This is to certify that the

dissertation entitled

Genotypic Variation in Response to Elevated Atmospheric Carbon Dioxide in Two Populations of *Plantago lanceolata* L.

presented by

Dawn Jenkins Klus

has been accepted towards fulfillment of the requirements for

Ph. D. degree in Botany

Major professor

Date November 17, 1995

GENOTYPIC VARIATION IN RESPONSE TO ELEVATED ATMOSPHERIC CARBON DIOXIDE IN TWO POPULATIONS OF PLANTAGO LANCEOLATA L.

By

Dawn Jenkins Klus

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1995

ABSTRACT

GENOTYPIC VARIATION IN RESPONSE TO ELEVATED ATMOSPHERIC CARBON DIOXIDE IN TWO POPULATIONS OF PLANTAGO LANCEOLATA L.

By

Dawn Jenkins Klus

The nature and the magnitude of possible adaptive evolutionary changes in response to elevated atmospheric CO_2 will be determined by genetic variation in that response within populations and species. This study documented genotypic variation in the phenotypic response to elevated CO_2 for maternal families of two populations of *Plantago lanceolata* grown in open topped chambers. Three groups of traits were measured over the course of one growing season: physiology, growth and biomass allocation.

The overall effect of elevated CO_2 did not appear to affect plant size traits as much as the allocation of photosynthate due to increased assimilation rates in the elevated CO_2 environment. Root: shoot ratios and assimilation rates increased for the elevated CO_2 grown plants; specific leaf area and stomatal conductance declined; nitrogen allocation to aboveground tissues increased. It appeared that *Plantago lanceolata* was flexible in terms of integrating its physiological processes with patterns of nutrient and biomass allocation within the plant. In ambient and elevated CO_2 environments, there were highly significant population level differences for most variables. Significant CO_2 x population

interactions were detected for physiological and growth traits. Significant CO_2 x family interactions were detected for nutrient allocation, physiological and growth traits. Individual families varied greatly in the magnitude and direction of response to elevated CO_2. Some families responded positively, some negatively, and some not at all to the elevated CO_2 environment. Genotype x environment interactions resulted in families changing rankings with respect to one another across ambient and elevated CO_2 environments, thus rendering it difficult to predict the performance of a family in elevated CO_2 from its performance in ambient CO_2. Because the response of the populations and the families of *P. lanceolata* to elevated CO_2 was not uniform, some or all of the changes in physiology and allocation patterns may ultimately help certain families of *Plantago lanceolata* to survive, compete, and reproduce with greater success in an elevated CO_2 world. Such variety in response to elevated CO_2 at the family level has implications for both intra- and interspecific competitive ability in elevated CO_2 and suggests that elevated atmospheric CO_2 may have the potential to act as an agent of natural selection.

Dedicated to

my parents, Ruth and Louis Jenkins

my husband, John Klus

and to my son, Nicholas Klus

ACKNOWLEDGEMENTS

I would like to acknowledge Susan Kalisz, Steve Tonsor, Peter Curtis and James Teeri for their support: moral, material, and financial. Without their collaboration, this project would not have been a success.

Everyone at the W. K. Kellogg Biological Station, past and present, has inspired me, encouraged me, and given me valuable assistance whenever I needed it. My thanks to all of you, but especially to:

Denise Thiede	Paco Moore	Ann O'Neill	Kay Gross
Kim Thompson	Jon Ervin	Bryan Foster	Phil Robertson
Pam Woodruff	Nina Consolatti	Andy Turner	Hal Collins
Sandy Halstead	Deb Walker	Pete Smith	Carolyn Miller
Helen Garchow	Carolyn Hammarsjkold	Char Adams	Alice Gillespie

"On location" thanks go to Brenda Casper, Kel Wiederman, and Scott Starr for making a place for me in Philadelphia.

My new friends among the faculty and staff of Sayre School in Lexington, Kentucky, have provided me with constant encouragement. Thank you.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

Table	Page

Table	Page

LIST OF FIGURES

Figure Page

Figure Page

LIST OF ABBREVIATIONS

Assimilation (μmol CO_2 m^{-2} s^{-1})..A

Transpiration (mmol H_20 m^{-2} s^{-1})...E

Water Use Efficiency (mmol CO_2 mol^{-1} H_20)....................................WUE

Stomatal Conductance (mol H_20 m^{-2} s^{-1})...G_s

Intercellular CO_2 (μliter $liter^{-1}$)...C_i

Specific Leaf Area (cm^2 g^{-1} dry weight)...SLA

Relative Growth Rate (g biomass g^{-1} day^{-1})..RGR

INTRODUCTION

Evidence is now unequivocal that atmospheric CO_2 levels are increasing worldwide (Keeling, 1986). Predictions indicate that the increase will continue well into the next century, with a doubling of current CO_2 levels by 2050 being likely (Strain, 1987). Numerous studies have established what is now considered a picture of the "typical" whole plant response to elevated CO_2, at least in the short term. Exposure to elevated CO_2 results in increased photosynthetic rates, decreased transpiration and stomatal conductance, and increased water use efficiency (see Bazzaz, 1990, for a review). Accompanying these physiological changes, alterations in tissue characteristics and biomass allocation often result in decreased specific leaf area (SLA), increased relative growth rate (RGR), and increased root: shoot ratios (Bazzaz, 1990).

However, as the variety of species studied and length of experiments have increased, considerable interspecific variation in the magnitude and duration of this "typical" response to elevated CO_2 has been documented. Assimilation rates may undergo negative acclimation, declining over time to a point equivalent to or even lower than that of plants photosynthesizing in ambient CO_2 (DeLucia *et al.*, 1985). Decreased stomatal conductance (G_s) may bring about increased leaf temperatures and vapor pressure deficits at the leaf surface, resulting in increases in transpiration (Bowes, 1993). Short-term increases in relative growth rate (RGR) may decline over time; early gains in biomass in

plants exposed to elevated CO_2 may not be maintained (Larigauderie *et al.*, 1988; but see Poorter *et al.*, 1988). Biomass allocation patterns in response to elevated CO_2 appear to species-specific, with certain species tending to increase root: shoot ratios in an elevated CO_2 environment, while other species do not (see Woodward *et al.*, 1991, and Stulen and den Hartog, 1993, for reviews).

Complicating the picture even further is the fact that environmental conditions also affect the magnitude and duration of response to elevated CO_2. For example, high nutrient environments may magnify and prolong CO_2 - mediated changes in physiological and growth processes, while low nutrient conditions may have the opposite effect (Patterson and Flint, 1982; Larigauderie *et al.*, 1988). Moreover, the nature of the response to elevated CO_2 may be an intrinsic, genetically determined trait of certain species. For example, plant species from less productive communities such as serpentine grasslands or the arctic tundra do not appear to respond as strongly to elevated CO_2 as plants from more productive environments (Williams *et al.*, 1988; Oechel and Strain, 1985).

The ability of a plant species to maintain a positive response to elevated CO_2 over the long term may depend upon its life history and its ability to maintain appropriate internal source-sink relationships. Negative photosynthetic acclimation may not occur, or may not proceed as rapidly, when plants possess adequate sinks for carbohydrate in the form of roots, fruits, or other storage products (Woodward *et al.*, 1991; Ziska and Teramura, 1992). Similarly, woody plants that can allocate carbohydrate to structural tissues or herbaceous plants that can export photosynthate to belowground storage in tubers or roots may have a greater capacity to maintain a positive long-term response to elevated CO_2 (O'Neill *et al.*, 1987; Bhattacharya *et al.*, 1985; Arp and Drake, 1991).

Species-specific variation in ability to respond to elevated CO_2 may affect community level interactions. Exposure to elevated CO_2 may act as a release from carbon limitation for C_3 species, putting them at a competitive advantage compared to C_4 species which are not carbon-limited at current ambient CO_2 levels (Patterson *et al.*, 1984; Carter and Peterson, 1983). Intrinsic differences in species' abilities to allocate tissues to roots to increase the acquisition of nitrogen or water, or to reallocate nitrogen away from photosynthesis to other critical processes within the tissues, may increase the ability of some species to utilize these limiting nutrients at the expense of others (Arp, 1991). Since elevated CO_2 can affect early growth parameters such as germination and seedling size (Wulff and Alexander, 1985), species which are able to take advantage of additional carbon early in the growing season may alter the plant community in terms of plant density and species diversity (Goldberg and Miller, 1990). Thus, elevated CO_2 may affect plant species interactions with the abiotic environment and with other members of the community (Tilman, 1993).

The discovery of intraspecific variation in the ability to respond to elevated CO_2 has provoked interest in determining whether plants may evolve in response to elevated CO_2 (Geber and Dawson, 1993). Physiological traits display genetic variation and are heritable (Scheiner *et al.*, 1984; Tonsor and Goodnight, 1996). Studies of agricultural crops have revealed that genetic variation in response to elevated CO_2 affects traits such as seed number and seed size, aspects of yield that are comparable to evolutionary measures of fitness (E.g., Ziska and Teramura, 1992). Very few studies have been conducted that examine the extent of intraspecific variation in response to elevated CO_2

for native plant populations, but the results of those studies indicate that heritable intraspecific variation in fitness traits in response to elevated CO_2 exists (Garbutt and Bazzaz, 1984; Curtis *et al.*, 1994). If elevated CO_2 will act as an agent of natural selection, intraspecific variation in physiological response to elevated CO_2 must translate into variation in such fitness components as survivorship, size, and fecundity (Geber and Dawson, 1993). Under elevated CO_2, increased rates of photosynthesis have been demonstrated to have a positive effect on growth and biomass accumulation, resulting in predictions of increased crop yield of 30-40 % (Cure and Acock, 1986). Yet the mechanistic connection between physiological traits and growth remains unclear (Pereira, 1994). Relative growth rate (RGR) is expressed as the product of net assimilation rate (NAR) and leaf area ratio (LAR): RGR = NAR x LAR. NAR is usually positively correlated with photosynthetic rate, while LAR is usually positively correlated with specific leaf area (SLA) (Konings, 1989). Under current ambient CO_2 conditions, high RGR appears to be more strongly associated with high SLA than high photosynthetic rates (Shipley, 1995). Because exposure to elevated CO_2 often results in increased photosynthetic rates, but decreased SLA, the net effect of elevated CO_2 on size and yield (surrogates for fitness) will depend upon how members of a species integrate these two potentially counteracting responses to elevated CO_2 into growth.

Because the interaction of a plant's genotype with its environment determines its phenotype, exposure of the genotype to a novel environment, such as twice-ambient CO_2, may result in the expression of a different phenotype. A novel environment may bring about new interactions among genes, potentially resulting in unexpected phenotypic outcomes. That is, the relative contribution of various genes to a given trait, such as growth rate, may vary in different environments (Wright, 1969). Variation in gene interactions may result in shifts in the genetic correlations between traits, which in turn can change the constraints on the independent evolution of correlated traits (Via and Lande, 1983). Changes in genetic correlations as a response to elevated CO_2 may result in a release of genetic variation currently masked by the ambient CO_2 environment, and may make short-term evolutionary response to elevated CO_2 possible (Bradshaw and McNeilly, 1991). Therefore, documenting the extent of phenotypic family level variation in response to elevated CO_2 is a necessary first step in determining the extent of genetic variation upon which elevated CO_2 may act as an agent of natural selection.

A pilot experiment I conducted in 1991 at the Duke University Phytotron indicated that just such intraspecific variation in response to elevated CO_2 existed for maternal families in two populations of *Plantago lanceolata* (see Appendix). Multivariate analysis of the physiological traits of assimilation, transpiration, and stomatal conductance revealed significant CO_2 environment by family interactions in nutrient-rich, controlled greenhouse conditions. Family level variation was also detected for aboveground and belowground biomass, but not for root: shoot ratios. The results of the 1991 experiment led me to conduct another set of experiments in 1992 in the more natural conditions offered by open-topped environmental chambers set up in an old field at the W. K. Kellogg Biological Station in Hickory Corners, Michigan. The goals of the 1992 experiment were three-fold: (1) to document the extent of genotypic variation in response to elevated CO_2 for physiological, growth, and biomass allocation traits, (2) to determine the nature of the duration of response to elevated CO_2 by comparing growth, allocation, and physiological traits after short-term exposure to elevated CO_2 with the same traits after exposure to elevated CO_2 for an entire growing season, and (3) to explore how source-sink relations in an herbaceous perennial such as *Plantago lanceolata* might affect the ability of members of the species to integrate long-term response to elevated CO_2 with respect to physiology, growth and biomass allocation.

CHAPTER ONE

INTRASPECIFIC VARIABLE RESPONSES TO ELEVATED ATMOSPHERIC CO_2: RESOURCE PARTITIONING IN ABOVE- AND BELOWGROUND TISSUES IN PLANTAGO LANCEOLATA L.*

*This chapter was co-written with Susan Kalisz, Stephen J. Tonsor, Peter S. Curtis, and James A. Teeri for submission to *Oecologia*.

INTRODUCTION

Recent increases in atmospheric CO_2 concentration have been clearly documented (Neftel *et al.*, 1985; Keeling, 1986). Extrapolation from the current pattern indicates that this increase will continue, with a doubling of current CO_2 levels being likely by the middle of the next century (Strain, 1987). Numerous studies have established what is now considered a picture of the "typical" whole plant response to elevated CO_2, at least in the short term. In C_3 species whole plant biomass and yield typically increase (Kimball *et al.*, 1993; Poorter, 1993; Cure and Acock, 1986), and patterns of biomass and resource allocation within the plant are often altered. For example, root:shoot ratios generally increase under elevated CO_2. Despite increases in root biomass, nitrogen uptake may not keep up with carbon supply, resulting in increased carbon: nitrogen ratios (see Bowes, 1993; Woodward *et al.*, 1991; Bazzaz, 1990 for reviews).

However, as the variety of species studied and the length of experiments have increased, considerable interspecific variation in the magnitude and duration of this "typical" response to elevated atmospheric CO_2 has been documented. While many reviews cite an increase in root:shoot ratio under most conditions (Bowes, 1993; Woodward *et al.*, 1991; Bazzaz, 1990), some studies have documented no increase in root:shoot ratio (see Stulen and den Hartog, 1993, for a review). Some reports have shown that the initial increase in biomass of elevated CO_2 grown plants compared to ambient grown plants was maintained throughout the experiment (Poorter *et al.*, 1988;

Smith *et al.*, 1987); in other studies early differences in biomass among CO_2 environments disappeared by the end of the experiment (Larigauderie *et al.*, 1988; Norby *et al.*, 1987).

There are several possible explanations for this variety of experimental results. Growth conditions may affect the magnitude and duration of response to elevated atmospheric CO_2. High nutrient environments may magnify and prolong CO_2-mediated increases in overall biomass, with no change in root:shoot ratio (Patterson and Flint, 1982). Low nutrient conditions may dampen the magnitude or curtail the duration of a positive biomass response to high CO_2 as other resources besides carbon become limiting, while shifting the allocation of biomass to roots at the expense of shoots (Larigauderie *et al.*, 1988).

While environmental conditions play a role in governing the response of plants to elevated CO_2, some of the variation in the magnitude and duration of response may be related both to the nature of the environment to which the species is adapted, and to the life history and phenology of the species. That is, the ability to respond to elevated CO_2 may be an intrinsic, genetically determined trait of certain species. For example, plant species from low resource communities such as serpentine grasslands or the arctic tundra do not appear to respond as strongly as plants from more productive environments (Williams *et al.*, 1988; Oechel and Strain, 1985). The ability of a plant species to maintain a positive response to elevated CO_2 over the long term may depend upon its life history and its ability to maintain appropriate source-sink relationships (Bowes, 1993; Arp, 1991). In nutrient-rich agricultural systems, crops with yield components primarily aboveground such as soybeans and cotton generally do not increase root:shoot ratio in response to elevated CO_2 (Idso *et al.*, 1988). On the other hand, root crops such as radishes, carrots and sweet potatoes generally allocate more to root growth and maintain a positive response to elevated CO_2, presumably because they have ample sink strength in underground organs for carbohydrate (Idso *et al.*, 1988; Bhattacharya *et al.*, 1985). In a salt marsh community, perennial herbaceous species that were able to maintain an adequate sink for carbohydrate belowground maintained a positive response to elevated CO_2 for more than four years (Curtis *et al.*, 1989; Arp and Drake, 1991). Thus, elevated atmospheric CO_2 would seem to affect species which allocate biomass significantly to belowground tissues differently than species which do not.

