain. Farquhar and Sharkey (29) claim that the widely used resistance analog mode for CO_2 transport (diffusion) (36) usually substantially over estimates the limitations imposed on net

photosynthesis by reduced stomatal conductance at normal atmospheric CO_2 levels and that such limitations are generally small. At higher CO_2 levels, any limitations would presumably be smaller. The lower stomatal conductance at elevated CO_2 levels is often cited as a factor in the smaller enhancement of net photosynthesis in C_4 species (88, 39). However, Farquhar and Sharkey (29) state that such reduced conductance does not limit net photosynthesis in C_4 species since Rubisco is already CO_2 saturated at atmospheric CO_2 levels (340 µl/liter).

CO_2 enrichment decreases conductance and reduces the transpiration rate, yet increases photosynthesis of C_3 species (29, 91, 107). In C_3 or C_4 species, the photosynthesis/transpiration ratio or 'water use efficiency' is often increased (91, 107). Greater water use efficiency in enriched plants can lead to an overall increase in net assimilation by decreasing or avoiding water stress effects on net photosynthesis (91). Water stress avoidance may be the most significant impact of decreased stomatal conductance on net photosynthesis at enriched CO_2 levels.

III. Effect of Enrichment on the Rate of Net Photosynthesis

A. Single Leaf Response

There is considerable variation in the response of net photosynthesis to CO_2 enrichment. Factors contributing to this variation include the species measured, environmental conditions, the CO_2 levels used, leaf age, plant developmental stage and sink-source relations (59). The environmental effects on photosynthesis, which also apply to photosynthesis at high CO_2 levels, were recently reviewed by Berry and Downton (8). In examining the effect of CO_2 enrichment on photosynthesis, a strong response determining factor is the duration of exposure to high CO_2 levels. For the purpose of this discussion, exposure to elevated CO_2 levels for less than 1 day will be considered short term , whereas long term will refer to any exposure in excess of 1 day.

In general, short term enrichment at sub toxic levels (less than 2000 µl CO_2/liter) will result in greater net photosynthesis per unit area relative to controls. The magnitude of such increases in C_3 species typically range from 1.3-4.0 fold (e.g., C_3 legumes in Table 2.2). This is consistent with the interpretation that the increase in net photosynthesis by short term enrichment is mainly due to enhancement of carboxylation and inhibition of photorespiration. The fact that short term enrichment of C_4 species usually increases photosynthesis by only 10% to 30% (3, 39) further supports this interpretation.

A wider range of net photosynthetic response to long term CO_2 enrichment is reported. Four general responses can be distinguish-

Table 2.2. CO_2 enrichment effects on net photosynthesis (Pn) of legumes.

Plant	Culture & Enrichment Treatments: GH[1] or GC	CO_2 (μl/liter): Enriched	CO_2 (μl/liter): Control	Duration[2] (Days)	Growth[3] Stage	Pn Sampling: CO_2 prior to Pn sampling[4]: Enriched	CO_2 prior to Pn sampling[4]: Control	PPFD μmde $m^{-2}s^{-1}$	Enriched Pn: CO_2 (μl/liter)	Enriched Pn: $mgCO_2m^{-2}s^{-1}$	Control Pn: CO_2 (μm/liter)	Control Pn: $mgCO_2 m^{-2}s^{-1}$	Ratio: Enriched/Control Pn: Initial Ratio	Initial Days[5]	Subsequent Ratio	Subsequent Days[5]	Ref.
Bean																	
Phaseolus vulgaris																	
'Satisfaction'	GH	1500	300	30	V	1500	300		1500	1.39	300	0.82	1.69	30			78
'Nanus'	GH		300		V	300	300	472	1700	0.38	340	0.25	1.53				10
'Hawkesbury wonder'	GH		300		V	330	330	1500	700	1.20	300	0.92	1.30				102
'Berna'	GH		300		V	300	300		500	0.51	300	0.40	1.275				39
Soybean																	
Glycine Max L. Merril																	
'Fiskeby V'	GC	1000	350	35	R	100	300	600	1000	NA	300	NA	3.33	4	2.00	30	17
						300	1000	600	300	NA	1000	NA	0.28	4	0.14	30	17
'Bragg'	GH	630	330	55-125	V & R	630	330	1800-2000	630	0.88	330	0.57	1.54	69-73			74
						330	630	1800-2000	330	0.57	630	0.75	0.76	69-73			74
'Harosoy NL'	GC		300	50-60	R	600	300			NA			1.53				24
'Harosoy'	GC		300	50-60	R	600	300			NA			1.84				24
'Wayne'	GC		300	50-60	R	600	300			NA			1.75				24
'Clark'	GC		320	28	V	1000	320	700	1000	NA	320	NA	1.87	14	1.76	28	104
'Hark'	GC		300	21	V	300	300		1670	2.24	300	0.56	4.00				11
Pasture Legumes																	
Vigna luteda (Jacq.) Benth.																	
'Dalrymple'	GC		300			300	300		1350	3.96	350	1.50	2.64				63
Calopogonium mucunoides Desv.			300						1350	3.16	350	1.00	3.16				63
Phaseolus atropurpureus D.C.																	
'Siratro'			300						1350	2.31	350	0.92	2.51				63
Desmodium paniculatum	GC	1000	350	49	R	1000	350	1300	1000	0.70	300	0.75	0.93	49			108
									300	0.47	300	0.75	0.63	49			108
									1000	0.70	1000	1.12	0.63	49			108
									300	0.47	1000	1.12	0.42	49			108

1. GH = Green House; GC = Growth Chamber
2. Duration: length of experiment or enrichment where available
3. V = vegetative; R = reproductive
4. If different than concentration plant was grown in, usually less than two hours.
5. Days after beginning of experiment when Pn measurement made.

ed. The first response type is characterized by a substantial, maintained enhancement of net photosynthesis relative to controls (1.3-3.0 fold in magnitude). The second type is characteristic of C_4 species in which photosynthetic rates are not affected or only increased less than 30%. A third type of response is that net photosynthesis of enriched leaves is greater when measured at high CO_2 levels but is less than that of controls when measured at normal atmospheric levels. The fourth response type is characterized by photosynthetic rates of enriched leaves measured at high CO_2 levels that are lower than rates of control leaves measured at control CO_2 levels. The most common response is the maintained substantial increase in net photosynthesis. The magnitude of the increase may decline in coincidence with normal leaf ageing and senescence. Such prolonged increases in photosynthesis have been reported for a wide range of plants including tomato (47), cotton (45, 107) and soybean (74, 17, 104).

There are noticibly few reports of long term CO_2 enrichment effects on photosynthetic rates of C_4 species. However, based on available evidence, long term enrichment effects on photosynthetic rates are similar to short term effects. Mauney et al. (73) reported net photosynthesis of leaves of sorghum enriched at 650 µl CO_2/liter was only 2% higher than that of controls. Similarly, enrichment of corn up to 900 µl CO_2/liter failed to increase net photosynthesis (91). However, in other experiments, long term enrichment of corn at 600 µl CO_2/liter increased net photosynthesis by 20% (107) and 15% (12).

Several investigators have reported that photosynthetic rates of leaves of long term enriched plants were greater than controls when measured at elevated CO_2 levels, but less than controls when measured at normal atmospheric CO_2 levels. Crops showing this type of response were cotton, sunflower, sorghum (74), grapes (62) and soybeans (49, 17). The fact that soybeans displayed this response in one experiment (17) but not in another (74) suggests the occurrence of this response is dependent on other factors in addition to long term enrichment. The decline in photosynthesis in soybeans (49) and grape (62) was linked to increased residual resistance associated with starch accumulations in soybean and also anatomical changes and increased leaf thickness as in grape, while Clough et al. (18) suggested decreased sink demand may have triggered the decline in photosynthesis.

Recently, there have been reports of photosynthetic rates of leaves of long term enriched plants measured at high CO_2 levels being less than control leaf photosynthetic rates measured at normal ambient concentrations. Aoki and Yabuki (4) reported that photosynthetic rates of lower leaves of cucumber plants enriched for 15 days at 1200, 2400 and 5500 μl CO_2/liter were only 64%, 47% and 39% of the controls, respectively. However, enrichment enhanced photosynthesis of upper leaves, suggesting that leaf age and irradiance contributed to this differential response. Under high light intensity, the photosynthetic rate of leaves of enriched Desmodium paniculatum were slightly less than those of controls when measured at the respective CO_2 levels in which plants were grown (108). At

lower light intensities, net photosynthesis of enriched leaves was higher than that of controls. The decrease in photosynthesis in enriched plants was correlated to reduced grana formation and large starch accumulation. Enrichment also caused a 20% to 30% decline on the net photosynthetic rates of tobacco leaves relative to controls (87). This reduction was accentuated by a low nitrogen treatment. Wong (107) reported leaf photosynthetic rates of CO_2 enriched corn was 22% lower than control rates when grown at low nitrogen but was 20% greater when grown at high nitrogen.

B. Whole Plant Response

The idea that whole plant net photosynthesis responds to high CO_2 levels in the same way as single leaves has been a popular assumption behind many applications of CO_2 enrichment. It has been the basis for enrichment of commercial greenhouses (106) and for experiments using enrichment to study the influence of increased photosynthesis on plant growth and yield (43). Yet, whole plant net photosynthesis of enriched plants has not been extensively investigated (77). Canopy photosynthesis of tomato did respond to high CO_2 in a manner similar to that of a single leaf (42). Further support is provided by investigations which measured net assimilation rate (NAR) which is the gain in plant dry weight per unit leaf area per unit time. This approximates the daily net photosynthetic rate of the whole plant. Net assimilation rates for enriched soybeans range from 1.3 to 1.6 fold greater than those of controls (73, 82, 18). These increases are of similar magnitude to those for single leaf photosynthesis of soybeans under long term enrichment (Table 2.2).

The NAR of CO_2 enriched corn (C_4 species) was either not increased (82) or enhanced by only 25% (96) relative to respective controls. These responses are very similar to those of net photosynthetic rates of enriched corn leaves, discussed earlier (12, 91 107).

While the direction of the response of photosynthetic rates to CO_2 enrichment of single leaves and the canopy often are similar, a number of factors could cause considerable difference between these rates. Shade, source-sink relations and a wide range of leaf age within the canopy can contribute to a discrepancy between whole plant and single leaf photosynthetic rates (84). In addition, a considerable amount of non-photosynthesizing tissue is often included in measurement of whole plant photosynthesis, increasing the potential for differences between canopy and single leaf photosynthesis rates. However, since CO_2 enrichment often increases total plant leaf area (59) and can enhance net photosynthesis even at low irradiance (42), it may compensate for shading and age effects resulting in closer correlations between single leaf and canopy photosynthetic rates than may exist at normal atmospheric CO_2 levels. Yet, Aoki and Yabuki (4) showed that considerable variation exists in leaf photosynthetic rates within the canopy of CO_2 enriched cucumbers.

IV. Effect of CO_2 Enrichment on Dry Matter Accumulation

A. Plant Dry Weight

A representative compilation of reported enrichment effects on total plant dry weight is presented in Table 2.3. In the C_3 species represented, enrichment resulted in large initial increases in plant dry weight, ranging from 1.2 to 4.6 fold with a mean increase for all reports of 2.1 fold. Although enriched plants maintained early weight gains, at subsequent measurements the ratio of enriched to control plant dry weight was often smaller. In 67% of the cases presented in Table 2.3, the ratio of enriched/control plant dry weight decreased between the initial and a later sampling. The ratio increased in 28% of the cases and was unchanged in the remaining 5%. In the 3 C_4 species represented, enrichment for 10-30 days increased plant dry weights from 1.2 to 2.1 fold with a mean of 1.6 fold. However, after 40 to 60 days of enrichment, the initial increases had diminished, and in 4 of 7 cases, dry weight of enriched C_4 plants was less than that of controls.

B. Net Assimilation Rate

The enrichment induced increase in plant dry weight in both C_3 and C_4 species presumably resulted from greater whole plant photosynthesis, which is a function of increased net photosynthesis per unit leaf area and/or greater leaf area per plant. The net assimilation rate is equivalent to the whole plant net photosynthesis minus respirational losses. Thus, barring large changes in dark respiration, NAR reflects the direction of response of whole plant

Table 2.3 CO_2 enrichment effect on plant dry weight.