Because CO_2 can have a direct effect on plant performance and since the response of plants to elevated atmospheric CO_2 varies with species, there is growing interest in determining whether plant species will be capable of an evolutionary response to increased CO_2 (Geber and Dawson, 1993). If a species' ability to respond to elevated atmospheric CO_2 is genetically determined, intraspecific genetic variation in response to CO_2 may also exist. Clearly, if CO_2 is to be expected to act as an agent of natural selection, the species must exhibit heritable variation in the magnitude of response to elevated CO_2, and the response of a species to CO_2 must be of lasting duration and affect plant fitness in some way. Studies of agricultural crops have revealed genotypic variation in elevated CO_2 effects in characteristics such as seed number and seed size, aspects of yield that are comparable to evolutionary measures of fitness (e.g., Ziska and Teramura, 1992).

In wild plant species, elevated CO_2 may affect traits both directly and indirectly associated with fitness. For example, size at the end of one growing season may be associated with reproductive success in the next season in perennial species (Primack, 1979). Very few studies have been conducted that examine the extent of intraspecific variation in fitness traits in response to CO_2 explicitly (Garbutt and Bazzaz, 1984; Curtis *et al.*, 1994), but the results of those studies indicate that heritable intraspecific variation in native plant populations in response to CO_2 exists.

The purpose of this experiment was two-fold: (1) to document the extent of intraspecific variation in whole plant traits associated with fitness in response to elevated atmospheric CO_2 in a naturally occurring plant species; and (2) to explore the ways in which aboveground and belowground tissues are allocated in response to elevated CO_2 in a perennial herbaceous species that has a significant belowground sink for carbohydrate. We selected two populations of the short-lived herbaceous perennial, *Plantago lanceolata*, and measured overall biomass of maternal families from those populations after exposure to ambient and elevated CO_2 for one full growing season. Overall biomass is a trait that has been associated with lifetime fitness in this species (Primack and Antonovics, 1982). We also examined how that biomass was allocated between aboveground and belowground components (root:shoot ratio). Finally, we measured tissue chemical composition in leaves and roots for parameters hypothesized to be associated with the maintenance of a positive response to elevated CO_2: soluble sugar and starch content, and percent carbon and percent nitrogen content.

MATERIALS AND METHODS

Plantago lanceolata has been used extensively in physiological, ecological and genetic studies (Tonsor and Goodnight, 1996; Kuiper and Bos, 1992; Tonsor, 1985, 1990; Teramura, 1983). Populations of this species grow in a variety of habitats, and have been found to exhibit both local genetic variation (Tonsor, 1985, 1990; Tonsor *et al.*, 1993;

Teramura 1983; Teramura and Strain, 1979) and phenotypic plasticity (Teramura and Strain, 1979; Antonovics and Primack, 1982). The species is known to be genetically variable for physiological and morphological traits (Tonsor and Goodnight, 1996; Teramura, 1983; Teramura and Strain, 1979), and is capable of undergoing rapid evolutionary change (Wolff and Van Delden, 1989; Wu and Antonovics, 1976). Moreover, the species maintains a significant belowground sink for carbohydrate in the form of a rhizome (Teramura, 1983) and is capable of shifting allocation between aboveground and belowground tissues under changing nutrient conditions (Van der Aart, 1985). Studies of the effects of elevated CO_2 on *P. lanceolata* have revealed genetic variation in allelochemical content (Fajer *et al.*, 1992) and early growth parameters (Wulff and Alexander, 1985).

The study populations of *Plantago lanceolata* were chosen to represent two distinct habitats. The Ely Lake (EL) population (Allegan County, Michigan) grows on exposed sandy soil on a sunny lakeshore, experiencing high irradiance, low nutrient availability, and periodic water stress. The Kellogg Field (KF) population (Kalamazoo County, Michigan) grows in partial shade on the edge of a mown field. The Kellogg Field soil is higher in both organic matter and water availability.

A total of 24 families, twelve randomly selected from each of the two populations, were used in the experiment. On June 5, 1992, all seeds from each family were divided into two equal groups, planted in separate flats, and placed in either ambient or twice-ambient (hereafter referred to as elevated) CO_2 to germinate. Germination took place over 7 - 10 days. On June 17, six maternal siblings from each family in each CO_2 environment were transplanted into separate 30-cm high pots made from 10-cm diameter

PVC pipe with mesh screen bottoms. The day of transplanting was considered Day 1 of the experiment. The pots were filled with a 50-50 mixture of low organic matter field soil (Kalamazoo loam) and sand. 144 pots (6 pots x 12 families x 2 populations) were distributed randomly among four replicate outdoor open-top chambers in each CO_2 environment. Both ambient and elevated CO_2 chambers had one-meter square internal dimensions, contained 36 pots each, and were constructed following the protocol of Curtis and Teeri (1992). To determine the effect of the chambers themselves, 72 additional seedlings from each population were planted in individual pots and distributed randomly among four 1 m^2 unchambered control sites. The chambers and unchambered control sites were arrayed in four randomized blocks in a recently abandoned field adjacent to the Terrestrial Field Laboratory at Kellogg Biological Station, Hickory Corners, Michigan. Each block contained one ambient CO_2 chamber, one elevated CO_2 chamber, and one unchambered control site. Prior to the experiment, the site was cleared, herbicided, and disked to smooth out uneven patches. The experimental site experienced full sun throughout the day.

The plants were watered as needed, usually twice daily. There was no fertilizer supplementation. Pure CO_2, mixed with ambient air by ventilation fans, was supplied 24 hours per day to the elevated chambers. Ambient air was circulated within the ambient chambers by the same type of fan. CO_2 levels were monitored continuously and levels were recorded on a computer at three-minute intervals (Curtis and Teeri, 1992). Mean daytime (0700-1900 hours) CO_2 partial pressure inside the elevated chambers was 72 ± 6 Pa ($\pm$ s.d.), and 36 ± 3 Pa inside the ambient chambers. Quantum sensors and shaded thermocouples attached to a LI-1000 datalogger (LICOR Inc., Lincoln NB, USA) recorded irradiance levels and temperature. Daytime temperatures were 1.7 ± 0.6 °C higher inside the chambers than in the unchambered control sites, with no significant difference in temperature between ambient and elevated chambers. Three weeks into the experiment the young plants were exhibiting symptoms of light stress (prostrate growth and red pigmentation of the leaf bases) so all chambers and control sites were covered with neutral density shade cloth. The shade cloth reduced ambient light by 68%, and the plants recovered their normal phenotype.

Leaf number was counted for each plant on Day 1, 30, 55, and 127 of the experiment. On October 21, after 127 days of growth, and following several frosts and a snowfall, the plants were harvested. The roots and leaves (shoots) were separated at the soil line, dried at 60°C for five days, and weighed. For a subset of three families, shoot and root tissue were analyzed separately for soluble sugar, starch, % carbon, and % nitrogen content. For this part of the analysis, we selected three families having a complete sample size of six individuals in each CO_2 environment at the end of the experiment; we deliberately selected families which appeared to be thriving in both CO_2 environments, but whose overall appearance was qualitatively distinct. Sample size for each of the two CO_2 treatments for tissue content analysis was 18: n = 6 plants per family x (3 families). Plants from the unchambered control sites were not included in the tissue analysis. The shoot and root tissues of each plant were analyzed separately for % carbon and % nitrogen content with a Carlo Erba NA1500 Series II CHN analyzer (Fison's Instruments, Paramus NJ, USA). Starch content and the combined concentration (as glucose units) of sucrose, glucose and fructose (mg soluble sugar/g dry weight) were analyzed enzymatically in shoot and root tissue (Jones *et al.*, 1977).

Statistical Analysis

Data from the main experiment and the subset of three families used for tissue chemical composition analysis were analyzed by analysis of variance (PROC GLM) using SAS (SAS Institute, Inc., 1988). The model for the analysis of the main experiment was:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_{kl} + (\beta\gamma)_{jk} + (\beta\delta)_{jkl} + \epsilon_{ijkl}.$$

Block (α), CO_2 level (β), population (γ), and family nested within population (δ) were main effects in the model. CO_2 x population ($\beta\gamma$) and CO_2 x family nested within population ($\beta\delta$) were interaction terms. Sample size for each family in each CO_2 environment was ranged from 2 - 6. Of the 24 families used in the main experiment, 6 families were excluded from analysis because 2 or fewer individuals germinated in one or both CO_2 environments.

The model for the analysis of the three families used for tissue chemical composition analysis was: $Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ij}$. CO_2 level (α) and family (β) were main effects in the model; CO_2 x family was the interaction term. Because only one family from population EL was included in the tissue composition analysis, a population effect could not be determined. All variables in both analyses were normally distributed except root:shoot ratio. A log transformation of root:shoot ratio to achieve normality did not change significance levels and the untransformed data were used in the overall ANOVA.

Because a comparison of family means was intended initially, all main effects and interactions in the model were considered to be fixed (Gill, 1978). A statistically significant population or family (population) value demonstrated that these populations or families could be distinguished from one another in their response to elevated CO_2. A statistically significant interaction term indicated that the populations or families differed in the magnitude and/or direction of response to the elevated CO_2 environment. To quantify further the nature of population and family level differences, each population and each family was tested individually for its response to CO_2 environment, using one-way ANOVAs. Because of the small sample sizes ($n \leq 6$) for each family we used both $p < 0.05$ and $p < 0.1$ level of statistical significance, as recommended by Gill (1978).

RESULTS

BIOMASS ALLOCATION

-CO_2 Environment Effects-

Plants grown in both ambient and elevated chambers had larger biomass and smaller root:shoot ratios than plants in the unchambered control sites (data not shown). Because the chambers had similar effects on both ambient- and elevated-CO_2 grown plants, the chamber effect was not incorporated into the analysis of experimental results and the unchambered control treatment will not be considered further.

Plants grown in elevated CO_2 had significantly greater belowground biomass, whole plant biomass and root:shoot ratio than ambient CO_2 grown plants (Table 1; Figure 1). Final aboveground biomass did not show a significant CO_2 response, although leaf number was significantly greater ($p<.05$) for the plants grown in elevated CO_2 earlier in the experiment (Days 27 and 59, data not shown).

-Population Level Effects-

Both populations EL and KF had significantly greater belowground biomass and root:shoot ratios in elevated CO_2 compared to the ambient CO_2 environment (Figure 1).

Table 1. Analysis of variance for biomass variables. Overall model is presented.

		Aboveground Biomass			Belowground Biomass			Overall Biomass			Root: Shoot Ratio		
Source	df	MS	F	*P*	MS	F	*P*	MS	F	*P*	MS	F	*P*
Block	3	64.5	2.6	0.0543	1.9	0.3	0.8093	61.7	1.3	0.2871	0.1	5.3	0.0018
CO_2	1	62.6	2.5	0.1143	86.6	15.0	0.0002	296.5	6.1	0.0146	0.3	13.0	0.0004
Population	1	333.6	13.4	0.0003	68.3	11.8	0.0008	703.8	14.5	0.0002	0.0005	0.03	0.8682
Family (Population)	16	56.6	2.3	0.0052	15.2	2.6	0.0011	119.9	2.5	0.0024	0.05	2.4	0.0030
CO_2 x Population	1	6.3	0.3	0.6156	2.6	0.5	0.5000	17.1	0.4	0.5544	0.0006	0.03	0.8653
CO_2 x Family(Pop)	16	19.9	0.8	0.6831	4.7	0.8	0.6692	39.1	0.8	0.6801	0.01	0.7	0.7554
Error	152	24.8	-	-	5.8	-	-	48.6	-	-	0.02	-	-

Figure 1. Comparison of aboveground biomass, belowground biomass, and root: shoot ratio by CO_2 treatment. For each category (overall, population, family) means for ambient CO_2 appear to the left of means for elevated CO_2. Vertical bars indicate one standard error. Aboveground biomass, belowground biomass, and root: shoot ratios were considered separately for each category. Scale for root: shoot ratio appears to the right. Significance levels appear above bars for aboveground biomass, below bars for belowground biomass, and immediately adjacent symbol for root: shoot ratio. Whole plant biomass differed significantly across CO_2 treatment for overall data (*), population KF (*), family EL 7 (*1), family EL 9 (*), and family KF 31 (*). Significance levels: *1 $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

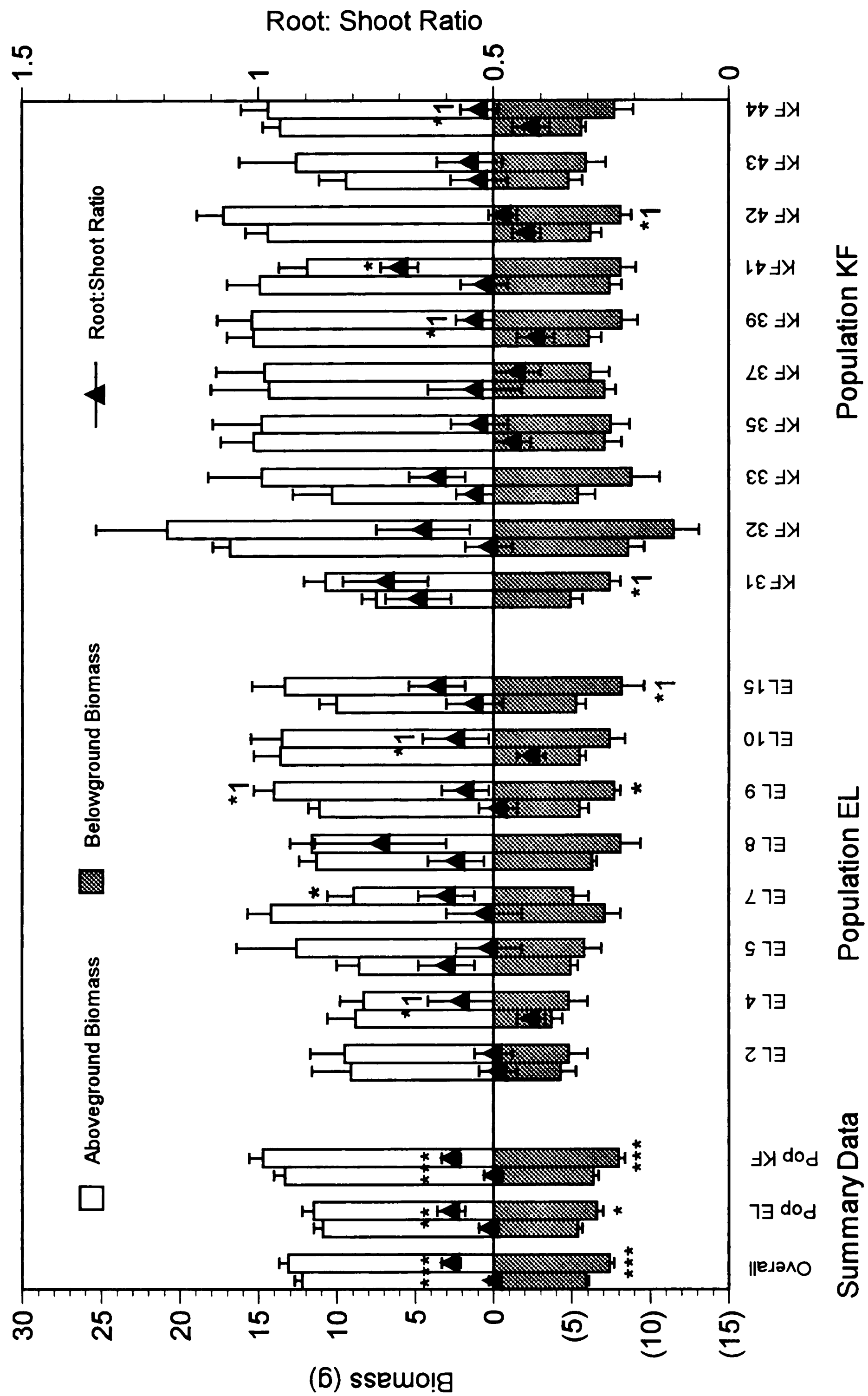

Root: Shoot Ratio
1.5
1
0.5
0
Aboveground Biomass
Belowground Biomass
Root:Shoot Ratio
Biomass (g)
30
25
20
15
10
5
0
(5)
(10)
(15)
Overall
Pop EL
Pop KF
EL 2
EL 4
EL 5
EL 7
EL 8
EL 9
EL10
EL15
KF31
KF 32
KF 33
KF 35
KF 37
KF 39
KF41
KF 42
KF 43
KF 44
Summary Data
Population EL
Population KF

Figure 1

Aboveground biomass did not differ significantly for either population across CO_2 treatments. Only population KF had significantly greater whole plant biomass ($p < .05$) in elevated CO_2. Within the ambient CO_2 environment, there were significant differences between populations EL and KF for all biomass variables except root:shoot ratio (Figure 1). Plants from Population KF were significantly larger than those from population EL for aboveground biomass, belowground biomass and total biomass ($p < .05$). Although there was no CO_2 x population interaction (Table 1), population KF responded proportionally more to elevated CO_2 for belowground and whole plant biomass than did population EL. Belowground biomass for population KF was 25% greater in the elevated than in the ambient CO_2 environment; for population EL the increase in belowground biomass in the elevated CO_2 environment was 22%. Whole plant biomass in the elevated CO_2 environment was 16% greater for population KF and 11% greater for population EL relative to the ambient CO_2 environment.

-Family Level CO_2 Effects-

In contrast to the overall positive response to elevated CO_2 at the population level, the family means showed significant variation in direction and magnitude of response (Figure 1). Three types of responses emerged when family means in the ambient and elevated CO_2 environments were compared (Figure 1). **(1) Increase in biomass** - Across both populations, nine families showed significant increases in one or more biomass variables in the elevated CO_2 environment compared to the ambient environment. Family EL 9 exhibited increased aboveground, belowground and whole plant biomass in elevated CO_2. Family KF 31 had increased belowground and whole plant biomass under elevated CO_2. Two families (EL 15 and KF 42) only increased belowground biomass under elevated CO_2. Five families (EL 4, EL 10, KF 39, KF 41, and KF 44) increased their root: shoot ratios under elevated CO_2. **(2) No change in biomass -** Eight families showed no significant response to the elevated CO_2 environment for any of the biomass variables. **(3) Decrease in biomass -** One family, EL 7, was significantly smaller in aboveground and whole plant biomass in the elevated compared to the ambient CO_2 environment. These three types of responses reveal how the families in these populations differed in which portions of their biomass, if any, responded to the elevated CO_2 environment. It is clear that there is no consistent, generalized response to elevated CO_2 at the family level for these biomass variables.

When relative allocation to aboveground and belowground tissues (that is, root: shoot ratio) was compared among CO_2 environments for the individual families, the general response was toward increased root: shoot ratios. However, the means by which the individual families achieved this response to elevated CO_2 varied considerably. Of the five families with significantly greater root:shoot ratios in elevated CO_2, two routes to achieve this response were detected: **(1)** little change in aboveground biomass accompanied by a tendency to increase belowground biomass (families EL 4, EL 10, KF 39, and KF 44); and **(2)** aboveground biomass being smaller in elevated CO_2 than in ambient CO_2 with little change in belowground biomass (family KF 41). Thirteen of the eighteen families analyzed did not significantly increase root: shoot ratios in elevated CO_2, although the trend in nine of these families was toward greater root: shoot ratios in elevated CO_2. There was not always sufficient statistical power for resolving small differences in family mean responses among CO_2 environments for small sample sizes ($n \leq 6$ for each family with each CO_2 environment).

Family performance in ambient CO_2 was not a good predictor of family performance in elevated CO_2. Several families which were either the largest or smallest for a particular biomass variable in the ambient CO_2 environment changed rankings with respect to the other families when in an elevated CO_2 environment. Families EL 5 and KF 33 had comparatively small aboveground biomass in ambient CO_2 but were close to the largest families in aboveground biomass in elevated CO_2, while families EL 7 and KF 41 decreased in aboveground biomass in elevated CO_2 relative to the other families (Figure 1). Similarly, families EL 15 and KF 33 moved up in ranking for belowground biomass in the elevated CO_2 environment while families EL 7, KF 35 and KF 37 moved down. By contrast, certain families did not change biomass ranking when exposed to elevated CO_2 . For example, family KF 32 had the largest aboveground, belowground, and whole plant biomass and family KF 31 had the highest root:shoot ratio of any family in population KF in ambient and elevated CO_2.

TISSUE CHEMICAL COMPOSITION

-CO_2 Environment Effects-

For families EL 15, KF 32 and KF 35, there were significant overall differences in soluble sugar content, carbon content (%DW) , nitrogen content (%DW), and carbon:nitrogen ratios between ambient- and elevated-CO_2 grown plants. Overall, whole plant soluble sugar was 15% higher in the elevated CO_2 grown plants (59.3 mg/g dry weight vs. 51.8 mg/g, $p<.005$) with this increase present in both aboveground and belowground tissues (Table 2, Figure 2). Starch content was less than 1% for all individuals in the three families in both environments. Whole plant % carbon was slightly, although significantly, higher in the elevated compared to the ambient CO_2 environment

Table 2. Analysis of variance for tissue composition components for three families (EL 15, KF 32, KF 35). Overall model for each variable is presented.

		Aboveground Soluble Sugar			Aboveground % Carbon			Aboveground % Nitrogen			Aboveground C: N Ratio		
Source	df	MS	F	*P*	MS	F	*P*	MS	F	*P*	MS	F	*P*
CO_2	1	912.0	7.0	0.0131	41.3	9.8	0.0039	0.5	6.5	0.0160	155.1	4.4	0.0438
Family (Population)	2	192.2	1.5	0.2470	7.2	1.7	0.1965	0.9	12.5	0.0001	386.9	11.1	0.0003
CO_2 x Family(Pop)	2	249.5	1.9	0.1667	2.8	0.7	0.5179	0.4	6.0	0.0065	167.8	4.8	0.0156
Error	30	131.1	-	-	4.2	-	-	0.1	-	-	35.0	-	-

		Belowground Soluble Sugar			Belowground % Carbon			Belowground % Nitrogen			Belowground C: N Ratio		
Source	df	MS	F	*P*	MS	F	*P*	MS	F	*P*	MS	F	*P*
CO_2	1	227.5	3.3	0.0792	2.4	0.3	0.5903	0.2	6.0	0.0203	134.2	11.7	0.0019
Family (Population)	2	394.2	5.7	0.0078	14.8	1.8	0.1762	0.1	2.1	0.1393	58.9	5.1	0.0122
CO_2 x Family(Pop)	2	25.1	0.4	0.6979	1.1	0.1	0.8778	0.0	0.0	0.9824	4.2	0.4	0.6989
Error	30	68.8	-	-	8.0	-	-	0.0	-	-	11.5	-	-

Figure 2. Comparison of soluble sugar, % carbon content, % nitrogen content and carbon: nitrogen ratio by CO_2 treatment. For each category (overall and family) means for ambient CO_2 appear to the left of means for elevated CO_2. Vertical bars indicate one s.e. Aboveground and belowground means were considered separately for each variable. Significance levels appear above bars for aboveground means and below bars for belowground means. Significance levels: *1 $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

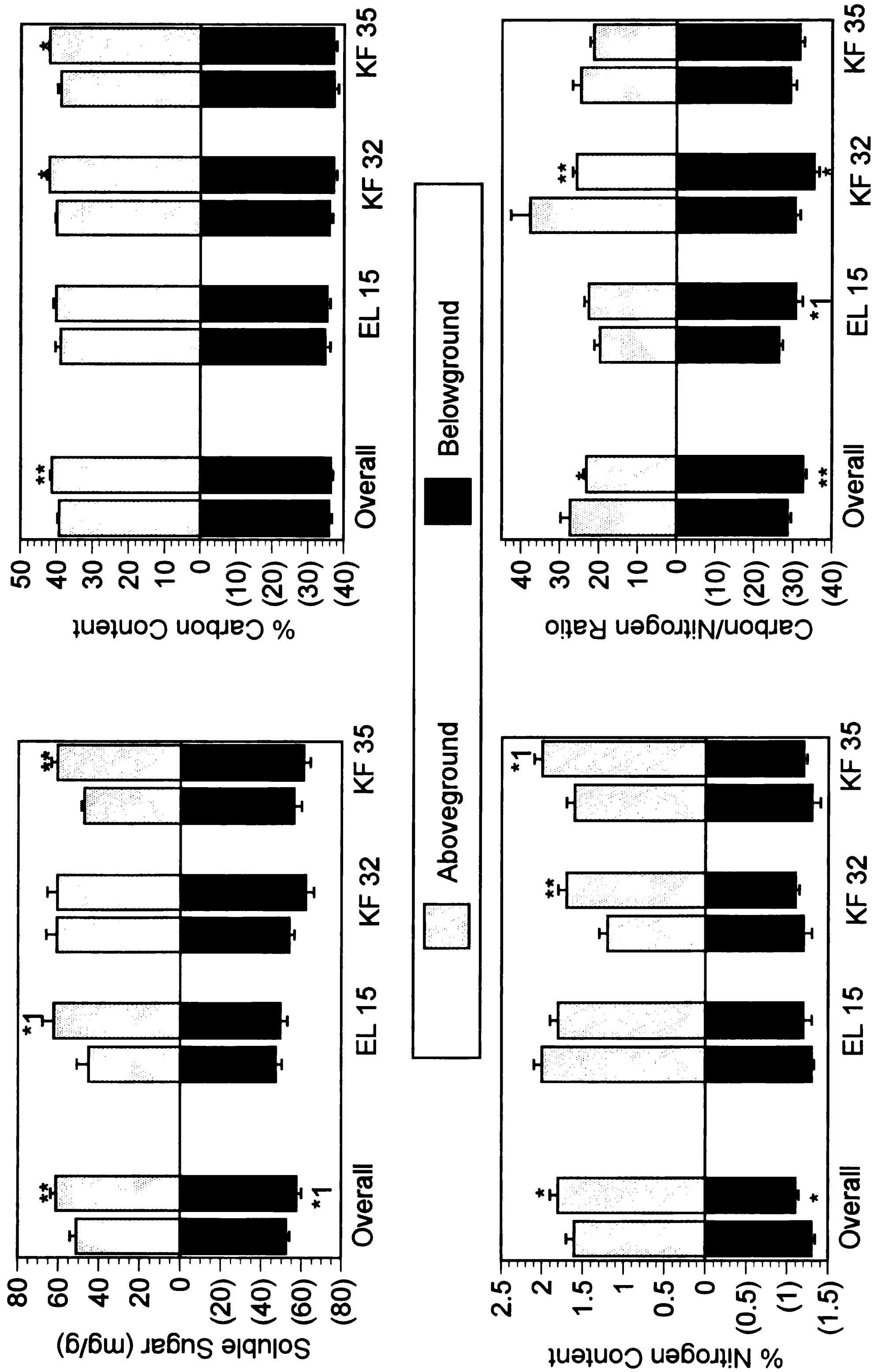

Soluble Sugar (mg/g)
% Carbon Content
% Nitrogen Content
Carbon/Nitrogen Ratio
Overall
EL 15
KF 32
KF 35
Aboveground
Belowground

Figure 2

(38.9 % vs. 37.5 %, $p < .05$). The increase in whole plant % carbon was due entirely to an increase in carbon content in aboveground tissues in the elevated CO_2 environment, as belowground tissues did not differ significantly in % carbon content (Figure 2).

Whole plant nitrogen content (%DW) showed no CO_2 response, although the distribution of nitrogen between aboveground and belowground tissues was affected by CO_2 environment. In elevated CO_2, % nitrogen content was significantly higher in aboveground tissues, but lower in belowground tissues compared to plants in ambient CO_2 (Figure 2). Carbon: nitrogen ratios were lower in aboveground and higher in belowground tissues under elevated CO_2 (Figure 2).

-Family Level CO_2 Effects-

There were significant differences among the three families EL 15, KF 32, and KF 35 in response to elevated CO_2 for soluble sugar content, % carbon, % nitrogen, and carbon:nitrogen ratios (Figure 2). Families EL 15 and KF 35 had significantly more aboveground (and whole plant) soluble sugar under elevated CO_2 , while the soluble sugar content in family KF 32 was not affected by CO_2 treatment (Figure 2). There were small but significant increases in aboveground % carbon content in families KF 32 and KF 35 in the elevated CO_2 environment, but no increase in % carbon content in family EL 15 (Figure 2). Belowground soluble sugar and % carbon content did not change significantly with CO_2 environment for any individual family.

There were significant family level and CO_2 x family interactions for aboveground % nitrogen content and aboveground carbon: nitrogen ratio (Table 2). Families KF 32 and KF 35 had higher aboveground % nitrogen content in the elevated CO_2 environment than in the ambient, while family EL 15 showed no change. Belowground % nitrogen was not affected by CO_2 in any of the three families, but families KF 32 and KF 35 both contained significantly less nitrogen belowground relative to aboveground under elevated CO_2. In family KF 32, the carbon: nitrogen ratio decreased aboveground, but increased belowground under elevated CO_2, while family EL 15 showed increased belowground carbon: nitrogen ratio only. Family KF 35 showed no response to CO_2 in above- or belowground carbon: nitrogen ratio.

DISCUSSION

Our results demonstrate significant within and among population variation in *Plantago lanceolata* for response to elevated CO_2. Belowground biomass, whole plant biomass, and root:shoot ratios increased in the elevated CO_2 environment, but we found considerable variation in the direction and magnitude of response to elevated CO_2 at the level of the population and family. Populations EL and KF responded to elevated CO_2 similarly by increasing biomass. Belowground biomass was most responsive to elevated CO_2. This differed from the 1991 experiment in which aboveground biomass was also larger in the elevated CO_2 environment under more productive greenhouse conditions (see Appendix). Increases in belowground biomass in the 1992 experiment resulted in increased root:shoot ratio in both populations in the elevated CO_2 environment. Yet the two populations were not identical in their responses to elevated CO_2 (Figure 1). Plants from Population KF were larger than those from population EL for all biomass variables except root:shoot ratio, and Population KF showed a significantly greater response to the elevated CO_2 environment for belowground and whole plant biomass than Population EL.

The family responses to elevated CO_2 in biomass and biomass allocation were far

more complex than the population-level analyses suggest. Depending upon the family, either aboveground biomass, belowground biomass, both biomass components, or neither biomass component showed a response to elevated CO_2 (Figure 1). In general, though, belowground biomass was more responsive to elevated CO_2. While only eight families maintained a greater than 10% increase in aboveground biomass for the entire growing season, a much larger number of families (thirteen out of eighteen) maintained a greater than 10% increase in belowground biomass in the elevated CO_2 environment. This resulted in the general response of increased root: shoot ratios in the families grown in elevated CO_2. However, different families may well have followed different physiological and developmental patterns to achieve a similar response to elevated CO_2, illustrated by the different routes taken to achieve increased root: shoot ratio by the individual families.