Plant	Treatment CO_2 (µl/liter)	Treatment Duration (Days)	Plant Dry Weight Enriched/Control Initial Ratio	Initial Days	Subsequent Ratio	Subsequent Days	Ref.
C4 Species							
Corn	1000	42	NS	21	0.78	42	31
Corn	600	45	1.17	12	0.98	45	82
Corn	1000	45	1.21	12	0.88	45	82
Corn	675	45	1.50	28	1.25	43	96
Itchgrass	600	45	2.05	12	1.21	45	82
Itchgrass	1000	45	1.96	12	0.98	45	82
Sorghum	630	65	1.73	10-30	1.06	30-65	73
C3 Species							
Barley	1000	42	1.44	21	1.29	42	31
Bean	1000	21	1.71	21			100
Cotton	630	110	2.65	10-30	1.00	30-70	73
Cucumber	1200	24	2.35	7	1.66	24	4
Desmodium paniculatum	1000	33	1.50	10	1.90	33	108
Kale	1000	42	1.44	21	1.48	42	31
Leea brunoniana	850-1000	28	2.50	28			62
Radish	675	26	2.00	11	1.17	21	96
Soybean	1000	21	2.40	9	3.33	15	49
Soybean	630	110	4.55	10-30	6.76	30-70	73
Soybean	600	45	1.53	12	1.23	45	82
Soybean	1000	45	1.80	12	1.72	45	82
Soybean	1000	25	1.84	25			18
Soybean	675	75	1.88	28	1.60	73	96
Sugarbeet	1000	42	1.26	21	1.23	42	31
Sugarbeet	600-850	99	1.31	99			109
Sugarbeet	1000	10	2.80	10			109
Sugarbeet	675	60	1.33	32	2.00	52	96
Sunflower	630	90	2.08	10-30	1.12	30-70	73
Tomato	1000	21	2.03	21			100
Tomato	1000	35	1.53	12	1.15	35	68
Velvet leaf	600	45	3.28	12	1.36	45	82
Velvet leaf	1000	45	2.47	12	1.54	45	82
Grape	850-1000	14	3.50	14			62

photosynthesis to enrichment. A compilation of CO_2 enrichment effect on NAR is presented in Table 2.4, including many of the same reports as presented in Table 2.2. An attempt is made to classify NAR responses into 5 general types: 1) sustained increases in NAR of enriched plants relative to controls, 2) an initial increase in NAR followed by a decline, but yet higher than that of controls, 3) initial increase of NAR followed by a decline below that of the control, 4) no significant change in NAR and 5) NAR of enriched plant is lower than that of the control with no initial increase evident. In general, NAR of C_3 species show greater positive response to enrichment than C_4 species with responses 1 and 2 dominating. In C_4 species, response types 4 and 5 predominated. These differences between C_3 and C_4 plants are similar to those reported for plant dry weight. Closer examination of Table 2.4 shows that considerable variation exists in NAR response within a crop species (e.g., corn, soybeans) possibly reflecting genotypic difference. Yet, enrichment of the same corn cultivar ('Dekalb xl 395') at 600 and 1000 µl CO_2/liter resulted in response types 4 and 5, respectively, suggesting that the higher CO_2 level may have had a toxic effect (82).

In C_4 species, enrichment increases plant dry weight largely through an increase in leaf dry weight, i.e., presumably greater photosynthetic surface, rather than higher photosynthetic rates. This is supported by the lack of higher NAR (Table 2.4). Greater plant dry weight due to enrichment was attributed to higher leaf areas in corn (82, 91), sorghum (73) and itchgrass (82). In some C_3

Table 2.4. CO_2 enrichment effect on net assimilation rate (NAR)

Plant	Response Type	Treatment CO_2 (µl/liter)	Treatment Duration (Days)	NAR: Enriched/Control Initial Ratio	Initial Days	Subsequent Ratio	Subsequent Days	Ref.
C4 Species								
Corn	4	600	45	NS	12-24	NS	24-45	82
Corn	5	1000	45	NS	12-24	0.89	24-45	82
Corn	2	675	40	1.25	26-33	NS	33-44	96
Itchgrass	4	600	45	NS	12-24	NS	24-45	82
Itchgrass	5	1000	45	NS	12-24	0.92	24-45	82
Sorghum	3	630	65	1.42	10-30	0.80	30-65	73
C3 Species								
Barley	1	1000	42	1.10	28	1.08	42	31
Bean		1000	12	1.74	12			100
Cotton	2	630	110	1.50	10-30	1.08	70-110	73
Desmodium paniculatum	1	1000	33	1.20	1-10	1.40	23-33	108
Kale		1000	28	1.23	28			31
Radish	1	675	30	3.00	9-13	2.00	27.30	96
Soybean	3	630	110	1.48	10-30	0.33	70-110	73
Soybean	2	600	45	1.22	12-24	1.02	24.45	82
Soybean	1	1000	45	1.29	12-24	1.34	24-45	82
Soybean		1000	24	1.60	25			18
Sugarbeet		1000	49	1.08	28	1.16	49	31
Sugarbeet		1000	10	1.56	10			109
Sugarbeet	1	675	85	1.85	31-38	1.60	38-57	96
Sunflower	3	630	90	1.28	10-30	0.36	70-90	73
Tomato		1000	20	1.20	20			100
Tomato	1	1000	35	2.25*	19	1.31*	35	51
Velvet leaf	2	600	45	1.16	12-24	1.02	24-45	82
Velvet leaf	2	1000	45	1.23	12-24	1.11	24-45	82
Grape		850-1000	14-28	2.37	14			62
Wheat	1	1300	28	3.30*	7-14	1.40	21-28	65.66
Wheat	3	800	24	1.55	10	0.93	24	76

*Control other than 350 ± 50 ppm CO_2

species, the gain in plant dry weight due to enrichment is the result of both increased NAR and greater leaf area. Examples of this include bean and tomato (100), soybean (82) and D. paniculatum (108). In other experiments with C_3 species, increases in plant dry weight were mainly due to higher NAR since leaf area was not influenced by enrichment. These reports include work with soybean (18, 96), sugarbeet (96), wheat (76), Vitis vinifera (62) and velvet leaf (82).

C. Root/Shoot Ratio

The increased photosynthate (dry matter) produced in response to enrichment may be generally distributed throughout the plant or differentially partitioned to certain organs or tissues. In addition, enrichment may alter the existing distributional pattern. Evaluation of the root/shoot ratio of enriched plants, where possible, is one way to assess the enrichment effect on dry matter partitioning. Representative examples of enrichment effects on root/shoot ratios reported in the literature are summarized in Table 2.5. Ratios presented are for root/shoot ratio of enriched plants divided by those of controls. Enrichment did not result in any change in the root/shoot ratio of C_4 species, corn or itchgrass, thus indicating the extra dry matter was generally distributed throughout these plants. The root/shoot ratio of C_3 species in response to enrichment, was variable. Values ranged from a 0.4 fold decrease in D. paniculatum (108) to a 1.8 fold increase in sugarbeet. The 1.4 fold increase in radish and 1.8 fold greater root/shoot ratio in sugar beet (96) suggest root crops may preferen

Table 2.5. CO_2 enrichment effect on root/shoot ratio

Plant	Treatment CO_2 (µl/liter)	Treatment Duration (Days)	Root/Shoot Ratio Enriched/Control	Root/Shoot Ratio Day Measured	Ref.
C4 Species					
Corn	1000	45	NS	45	82
Corn	675	58	NS	58	96
Itchgrass	1000	45	NS	45	82
C3 Species					
Barley	1000	28	0.86	28	31
Bean	1000	21	1.39	12	100
Desmodium paniculatum	1000	33	0.62	22	108
Kale	1000	28	0.86	28	31
Okra	1000	70	1.18	70	95
Radish	675	55	1.42	55	96
Soybean	1000	45	1.15	45	82
Soybean	1000	25	NS	25	18
Soybean	675	70	NS	58	96
Sugarbeet	1000	28	NS	28	31
Sugarbeet	850	99	NS	99	109
Sugarbeet	675	55	1.80	55	96
Tomato	1000	21	NS	20	100
Velvet leaf	1000	45	1.19	45	82
Wheat	675	92	1.33	92	94

tially partition 'extra dry matter' to their predominant root sink. However, in other experiments, root/shoot ratios of enriched sugarbeets were not greater than controls (31, 109). Similar variation in root/shoot ratio within a species in response to enrichment can be seen in soybeans (Table 2.5). This variation may have been due to a number of factors including: different cultivars, environmental conditions and growth stage.

V. CO_2 Enrichment Effect on Yield

A. Introduction

Increasing crop yield is the common goal of many agriculturists. In this thesis, yield is defined as the saleable portion of the crop. This is commonly termed economic yield as opposed to biological yield, the total biomass which is produced by a crop. Yield is the sum product of many physiological processes and their response to a dynamic environment. In some instances, a single factor, for example, water stress, a disease or mineral deficiency, may be observed to limit yield. Under less severe conditions, yield is limited by photosynthate production and distribution. The frequently expressed notion that yield is not dependent on photosynthesis stems from lack of correlation between net photosynthesis per unit leaf area and yield (25). However, in refuting this misconception, Zelitch (110) points out that such a correlation based on instantaneous photosynthetic measurements of single leaves would be very fortuitous. He then reviewed evidence supporting a close relationship between yield and photosynthesis including correlations of canopy photosynthesis and yield in various crops and CO_2 enrichment induced yield increases.

The purpose of this review is to examine experiments in the literature where direct effects of CO_2 enrichment on yield were measured. It is recognized that other factors such as plant water status (37, 93) and mineral nutrition (15, 93) can modify the yield response to enrichment, but unless noted otherwise, it is assumed

the reported yield responses are principally due to elevated CO_2 levels.

B. Crop Yield in General

Crops are often classified according to which part of the plant comprises the yield. Although such classifications contain come measure of arbitrariness and ambiguity, they do serve to provide some logical basis from which to analyze yield and environmental factors and physiological processes affecting it. In this discussion, plants are classified into these groups: fiber, leaf, root, fruit, grain and seed legume crops. This system is adopted to facilitate easy reference to the statistical analysis of CO_2 enrichment effects on yield by Kimball (58).

According to Kimball (58), the literature reports an overwhelming positive response to CO_2 enrichment. He reviewed 437 experiments or observations of 37 species extracted from 70 reports published during the past 64 years and concluded that CO_2 enrichment increased yield an average of 28%. Kimball's review is an exhaustive and valuable reference. Yet, deficiencies in the data base should be pointed out lest it be concluded that all is known concerning enrichment effects on yield. The 437 observations contain only one unsubstantiated report on a C_4 species. The observations include a wide range of enrichment regimes, in terms of duration (7 to 120 days) and CO_2 concentrations (525 to 6000 μl CO_2/liter). Many older observations cited did not report CO_2 concentrations. Therefore, averaging may be an inappropriate

evaluation for such data. Kimball (58) also provided a second analysis of a smaller number of observations. This analysis used data from 38 experiments which met more rigid criteria for enrichment duration and concentration. Based on this analysis, enrichment during the whole crop cycle resulted in an average yield increase of 33% at 600 µl CO_2/liter and 67% at 1200 µl CO_2/liter.

A summary of enrichment effects on yields of representative crops from each crop classification is presented in Table 2.6. The measure of enrichment effect is the ratio of yield obtained from enriched plants to that of control plants at ambient CO_2 concentrations. The data in Table 2.6 show consistent yield increases in response to enrichment which supports Kimball's (58) conclusion. Kimball also calculated the mean yield increase within each crop classification, thus providing a response ranking by crop type. The most to least responsive crop types are ordered as follows: fiber, seed legume, root, leaf, grain and fruit.

C. Legume Yield

Seed legume yield components are the number of pods per plant, number of seeds per pod and seed dry weight per seed. Yield components of snap beans are the number of pods per plant, weight per pod and a number of quality factors including pod diameter (sieve size), pod length and shape. The number of pods per plant is the primary yield component in both snap and dry beans (1). The principal regulation of the number of pods per plant is via abscission. Abscission rates are dependent on pod load, water deficits, extreme temperatures, irradiance levels and other factors. (72). For

Table 2.6. CO_2 enrichment effect on yield

Yield Type	CO_2 Enrichment Regime: CO_2 (µl/liter)	Development Stage	Duration (Days)	Reported Yield Parameter & Units	Yield Ratio: Enriched / Control	Ref.
Root Crops						
Radish	unknown		26	root f.w. (g)	1.47	21
Sugarbeet						
cv.Alt-10	600-850		158	root d.w. (kg/plot)	1.32	109
Leaf Crops						
Lettuce						
cv.Bibb	800-2000	thru harvest	40-70	f.w./10 heads (lbs)	1.58	106
cv.Chestnut	"	"	"	"	1.75	106
cv.Grand Rapids	"	"	"	"	2.00	106
Swiss Chard						
cv.Flavercens D.C.	600	thru harvest	24	f.w./plant (g)	0.93	52
"	900	"	"	"	2.20	52
"	1500	"	"	"	3.81	52
Fruit Crops						
Tomato						
cv.Tuckercross	800-2000	thru harvest	up to 220	f.w./plant (kg)	1.42	106
mean of 3 cv.	NA	thru harvest	up to 220	f.w./plant (lbs)	1.23	61
cv.Revermun	1000	thru harvest	97		1.47	69
cv.Vendor	800-1000	'til just prior to fruit maturity	110	f.w./plant (g)	1.42	47
Cucumber						
cv.Elem	900	thru harvest	175	fruit f.w. (kg/m^2)	1.25	26
"	1500	"	"	"	1.18	26
"	3000	"	"	"	1.26	26
cv.Sylvia	1000	unknown	unknown	f.w. (kg/plant)	1.36	105
Fiber Crop						
Cotton						
cv.DPL 16	630	thru harvest	115	lint yield (g/plant)	2.79	73
"	"	"	"	seed yield (g/plant)	2.40	73

Table 2.6. (continued) CO_2 enrichment effect on yield

Yield Type	CO_2 Enrichment Regime: CO_2 (µl/liter)	Development Stage	Duration (Days)	Reported Yield Parameter & Units	Yield Ratio: Enriched / Control	Ref.
Grain Crops						
Wheat						
cv.Era	600	preflower	32	grain d.w. (g/plot)	1.06	60
"	"	flower	33	"	1.15	60
"	"	grainfill	28	"	1.14	60
cv.8037	600	preflower	32	"	1.03	60
"	"	flower	33	"	1.18	60
"	"	grainfill	28	"	1.37	60
cv.Gabo	590	thru harvest	99	grain d.w. (kg/m^2)	1.53	37
cv.WW15	590	thru harvest	99		1.32	37
cv.GW01809	1000	thru harvest	80	grain d.w. (g/plant)	1.62	93
Legumes						
Beans						
cv.Porillo Sintetico	1200	flowering	-5 to +15	seed d.w. (g/m^2)	1.40	15
"	"	"	-5 to +35	"	1.43	15
cv.unreported	unknown	thru harvest	67	seed f.w. (g/plant)	1.83	21
cv.unreported	1000-1500	flowering thru harvest	not reported	seed d.w. (kg/ha)	1.59	44
Soybeans						
cv.Hark	1350	thru harvest	92	seed d.w. (g/plant)	1.57	19
cv. Chippewa-64	1350	thru harvest	92	seed d.w. (g/plant)	1.41	19
cv.Hark	1200	preflower	33	seed d.w. (g/plant)	0.95	43
"		flowering	36	"	1.02	43
"		post flowering	27	"	1.24	43
"		thru harvest	96	"	1.37	43
cv.unreported	1000-1500	flowering thru harvest	not reported	seed d.w. (kg/ha)	1.98	44

example, under water stress legumes are reported to have the following abscission rates: 20% to 55% in snap beans (89, 34), 36% to 47% in lupines (9) and up to 80% in soybeans (92).