There are two possible explanations for the greater responsiveness of the belowground biomass component to the elevated CO_2 environment. The first explanation draws upon the model of balanced carbon and nitrogen allocation between shoots and roots (Davidson, 1969; Thornley, 1972: Johnson, 1985). This model predicts that as aboveground tissues experience an increase in carbon supply, allocation to belowground tissues increases to balance the nutrients within the plant. The results of the tissue composition analysis were not consistent with this model. When the three families, KF 32, KF 35, and EL 15, were analyzed for carbon and nitrogen content, we found that families KF 32 and KF 35 increased nitrogen allocation to the shoots under elevated CO_2, without significantly increasing root: shoot ratio. Family EL 15, on the other hand, had a significantly greater root: shoot ratio in elevated CO_2, but did not increase % nitrogen content aboveground. Thus, an increased root: shoot ratio did not result in increased

allocation of nitrogen aboveground. However, because such a small number of families was included in this portion of the experiment, it is clear that a much larger scale study is needed to explore the relationship between biomass, carbon and nitrogen allocation fully.

A second explanation for an increase in allocation to belowground biomass under elevated CO_2 relates to the life history of this particular species. *Plantago lanceolata* can increase carbon allocation to belowground biomass in order to maximize survival and reproductive potential in subsequent growing seasons (Van der Aart, 1985; Teramura, 1983). When all eighteen families were measured for changes in biomass allocation under elevated CO_2, our experiment revealed that a greater carbon supply (in the form of increased CO_2), coupled with a large belowground sink for carbon, makes the pattern of biomass allocation at high CO_2 in *Plantago lanceolata* consistent with results from root crops, in which root:shoot ratios increased in elevated CO_2 environments regardless of the availability of other resources (Idso *et al.*, 1988). Similarly, *Bromus mollis*, a naturally occurring perennial species, also accumulated significant belowground biomass in an elevated CO_2 environment, even in soils that were not resource limited (Larigauderie *et al.*, 1988).

In an elevated CO_2 world, it is possible that the population structure and community interactions of this species may change. In our experiment, it was not possible to predict how a family would respond to elevated CO_2 simply by examining its performance in ambient CO_2. Several families changed rankings relative to one another in the elevated CO_2 environment, becoming either relatively larger or relatively smaller in the elevated CO_2 environment than members of the same family in the ambient CO_2 environment (Figure 1). Under an elevated CO_2 atmosphere in the natural community in which *Plantago lanceolata* grows, changes in allocation like those documented in this study can be expected to alter the competitive interactions both among conspecifics and among species. Although most families did respond to elevated CO_2, it is also important to note that certain families did not respond to the elevated CO_2 environment by increasing biomass or root:shoot ratios. The lack of response in these families to the increase in carbon supply may ultimately result in these families being at a competitive disadvantage compared to other families in a world of consistently increasing atmospheric CO_2. In fact, it is hard to imagine how the families that did not respond to elevated CO_2 would be able to interact successfully in a community context in an elevated CO_2 world. If competitive success depends on allocation properties, as has been widely suggested (for example, Grime, 1977, Goldberg, 1991), this study suggests that families that allocate more biomass belowground may be at a competitive advantage in an elevated CO_2 world, but which families will respond in this way to elevated CO_2 cannot be predicted by their performance under current ambient CO_2 conditions.

- Implications for Adaptive Response to CO_2

Our results demonstrate sufficient variation in response to CO_2 at the family level to indicate that CO_2 by itself may act as an agent of natural selection in natural plant populations. *Plantago lanceolata* has been shown to respond evolutionarily to human-induced selective forces of recent occurrence and moderate strength. For example, Wu and Antonovics (1976) documented increased lead tolerance in roadside populations of *Plantago lanceolata* growing on higher lead soils near the source of automobile emissions, compared to populations away from the road. Rapid evolution in *P. lanceolata* was also demonstrated by Wolff and Van Delden (1989), who obtained a response to selection for leaf angle in *P. lanceolata* in one generation. Other studies have found genotypic variation in this species in elevated CO_2 environments for ecologically important traits such as germination rate and seedling size (Wulff and Alexander, 1985). In our experiment, families varied in biomass accumulation and allocation. Primack and Antonovics (1982) have shown that biomass accumulation and allocation patterns are associated with lifetime survival and reproductive potential (fitness) in *Plantago lanceolata*, and Tonsor and Goodnight (1996) have shown significant narrow sense heritability for plant size in this species. Thus, it is likely that *Plantago lanceolata* has the potential for an evolutionary response to increasing levels of atmospheric CO_2.

In this experiment, the entire range of responses to elevated CO_2 (positive response, negative response, no response) that has previously been documented at the species level (e.g., Bazzaz, 1990) was seen within *Plantago lanceolata* at the family level. This range of responses occurred in a variety of traits, including biomass, root:shoot ratios, % nitrogen content and C:N ratios. Which biological traits are acted upon by natural selection will depend upon the ecological context in which families and populations are found. For example, in the presence of elevated CO_2, certain traits may experience strong selection in low nutrient or dry environments.

One further implication of the great variation in response to elevated CO_2 among the families in this study relates to recent developments in the measurement of selection (Arnold and Wade, 1984; Kalisz, 1986; Wade and Kalisz, 1990). When measuring the opportunity for selection, a comparison of means is not necessarily the most illuminating procedure to use in situations in which phenotypic variation increases in one environment compared to another. In this study, the range of family mean values (largest family mean

minus smallest) for all of the biomass variables (aboveground, belowground and whole plant biomass, and root: shoot ratio) was greater in elevated CO_2 than in ambient CO_2 by an average of 33%. Because these measures of plant size are correlated with fitness in *Plantago lanceolata* (Primack and Antonovics, 1982; Tonsor and Goodnight, 1996), a broader range of mean values for biomass traits might translate into a greater range in relative fitness among the families in these populations in an elevated CO_2 environment. Under these circumstances, those families exhibiting extreme responses (either very large positive responses or those in a direction opposite of expectation) may provide more information about the possible role natural selection may play in an elevated CO_2 atmosphere than families which exhibit more "typical" responses. An increased variance in relative fitness among genotypes in an elevated CO_2 environment would provide a greater opportunity for selection to occur, as defined by Arnold and Wade (1984).

Whether shifts in species composition of a community or shifts in species function within a community are the predominant responses to elevated CO_2 is largely a question of relative rates of response at the intraspecific genetic and the interspecific community level. It will be important to incorporate the complexity of genetic variation in response to elevated atmospheric CO_2 into future studies directed toward understanding the impact of this aspect of environmental change on plant species and communities.

CHAPTER TWO

PHYSIOLOGICAL VARIATION IN RESPONSE TO ELEVATED CARBON DIOXIDE IN PLANTAGO LANCEOLATA

INTRODUCTION

Carbon dioxide has been determined to be a limiting resource to plants which photosynthesize by means of the C_3 pathway (Bowes, 1991). Atmospheric CO_2 levels are currently increasing (Keeling, 1986); predictions indicate that the increase will continue well into the next century, with a doubling of current CO_2 levels by 2050 likely (Strain, 1987). Elevated atmospheric CO_2 has direct effects on plant life (Strain and Cure, 1985). Exposure to elevated CO_2, at least in the short term, stimulates photosynthetic, or CO_2 assimilation, rates (A, expressed as μmol CO_2 fixed m^{-2} sec^{-1}). Transpiration rates (E, mmol H_2O m^{-2} s^{-1}) often decline. The increase in assimilation, accompanied by a decrease in transpiration, results in increased instantaneous water use efficiency (calculated as A/E) (Bazzaz, 1990). Accompanying these changes in assimilation and transpiration are declines in stomatal conductance (G_s, expressed as mol H_2O m^{-2} s^{-1}) and decreases in specific leaf area (SLA, cm^2 area g^{-1} dry weight) (Bazzaz, 1990). Often, relative growth rates increase and plants in elevated CO_2 may accumulate more biomass than plants growing in ambient levels of CO_2, especially when other nutrients are not limiting (Klus, Ch. 1; Bazzaz, 1990; Kimball *et al.*, 1993).

However, these initial changes in physiology and growth in response to elevated CO_2 may not be maintained. The capacity of a plant to maintain an overall positive response to elevated CO_2 depends upon a complex interaction of many physiological traits. For example, the maintenance of increased water use efficiency results from the interaction of the internal processes of assimilation, transpiration, and stomatal conductance. After prolonged exposure to elevated CO_2, decreases in stomatal conductance may result in increased leaf temperatures and vapor pressure deficits at the leaf surface. Under these conditions, transpiration rates may not decline, offsetting potential gains in water balance due to lower G_s (Bowes, 1993: Schulze *et al.*, 1987). Other physiological processes, such as assimilation and stomatal conductance, may undergo negative acclimation. Assimilation, in particular, may decline over time to a point equivalent to or even lower than that of plants photosynthesizing in ambient CO_2 (Wulff and Strain, 1982; DeLucia *et al.*, 1985).

Photosynthetic acclimation to elevated CO_2 has been widely investigated. Most of the mechanisms proposed to explain negative photosynthetic acclimation recognize that an imbalance between carbon and other plant nutrients, such as nitrogen, may occur as supplies of carbon in the form of CO_2 increase (Arp, 1991; Bowes, 1991). Much of a plant's nitrogen is tied up in the carbon-fixing enzyme Rubisco (ribulose bisphosphate carboxylase-oxygenase), the single most abundant enzyme in the world. Rubisco comprises 30- 50% of a plant's leaf protein content in C_3 species (Bowes, 1991). Bowes (1991) has summarized three nonexclusive mechanisms by which negative photosynthetic acclimation to elevated CO_2 may occur. First, plants may reallocate nitrogen from Rubisco by lowering either the quantity or the activation state of Rubisco, slowing down the accumulation of the products of photosynthesis (Arp, 1991) . Second, the rate of regeneration of ribulose bisphosphate (RuBP) or inorganic phosphate (P_i) may lag behind the supply of carbon to Rubisco, again reducing the rate of carbon fixation (Sharkey, 1985). Third, starch may accumulate as an end product of photosynthesis and may physically interfere with the diffusion of CO_2 into the thylakoids or otherwise disrupt the function of the carbon fixation pathway (Wulff and Strain, 1982; DeLucia *et al.*, 1985).

All of these mechanisms proposed to explain the negative acclimation of photosynthesis under exposure to elevated CO_2 may involve an imbalance in the supply of two critical nutrients, carbon and nitrogen, or an imbalance between the source of carbon compounds in the plant (photosynthesis) and the destination of those compounds (the sink) (Arp, 1991; Poorter, 1993). If nitrogen supply cannot keep up with carbon supply, or if photosynthate accumulates because the plant does not possess adequate sinks for carbohydrate, the rate of photosynthesis may decrease. Experiments conducted to explore the relationship between source and sink for carbohydrate under elevated CO_2 suggest that negative acclimation may not occur, or may not proceed as rapidly, when plants possess adequate sinks for carbohydrate in the form of roots, fruits or other storage products (Arp, 1991; Woodward *et al.*, 1991; Ziska and Teramura, 1992).

The ability of a plant to modify physiological pathways and shift patterns of nutrient allocation and acquisition in response to elevated CO_2 may be determined by the plant's life history and intrinsic growth properties. Poorter (1993) reviewed growth responses of 156 species to elevated CO_2 and concluded that the plants which may be most successful in integrating physiological and allocational responses to elevated CO_2 may be those with large source-sink strength and/or those with intrinsically high relative growth rates. Plants that export photosynthate to belowground storage products such as sweet potatoes (Bhattacharya *et al.*, 1985), carrots, or radishes (Idso and Kimball, 1989) typically maintain long-term positive responses to elevated CO_2. Woody plants (O'Neill *et al.*, 1987) or herbaceous plants with large root systems may also have a greater capacity to integrate physiological and morphological processes to maintain a long-term positive response to elevated CO_2 (O'Neill *et al.*, 1987; Larigauderie *et al.*, 1988; Arp and Drake, 1991).

Intrinsic differences in species' abilities to allocate tissues to roots to increase the acquisition of nitrogen or to reallocate nitrogen within tissues, may alter ecological interactions among species. Differences in the magnitude and duration of the CO_2 response may increase the ability of some species to utilize limiting nutrients at the expense of others (Arp, 1991). Interspecific variation in the ability to respond in this way to elevated CO_2 may alter how a plant interacts with its abiotic environment and with the other members of its community (Tilman, 1993).

The discovery of intraspecific variation in the ability to respond to elevated CO_2 has provoked interest in determining whether plants may evolve in response to elevated CO_2 (Geber and Dawson, 1993). Physiological traits display genetic variation and are heritable (Scheiner *et al.*, 1984; Geber and Dawson, 1990; Radin *et al.*, 1994; Tonsor and Goodnight, 1996). To date, however, no experiments have explicitly documented the extent of genotypic variation in physiological traits in response to elevated CO_2. The nature of the intraspecific variation in physiological response to elevated CO_2 may include variation in the instantaneous capacity to respond positively to elevated CO_2, and may also include variation in the ability to maintain a positive response to elevated CO_2 over long time periods.

If elevated CO_2 will act as an agent of natural selection, intraspecific variation in physiological response to elevated CO_2 must translate into variation in fitness components such as survivorship, size, and fecundity (Geber and Dawson, 1993). Under elevated CO_2 increased rates of photosynthesis have been demonstrated to have a positive effect on growth and biomass accumulation, resulting in predictions of increased crop yield of 30 - 40% (Kimball, 1983; Cure and Acock, 1986). Yet the mechanistic connection between the physiological behavior of a plant and its growth remains unclear (McGraw and Wulff, 1983; Poorter, 1989; Pereira, 1994). Elevated CO_2 often results in increased relative growth rates initially, but those early increases may decline under prolonged exposure (Bazzaz, 1990). Early gains in biomass, though, due to higher initial growth rates, may be maintained over the long term (Wulff and Strain, 1982; Poorter *et al.*, 1988) and may be key in determining the outcome of competitive interactions. Under ambient CO_2 conditions high relative growth rate appears to be more strongly associated with high SLA than high photosynthetic rates (Shipley, 1995). Because exposure to elevated CO_2 often results in increased photosynthetic rates, but decreased SLA, the net effect of elevated CO_2 on size and yield (surrogates for fitness) will depend on how members of a species integrate these two potentially counteracting responses.

The goal of this experiment was to determine, for two populations and twenty-four families of *Plantago lanceolata*, the extent of genotypic variation in physiological response to elevated CO_2. We measured traits which are known to be involved with both the instantaneous capacity for response to elevated CO_2 and with the long-term maintenance of positive response to elevated CO_2: assimilation, transpiration, stomatal conductance, and water use efficiency. We also measured specific leaf area, in an attempt to connect the physiological behavior of the plants with one aspect of their growth.

We investigated variation in the capacity to respond physiologically to elevated CO_2 by measuring each plant at both CO_2 growth levels, ambient CO_2 and twice ambient ("elevated") CO_2. This allowed us to compare the long-term physiological response of

plants grown in elevated CO_2 to the instantaneous physiological response of plants grown in ambient CO_2 but exposed temporarily to elevated CO_2 and *vice versa.* We also measured physiological traits two times during the growing season, middle and late, to detect whether the physiological response to elevated CO_2 changed as the growing season progressed.