A lack of available photosynthate during flowering and early podfill may be a major contributing factor to pod abscission and therefore, a limiting factor of yield in beans (1, 99, 103) and soybeans (43). The involvement of photosynthate in pod set is supported by experimental manipulation of the source/sink ratios in legumes. Increasing the source/sink ratio through pod removal (35, 103) or CO_2 enrichment (14, 15, 44, 192, 43) has been shown to improve pod set; whereas, it was reduced by shading (71) or leaf removal (99) which lowered the source/sink ratio.

CO_2 enrichment effects on yield components of seed legumes are reported in Table 2.7 as the ratio of enriched to control yields. The notable absence of data on enrichment effects on snap beans requires that this discussion will involve mostly seed legumes. In all experiments in Table 2.7, the enriched CO_2 concentrations only range from 1000 to 1500 µl CO_2/liter, thus allowing comparisons between studies to be more confidently used.

1. Number of pods

Enrichment treatments that continued throughout the crop life cycle generally resulted in the greatest yields. Such treatments increased number of pods per plant or unit area by 60% in dry beans (21) and 33% to 75% in soybeans (19, 43). The magnitude of yield increase varied with cultivar, thus the yield of 'Hark' soybean increased 75% and that of 'Chippewa-64', 33% (19).

Table 2.7. CO_2 enrichment effects on legume yield components

Yield Type	CO_2 Enrichment Regime: CO_2 (µl/liter)	Development Stage	Duration (Days)	Reported Yield Parameter & Units	Yield Ratio Enriched/Control	Ref.
Beans						
cv. Porillo Sintetico	1200	Flowering	-5 to +15	No. pods/m^2	1.19	15
"	"	"	"	Seed d.w. (g/m^2)	1.40	15
"	"	"	"	No. beans/pod	1.10	15
"	"	"	"	Mean seed d.w. (mg/seed)	1.05	15
"	"	"	-5 to +35	No. pods/m^2	1.33	15
"	"	"	"	Seed d.w. (g/m^2)	1.43	15
"	"	"	"	No. beans/pod	1.05	15
"	"	"	"	Mean seed d.w. (mg/seed)	1.01	15
cv. unreported	uk	All	67	Pods/plant	1.61	21
"	"	"	"	Seed f.w./plant(g)	1.83	21
"	"	"	"	No. seeds/plant	1.45	21
cv. unreported	1000-1500	Anthesis thru final harvest	not rptd	Seed d.w. (Kg/Ha)	1.59	44
Soybean						
cv. unreported	1000-1500	Anthesis thru final harvest	not rptd	Seed d.w. (Kg/Ha)	1.98	44
cv. "Hark"	1200	Preflower	33	No. pods/plant	0.94	43
"	"	"	"	Seed d.w. (g/plant)	0.95	43
"	"	"	"	Mean seed d.w. (g/100 seed)	0.95	43
"	"	Flowering	36	No. pods/plant	1.20	43
"	"	"	"	Seed d.w. (g/plant)	1.02	43
"	"	"	"	Mean seed d.w. (g/100 seed)	0.92	43
"	"	Podfill	27	No. pods/plant	1.06	43
"	"	"	"	Seed d.w. (g/plant)	1.24	43
"	"	"	"	Mean seed d.w. (g/100 seed)	0.99	43
"	"	Continuous	96	No. pods/plant	1.41	43
"	"	"	"	Seed d.w. (g/plant)	1.37	43
"	"	"	"	Mean seed d.w. (g/100 seed)	0.81	43
cv. "Hark"	1350	All	92	No. pods/plant	1.75	19
"	"	"	"	Seed d.w. (g/plant)	1.57	19
"	"	"	"	No. seeds/plant	1.09	19
cv. Chippewa-64	1350	All	92	No. pods/plant	1.33	19
"	"	"	"	Seed d.w. (g/plant)	1.41	19
"	"	"	"	No. seeds/plant	1.04	19

In two experiments, enrichment treatments were imposed only during specific developmental stages. These experiments were designed to test the effect of increasing available photosynthate at different developmental stages on yield. Hardman and Brun (43) tested the effect of 4 different periods of CO_2 enrichment on yield of soybeans. The treatments included enrichment at 1200 µl CO_2/liter during preflowering, flowering, post-flowering stages, all three development stages (continuous), plus appropriate ambient controls. The flowering and continuous enrichments resulted in yield increases of 20% and 40%, respectively. The difference in yield between the 2 treatments was attributed to the increased pod abscission which occurred when enrichment ceased in the flowering treatment. Enrichment during the preflower and post-flower stages did not affect pod number.

In a study at CIAT (15), dry beans were enriched for either 10 or 30 days, beginning 5 days before flowering. The longer enrichment resulted in 33% more pods than the control, while the shorter duration increased pod yield by only 19%. The yield differences between enrichment treatments was again partially attributed to abscission of young pods when the enrichment was halted in the shorter experiment. The increased number of pods under enrichment supports the hypothesis that photosynthate is limiting pod set during the flowering and early podfill stages of legume development.

2. Seed Yield

Enrichment resulted in substantial increases in soybean seed dry weight yield on a per plant or area basis (Table 2.7) in

experiments where enrichment was used during the podfill or post-flowering development stage (19, 43). Hardman and Brun (43) reported no increase in seed yield when plants were enriched only during flowering. However, work at CIAT (15) found that enrichment just prior to and during flowering in beans caused a 40% increase in seed dry weight.

Enrichment did not significantly affect bean seed size (15). However, Hardman and Brun (43) reported that continuous enrichment or just at flowering resulted in an 18% decline in seed size of soybean. This was associated with increased number of pods per plant. These two facts support the interpretation that extra pods increased competition for photosynthate between pods as well as seeds and resulted in decreased seed size.

Chapter 3

PHOTOSYNTHESIS OF AN UPPER CANOPY LEAF OF CO_2 ENRICHED BEANS *PHASEOLUS VULGARIS* L., DURING REPRODUCTIVE DEVELOPMENT

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Additional index words. specific leaf weight, starch, conductance, transpiration.

Abstract. Bush snap beans ('Oregon 1604'), grown in the field or outside in containers, were exposed to CO_2 in open topped chambers at either 340 µl/liter (control) or 1250 µl/liter (enrichment) during daylight hours from flowering to seed maturity, a period of 45 (field) or 35 (container) days. A transient CO_2 depletion method was used to measure net photosynthesis of the penultimate mainstem leaf, which at the start of treatments was 50-60% of the final expansion reached a week later. Enrichment did not affect final leaf size, but increased mid-day leaf photosynthesis by 3-6 fold (field) or 2-5 fold (container) over controls from first flower to seed maturity. Enhancement of photosynthesis was maintained throughout the day. Maximum mean net photosynthesis of 2.6 mg CO_2 $m^{-2}s^{-1}$ was measured in the field 7-11 days after enrichment was initiated. Photosynthesis was still high at commercial pod maturity, even though the leaf accumulated starch to a level of 10.7 g m^{-2}. Starch levels of enriched penultimate leaves were 30%, 505% and 400%

greater than those of controls at the end of flowering, pod maturity and seed maturity, respectively. Specific leaf weight of enriched leaves was about 40% higher than that of controls at or before pod maturity in field plants, but the difference was not significant at seed maturity. Measurements on several dates in both field and container studies showed that under enrichment leaf conductance and transpiration of the penultimate leaf was reduced by 50% or more. However, in container plants at seed maturity, conductance and tranpiration of enriched leaves were higher than that of control leaves which show more advanced senescence. Delayed senescence in penultimate leaves of container plants under enrichment was evidenced by maintenance of stomatal conductance and photosynthetic capacity late in the season. A higher net photosynthesis/transpiration ratio, a measure of water use efficiency, was observed throughout the reproductive period in enriched leaves of container grown plants and was a function of both decreased transpiration and increased net photosynthesis.

Introduction

Effects of long term CO_2 enrichment on net photosynthesis of leaves of field grown beans have not been documented. Most studies on photosynthetic response of beans and soybeans to high CO_2 levels have measured effects on short term exposure (3, 34, 14, 11, 4, 35). Enrichment in these studies caused 1.2-4 fold increases in net photosynthesis. Long term CO_2 enrichment has been shown to influence dry matter accumulation and yield of field grown beans (6, 7) and soybeans (16, 17), presumably due to enhanced photosynthesis. However, Kramer (22) emphasized that greater photosynthesis on a leaf area basis in response to long term CO_2 enrichment is not a forgone conclusion. In some experiments after initial increases, net photosynthesis of enriched leaves declined to levels at or below those of control leaves (1, 32). Also, increased plant dry weight in response to enrichment may be due to larger leaf area, as well as to increased photosynthesis per unit leaf area (28, 32).

This report is part of a study on the effect of CO_2 enrichment during flowering and podfill on dry matter accumulation and yield components in beans. Photosynthesis and other parameters of a single leaf were monitored during the reproductive period in order to better interpret responses to enrichment. An upper canopy leaf was chosen for measurements because of its accessability and its role as a major supplier of photosynthate to developing pods in the cultivar used (27).

Materials and Methods

A field experiment was conducted in 1981 at the Oregon State University Vegetable Research Farm on a Chehalis silty clay loam soil. Prior to planting, the insecticide fonofos and herbicide trifluralin were incorporated into the soil and fertilizer was broadcast at rates of 90, 116 and 74 kg ha^{-1} of N, P and K, respectively. Seeds of the bush snap bean 'Oregon 1604' were hand planted at 13 cm intervals in 4 rows, each separated by 23 cm, giving an approximate plant population of 34 plants m^{-2}.

Carbon dioxide was supplied at rates of 340 ± 20 µl/liter (control) or 1250 ± µl/liter (enriched) to 8 chambers in each treatment. The wood framed chambers were 51 cm x 94 cm and 91 cm high with removable sides. The chamber top was left open to minimize temperature and RH build up (17, 18). Chambers stradled the 2 inner rows, enclosing 14 plants, with the outer rows serving as borders. Distance between chambers was sufficient to prevent shading. Each of 4 blower fan assemblies provided air via separate lengths of 10 cm diameter plastic dryer duct to 4 chambers. Perforated duct ran the length of the chambers between the rows. Air was supplied at 1.3 m^3 min^{-1}. Enriched chambers received supplimental CO_2 from cylinders of compressed CO_2 via Tygon tubing entering the air supply at the chamber base. The amount of CO_2 added to each chamber was controlled by fine metering valves. The flow necessary to maintain the required CO_2 concentration was determined empirically by varying flow and sampling the atmosphere just above the plant canopy. Samples were analyzed as described below. The CO_2 concentration

within each chamber was monitored bi-weekly throughout the treatment period and adjusted as needed. Chambers without sides were put in place several weeks before flowering to minimize chance of injury to plants. Sides were installed and blowers turned on 2 days before anthesis to allow preliminary calibrations. Enrichment was begun on the day of first flower, defined as the day anthesis occured at the lowest node of the terminal mainstem raceme. Enrichment was carried on daily between 0800 and 2000 hr. until seed maturity, a period of 45 days.

Net photosynthesis of single leaflets was measured using a transient CO_2 depletion method similar to that described by Clegg et al. (8). A hand-held Plexiglas hinged-box leaf chamber (volume = 0.530 liter) fitted with a 1.5-6.0 V fan was used. The jaws of the chamber were fitted with closed-cell foam rubber gaskets. A monofilament nylon web was used to keep the leaf surface flat inside the chamber. The chamber was equipped with 3 syringe ports, each fitted with a rubber serum stopper to facilitate sampling. Measurement at 340 ± 20 µl CO_2/liter began by enclosing a leaflet in the chamber with the fan running and within 5 seconds withdrawing a 10 ml gas sample with a plastic syringe. Precisely 30 seconds later, another 10 ml sample was collected. Syringes were sealed by inserting the needle into a silicone disk. A similar, but slightly modified, procedure was used to measure net photosynthesis of leaflets of enriched plants at 1250 µl CO_2/liter. Immediately after enclosing the leaflet in the chamber, approximately 8.4 ml of 5% CO_2 was injected into the chamber in order to raise the CO_2 concentration to

1250 µl/liter. The chamber atmosphere was sampled after 5 seconds and again after a 30 second period.

Measurements of net photosynthesis were made on the same central leaflet of the penultimate mainstem trifoliate (at node 5) of a single plant from each chamber throughout the reproductive period. To facilitate measurements, one side of each treatment chamber was removed just prior to the sampling. Photosynthesis measurements were initiated at 1030 hr and took 1 hr to complete. The outline of each measured leaflet was traced on paper on each occasion or until full expansion was reached. Using this tracing, the leaf area within the photosynthesis chamber was estimated with a LI-COR Model 3100 area meter (LI-COR, Lincoln, Nebraska). Photosynthetic photon flux density (PPFD) in the field was measured using the LI-COR Model LI-185B Quantum Radiometer Photometer. Leaf conductance and transpiration rates were measured with a LI-COR Model LI-1600 steady state porometer on the same leaves that were sampled for net photosynthesis.

Upon completion of all measurements, the samples were transported to the laboratory for analysis, which was completed within 4 hr of collection. Sample analysis and calculations of net photosynthesis were previously described (8). The flow through system employed utilized Nylaflow tubing, a flowmeter (Brooks Rotameter Model #1355CA 1A 1AAA), a Beckman Model 865 IRGA in the absolute mode, and a dual pen recorder (Soltec Model 233 or Linear Model 885). Prepurified N_2 at a flow rate of 1 liter/min was used as the standard carrier gas. The system was calibrated using either a

primary standard gas of 330 ± 3.3 µl CO_2/liter or a standard gas of 1250 ± 12.5 µl CO_2/liter (Matheson).

Photosynthesis data from the field study were analyzed by ANOVA. A split plot experimental design was employed with CO_2 treatments as mainplots and sampling dates as subplots. Appropriate LSD's were calculated for mean separation.

Penultimate mainstem leaves from 2 plants per chamber were removed on 15 (end of flowering), 22 (pod maturity) and 45 (seed maturity) days after first flower (DAFF). Pod maturity as used here indicates the stage for commercial snap bean harvest with 50% of the pods being in sieve size 1-4. Leaf areas and dry weights were obtained and specific leaf weight (SLW) were calculated. Leaf starch was hydrolyzed by amyloglucosidase and measured as glucose by a colorimetric method (15). Statistical analyses of these data included ANOVA as a split plot design with CO_2 treatments as mainplots and harvests as subplots.

In a second experiment, seeds were planted in 4 liter pots containing a silty loam-vermiculite (2/1, v/v) soil mix supplimented with fertilizers. Plants were grown outside and thinned to one per pot upon emergence of the third trifoliate. Ten plants each were placed into 2 treatment chambers and additional plants surrounded the chambers. The chambers either received air containing 340 ± 20 µl CO_2/liter (control) or 1250 ± 250 µl CO_2/liter (enriched) by the assembly previously described. Treatments were applied during daylight hours from first flowering to seed maturity. On 8 days during this period, photosynthesis of the central leaflet of the

penultimate mainstem trifoliate leaf was monitored on the same plants with each chamber. Measurements were made between 1000-1200 hr or soon after cloud cover broke in early afternoon. During most of the experiment, 6 plants per treatment were used, but the sample size was reduced to only 2 plants per treatment on 35 DAFF due to senescence or leaf injury. On 4 dates, leaf conductance and transpiration were measured on the same leaflets directly following measurement of photosynthesis.

On several occasions, net photosynthesis of penultimate leaves was also measured in the laboratory with a steady state system (2). At 0830 hr a single attached penultimate leaf was sealed in a semi-closed assimilation chamber at 25 ± 0.1°C, RH = 48 ± 2% and PPFD = 1.3 ± 0.2 mE $m^{-2}s^{-1}$. Measurements were made over a 1-2 hr period following a 1 hr equilibration period at either 359 µl CO_2/liter or 1250 µl CO_2/liter (enriched). When the lower set points (330 or 1000 µl/liter) were reached, CO_2 was automatically injected into the chamber to reestablish the initial concentrations.

Results

CO_2 enrichment of field grown plants resulted in a 3 to 6 fold increase in net photosynthesis of the penultimate leaf throughout the reproductive period (Figure 3.1). The maximum mean net photosynthesis rates of enriched plants (2.67 mg CO_2 $m^{-2}s^{-1}$; 96.1 mg $dm^{-2}hr^{-1}$) occured from 7-11 DAFF, during the peak flowering and pod set period. The penultimate leaf in both treatments was fully expanded by this time. Enriched plants showed a second peak in net photosynthesis at 24 DAFF concurrent with the beginning of rapid seed growth, just 2 days prior to the pod maturity harvest. The highest mean net photosynthesis rate of leaves of control plants was about 0.60 mg CO_2 $m^{-2}s^{-1}$. Photosynthesis rates of both control and enriched leaves showed declining trends during the experimental period.

Diurnal measurements made 21 DAFF show that elevated CO_2 levels enhanced net photosynthesis throughout the day with highest rates occuring in mid-afternoon (Figure 3.2). The net photosynthesis rate of control leaves was highest in mid-morning and declined thereafter. Similar diurnal patterns were observed on 11 and 30 DAFF (data not given).

In the experiment with container plants, grown outside, CO_2 enriched leaves again had higher net photosynthesis than control leaves, although the 2-3 fold increase was less than that observed in field grown plants (Figure 3.3). In this experiment, net photosynthesis of the penultimate leaf in both treatments was relatively constant over the first 3 sampling dates, but increased 20 by DAFF

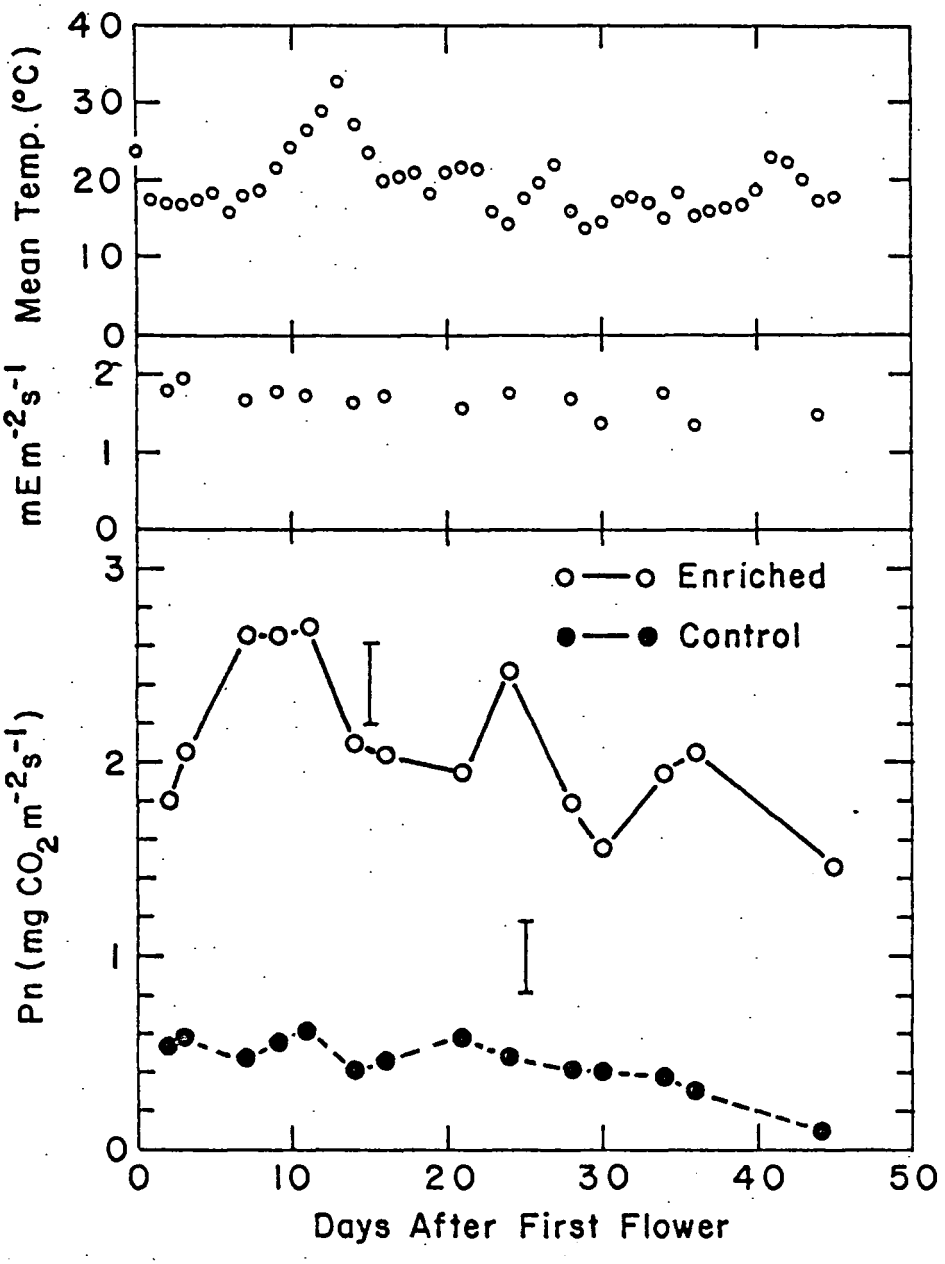

Fig. 3.1. Net photosynthesis (Pn) of enriched (1250 µl CO_2/liter) and control (340 µl CO_2/liter) penultimate mainstem leaves of field grown beans throughout the reproductive period measured between 1000 and 1200 hr. Each point represents mean Pn of 8 leaves. Verticle bars represent LSD at 0.05 level. Upper bar is for separation of same treatment means at different dates. Lower bar for separation of different treatment means at same or different dates. Also PPFD measured at time of Pn measurements and daily mean temperature are presented.

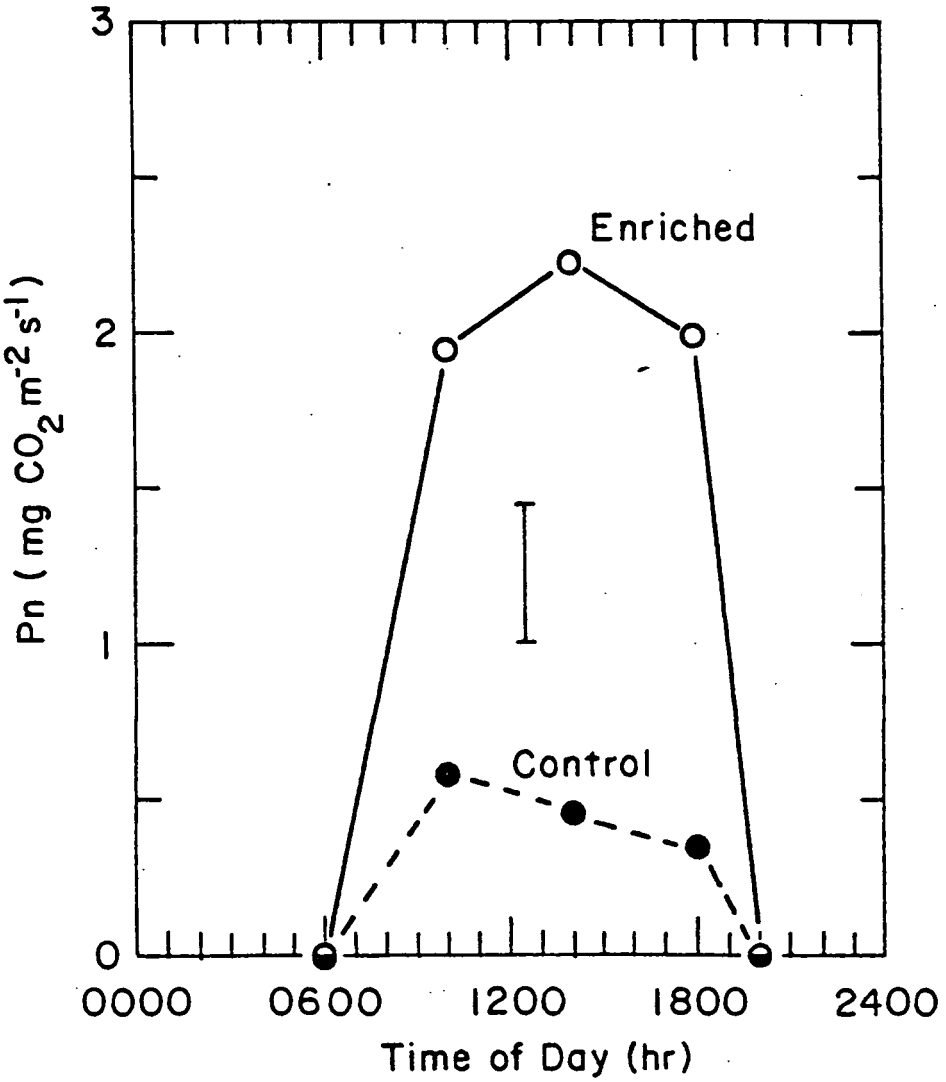

Fig. 3.2. Diurnal curve of net photosynthesis (Pn) of enriched (1250 µl CO_2/liter) and control (340 µl CO_2/liter) penultimate mainstem leaves of field grown beans measured on the 21st day after first flower. Each point represents mean of 8 leaves. Verticle bar equals LSD at 0.05 level.