MATERIALS AND METHODS

Plantago lanceolata has been used extensively in physiological, ecological and genetic studies (Tonsor and Goodnight, 1996; Kuiper and Bos, 1992; Tonsor, 1985, 1990; Teramura, 1983). The species is genetically variable for physiological and morphological traits (Teramura, 1983; Teramura and Strain, 1979); Tonsor and Goodnight (1996) found significant narrow sense heritabilities for transpiration rate, photosynthetic capacity, and specific leaf weight in a Michigan population of *Plantago lanceolata* under current ambient CO_2 levels, indicating that the potential exists for evolutionary change in physiological traits for this species in the field. Populations of *Plantago lanceolata* grow in a variety of habitats, and have been found to exhibit local genetic variation (Tonsor, 1985, 1990; Tonsor *et al.*, 1993; Teramura 1983; Teramura and Strain, 1979), phenotypic plasticity (Teramura and Strain, 1979; Antonovics and Primack, 1982), and the capacity to undergo rapid evolutionary change (Wolff and Van Delden, 1989; Wu and Antonovics, 1976). Studies of the effects of elevated CO_2 on *P. lanceolata* have revealed genetic variation in allelochemical content (Fajer *et al.*, 1992) and early growth parameters (Wulff and Alexander, 1985).

The study populations of *Plantago lanceolata* were chosen to represent two distinct habitats. The Ely Lake (EL) population (Allegan County, Michigan) grows on exposed sand on a sunny lakeshore, experiencing high irradiance and periodic water stress. The Kellogg Field (KF) population (Kalamazoo County, Michigan) grows in Kalamazoo loam in partial shade on the edge of a mown field.

A total of 24 families, twelve randomly selected from each of the two populations, were used in the experiment. On June 5, 1992, all seeds from each family were divided into two equal groups, planted in separate flats, and placed in either ambient or twice-ambient (hereafter referred to as elevated) CO_2 to germinate. Germination took place over 7 - 10 days. On June 17, six maternal siblings from each family in each CO_2 environment were transplanted into separate 30-cm high pots made from 10-cm diameter PVC pipe with mesh screen bottoms. The day of transplanting was considered Day 1 of the experiment. The pots were filled with a 50-50 mixture of Kalamazoo loam and sand. 144 pots (6 pots x 12 families x 2 populations) were distributed randomly among four replicate outdoor open-top chambers in each CO_2 environment. Both ambient and elevated CO_2 chambers had one-meter square internal dimensions, contained 36 pots each, and were constructed following the protocol of Curtis and Teeri (1992). To determine the effect of the chambers themselves, seventy-two additional seedlings from each population were planted in individual pots and distributed randomly among four 1 m^2 unchambered control sites. The chambers and unchambered control sites were arrayed in four randomized blocks in a recently abandoned field adjacent to the Terrestrial Field Laboratory at Kellogg Biological Station, Hickory Corners, Michigan. Each block contained one ambient CO_2 chamber, one elevated CO_2 chamber, and one unchambered control site. Prior to the experiment, the site was cleared, herbicided, and disked to smooth out uneven patches. The experimental site experienced full sun throughout the day.

The plants were watered as needed, usually twice daily. There was no fertilizer supplementation. Pure CO_2, mixed with ambient air by ventilation fans, was supplied 24 hours per day to the elevated chambers. Ambient air was circulated within the ambient chambers by the same type of fan. CO_2 levels were monitored continuously and levels were recorded on a computer at three-minute intervals (Curtis and Teeri, 1992). Mean daytime (0700-1900 hours) CO_2 partial pressure inside the elevated chambers was 72 ± 6 Pa ($\pm$ s.d.), with the mean daytime CO_2 partial pressure inside the ambient chambers being 36 ± 3 Pa. Quantum sensors and shaded thermocouples attached to a LI-1000 datalogger (LICOR Inc., Lincoln NB, USA) recorded irradiance levels and temperature. Daytime temperatures were 1.7 ± 0.6 °C higher inside the chambers than in the unchambered control sites, with no significant difference in temperature between ambient and elevated chambers. Three weeks into the experiment the young plants were exhibiting symptoms of light stress (prostrate growth and red pigmentation of the leaf bases) and all chambers and control sites were covered with shade cloth. The shade cloth reduced ambient light by 68%, and the plants recovered their normal phenotype.

Physiological measurements were made on all of the plants in the experiment in two separate periods: August 9 - 13 and September 4 - 10, 1992. Measurements were made in an open system using two portable infra-red gas analyzers (Models LCA-2 and LCA-3, Analytical Development Corporation, Hoddesdon, UK) and narrow Parkinson Leaf Chambers (Analytical Development Corporation, Hoddesdon, UK) at both ambient and elevated CO_2. Elevated CO_2 was provided from one of the elevated open-top chambers. The CO_2 source (ambient or elevated CO_2) was rotated to a different machine each day. The machines were set to have identical air flow rates (400 ml min^{-1}) and saturating light levels (1300 μE) for all measurements. Mean leaf chamber temperature was 28.5 °C (s.d. 2.5) in August and 26.8 °C (s.d. 1.7) in September.

Measurements of physiological traits were made on an intact, most recently fully expanded leaf of each plant. Each plant was measured at two CO_2 levels, first at its own growth CO_2 level (ambient or elevated), then at the other CO_2 level. For each measurement, the leaf was allowed to equilibrate within the leaf chamber for three minutes before readings were taken. After measurement at both CO_2 levels, the leaf was removed from the plant, the area of leaf inside the leaf chamber was excised (if the leaf did not entirely fill the leaf chamber), and all parts of the leaf were pressed, dried and weighed. Specific leaf area for each plant was calculated as the ratio of leaf area (cm^2) to the dry weight (g) of the leaf used for the physiological measurements. Calculations of CO_2 assimilation (μmol CO_2 m^{-2} s^{-1}), transpiration (mmol H_2O m^{-2} s^{-1}), stomatal conductance (mol H_2O m^{-2} s^{-1}) and intercellular CO_2 concentration (μbar) for both months were made using equations from Analytical Development Corporation (1992) and von Caemmerer and Farquhar (1981).

Statistical Analysis

For each month's measurements, preliminary analysis indicated that the four gas exchange variables (assimilation, transpiration, stomatal conductance, and internal leaf CO_2 concentration) differed according to experimental block, machine used for measurement, and date of measurement. The variables were adjusted for these differences by subtracting the mean for each block, machine and date from each individual measurement and adding back the grand mean for each month summed over all blocks, machines, and dates (Tonsor and Goodnight, 1996). This procedure removed deviations due to experimental block, intrinsic differences between the two infra-red gas analyzers, and variation in environmental conditions on different days, while retaining the differences due to CO_2 treatment and other main effects.

The four gas exchange variables were initially analyzed using a multivariate analysis of variance (MANOVA) to determine overall main and interaction effects. The multivariate analysis was followed by appropriate univariate mixed model analyses for the four primary physiological variables plus instantaneous water use efficiency (the ratio of assimilation to transpiration) and specific leaf area (cm^2/g dry weight of leaf tissue). The overall model for the analysis of the physiological variables included four fixed effects (CO_2 treatment, month, CO_2 measurement level , and population), one random effect (family nested within population), and two-way interactions for all main effects. Interaction terms containing the random effect were also treated as random effects. CO_2 measurement level was omitted from the ANOVA for specific leaf area, since measurement level was not a treatment effect for that variable. Expected mean squares for the main effects in the mixed model ANOVA were divided by the appropriate interaction terms to produce the F values for significance tests. Significance levels for the main effects in the multivariate analysis were interpreted using Roy's Greatest Root, recommended because of its statistical power and the fact that it is applicable to post hoc statistical comparisons (Scheiner, 1993). CO_2 treatment means for the chambers and unchambered control sites were individually tested for treatment differences, using

Bonferroni corrections for multiple tests of means. Sample size for each family in each CO_2 environment was $n \leq 6$. Of the 24 families used in the main experiment, 6 families were excluded from the analyses because 2 or fewer individuals germinated in one or both CO_2 environments. All variables in the analyses were normally distributed except transpiration (in August) and stomatal conductance. Log_{10} transformation of transpiration and square-root transformation of stomatal conductance improved normality.

Since each plant in the experiment was measured at two levels of CO_2 in each of two months, a repeated measures analysis was conducted. The repeated measures analysis is analogous to a split-plot analysis in which the repeated measure is considered to be a within-subject effect, and the experimental effects are considered between-subjects effects (Potvin *et al.*, 1990; von Ende, 1993). In this experiment, there were two classifications of repeated measures: CO_2 level (ambient and elevated), and month (August and September). Accordingly, we performed two separate repeated measures analyses: one to test the response of the physiological variables to the level of CO_2 measurement, and the other to test the response of the physiological variables across the two months. The repeated measures analysis produces two kinds of output: a "between-subjects" analysis, and a "within-subjects" analysis. The between-subjects analysis tests the significance of each main effect and interaction in the overall experimental summed over the repeated measure. The first line of the within-subjects analysis shows the significance of the repeated measure, summed over all of the main effects. The following lines of the within-subjects analysis tests the significance of the interaction of the repeated measure and each main effect of the experimental model. All four physiological variables were initially tested together in a multivariate repeated measures analysis, then were considered separately for their contribution to the multivariate results.

RESULTS

The CO_2 treatment responses showed significant variation for each of the physiological traits, more so for the elevated CO_2 measurement level than the ambient CO_2 level, and more in September than in August (Table 3).

CO_2 Treatment Effects

The ambient and elevated CO_2 chambers were compared using a multivariate analysis of variance of the four variables assimilation, transpiration, stomatal conductance and intercellular CO_2 concentration. Water use efficiency was not included in the overall multivariate analysis because it is a linear combination of assimilation and transpiration and would reduce the power of the multivariate analysis. The multivariate analysis revealed that all of the main effects were highly significant (Table 4. A). In addition, all of the two-way interaction terms involving CO_2 as a treatment effect were also highly significant.

Each of the four physiological variables used in the multivariate analysis was also tested using a univariate mixed model analysis of variance (Table 4. B); water use efficiency and specific leaf area were analyzed univariately as well. Assimilation rate and intercellular CO_2 concentration contributed the most to the overall significance of the CO_2 treatment effect in the multivariate analysis. Assimilation rates were higher for the elevated grown plants measured in elevated CO_2 than for the ambient grown plants measured in ambient CO_2 for both months. Intercellular CO_2 was higher in plants at the elevated CO_2 measurement level for all treatments. Water use efficiency and specific leaf area respond significantly to CO_2 treatment. Water use efficiency was higher when

Table 3. Means (s.e.) for CO_2 treatments for ambient and elevated CO_2 measurements. Values for August and September are presented. N = 89 - 99 for each variable. CO_2 treatment means were compared using univariate one-way ANOVAs. Letters represent significant differences for each variable measured at the same CO_2 level during the same month. Lowercase letters, differences between means at ambient CO_2. Uppercase letters, differences between means at elevated CO_2. Differences were considered to be significant at the $p < 0.025$ level (Bonferroni correction for multiple comparisons of means).

Table 3. A. August

Variable	Grown in ambient CO_2, measured in ambient CO_2	Grown in elevated CO_2, measured in ambient CO_2	Grown in unchambered sites, measured in ambient CO_2	Grown in ambient CO_2, measured in elevated CO_2	Grown in elevated CO_2, measured in elevated CO_2	Grown in unchambered sites, measured in elevated CO_2
Assimilation	12.86 (0.37)^a	13.27 (0.33)^a	13.21 (0.41)^a	26.17 (0.52)^A	24.48 (0.58)^A	25.61 (0.58)^A
Transpiration	7.37 (0.17)^a	7.31 (0.17)^a	7.64 (0.16)^a	6.94 (0.15)^B	7.56 (0.20)^A	7.45 (0.14)^A
Water Use Efficiency	1.76 (0.04)^a	1.84 (0.04)^a	1.73 (0.04)^a	3.81 (0.06)^A	3.33 (0.07)^B	3.47 (0.07)^B
Stomatal Conductance	1.23 (0.07)^a	1.09 (0.06)^a	1.19 (0.08)^a	0.87 (0.06)^A	1.05 (0.05)^A	1.02 (0.07)^A
Intercellular CO_2	251.16 (1.74)^a	247.01 (1.85)^a	249.31 (2.12)^a	523.10 (4.38)^B	552.82 (4.09)^A	536.45 (3.86)^B
Specific Leaf Area	213.66 (4.66)^a	188.54 (3.23)^b	198.25 (4.06)^b	-----	-----	-----

Table 3. B. September.

Variable	Grown in ambient CO_2, measured in ambient CO_2	Grown in elevated CO_2, measured in ambient CO_2	Grown in unchambered sites, measured in ambient CO_2	Grown in ambient CO_2, measured in elevated CO_2	Grown in elevated CO_2; measured in elevated CO_2	Grown in unchambered sites, measured in elevated CO_2
Assimilation	13.00 (0.50)^a	11.39 (0.47)^b	13.21 (0.46)^a	24.80 (0.71)^A	19.59 (0.80)^B	23.44 (0.77)^A
Transpiration	7.29 (0.22)^b	7.28 (0.24)^b	8.21 (0.27)^a	6.84 (0.20)^B	7.16 (0.27)^B	7.82 (0.27)^A
Water Use Efficiency	1.78 (0.05)^a	1.51 (0.04)^b	1.64 (0.04)^b	3.68 (0.07)^A	2.76 (0.10)^B	3.09 (0.07)^B
Stomatal Conductance	1.14 (0.07)^a	1.02 (0.07)^b	1.18 (0.06)^a	0.91 (0.06)^A	0.85 (0.05)^B	0.99 (0.06)^A
Intercellular CO_2	259.65 (2.41)^b	264.92 (2.35)^a	263.86 (1.97)^a	529.35 (4.39)^B	568.51 (8.42)^A	554.27 (5.20)^A
Specific Leaf Area	163.79 (3.66)^a	146.70 (3.07)^b	141.69 (3.47)^b	-----	-----	-----

Table 4. Multivariate analysis of variance for the main effects of CO_2 treatment, population, level, month, and family nested within population, and their two-way interactions. Part A. Overall MANOVA for four physiological variables: assimilation, transpiration, stomatal conductance, and intercellular CO_2 concentration. Part B. Summary of univariate mixed model analysis of variance for each of the four physiological variables used in the MANOVA, plus instantaneous water use efficiency and specific leaf area. CO_2 measurement level was not a main effect in the analysis of variance for specific leaf area.

Table 4. A. Overall multivariate analysis.

Source	Numerator df	Denominator df	Roy's Greatest Root	F value	p > F
CO_2 Treatment (**CO_2**)	4	618	0.1527	23.5890	0.0001***
Population (**Pop**)	4	618	0.0223	3.4467	0.0085**
CO_2 Measurement Level (**Level**)	4	618	47.5262	7342.7930	0.0001***
Month (**Month**)	4	618	0.0903	13.9519	0.0001***
Family (Population) (**Fam(Pop)**)	16	621	0.1221	4.7389	0.0001***
CO_2 * Pop	4	618	0.0156	2.4074	0.0483*
CO_2 * Level	4	618	0.0852	13.1579	0.0001***
CO_2 * Month	4	618	0.0322	4.9715	0.0006***
CO_2 * Fam(Pop)	16	621	0.0808	3.1367	0.0001***
Pop * Level	4	618	0.0015	0.2390	0.9163
Pop * Month	4	618	0.0026	0.3961	0.8115
Level * Month	4	618	0.0140	2.1695	0.0710
Level * Fam(Pop)	16	621	0.0247	0.9596	0.5001
Month * Fam(Pop)	16	621	0.0603	2.3396	0.0023**

Table 4. B. Summary of probabilities from univariate mixed model ANOVAs. CO_2 measurement level was not included in the model for SLA.