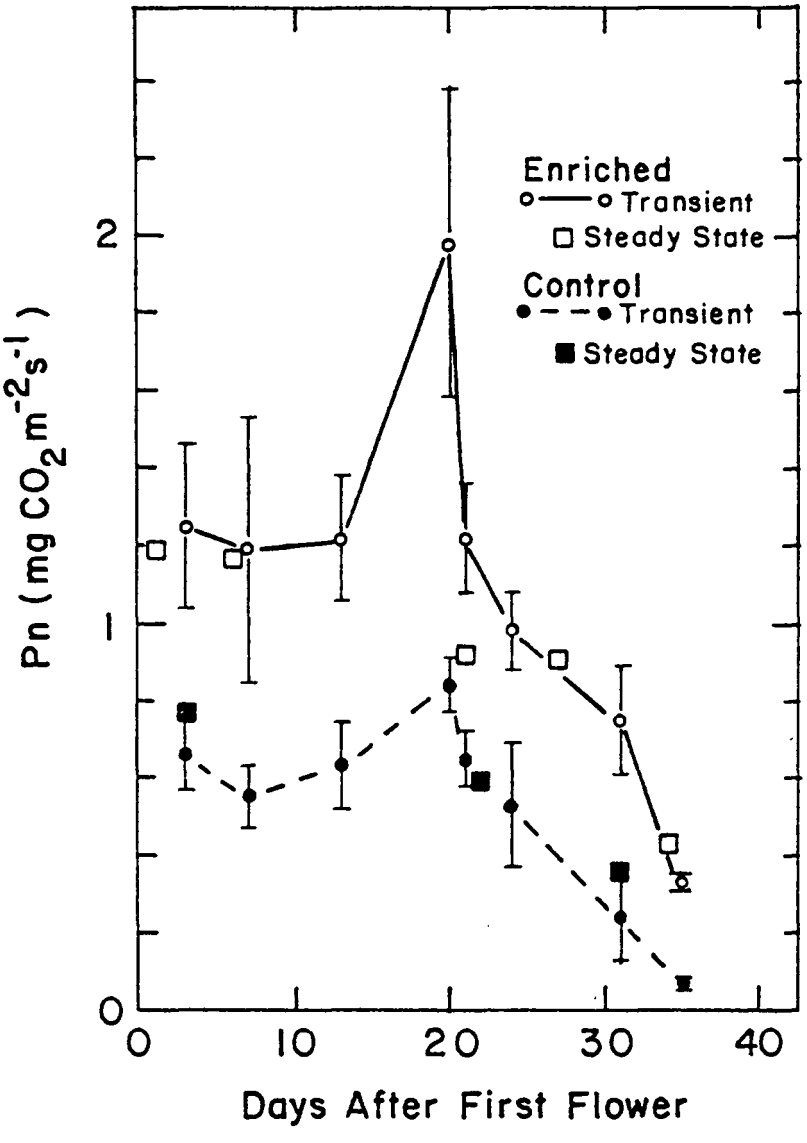

Fig. 3.3. Net photosynthesis (Pn) of enriched (1250 µl CO_2/liter) and control (340 µl CO_2/liter) penultimate mainstem leaves of container grown beans measured by transient and steady state CO_2 depletion methods throughout the reproductive period. Vertical bar equals ± SE.

to maximum rates of 1.98 and 0.83 mg CO_2 $m^{-2}s^{-1}$ for enriched and control leaves, respectively. Rapid seed growth was initiated at about this time. Photosynthesis in both treatments markedly declined over the final 15 day period.

Measurement of net photosynthesis by the steady state and transient methods generally gave similar values (Figure 3.3). This reinforces the validity of the transient method.

Leaf conductance and transpiration of field grown beans were determined on 2 dates (Table 3.1). Enrichment decreased conductance of the penultimate leaf to less than half that of control leaves causing reductions in transpiration or 36% and 56% on 27 and 37 DAFF, repectively.

With container plants, leaf conductance in the control was nearly 4 fold greater than that of enriched plants on 3 DAFF, but the difference lessened with time and similar values were obtained on 13 DAFF (Table 3.2). However, the relationship was reversed on 35 DAFF when leaf conductance of enriched leaves was over 4 fold higher than that of the more yellow control leaves. A similar pattern was reflected in transpiration rates (Table 3.2). The net photosynthesis/transpiration ratio, used as a measure of water use efficiency, expresses the amount of CO_2 fixed per unit water transpired. Due to initially lower transpiration rates and consistently higher rates of net photosynthesis, the penultimate leaf of enriched plants always had a higher net photosynthesis/transpiration ratio than control leaves on a given date (Table 3.2). However, the dif-

Table 3.1. Effects of enrichment on leaf conductance and transpiration of the penultimate mainstem leaf of field grown beans.

Days after first flower	Conductance ($m\ s^{-1}$) x 1000		Transpiration ($mg\ H_2O\ m^{-2}s^{-1}$)	
	Control	Enriched	Control	Enriched
27	12.50	5.88*	3712	2366*
37	6.30	2.66*	1867	807*

*Significant at 0.05 level

Table 3.2. Effects of CO_2 enrichment during the reproductive period on stomatal conductance, transpiration and the net photosynthesis/transpiration (Pn/E) ratio of the penultimate mainstem leaves of container grown beans.

Days after first flower	Conductance ($m\ s^{-1}$) x 1000		Transpiration ($mg\ H_2O\ m^{-2}s^{-1}$)		Pn/E ($mg\ CO_2/mg\ H_2O$) x 10,000		Enriched Pn/E
	Control	Enriched	Control	Enriched	Control	Enriched	Control Pn/E
3	9.09	2.33*	1529	651*	4.31	19.23	4.46
7	7.91	6.41	1618	1447	3.39	8.29	2.44
13	2.74	2.30	501	642	12.57	19.01	1.51
35	0.53	2.27*	184	768	3.80	4.29	1.13

*Significant at 0.05 level.

ference between treatments continually decreased during the experiment, mainly a result of declining transpiration in control leaves.

Enrichment increased the dry weight of the penultimate leaf of field grown beans by 79% and 66% at the end of flowering and pod maturity, respectively (Table 3.3). Specific leaf weight was also higher in enriched plants at these two stages. Neither dry weight nor SLW of enriched and control leaves differed significantly at seed harvest. The higher value of SLW for the control at 35 DAFF may have been due to sampling error or other unknown causes. Under enrichment the penultimate leaf accumulated much higher levels of starch per unit leaf area at all 3 harvests (Table 3.3). Leaf starch increases of 30% and 505% accounted for 9% and 31% of gain in SLW at flowering and pod maturity. On the basis of percent leaf dry weight, starch levels of enriched leaves were 9.7%, 15.0% and 5.0% and control values 9.9%, 3.4% and 1.5% at end of flowering, pod maturity and seed maturity, respectively.

Table 3.3. Effect of enrichment during the reproductive period on dry weight, specific leaf weight and starch of the penultimate mainstem leaf at 3 harvests in field grown beans.

	Harvests					
	End of Flowering		Pod Maturity		Seed Maturity	
	Control	Enriched	Control	Enriched	Control	Enriched
Leaf dry wt.(g)	0.731	1.308*	0.776	1.284*	0.478	0.476
Specific leaf wt. ($g\ m^{-2}$)	46.5	63.6*	47.8	67.1*	57.9	50.9
Starch ($g\ m^{-2}$)	4.78	6.30*	1.75	10.71*	0.91	3.67*

*Significant at 0.05 level.

Discussion

Continuous enrichment with 1250 µl CO_2/liter stimulated net photosynthesis of the penultimate leaf 2-6 fold over the entire 35-45 day reproductive period. These very high rates were maintained throughout the day as well as the season. Such large increases in net photosynthesis in response to brief exposure (1 day) to high CO_2 levels (1000 µl/liter) have been reported for soybean (4, 9) and pasture legumes (24). However, increases in net photosynthesis due to enrichment of legumes are more commonly 1.2-2 fold (3, 11, 14, 28, 35). The maximum mean net photosynthetic rates for enriched penultimate mainstem leaves of 'Oregon 1604' were nearly twice as great as those reported for Phaseolus vulgaris in response to either short term (1 day) (34) or long term (30) exposure to high CO_2 levels. However, such comparisons between experiments are complicated by the dependency of net photosynthesis on such factors as: position, age and developmental stage of leaves assayed; environmental conditions prior to and during measurements; and internal physiological conditions such as the plants' nutritional or water relations status.

Several factors may have contributed to maintenance of high photosynthetic rates by the penultimate leaf under enrichment. As an upper canopy leaf, it was exposed to the prevailing full sunlight during development. Furthermore, during measurements the leaf was oriented to receive full sunlight which was always above 1.4 mE $m^{-2}s^{-1}$ (Figure 3.1). The positive interaction between irradiance and CO_2 levels is well known. Photosynthetic response to CO_2

enrichment is also dependent on leaf developmental stage. When enrichment was initiated at first flower, the penultimate leaf was only 50-60% of the full expansion reached within 7 days. While final leaf area was not affected, enrichment may have induced modification within the leaf which contributed to maintenance of high rates of net photosynthesis. Hicklenton and Jolliffe (20) reported that enrichment (1000 µl CO_2/liter) of tomato plants increased net photosynthesis of a leaf at an early, but not at a later developmental stage. This increase in photosynthesis was associated with lower values of mesophyll resistance, CO_2 compensation point, photorespiration, and glycolic acid oxidase activity; but higher activity of ribulose-1,5-bisphosphate carboxylase.

The fact that the increase in leaf starch only accounts for a small percentage of the substantial increase in SLW in enriched leaves at the end of flowering suggests that anatomical modifications in the leaves occured. Adaptation of leaves to enrichment such as increases in density of palisade cells (21), number of palisade layers (33), palisade cell length and number of mesophyll layers (23) have been reported and likely contribute to their greater photosynthetic activity per unit leaf area.

The penultimate leaf maintained high photosynthetic rates even though it accumulated starch to levels several times greater than that in the controls. Starch levels may have even been higher in the afternoon, yet no decline in photosynthetic rates was evident (Figure 3.2). The starch level of 10.7 g m^{-2} in the enriched penultimae leaf at pod maturity is above the lower limit at which

Nafziger and Koller (29) reported starch inhibition of photosynthesis in soybeans grown at 0.5 mE $m^{-2}s^{-1}$ PPFD. On a percent leaf dry weight basis, the 15% starch content in enriched leaves at pod maturity is greater than the 12% starch concentrations reported to inhibit photosynthesis in tomatoes (26). This inhibition was associated with deformation of chloroplasts (25, 26). Cave et al. (5) reported that a 5% starch content in leaves of Trifolium subterraneum grown in 1000 µl CO_2/liter resulted in disruption of chloroplast structure and reduction in chlorophyll when compared to leaves of controls which had a 2.6% starch content. Hofstra and Hesketh (21) concluded that supression of net photosynthesis in leaves of enriched soybeans was mostly due to increased mesophyll resistance only part of which could be attributed to their starch content which ranged from 27%-47%. Thus, it appears there is considerable variation among species in the amount of starch that can be accomodated in leaves before adversely affecting photosynthesis. Despite the relatively large accumulations of starch in leaves of 'Oregon 1604', high rates of net photosynthesis were maintained.

Two factors which can affect starch accumulation in response to enrichment are the sink demand on the leaves and the CO_2 levels used in enrichment (26). The penultimate leaf is a primary source for the terminal mainstem raceme in 'Oregon 1604' which is a major pod bearing raceme (27). The decline in starch after pod maturity even though net photosynthesis remained high suggests that the sink demand was indeed high at the node. Madsen (25, 26) found that

enrichment of tomatoes at or above 1500 µl CO_2/liter resulted in both high leaf starch levels and reduced photosynthesis, whereas enrichment at 1000 µl CO_2/liter also caused large amounts of starch accumulation but photosynthesis was not inhibited. Inhibition of photosynthesis in tomatoes enriched at levels greater than 1000 µl CO_2/liter was linked to low ribulose-1,5-bisphosphate carboxylase activity (19). This suggests that a variance of a few hundred µl CO_2/liter may make the difference between enhancement and inhibition of photosynthesis under enrichment. However, the inhibiton range of CO_2 concentrations probably varies between species and in response to sink-source relations as well as other factors. Aoki and Yabuki (1) reported that after 9 days of enrichment of cucumber at 2400 and 5500 µl CO_2/liter, upper canopy leaves showed higher, but lower leaves lower, rates of net photosynthesis than comparable control leaves. The difference in response between upper and lower leaves may be partially attributable to differences in leaf age as well as light interception. Our results indicate enrichment at 1250 µl CO_2/liter under the conditions in the 2 outdoor experiments was below any hypothetical inhibitory CO_2 level for the penultimate leaves in beans.

In the field experiment, the rate of net photosynthesis of enriched leaves increased at the peak flowering period and shortly after pod maturity, a time when rapid seed filling begins (Figure 3.1). Other investigators have reported high rates of photosynthesis at flowering (13) and podfill (31) in beans and during podfill in soybeans (12). Also, in both field and container grown

beans, leaves of both treatments were only 50-60% expanded at 3 DAFF but reached full expansion by 7 DAFF. Maximum leaf photosynthetic rates often accompany full expansion. Therefore, the peak photosynthetic rates 7-11 DAFF of leaves of enriched field grown beans may have reflected this. However, sharp increases in photosynthetic rates were not observed in field grown controls nor during flowering of container plants making it difficult to attribute peak photosynthetic rates in these experiments to developmental events such as leaf expansion, flowering and/or seed filling. Interpretation of the peak photosynthetic rates is further complicated by the difficulty in dismissing the possible contribution of environmental factors.

The more than 50% reduction in leaf conductance observed in field grown beans under enrichment is comparable to the decreases reported for soybeans grown under elevated CO_2 levels (21, 33). In contrast, leaf conductance in tomato is essentially unaffected by enrichment (19, 20). Davis and McCree (10) showed that leaf conductance of bean leaves declines continuously from full expansion to death. This pattern is reflected in the decrease in conductance of control leaves of container plants throughout the reproductive period, whereas the conductance of enriched leaves was relatively stable. The different patterns may relate to the delay of senescence of the penultimate leaf under enrichment. Since the rate of transpiration mirrored leaf conductance, it was usually lower under enrichment which in turn contributed to a higher photosynthesis/transpiration ratio at the elevated CO_2 level. The

higher ratio was, however, more a result of a higher photosynthetic rate so that even when transpiration of enriched leaves was greater than controls, as on 35 DAFF in the container study, the photosynthesis/transpiration ratio was still greater. The higher ratio may have reduced water stress in enriched plants similar to the observation of Rogers et al. (33) with enrichment of soybean, corn and sweetgum. Reduced water stress under enrichment may have contributed to the maintenance of high photosynthetic rate throughout the reproductive period.

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Chapter 4

EFFECTS OF CO_2 ENRICHMENT DURING FLOWERING AND PODFILL ON DRY MATTER ACCUMULATION AND YIELD IN FIELD GROWN BEANS, *PHASEOLUS VULGARIS* L.

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Additional index words. specific leaf weight, starch.

Abstract. In the first of 2 field experiments 'Oregon 1604' (snap bean) and 'Royal Red' (kidney bean) were enclosed in open top chambers just prior to flowering and exposed for 12 hr during the day to either CO_2 enriched (1250 µl CO_2/liter) or ambient air (340 µl/liter CO_2) during either flowering, podfill or flowering plus podfill (continuous). At other times plants received ambient air. Enrichment during flowering increased shoot dry weight of 'Oregon 1604' and 'Royal Red' by 38% and 30% respectively, at the end of flowering. By pod maturity the difference between continuously enriched and controls was no longer significant. Enrichment increased the number of pods per plant at the end of flowering in both cultivars but these were not maintained at pod maturity. CO_2 enrichment did not significantly affect seed yield or yield components. In a second experiment plants of 'Oregon 1604' were either enriched or received ambient air continuously during

flowering and podfill. Enrichment increased shoot dry weight by 67%, 42% and 22% at end of flowering, pod maturity and seed maturity, respectively. Greater vegetative (leaf and stem) dry weight accounted for 47%, 59% and 32% of the shoot dry weight increase at the 3 harvests, respectively. Dry weight increases of enriched leaves were associated with approximate 25% greater specific leaf weights. Starch accounted for about 50% of the observed increases in specific leaf weight for enriched plants at the pod and seed maturity harvests. Continuous enrichment increased the number of filled pods at end of flowering, pod maturity and seed maturity. Higher number of pods did not translate into increased seed dry weight per plant due to fewer seeds per pod. Weight per seed was not affected. The decline in difference between shoot dry weight of enriched and control plants by pod maturity in both experiments suggests that the enhancement of net photosynthesis by continuous enrichment during flowering was not maintained during the entire post flowering period. The greater number of pods due to enrichment during flowering strongly supports the hypothesis that available photosynthate is a limiting factor to initial pod set.

Introduction

Available photosynthate (source) during flowering and early podfill may be the primary limiting factor of yield of beans (1, 25, 27) and soybeans (12). Overlap of vegetative and reproductive growth is a major cause of the photosynthate deficiency (12, 15, 27). The number of pods per plant is the most limiting and sensitive yield component (1). The principal regulation of the number of pods per plant is via abscission. Abscission rates are dependent on pod load, water deficits, extreme temperatures, irradiance levels and other factors (18). Many of the above factors directly or indirectly affect photosynthesis which implies a probable relationship. Experimental manipulation of the source/sink ratios in legumes also suggests the involvement of photosynthesis. Increasing the source/sink ratio through pod removal (10, 23, 27) or CO_2 enrichment (6, 7, 8, 12, 13) has been shown to improve pod set whereas it was reduced by shading (17) or leaf removal (25), which lowered the source/sink ratio.

Adjustment of pod numbers to insufficient assimilate during flowering and early podfill may result in beans being sink limited during seed development (4, 25). Alleviating the source limitation during flowering and pod development should increase number of pods retained and result in greater seed yield.

The object of this research was to improve the photosynthate supply during flowering and/or podfill by CO_2 enrichment in order to evaluate its role as a limiting factor in dry matter accumulation, pod set and seed yield in beans.

Materials and Methods

Field experiments were conducted in 1980 and 1981 at the Oregon State University Vegetable Research Farm on a Chehalis silty clay loam soil. Prior to planting, the insecticide fonofos and the herbicide trifluralin were incorporated into the soil and fertilizer broadcast at the rates of 90, 116 and 74 kg ha^{-1} of N, P and K, respectively. Two determinant cultivars, 'Oregon 1604', a snap bean, and 'Royal Red', a kidney bean, were grown in 1980, but only 'Oregon 1604' in 1981. Seeds were hand planted at 13 cm intervals in 4 rows, each separated by 23 cm resulting in an approximate plant density of 34 plants m^{-2}. Sprinker irrigation was provided as needed, about 3.6 cm water every 7-10 days.

1980. Portions of 2 middle rows were enclosed in 32 separate wood framed chambers (51 cm x 71 cm and 91 cm high) on July 18, just prior to first flower (defined as the day anthesis occured at the lowest node of the terminal mainstem raceme). The sides of the chamber were covered with clear plastic but tops were open to minimize temperature and RH build up. Chambers elevated daylight air temperatures by only 1-3°C. The outer plant rows bordered the chambers, each of which enclosed 10 plants. Each of 4 blower fan assemblies provided air via separate lengths of 10 cm diameter plastic dryer duct to 8 chambers. Perforated duct ran the length of each chamber between the rows and supplied air at approximately 1.2 $m^3 min^{-1}$. Half the chambers received supplimental CO_2 from cylinders of compressed CO_2 via Tygon tubing entering the air supply at the

chamber base. The amount of CO_2 added to each chamber was controlled by separate fine metering valves.

Treatments consisted of exposing enclosed plants to 1250 ± 250 µl CO_2/liter between 0800 and 2000 hr daily, during different developmental stages as follows: (a) flowering, from first flower to the end of flowering; (b) podfill, from end of flowering through seed maturity; (c) both flowering and podfill (continuous); and (d) control, not enriched. When not enriched, plants received ambient air of 340 ± 20 µl CO_2/liter, as did control plants. Plants were harvested with respect to days after first flower (DAFF), as follows: preflowering (0 days) just before enclosing plants; end of flowering (16 days); pod maturity (27 days); and seed maturity (53 days). Pod maturity as used here indicates the stage for commercial snap bean harvest with 50% of the pods being in sieve sizes 1-4. Seed maturity describes seeds in undehisced, non-green, dry pods which have a moisture level in the ranges of 10-15%. At the preflowering harvest, 2 plants adjacent to each chamber were harvested. At each of the 3 harvests, 2 plants on opposite sides of the air duct within each chamber were harvested. The first, third and fifth plant pairs were harvested sequentially, starting at either end wall as chosen randomly for each chamber. The second and fourth plant pairs remained as internal borders throughout the experiment.

Prior to 0900 hr plants were cut off at the soil surface, labelled, transported on ice to the laboratory and separated into leaves, stem, flowers and pods. Counts were made of the number of: branches per mainstem node, nodes per branch, leaves per plant, pods

per node, filled pods per node and flowers per node. "Filled pods" were defined as: pods having at least one developing seed at pod maturity and pods containing one or more mature seeds at seed maturity. Leaf area per plant was measured using a LI-COR Model 3100 Area Meter (LI-COR, Lincoln, Nebraska). Tissues were weighed after drying at 80°C for 48 hr. Specific leaf weight (SLW) was obtained by dividing plant leaf dry weight by leaf area per plant. The number of seeds per node was counted at the seed maturity harvest.

1981. 'Oregon 1604' was exposed to 340 ± 20 µl CO_2/liter (control) or 1250 ± 250 ul CO_2/liter (enriched) from first flower through seed maturity. CO_2 was administered as in 1980 except that each of the 8 chambers per treatment was 20 cm longer, enclosed 14 plants and received air at $1.3 m^3 min^{-1}$. Two plants per chamber were harvested at each of the 4 following stages presented with DAFF: preflower (0 days, July 28), end of flowering (15 days), pod maturity (22 days) and seed maturity (45 days). Harvest procedures were similar to 1980 with analagous data collected.

To facilitate analysis of leaf starch, all leaves from each harvested plant were dried, combined and ground. This tissue was extracted with chloroform:methanol:water (12:5:3 by volume) (11). The residue was incubated with amyloglucosidase and the liberated glucose was determined using an enzymatic (glucose oxidase and peroxidase) colorimetric method (Sigma Chem. Co.).

Analysis of variance was used for statistical evaluation of the data. In 1980, a split split plot design was used with cultivars as

the mainplot, CO_2 treatments as subplots and harvests as sub-subplots. In 1981, a split plot design was used with CO_2 treatments as the mainplot and harvests as subplots. LSD's were used for mean separation.

Results

Dry Matter Accumuation

1980. Enrichment for 16 days during flowering increased shoot dry weight of both cultivars (Table 4.1). In 'Oregon 1604' the 38% increase was due to dry weight increases of 56% in leaves and 28% in stems, whereas in 'Royal Red' enrichment enhanced the dry weight of leaves and stem by approximately 30% each.

Enrichment treatments had little significant effect on dry weight of plant parts harvested at either pod or seed maturity (Table 4.1). Variability was greater than expected and insufficient replication was used in the experiment. Although not significant, shoot dry weight of plants enriched continuously for 27 days (flowering and podfill) was 21% ('Oregon 1604') and 32% ('Royal Red') greater than controls. These differences diminished to 8% and 11%, respectively, at seed maturity.

Vegetative growth continued during flowering as total leaf area of 'Oregon 1604' increased 16% for controls and 45% for enriched plants. Although not significant, leaf area of enriched 'Oregon 1604' was 38% greater than controls at the end of flowering but by pod maturity leaf area was similar for all treatments. Leaf area of 'Royal Red' increased by over 90% during flowering but there was no difference between treatments at any harvest. In both cultivars maximum leaf area was measured at the end of flowering. Leaf senescence began before flowering with the primary leaves and progressed acropetally. By seed maturity both cultivars had less than 20% of their maximum leaf area. There was no other indication that enrich

Table 4.1. Effect of CO_2 enrichment on leaf, stem, pod and shoot dry weight of 'Oregon 1604' and 'Royal Red'.

		'Oregon 1604'			'Royal Red'		
		Harvest			Harvest		
Plant part	Treatment	End of Flowering	Pod Maturity	Seed Maturity	End of Flowering	Pod Maturity	Seed Maturity
		dry wt.(g)/plant					
Leaf	Control	6.6	6.5	0.9	9.0	8.5	2.5
	Flowering	10.3*	6.3	1.7	11.9*	8.6	2.9
	Podfill		6.2	0.9		8.6	3.2
	Continuous		9.4	1.7		11.7*	2.3
Stem	Control	4.0	4.3	3.0	5.2	5.9	5.5
	Flowering	5.1*	4.4	3.3	6.7*	6.2	5.5
	Podfill		3.7	3.2		5.0	6.0
	Continuous		5.1	3.5		8.1	5.5
Pod	Control	3.0	10.5	22.4	1.4	5.6	26.4
	Flowering	3.2	9.5	24.0	1.7	6.8	30.9
	Podfill		9.6	23.2		4.3	34.0
	Continuous		11.3	23.8		8.0	29.4
Shoot	Control	12.5	21.3	26.4	15.5	21.0	34.3
	Flowering	18.6*	20.1	29.0	20.3*	21.6	38.5
	Podfill		19.5	27.3		17.8	43.2*
	Continuous		25.8	28.9		27.8	37.1

*Significantly different from control at 0.05 level.

ment increased plant size as determined by plant height, number of leaves, branches, mainstem nodes or branch nodes.

1981. Continuous enrichment during the reproductive period of 'Oregon 1604' increased shoot dry weight over the control by 67% at end of flowering, 42% at pod maturity and 22% at seed maturity (Figure 4.1). Greater vegetative (leaf and stem) dry weight accounted, respectively, for 47%, 59% and 32% of the shoot dry weight increase at these harvests (Figures 4.2, 4.3), remainder was the result of higher pod dry weight.

Specific leaf weight declined in both treatments during the reproductive periods (Figure 4.4). However, SLW of enriched plants was about 26% greater than that of the controls at the end of flowering and pod maturity harvests. Leaf starch levels of enriched plants increased rapidly after the end of flowering and were 7 fold greater than the controls at pod maturity (Figure 4.4). However, by seed maturity starch levels of enriched leaves substantially declined but were still 3 fold greater than the controls. Leaf starch accounted for less than 3% of the difference in SLW between enriched and control plants at the end of flowering harvest. However, about 50% of the observed increases in SLW for enriched plants at the pod and seed maturity harvests was due to higher leaf starch.

During the period between first flower and pod maturity, leaf area of enriched plants increased by 75% versus 49% for the controls. Although not significant, enriched plants had a 24% greater maximum leaf area than the controls at pod maturity. Leaf area then declined and less than 10% of the maximum leaf area

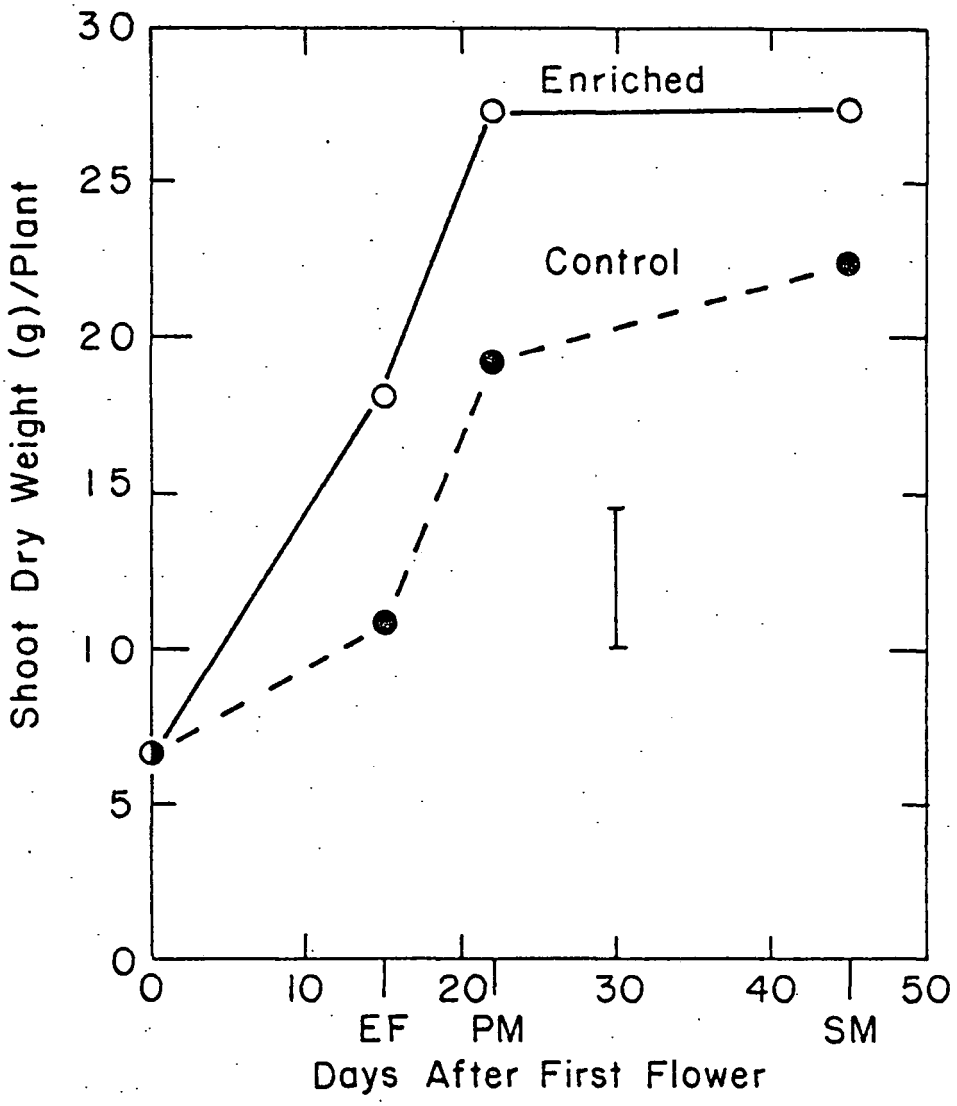

Fig. 4.1. Effect of CO_2 enrichment of 'Oregon 1604' from flowering through seed maturity on shoot dry weight per plant. Each point represents mean of 16 plants. EF, PM and SM point out the end of flowering, pod and seed maturity harvests, respectively. Vertical bar equals LSD at 0.05 level.