Source	df	A	E	G_s	C_i	WUE	SLA
CO_2 Treatment (**CO_2**)	1	0.0018**	0.1088	0.2260	0.0001***	0.0001***	0.0001***
Population (**Pop**)	1	0.1703	0.6376	0.5384	0.1697	0.0883	0.9861
CO_2 Measurement Level (**Level**)	1	0.0001***	0.0070**	0.0001***	0.0001***	0.0001***	-----
Month (**Month**)	1	0.0062**	0.7352	0.1282	0.0001***	0.0001***	0.0001***
Family (Population) (**Fam(Pop)**)	16	0.3787	0.2690	0.4497	0.1260	0.2831	0.2231
CO_2 * Pop	1	0.5093	0.3145	0.6446	0.4202	0.1040	0.9667
CO_2 * Level	1	0.0027**	0.0864	0.0130*	0.0001***	0.0001***	-----
CO_2 * Month	1	0.0027**	0.8770	0.0324*	0.1458	0.0001***	0.1436
CO_2 * Fam(Pop)	16	0.0046**	0.0379*	0.4392	0.0491*	0.0219*	0.2168
Pop * Level	1	0.9012	0.1787	0.6575	0.4460	0.5780	-----
Pop * Month	1	0.7645	0.8085	0.8674	0.4372	0.8737	0.7421
Level * Month	1	0.0148*	0.4468	0.9616	0.9736	0.0265*	-----
Level * Fam(Pop)	16	0.9268	0.9995	0.9892	0.8024	0.4179	-----
Month * Fam(Pop)	16	0.0343	0.0026**	0.0162*	0.3277	0.4406	0.4803

measured in elevated CO_2 for all CO_2 treatments. Specific leaf area was greater for the ambient CO_2 grown plants than for either the elevated CO_2 grown plants or the unchambered control plants. At least one two-way interaction, including the only interaction that did not involve a repeated measure, CO_2 treatment x family nested within population, was significant in the univariate analyses for assimilation, transpiration and intercellular CO_2 concentration. This indicates that the families responded differentially to the CO_2 treatment.

Chamber Effects

The open-topped chambers were compared with the unchambered control sites in order to determine the extent of a chamber effect. Plants in the ambient chambers did not differ from the plants in the unchambered control sites with respect to assimilation and stomatal conductance in August and September, at either CO_2 measurement level (Table 3). At the elevated CO_2 measurement level in August, plants in the ambient CO_2 chambers had lower transpiration and higher water use efficiency than the unchambered control plants. Specific leaf area was higher for the ambient CO_2 chamber plants than for the unchambered controls in August and September.

Contrary to expectation, the elevated CO_2 chambers did not differ from the unchambered control sites in the same way as the ambient CO_2 chambers did. There were no significant physiological differences between the plants measured in the elevated CO_2 chambers and the unchambered control sites in August, except for intercellular CO_2 (higher for the elevated CO_2 chamber) (Table 3). In September, plants in the unchambered control sites had greater assimilation rates, transpiration rates, and stomatal conductance at both CO_2 measurement levels than did plants in the elevated CO_2 chambers

(Table 3), but water use efficiency and specific leaf area did not differ for the unchambered control plants and the plants in the elevated CO_2 chambers.

Repeated measures analysis of CO_2 measurement levels

The main effects and interactions involving the physiological measurements at each CO_2 level were further examined using a repeated measures analysis of variance, which takes into account the correlation between repeated measurements on the same plant. The repeated measures analysis was used to indicate the presence of an interaction of the two CO_2 levels with the treatment effect, to determine whether the variables responded differently at the two CO_2 measurement levels, and to determine whether there was an overall difference between the two CO_2 measurement levels, pooled over all treatment effects.

Effects of CO_2 measurement level in August

In August, the multivariate tests were significant for both the CO_2 treatment effect and the family nested within population effect (Table 5. A). The level of measurement had a significant effect on the physiological variables. For each of the variables tested individually, the interaction of CO_2 measurement level and CO_2 treatment was significant. Thus, the direction of response for each of the variables for the two CO_2 growth environments differed between the ambient and elevated levels of measurement. Transpiration rate was similar for the two CO_2 treatments at the ambient CO_2 measurement level. At the elevated CO_2 measurement level, however, the transpiration rate for the ambient CO_2 grown plants was lower, while that of the elevated CO_2 grown plants was higher (Figure 3). Stomatal conductance was similar for the elevated CO_2 grown plants at both CO_2 measurement levels in August; stomatal conductance for the

Table 5. Repeated measures analysis for variables measured at the ambient and elevated CO_2 measurement level (identified as Level in the model). The analysis was conducted separately for each month, first in an overall multivariate analysis, then for each physiological variable separately. Significance levels: *1 $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 5. A. August.

				Physiological Variables:			
Between Subjects Effects:	Num df, Den df	Overall	df	**A**	**E**	**G_s**	**C_i**
CO_2	4, 130	*	1	NS	NS	NS	**
Pop	4, 130	NS	1	NS	NS	NS	NS
Fam(Pop)	16, 133	***	16	*	**	NS	NS
CO_2*Pop	4, 130	NS	1	NS	NS	NS	NS
CO_2*Fam(Pop)	16, 133	NS	16	NS	NS	NS	NS
Within Subjects Effects:							
Level	4, 130	***	1	***	NS	***	***
Level*CO_2	4, 130	***	1	**	***	***	***
Level*Pop	4, 130	NS	1	NS	NS	NS	NS
Level*Fam(Pop)	16, 133	*	16	NS	NS	NS	NS
Level*CO_2*Pop	4, 130	NS	1	NS	NS	NS	NS
Level*CO_2*Fam(Pop)	16, 133	NS	16	NS	NS	NS	NS

Table 5. B. September.

Between Subjects Effects:	Num df, Den df	Overall	df	A	E	G_s	C_i
				Physiological Variables:			
CO_2	4, 118	***	1	***	NS	NS	***
Pop	4, 118	NS	1	NS	NS	NS	*1
Fam(Pop)	16, 121	*	16	NS	NS	NS	NS
CO_2*Pop	4, 118	NS	1	NS	NS	NS	NS
CO_2*Fam(Pop)	16, 121	NS	16	NS	NS	NS	NS
Within Subjects Effects:							
Level	4, 118	***	1	***	***	***	***
Level*CO_2	4, 118	***	1	***	*	NS	***
Level*Pop	4, 118	NS	1	NS	*1	NS	NS
Level*Fam(Pop)	16, 121	*	16	NS	NS	NS	NS
Level*CO_2*Pop	4, 118	NS	1	NS	NS	NS	NS
Level*CO_2*Fam(Pop)	16, 121	*1	16	NS	NS	NS	NS

Figure 3. Comparison of overall means (± s.e.) for assimilation, transpiration, stomatal conductance, and intercellular CO_2 concentration for August and September. In each month, plants grown in each CO_2 treatment were measured at both CO_2 levels, ambient CO_2 and elevated CO2. For intercellular CO_2 concentration error bars may be obscured by symbol.

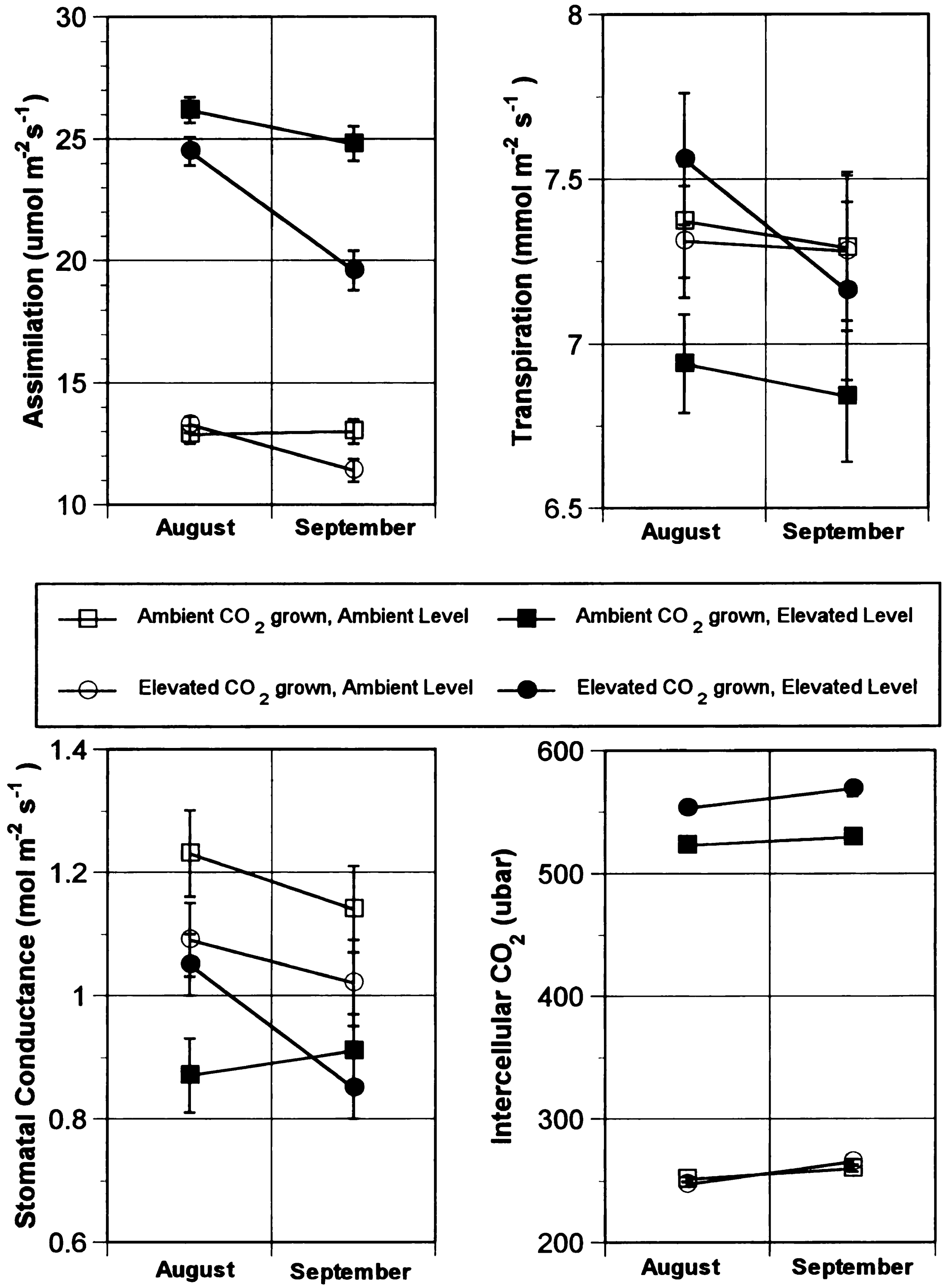

30
25
20
15
10
Assimilation (umol m-2s-1)
August
September
8
7.5
7
6.5
Transpiration (mmol m-2 s-1)
August
September
Ambient CO2 grown, Ambient Level
Ambient CO2 grown, Elevated Level
Elevated CO2 grown, Ambient Level
Elevated CO2 grown, Elevated Level
1.4
1.2
1
0.8
0.6
Stomatal Conductance (mol m-2 s-1)
August
September
600
500
400
300
200
Intercellular CO2 (ubar)
August
September

Figure 3

ambient CO_2 grown plants was higher than that of the elevated CO_2 grown plants measured in ambient CO_2, but lower than the elevated CO_2 grown plants measured in elevated CO_2 (Figure 3). The between-subjects test for the overall CO_2 treatment effect was only statistically significant for intercellular CO_2; the intercellular CO_2 concentration for the elevated CO_2 grown plants was higher than that for the ambient CO_2 grown plants. CO_2 measurement level was a significant effect for each of the physiological variables (Table 5. A); the response of each variable in ambient CO_2 differed from the response of each variable in elevated CO_2. For instance, both assimilation rate and intercellular CO_2 concentration were higher at the elevated CO_2 measurement level for both ambient and elevated CO_2 grown plants. In all cases, the pattern of response for water use efficiency was similar to that for assimilation rate. Assimilation rate and water use efficiency were highly correlated (overall $r^2 = 0.75$; $p < .0001$), while transpiration and water use efficiency were not as strongly correlated ($r^2 = -0.21$; $p < .0001$).

Effects of CO_2 measurement level in September

In September, both the CO_2 treatment and family nested within population effects were significantly different in direction and magnitude in the multivariate analysis (Table 5. B). When the variables were examined separately, the assimilation and intercellular CO_2 concentration results were similar. Both assimilation and intercellular CO_2 concentration were higher at the elevated CO_2 measurement level than in ambient CO_2, but the plants grown in elevated CO_2 had lower assimilation rates and higher intercellular CO_2 concentrations in elevated CO_2 than did the ambient CO_2 grown plants (Figure 3). For transpiration, there was a treatment x CO_2 level interaction; the elevated CO_2 grown plants maintained the same transpiration rate at both ambient and elevated CO_2 measurement levels, but the ambient grown plants transpired less in elevated CO_2 than they did in ambient CO_2. The results of the individual tests for stomatal conductance were nonsignificant, except for the effect of CO_2 level of measurement; stomatal conductance was lower for both ambient and elevated CO_2 grown plants in elevated CO_2 than in ambient CO_2.

Repeated measures analysis for August and September

A separate repeated measures analysis was conducted for the physiological measurements made during August and September, to take into account the correlation between measurements made at two separate times on the same plant. The repeated measures analysis was used to indicate the presence of an interaction of the two measurement times with the treatment effect, to determine whether the variable responded differently during August and September, and to determine whether there was an overall difference between the two months, pooled over all treatment effects.

Differences between August and September at the ambient CO_2 level

When the months were compared at the ambient CO_2 measurement level, the multivariate tests were significant for the CO_2 treatment, family nested within population, and treatment x family (population) interaction effects (Table 6. A). Only assimilation rate showed significant results for both the direction and magnitude of response across the months when the variables were examined individually. The assimilation rate in September tended to be lower for the elevated CO_2 grown plants in ambient CO_2 than the ambient CO_2 grown plants (Figure 3). Likewise, water use efficiency was lower for the elevated grown plants in September at the ambient CO_2 measurement level than in August (Table 3; Figure 3). For transpiration and intercellular CO_2 concentration, the

Table 6. Repeated measures analysis for variables measured in August and September. The analysis was conducted separately for each CO_2 measurement level, first in an overall multivariate analysis, then for each physiological variable separately. Significance levels: *1 $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 6. A. Ambient CO_2 measurement level.

				Physiological Variables:			
Between Subjects Effects:	Num df, Den df	Overall	df	**A**	**E**	**G_s**	**C_i**
CO_2 Trt	4, 115	*	1	*1	NS	*	NS
Pop	4, 115	NS	1	*	NS	NS	*1
Fam(Pop)	16, 118	**	16	*	NS	NS	*
CO_2*Pop	4, 115	NS	1	NS	NS	NS	NS
CO_2*Fam(Pop)	16, 118	**	16	NS	NS	NS	NS
Within Subjects Effects:							
Month	4, 115	***	1	*	NS	NS	***
Month*CO_2 Trt	4, 115	*	1	*	NS	NS	NS
Month*Pop	4, 115	NS	1	NS	NS	NS	NS
Month*Fam(Pop)	16,118	**	16	NS	NS	NS	NS
Month*CO_2*Pop	4, 115	NS	1	NS	NS	NS	NS
Month*CO_2*Fam(Pop)	16, 118	**	16	NS	NS	NS	NS

Table 6. B. Elevated CO_2 measurement level.