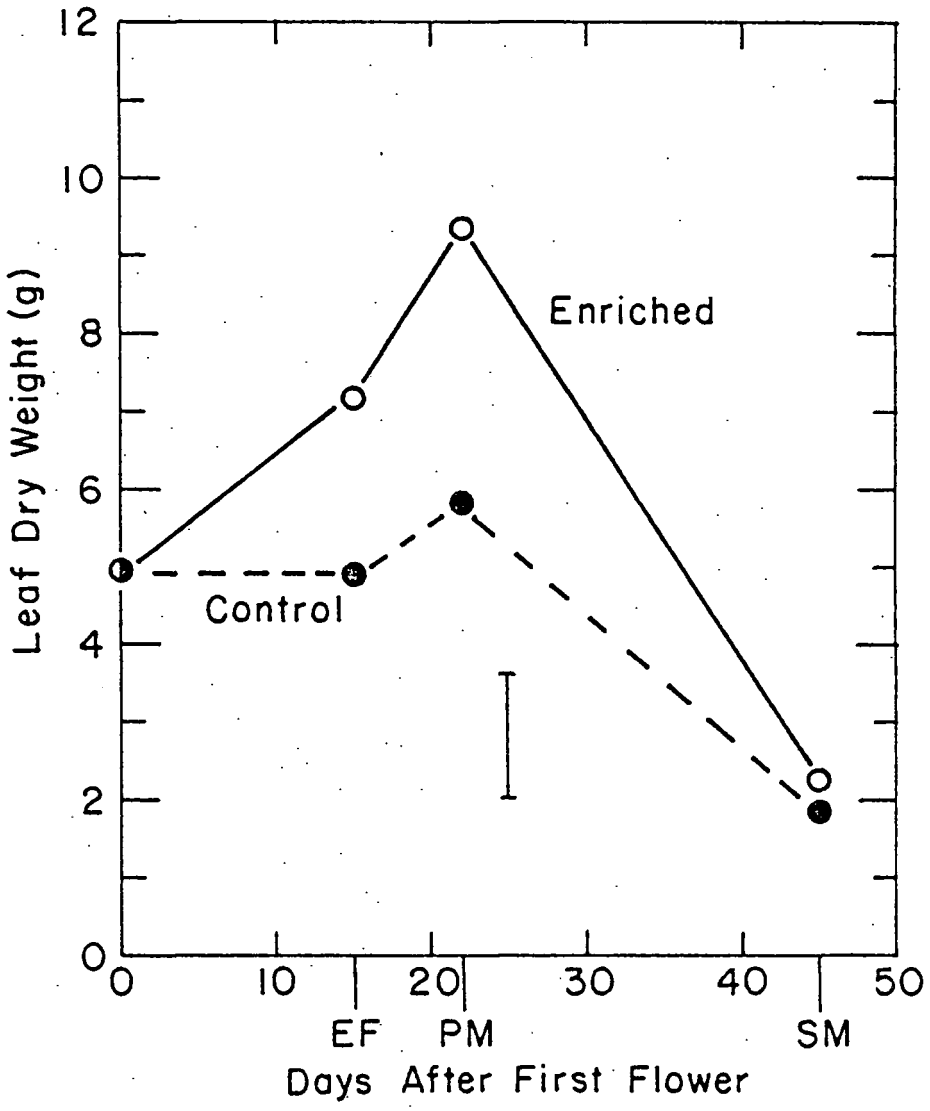

Fig. 4.2. Effect of CO_2 enrichment of 'Oregon 1604' from flowering through seed maturity on leaf dry weight per plant. Each point represents mean of 16 plants. Vertical bar equals LSD at 0.05level.

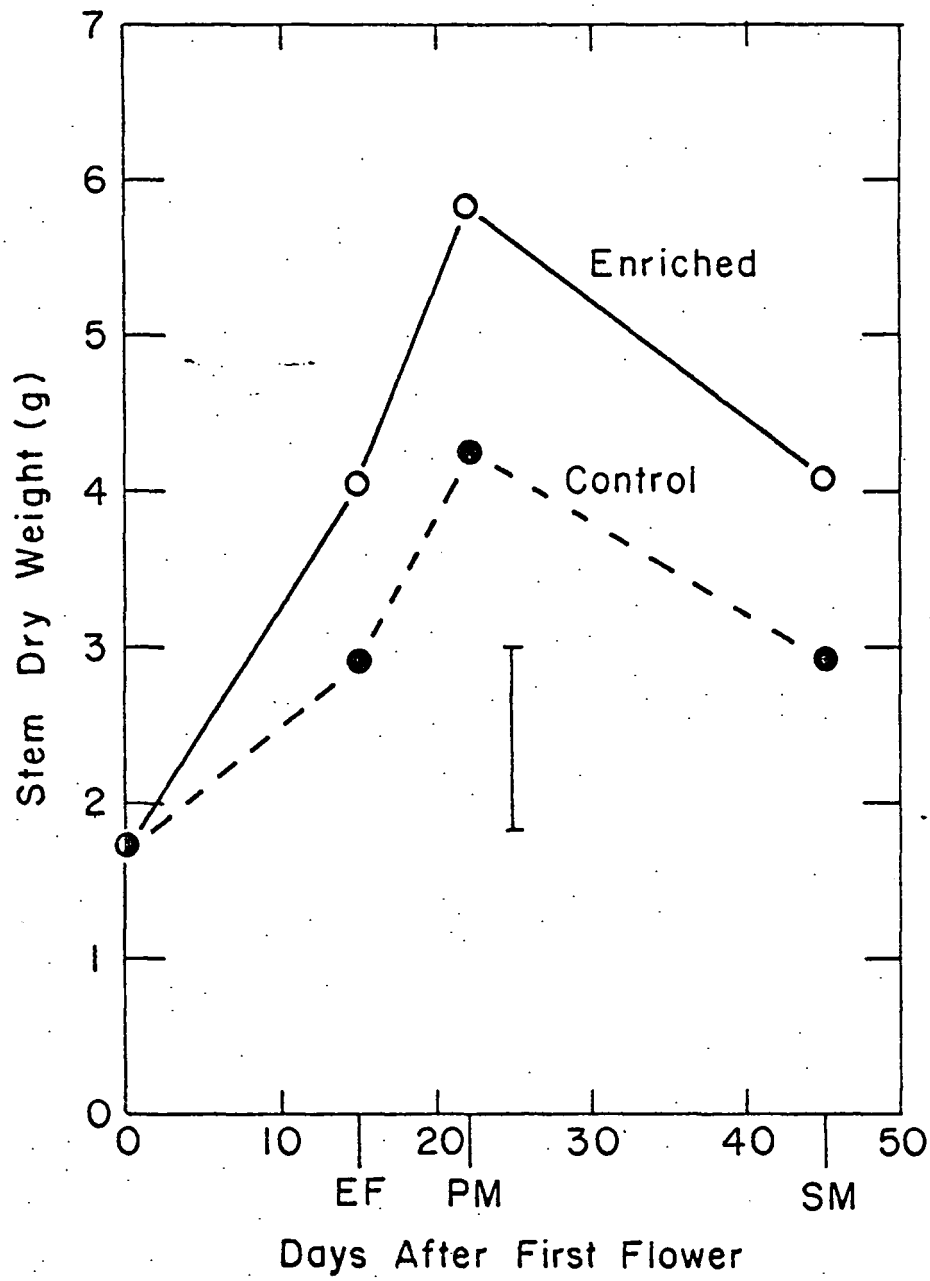

Fig. 4.3. Effect of CO_2 enrichment of 'Oregon 1604' from flowering through seed maturity on stem dry weight per plant. Each point represents mean of 16 plants. Vertical par equals LSD at 0.05 level.

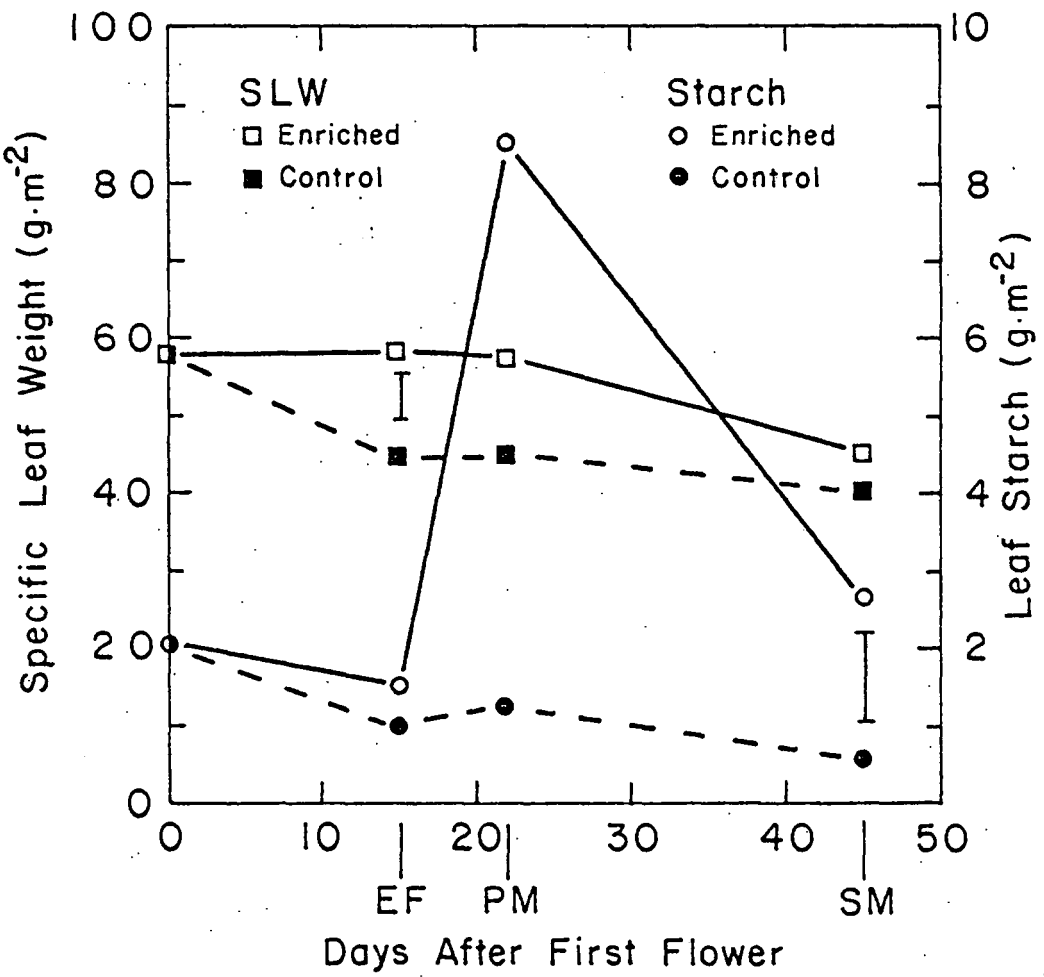

Fig. 4.4. Effect of CO_2 enrichment of 'Oregon 1604' from flowering through seed maturity on specific leaf weight and leaf starch. Each point represents mean of 16 plants. Vertical bar equals LSD at 0.05 level.

remained at seed maturity. Enrichment did not significantly affect plant height, number of leaves, number of branches or number of mainstem or branch nodes. Thus, the observed increases in vegetative dry weight were principally due to secondary growth and/or the accumulation of reserve materials in existing tissues.

Yield Components

1980. Enrichment during flowering caused a significant increase in the number of pods per plant at the end of flowering for both cultivars relative to the controls (Table 4.2). Continuous enrichment during flowering and podfill resulted in apparent but non-significant increases of total number of pods per plant of 17% ('Oregon 1604') and 23% ('Royal Red') at pod maturity compared to controls. Plants enriched only during flowering did not maintain their high pod number at seed maturity. In plants of 'Oregon 1604' enriched during flowering, nearly all the pod drop occurred between the end of flowering and pod maturity; whereas in comparable plants of 'Royal Red', pod loss was greatest after pod maturity. Number of pods per plant decreased in all treatments throughout the experiment. Enrichment only during podfill did not increase the number of pods or filled pods at either pod or seed maturity harvests. Enrichment treatments did not significantly affect pod dry weight per plant in any cultivar at any harvest.

Enrichment of 'Royal Red' during podfill increased by 30% both total number and weight of seeds per plant; however, these increases were not statistically significant (Table 4.3). Enrichment did not

Table 4.2. Effects of CO_2 enrichment during developmental stages on number of total pods per plant, number of filled pods per plant and pod dry wt. per plant at 3 harvest dates in 1980.

		'Oregon 1604'			'Royal Red'		
		Harvest			Harvest		
	Treatment	End of Flowering	Pod Maturity	Seed Maturity	End of Flowering	Pod Maturity	Seed Maturity
Total number of pods/plant	Control	36.6	37.4	25.5	20.8	23.5	17.0
	Flowering	42.1*	25.5	27.1	26.8*	23.8	17.6
	Podfill		37.4	23.8		17.9	19.3
	Continuous		43.6	24.9		28.9	17.1
	Mean	39.4	36.0	25.3	23.8	23.5	17.8
Number of filled pods /plant	Control		19.6	16.0		12.0	11.5
	Flowering		18.3	15.9		13.8	14.3
	Podfill		17.5	15.0		7.9	14.8
	Continuous		21.9	16.8		14.1	12.7
	Mean		19.3	15.9		11.95	13.3
Pod dry wt.(g) /plant	Control	3.0	10.5	22.4	1.4	5.6	26.4
	Flowering	3.2	9.5	24.0	1.7	6.8	30.9
	Podfill		9.6	23.2		4.3	34.0
	Continuous		11.3	23.8		8.0	29.4
	Mean	3.1	10.2	23.4	1.6	6.2	30.2

*Significant at 0.05 level.

Table 4.3. Effects of CO_2 enrichment on seed yield components of 'Oregon 1604' and 'Royal Red' at maturity in 1980.

	Yield Components	Treatments			
		Control	Flowering	Podfill	Continuous
'Oregon 1604'	Number of seeds/plant	73.3	69.2	76.5	74.8
	Dry wt.(g)/seed	0.23	0.25	0.24	0.22
	Seed dry wt.(g)/plant	16.72	17.32	16.62	16.23
	Number of seeds/filled pod	4.6	4.4	4.7	4.5
	Seed dry wt.(g)/filled pod	1.04	1.09	1.11	0.97
'Royal Red'	Number of seeds/plant	38.9	44.9	52.0	45.5
	Dry wt.(g)/seed	0.46	0.46	0.44	0.44
	Seed dry wt.(g)/plant	17.75	20.66	23.00	19.97
	Number of seeds/filled pod	3.4	3.1	3.5	3.6
	Seed dry wt.(g)/filled pod	1.54	1.44	1.55	1.56

affect the number or weight of seeds per plant for 'Oregon 1604', nor was seed size, as determined by the dry weight per seed, affected by any enrichment treatment.

1981. Enrichment caused a non-significant increase in the total number of pods per plant at all 3 harvests (Table 4.4). Number of filled pods per plant at the end of flowering was 13% greater in enriched beans relative to the controls. Continued exposure of plants to 1250 µl CO_2/liter increased number of filled pods per plant by 30% and 34% at the pod and seed maturity harvests, repectively. The larger number of filled pods increased pod dry weight per plant by 148%, 35% and 20% at the end of flowering, pod maturity and seed maturity harvests, respectively.

Enrichment failed to significantly increase the number or dry weight (yield) of seed per plant (Table 4.5). The number of seeds per filled pod declined 15% under enrichment, which caused a significant decrease in seed dry weight per filled pod. Enrichment had no effect on dry weight per seed.

Table 4.4. Effects of enrichment on number of pods per plant, number of filled pods per plant and pod dry wt. per plant of 'Oregon 1604' at 3 harvests in 1981.

	Harvest					
	End of Flowering		Pod Maturity		Seed Maturity	
	Control	Enriched	Control	Enriched	Control	Enriched
Number of pods/plant	25.8	29.1	33.6	37.7	19.0	23.7
Number of filled pods/plant			18.7	24.3*	11.9	16.0*
Pod dry wt.(g)/plant	2.24	5.56*	8.07	10.92	17.17	20.60*

*Significant at 0.05 level.

Table 4.5. Effects of CO_2 enrichment on seed yield components of 'Oregon 1604' at seed maturity in 1981.

Yield Component	Control	Enriched
Number of seeds/plant	54.1	63.6
Dry wt.(g)/seed	0.21	0.20
Seed dry wt.(g)/plant	11.53	12.85
Number of seeds/filled pod	4.58	4.01
Seed dry wt.(g)/filled pod	0.97*	0.81

*Significant at 0.05 level.

Discussion

CO_2 enrichment substantially increased shoot dry weight of 'Royal Red' (1980) and 'Oregon 1604' (1980, 1981) at the end of flowering. However, by seed maturity the magnitude of the increases in response to continuous enrichment was much less, only 8% to 22%. These dry weight gains due to CO_2 enrichment are considerably less than those reported by others for field grown enriched beans and soybeans (7, 12, 13). For example, enrichment of beans just before and during flowering or through podset increased plant dry weight 35% and 41%, respectively, at seed maturity (7).

Possible explanations for the apparent failure of enrichment to maintain the higher shoot dry weight observed at the end of flowering until seed maturity include: greater loss of dry weight by leaf and/or pod abscission with enrichment, increased partitioning to roots, higher rates of dark respiration and/or a decline in whole plant photosynthesis. Enrichment did not enhance pod or leaf abscission enough to account for the subsequent loss of dry weight gained during flowering. Increased partitioning of dry matter to the roots remains a possibilty because the efforts to quantitatively recover roots from the field grown beans were unsuccessful. There are several reports of increased root/shoot ratios in response of legumes to CO_2 enrichment (20, 26). Hardman and Brun (12) suggested that greater partitioning to the root may be a possible explanation for the absence of shoot dry weight increases in soybeans enriched prior to flowering. They also suggested higher respiration may account for this lack of an enrichment effect on shoot dry weight.

Using CO_2 levels to alter photosynthetic rates, Ludwig et al. (16) found that the rate of dark respiration of a tomato leaf increased linearly with a rise in the previous net photosynthetic rate integrated over an 8 hr photoperiod. Kendall and Thomas (15) reported that loss of carbon by dark respiration accounted for much of the extra carbon fixed during grain growth in CO_2 enriched wheat. Since the field grown plants in the present study were nodulated, enrichment could have stimulated greater partitioning to roots through increases in nodule formation and activity. Long term CO_2 enrichment of soybean (9) and pea (21) increased total nodule activity, and Williams et al. (28) reported that enrichment (1000 µl CO_2/liter) for 5 days stimulated root plus nodule respiration as well as N_2 (C_2H_5) fixation.

The other explanation for convergent plant dry weights of enriched and control plants at pod and seed maturity is that CO_2 enrichment may not have enhanced net photosynthesis on a whole plant basis for the entire period between end of flowering and seed maturity. This seems to be contradictory to the 3-6 fold increases in net photosynthesis of the penultimate leaf of enriched plants relative to the controls that were maintained throughout the reproductive period (Chapter 3). However, this leaf was an upper canopy leaf, was younger than the 'average' leaf and was oriented for maximum light interception during photosynthetic measurements. Therefore, photosynthetic rates of the penultimate leaf might not have been reflective of canopy photosynthetic rates. Prolonged enrichment could have reduced photosynthesis. Aoki and Yabuki (3) found

that the higher net photosynthetic rates of enriched (2400, 5500 µl CO_2/liter) upper canopy leaves of cucumber were maintained, whereas photosynthesis of lower canopy leaves, although initially enhanced, decreased below the rate of control leaves at 9 days (2400 µl CO_2/liter) and 14 days (5500 µl CO_2/liter) after enrichment had begun.

The large increase in starch levels in enriched leaves between the end of flowering and pod maturity in 1981 (Figure 4.4) suggests that photosynthesis was still higher in enriched than control plants for at least part of this period. High leaf starch levels have been correlated with declines in net photosynthesis of CO_2 enriched soybean leaves (14, 19), and have been associated with possible detrimental modifications in Desmodium paniculatum (29) and disruption in the chloroplast of Trifolium subterraneum (5) The starch level for enriched leaves at pod maturity of 8.5 g m^2 (14.6% leaf dry wt.) was lower than the inhibitory level of 10 g m^2 in soybeans reported by Nafziger and Koller (19) but higher than the disruptive levels of 5% dry weight in T. subterraneum reported by Cave et al. (5). However, the leaf starch level for the whole plant at pod maturity was less than the leaf starch level of 10.7 g m^2 (15.0% dry wt.) of the penultimate leaf in which high net photosynthesis was maintained throughout the reproductive period (Chapter 3).

Enrichment induced increases in number of total pods (1980), filled pods (1981), and pod dry weight (1981) at end of flowering support the hypothesis that available photosynthate during flowering is a limiting factor to pod set in beans. Photosynthate supply was

apparently most critical during the flowering period since limiting enrichment to a later period did not increase the pod load. Other investigators have reported greater pod set in response to CO_2 enrichment during flowering, as well as a positive effect of post-flowering enrichment (7, 12, 13). Enrichment of an indeterminant bean cultivar from 5 days before to 15 days after initial flowering increased the number of pods retained at seed maturity by 19% over the controls (7). But when enrichment continued into seed filling, the number of pods was 34% higher than comparable controls. Similarly, Hardman and Brun (12) reported increased number of pods per soybean plant of 46%, 20% and 61% in response to enrichment during flowering, post-flowering and both periods, respectively. The apparent additive effect supports a role of available photosynthate during flowering and post-flowering in determining the number of pods per plant in legumes. Following the termination of enrichment of 'Oregon 1604' at the end of flowering in 1980, number of pods per plant fell from 42 to 25 at pod maturity (Table 4.2). The failure to retain the extra pods set during flowering may have resulted from the presumed decrease in photosynthesis upon returning to ambient CO_2 levels. Abrupt termination of enrichment might also effect other processes important to pod set (e.g., water status and levels of growth regulators). When 'Oregon 1604' was enriched continuously to seed maturity, the number of pods per plant did not decrease between end of flowering and pod maturity (Tables 4.2,4.4).

The absence of higher seed yield (seed weight per plant) in response to any of the enrichment treatments in either year suggests

that yield was not limited by available photosynthate or that the enrichment treatments did not enhance the supply of available photosynthate to developing seeds. In 1980 the increase due to enrichment in the number of pods per plant at the end of flowering was already lost at the pod maturity harvest, 11 days later (Table 4.2). Since number of pods per plant is the primary yield component, little subsequent effect of enrichment would be expected. In 1981 continuous enrichment improved potential seed yield by increasing the number of filled pods per plant at seed maturity (Table 4.4). However, a compensatory decline in number of seeds per pod without an increase in seed size resulted in lower yield per pod (Table 4.5), preventing higher seed yield per plant. These results are in contrast to reports that enrichment during flowering and/or post-flowering increases seed yield of legumes (7, 8, 12). Work at CIAT (7) showed that enrichment at 1200 µl CO_2/liter for 20 days, starting 5 days before flowering, increased seed yield on an indeterminant bean cultivar by 40%. Enrichment for a further 20 days, however, resulted in only an additional 3% gain. These higher yields were attributed to increased number of seeds, due primarily to a greater number of pods per plant. Higher number of seeds was also the cause of the observed increase in soybean yield with enrichment (8, 12). As reported here, enrichment did not increase seed size in either cultivar. However, small increases, less than 10%, in the number of beans per pod contributed to higher seed yield of bean (7) and soybeans (8) under enrichment.

The decreased number of seeds per filled pod of enriched plants in the 1981 experiment suggests that a compensatory relationship exists between the number of filled pods per plant and the number of seeds per pod. Adams (1) adjusted the pod load of bean plants by removing young pods and found that both number of seeds per pod and dry weight per seed decreased as number of pods increased. He proposed that such compensation occurs among yield components in response to competition for a limited supply of photosynthate or other nutrients. Tanaka and Fujita (25) reported that number of seeds per pod declined in response to inadequate carbohydrate supply in bean plants prior to seedfill. They also found that the number of seeds per pod was established before seedfill began. It is difficult to attribute the decrease in seed number per pod in enriched 'Oregon 1604' to competition for insufficiant photosynthate since very high levels of starch accumulated in leaves during the period between end of flowering and pod maturity. However, large starch reserves in beans may not necessarily be available to developing seeds (2). Streeter et al. (22) found that starch stored in the leaf blade was relatively unavailable for seedfill in soybean. Yet, the decline in leaf starch (Figure 4.4) during seedfill suggests that it was available during this period. However, leaf starch may have been remobilized to stem and root tissues rather than to seeds. Tanaka and Fujita (25) found that the carbohydrate level in the stem of a determinant bean cultivar increased markedly during the seed growth stage. Stem dry weight also increased during that period, in contrast to the decline observed in 'Oregon 1604'

(Table 4.1, Figure 4.3) The decrease in the number of seeds per pod may have been in response to a deficiency of some other nutrient or metabolite. Alternatively, the number of seeds per pod may be regulated by hormone levels. Tamas et al. (24) linked the control of premature senescence and abscission of younger bean pods by older pods within a raceme to their endogenous ABA levels. Since cessation of seed development precedes pod abscission, seed number per pod may be regulated by ABA levels.

In conclusion, the increase in pod number at the end of flowering due to enrichment strongly supports the idea that available photosynthate is a limiting factor to bean yield during flowering. The failure to increase seed yield by CO_2 enrichment suggests factors other than photosynthate availability may be limiting seed set and growth.

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