				Physiological Variables:			
Between Subjects Effects:	Num df, Den df	Overall	df	**A**	**E**	**G_s**	**C_i**
CO_2 Trt	4, 126	***	1	***	*	NS	***
Pop	4, 126	NS	1	NS	NS	NS	NS
Fam(Pop)	16,129	**	16	NS	NS	NS	*1
CO_2*Pop	4, 126	NS	1	NS	NS	NS	NS
CO_2*Fam(Pop)	16, 129	NS	16	NS	NS	NS	NS
Within Subjects Effects:							
Month	4, 126	***	1	***	NS	*	*
Month*CO_2 Trt	4, 126	***	1	**	NS	***	NS
Month*Pop	4, 126	NS	1	NS	NS	*	NS
Month*Fam(Pop)	16, 129	*	16	NS	*1	NS	NS
Month*CO_2*Pop	4, 126	NS	1	NS	NS	NS	NS
Month*CO_2*Fam(Pop)	16,129	*	16	NS	NS	*	NS

performance of both ambient and elevated CO_2 grown plants measured in ambient CO_2 was virtually identical in August and September. Stomatal conductance, however, was higher for the ambient CO_2 grown plants than the elevated CO_2 grown plants in both August and September (Table 6.A).

Differences between August and September at the elevated CO_2 level

At the elevated CO_2 measurement level, the overall multivariate tests were significant for the CO_2 treatment and the family nested within population effects (Table 6. B); in addition, there was a significant CO_2 treatment x family (population) interaction in the direction of response in the two months. When examined univariately, both assimilation and stomatal conductance responded across the months in a nonparallel direction. Stomatal conductance was similar for the ambient CO_2 grown plants in both August and September, but it declined for the elevated CO_2 grown plants in September compared to August. The ambient CO_2 grown plants had higher assimilation rates, lower transpiration rates and lower intercellular CO_2 concentrations at the elevated CO_2 measurement level in both August and September than the elevated CO_2 grown plants (Table 3; Figure 3). Assimilation rates and stomatal conductance tended to be lower in September than in August; intercellular CO_2 concentration was higher in September than in August at the elevated CO_2 measurement level (Figure 3).

Population and Family Interactions with CO_2 Treatment

The multivariate analysis revealed a significant CO_2 treatment by population interaction for the physiological variables (Table 6. A), but the same interaction term was not significant for any of the physiological variables when examined individually (Table 6. B). Thus, overall the populations responded differently to CO_2 treatment. The repeated measures analysis indicated significant month by population interactions for assimilation rate at the ambient CO_2 measurement level (Table 6. A), and for stomatal conductance at the elevated CO_2 measurement level (Table 6. B). Ambient CO_2 grown plants in Population EL had lower stomatal conductance in September than in August (0.94 August v. 0.88 September), while ambient CO_2 grown plants from Population KF had higher stomatal conductance in September than in August (0.82 August v. 0.93 September).

The individual families showed significant interaction with CO_2 treatment. This was revealed in the multivariate analysis (Table 6. A), and in the univariate mixed model analyses for assimilation, transpiration, intercellular CO_2 concentration, and water use efficiency (Table 6. B). Figure 3 shows the individual family means for assimilation rate for both CO_2 treatments at both CO_2 measurement levels, in both months. Some families responded similarly to CO_2 measurement level, whether they were grown in ambient CO_2 or elevated CO_2 (for example, families EL 7, EL 9, KF 33 and KF 44 in August). Some families showed very different assimilation patterns in September compared to August (e.g., families EL 5, EL 9, KF 33, KF 37, KF 39, KF 43, and KF 44). Therefore, the pattern of response of the families for assimilation and other physiological traits was variable.

The repeated measures analysis revealed a significant interaction between month, CO_2 treatment and family for stomatal conductance at the elevated CO_2 measurement level (Table 6. B, Figure 4). As with assimilation, the types of response to CO_2 treatment with respect to stomatal conductance were extremely variable. Regardless of CO_2 growth environment, some families showed increased stomatal conductance in September compared to August, some families had decreased stomatal conductance in September,

Figure 4. Comparison of individual family means for stomatal conductance in August and September. (top) Plants grown in ambient CO_2, measured in elevated CO_2. (bottom) Plants grown in elevated CO_2, measured in elevated CO_2.

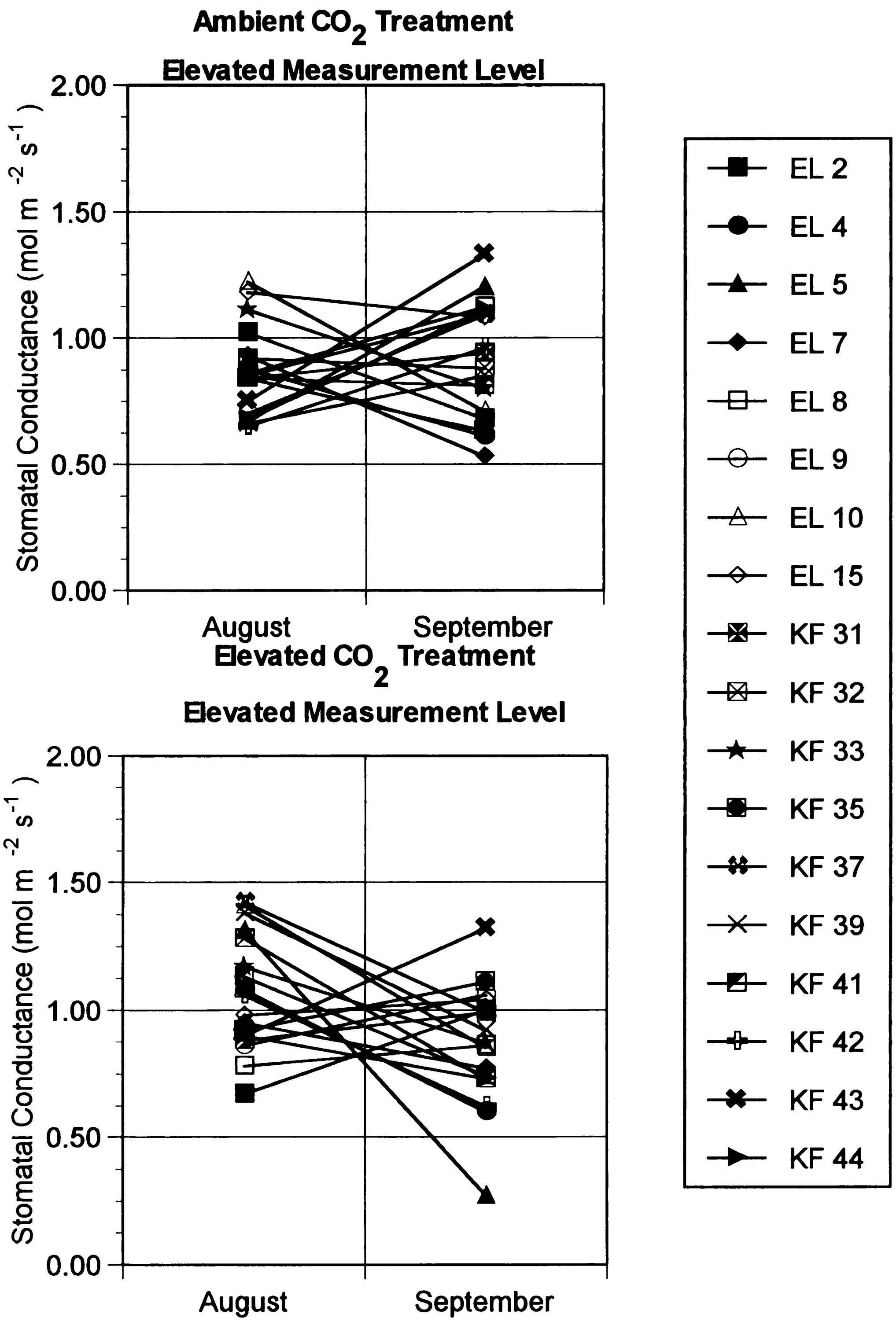

Ambient CO_2 Treatment
Elevated Measurement Level
Stomatal Conductance (mol m $^{-2}$ s $^{-1}$)
2.00
1.50
1.00
0.50
0.00
August
September
Elevated CO_2 Treatment
Elevated Measurement Level
Stomatal Conductance (mol m $^{-2}$ s $^{-1}$)
2.00
1.50
1.00
0.50
0.00
August
September
EL 2
EL 4
EL 5
EL 7
EL 8
EL 9
EL 10
EL 15
KF 31
KF 32
KF 33
KF 35
KF 37
KF 39
KF 41
KF 42
KF 43
KF 44

Figure 4

and still other families showed little change in stomatal conductance in September compared to August. This variation contributed to the nonparallel responses between August and September when compared by CO_2 treatment (Figure 3).

DISCUSSION

Overall, plants grown and measured in elevated CO_2 were significantly different physiologically from plants grown and measured in ambient CO_2. Plants grown and measured in elevated CO_2 had higher assimilation rates, higher water use efficiency, and lower stomatal conductance than plants grown and measured in ambient CO_2. Transpiration did not differ greatly between the ambient CO_2 and elevated CO_2 grown plants, so the increase in water use efficiency was directly related to the increase in assimilation rates in the elevated CO_2 grown plants. These results differed from the 1991 experiment, in which ambient CO_2 assimilation rates were more similar to elevated CO_2 assimilation rates, and where transpiration rates were lower for the elevated CO_2 grown plants (see Appendix). The failure of transpiration to decrease in 1992 as G_s declined may have been due to increased leaf temperatures of vapor pressure deficits in the elevated CO_2 plants, neither of which variables was measured in this experiment.

The differences between the plants in the two growth environments for assimilation, water use efficiency and stomatal conductance were not as marked in September as they were in August. Ambient CO_2 grown plants maintained the same physiological potential to respond to elevated CO_2 in September as in August, but assimilation and water use efficiency declined for the elevated CO_2 grown plants in September. Even though the physiological performance of the elevated CO_2 grown plants

declined in September, carbon fixation rates of the plants grown and measured in elevated CO_2 remained higher than carbon fixation rates of the plants grown and measured in ambient CO_2.

When compared to the unchambered control sites, some of the differences in the performance of plants in the ambient and elevated CO_2 chambers could be attributed to the presence of the chambers, while other differences seemed to be due to the action of elevated CO_2. The plants in both types of chambers (ambient CO_2 and elevated CO_2) had lower transpiration rates than the unchambered controls. This was probably due to reduced wind and increased relative humidity inside the chambers, a common occurrence in open topped chambers (Leadley and Drake, 1993). The leaves of plants inside the ambient CO_2 chambers had greater specific leaf area than the unchambered controls, but this was not the case for the plants inside the elevated CO_2 chambers. Since a reduction of specific leaf area is a well-known effect of increased CO_2 (Bazzaz, 1990), the increase in specific leaf area in the ambient chambers was probably a chamber effect which was offset in the elevated CO_2 chambers by the elevated CO_2.

Overall, the physiological results suggest that negative acclimation to elevated CO_2 was occurring in the elevated CO_2 grown plants. While assimilation rates were similar for plants measured in both CO_2 environments in August, the ambient CO_2 grown plants had lower transpiration and intercellular CO_2 concentration at the elevated CO_2 measurement level than the elevated CO_2 grown plants measured at the same level. The difference in performance of the elevated CO_2 grown plants compared to the ambient CO_2 grown plants was even greater later in the experiment. In September, the plants grown in elevated CO_2 had lower assimilation rates, lower stomatal conductance, and higher intercellular CO_2

concentrations than the ambient CO_2 grown plants at each measurement level. Both ambient CO_2 and elevated CO_2 grown plants declined in stomatal conductance and specific leaf area in September compared to August, occurrences common as the growing season progresses (Morison, 1987; den Hartog *et al.*, 1993). The decline in assimilation rate between August and September only occurred in the elevated CO_2 chambers, however, which indicates that this response was due more to long-term exposure to elevated CO_2 than to the progression of the growing season.

The decline in photosynthetic rates for the elevated CO_2 grown plants compared to the ambient CO_2 grown plants in September occurred at both CO_2 measurement levels, at equivalent intercellular CO_2 concentrations (Figure 5). Data from other investigations (von Caemmerer and Farquhar, 1981; DeLucia *et al.*, 1985; Sage *et al.*, 1989) have revealed that declines in photosynthetic rates prior to the CO_2 saturation point are generally due to a reduction either in the amount or in the activation state of Rubisco. Measurements made at ambient CO_2 corresponded to this pre-saturation level. In ambient CO_2, the elevated CO_2 grown plants measured in September were showing signs of Rubisco limitation (Figure 5). Measurements made in elevated CO_2 corresponded to saturating intercellular CO_2 concentrations. Again, the elevated CO_2 grown plants measured in September also showed the greatest signs of RuBP regeneration limitation of assimilation rates (Figure 5). Nitrogen concentrations in ambient and elevated CO_2 were measured for a subset of three families from the experiment. Per cent nitrogen was the same in the aboveground tissues of the elevated CO_2 grown plants compared to the ambient CO_2 grown plants (Klus, Ch. 1). Therefore, some of the nitrogen in the elevated CO_2 grown plants may have been reallocated from photosynthetic machinery to other

Figure 5. Assimilation versus intercellular leaf CO_2 concentration at both CO_2 measurement levels. At the ambient CO_2 measurement level, intercellular CO_2 concentration was close to 200 μbar. At the elevated CO_2 measurement level, intercellular CO_2 concentration was close to 500 μbar. Vertical bars indicate ± one s.e. for assimilation means. Horizontal bars indicate ± one s.e. for intercellular CO_2 means.

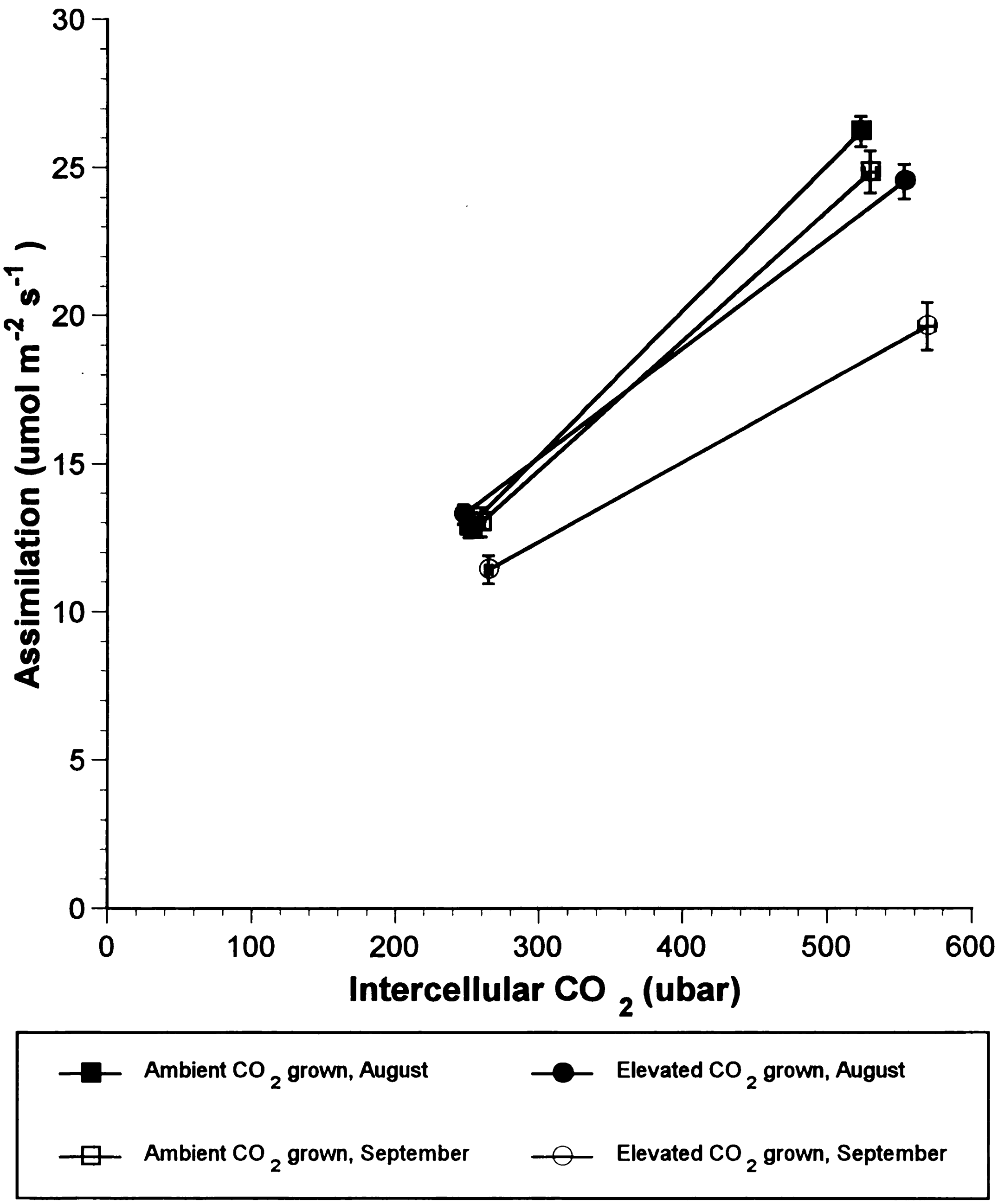

Assimilation (umol m-2 s-1)
30
25
20
15
10
5
0
0
100
200
300
400
500
600
Intercellular CO 2 (ubar)
Ambient CO 2 grown, August
Elevated CO 2 grown, August
Ambient CO 2 grown, September
Elevated CO 2 grown, September

Figure 5

molecules in the aboveground tissues.

Ecological and Evolutionary Implications

It is important to note that while the photosynthetic performance of the elevated CO_2 grown plants had declined from its maximum potential compared to the ambient CO_2 grown plants, the elevated CO_2 grown plants still assimilated at significantly higher rates in their own growth environment than the ambient CO_2 grown plants in ambient CO_2. These results are similar to those of Hollinger (1987) and Yelle *et al.* (1989) in which plants grown in elevated CO_2 maintained higher assimilation rates in their own environment. It is possible that as the growing season progressed, the plants in the elevated CO_2 environment were integrating their physiological response to elevated CO_2 by reallocating some nitrogen away from photosynthetic processes, possibly to other molecules in leaves or roots to increase their efficiency of nitrogen use and their capacity for nutrient uptake (Klus, Ch. 1).

The significant CO_2 by population interaction term in the overall multivariate analysis of the physiological variables (Table 6) indicated that the two populations responded differently to the CO_2 treatment. A key variable contributing to this interaction was water use efficiency (Table 6. B). Water use efficiency was higher for Population EL than Population KF in ambient CO_2 during both months (data not shown), but water use efficiency for Population KF increased to match that of Population EL in the elevated CO_2 treatment. This change in population response in the two CO_2 environments appeared to stem from the fact that, in several instances, assimilation rates were higher for Population EL than Population KF in ambient CO_2, but were similar for the two populations in elevated CO_2. The two populations may have been interpreting the ambient CO_2

environment in slightly different ways due to their habitats of origin. Population EL grows in sandy soil near a lake, and experiences high irradiance and periodic drought stress. Population KF grows in the moister, shadier habitat of a mown field. Depending upon the nature of local changes in rainfall and air temperature that may accompany increasing atmospheric CO_2 in the next 100 years, this difference in water use efficiency among the two populations in the elevated CO_2 treatment may be critical in determining the success of these populations in a novel environment.

Moreover, the intraspecific variation in instantaneous response to elevated CO_2 and the variation in the capacity to maintain a positive response to elevated CO_2 discovered in this experiment may mean that some families will be placed at an advantage or disadvantage relative to other families in an elevated CO_2 world. Families that are able to maintain lower stomatal conductance and higher water use efficiency may be better able to survive in a world of increased drought. Some families may be better than others at allocating resources so as to maintain high assimilation rates while also maximizing the use and acquisition of nitrogen. Under such circumstances, families showing such variation in response to elevated CO_2 may very well undergo changes in competitive rankings with respect to other members of their plant community. Depending upon the scale of interaction, this may result in changes in intraspecific and interspecific community dynamics.

Significant CO_2 x family interactions in physiological responses at both CO_2 measurement levels and for both months suggests the existence of intraspecific genetic variation in response to elevated CO_2. It was not possible to predict an individual family's performance in elevated CO_2 from its performance in ambient CO_2; therefore, families with higher assimilation rates, stomatal conductance, or water use efficiency at one CO_2 measurement level in August did not necessarily have equivalent rates at the other measurement level or during September. While the positive effects of CO_2 declined for some families over time, other families maintained a positive physiological response to CO_2 through the end of September.

If physiological variation somehow affects general plant performance (fitness) in the long term, then this physiological variation may result in variation in fitness upon which natural selection may act. The degree of variation in physiological response to elevated CO_2 at the family level in this experiment were similar to that of biomass accumulation and allocation previously described (Klus, Ch. 1). Yet the patterns of physiological response and biomass allocation for each family in elevated CO_2 did not correspond. Families grown in elevated CO_2 that expressed higher assimilation and water use efficiency, or higher specific leaf area, were no bigger on average than families with lower rates of assimilation, lower water use efficiency and lower specific leaf area.

It may be the case that exposure to elevated CO_2 initiates responses in plants that have contradictory growth outcomes. For example, in current ambient CO_2 conditions, high relative growth rate is often associated with high specific leaf area, but not necessarily with high photosynthetic rates (Lambers, 1987; Shipley, 1995). In an elevated CO_2 environment, plants generally increase photosynthetic rates, but other changes in morphology may prevent higher assimilation rates from being translated into higher growth rates. If excess carbohydrates formed by photosynthesis accumulate in the leaves, changes in leaf architecture may occur which might increase the diffusive resistance to CO_2 inside the leaf and increase dry matter content on an area basis (decrease specific leaf

area) (Dijkstra and Lambers, 1989). Although starch did not accumulate in the leaves of the plants in this experiment, there was a small increase in % carbon in aboveground tissues (Klus, Ch. 1), a decrease in specific leaf area, a decrease in stomatal conductance (Table 5), and a decrease in assimilation at equivalent intercellular CO_2 concentrations in the elevated CO_2 grown plants (Figure 5). An increase in the number of mesophyll cells devoted to photosynthesis (Shipley, 1995) or an increase in cell wall material, and an increase in diffusive resistance to internal CO_2 in the leaves of the plants grown in elevated CO_2 (Dijkstra and Lambers, 1989) might explain why the elevated grown plants showed some negative photosynthetic acclimation and why earlier increases in plant size were not maintained throughout the growing season.

The mechanisms by which plants translate their physiological response to elevated to the level of the whole plant remain little explored. The chain of events in response to elevated CO_2 by which plants integrate physiology with growth and biomass allocation must be more clearly elucidated before we will be able to predict the potential ecological and evolutionary impact of intraspecific variation in plant response to elevated atmospheric CO_2.

CHAPTER THREE

GROWTH RESPONSES TO ELEVATED CARBON DIOXIDE IN PLANTAGO LANCEOLATA

INTRODUCTION

Increased atmospheric CO_2 has direct effects on plant life (Strain and Cure, 1985). Physiological traits are altered in plants exposed to elevated CO_2, and growth often increases (reviewed in Bazzaz, 1990). Kimball (1983) and others (Cure and Acock, 1986) have predicted that increased growth in response to elevated CO_2 in the next one hundred years may result in as much as a 30% greater yield in agricultural crops. Depending upon experimental conditions, though, plant growth responses to elevated CO_2 have been found to be complicated by interactions with other environmental conditions (Bazzaz, 1990).

While the positive effects of elevated CO_2 on plant growth are most often seen when other nutrients are not limiting (Bazzaz, 1990), some studies have determined that CO_2 may have positive effects on growth even in nutrient-poor conditions (Norby *et al.*, 1986; Bowes, 1993). Often elevated CO_2 positively affects early growth rates (Wulff and Alexander, 1985), but those effects may decline over time (Wulff and Strain, 1982). Yet, despite the decline in the effect of elevated CO_2 on growth rate over time, early stimulation of growth may be sufficient to result in greater biomass in elevated CO_2 grown plants compared to ambient CO_2 grown plants (Wulff and Strain, 1982; Poorter *et al.*, 1988).

Important in determining the magnitude of growth response to elevated CO_2 is a plant's physiological response to CO_2 enrichment. It is generally agreed upon that physiological characteristics influence growth characteristics, but the connection between physiology and growth is not clear (McGraw and Wulff, 1983; Fichtner, 1994). The manufacture of plant tissue depends upon the supply of organic energy storage molecules and construction materials to the tissues manufactured by photosynthetic and biosynthetic pathways. Yet in agriculture, where plant growth and yield are critical issues, plants artificially selected for increased growth and yield often do not have increased photosynthetic rates (Evans, 1975). Accordingly, selection for increased photosynthetic rates does not always result in either increased growth or yield (Evans, 1975; McGraw and Wulff, 1983). Photosynthesis and growth may be integrated through other plant traits such as specific leaf area (Poorter, 1993).

Complicating attempts to understand the connection between photosynthesis and growth in response to elevated CO_2 is the fact that elevated CO_2 may affect individual plant traits in ways that may have contradictory effects on growth. For example, while elevated CO_2 is often associated with higher assimilation rates and greater biomass accumulation, elevated CO_2 also generally results in decreased specific leaf area, a condition associated with low growth rates (Konings, 1989; Garnier, 1992). Elevated CO_2 also affects other physiological processes such as stomatal conductance and transpiration which control water balance in the plant and interact with assimilation rates to determine overall water use efficiency (reviewed in Bazzaz, 1990). Therefore, the effect of elevated CO_2 on growth is mediated through assimilation rates and the interaction of assimilation rates with stomatal characteristics, water use, and biomass allocation.

It has been hypothesized that growth potential may be determined more by source-sink relations and the way a plant allocates photosynthate within and among its various organs to utilize and acquire other nutrients efficiently than by intrinsic photosynthetic capacity (Evans, 1975; Fichtner, 1994). Growth responses to elevated CO_2 are enhanced in plants which possess an adequate sink for the carbohydrate manufactured by photosynthesis (Poorter, 1993), for example, roots. Arp (1991) reviewed studies in which growth responses to elevated CO_2 declined over time, and determined that a lack of growth response was most pronounced when the plants were limited in the amount of biomass they could allocate to belowground tissues (usually because of small pot sizes). Plants that manufacture other sinks for carbohydrate, such as fruits or woody tissue, often show a greater growth response to elevated CO_2 than plants which do not possess such sinks (Poorter, 1993). Being able to allocate carbohydrate to sinks may help plants maintain an internal balance between the supply of carbon and the supply of nutrients, by moving the carbohydrate into storage organs, by using the carbohydrate to build more tissues to acquire nutrients, or by reallocating nutrients within the plants tissues to increase their effective use (Arp, 1991; Poorter, 1993).

Therefore, plants which are flexible in their ability to allocate organic resources among various types of tissue, for example, between aboveground and belowground tissues, seem to have a greater capacity for successfully translating physiological potential into growth than plants for which the relationship between physiology and growth is more canalized (Poorter, 1993). Interspecific variation exists in the extent to which plants can reallocate tissues or nutrients in response to changes in their abiotic environment. For example, *Plantago lanceolata* can change allocation to roots and shoots in response to changes in nitrogen availability, but *Plantago major* does not seem to be as flexible (Kuiper and Bos, 1992). Variation in growth responses to elevated CO_2 that are influenced by source-sink relationships have been documented for species possessing a variety of life histories (Poorter, 1993).

Understanding the way elevated CO_2 affects growth is critical in determining how plant life will respond to increasing levels of atmospheric CO_2 on a large scale. Increasing atmospheric CO_2 may have implications for plant-plant interactions (for example, competitive relationships) and evolutionary potential (Tilman, 1993; Geber and Dawson, 1993). Growth and biomass allocation patterns, not simple physiological potential, will ultimately determine whether plants will shift in competitive hierarchies within plants communities. Allocation of resources in response to elevated CO_2 for survival and reproduction will affect plant fitness, and therefore the capacity of plants to respond evolutionarily to elevated CO_2.

Experiments conducted in 1992 explored the effects of elevated CO_2 on biomass allocation (Klus, Ch. 1), physiology (Klus, Ch. 2) and growth traits in two populations of *Plantago lanceolata*. *Plantago lanceolata* is a species especially well-suited for a study of the effects of elevated CO_2 on growth traits. For herbaceous perennials such as *P. lanceolata*, size at the end of the first growing season is often an indicator of survival and fecundity in subsequent growing seasons (Primack, 1979; Solbrig, 1981). *P. lanceolata* also possesses an underground sink for carbon in the form of a corm, and is flexible in its allocation of biomass depending upon nutrient conditions (Kuiper and Bos, 1992). Another potential sink for carbohydrate are the vegetative side shoots which *Plantago lanceolata* produces under appropriate growth conditions (Teramura, 1983). To determine the response of early growth parameters to elevated CO_2 on two populations of *P. lanceolata*, I performed censuses throughout one growing season for the aboveground traits of leaf number, vegetative shoot number, and overall shoot diameter (the diameter of the base of the plant, including the side shoots, just above the soil surface). A subset of plants censused for growth traits was harvested midseason in order to determine biomass allocation patterns to above- and belowground tissues after exposure to elevated CO_2 for approximately one-half of the growing season. The plants harvested midseason were also measured physiologically and in terms of final biomass allocation to match the treatment of the plants in the larger experiment. By examining all three aspects of response to elevated CO_2, I hoped to gain insight into how physiological traits and the allocation of resources to tissues interact in determining whole plant growth responses to an increased supply of carbon resources.

MATERIALS AND METHODS

The study populations of *Plantago lanceolata* were chosen to represent two distinct habitats. The Ely Lake (EL) population (Allegan County, Michigan) grows on exposed sand on a sunny lakeshore, experiencing high irradiance and periodic water stress. The Kellogg Field (KF) population (Kalamazoo County, Michigan) grows Kalamazoo loam in partial shade on the edge of a mown field.

A total of 24 families, twelve randomly selected from each of the two populations, were used in the experiment. On June 5, 1992, all of the seeds from each family were divided into two equal groups, planted in separate flats, and placed in either ambient or twice-ambient (hereafter referred to as elevated) CO_2 to germinate. Germination took place over 7 - 10 days. On June 17, six maternal siblings from each family in each CO_2 environment were transplanted into separate 30-cm high pots made from 10-cm diameter

PVC pipe with mesh screen bottoms. The day of transplanting was considered Day 1 of the experiment. The pots were filled with a 50-50 mixture of relatively inorganic native field soil (Kalamazoo loam) and sand. 144 pots (6 pots x 12 families x 2 populations) were distributed randomly among four replicate outdoor open-top chambers in each CO_2 environment. Both ambient and elevated CO_2 chambers had one-meter square internal dimensions, contained 36 pots each, and were constructed following the protocol of Curtis and Teeri (1992). To determine the effect of the chambers themselves, seventy-two additional seedlings from each population were planted in individual pots and distributed randomly among four 1 m^2 unchambered control sites. The chambers and unchambered control sites were arrayed in four randomized blocks in Bailey Field, an abandoned agricultural site at Kellogg Biological Station, Hickory Corners, Michigan. Each block contained one ambient CO_2 chamber, one elevated CO_2 chamber, and one unchambered control site. In preparation for the experiment, the site was cleared, herbicided, and disked to smooth out uneven patches.

A smaller experiment (hereafter referred to as the growth experiment) was conducted in conjunction with the large-scale experiment (referred to as the main experiment) in order to follow early growth patterns and to determine biomass allocation and physiology in midseason without performing a destructive harvest within the main experiment. Seedlings from Populations EL and KF which had been germinated in ambient CO_2 or elevated CO_2 were transplanted into four-inch pots and placed on the ground between the larger main experiment pots. The growth experiment was set up in two ambient CO_2 chambers, two elevated CO_2 chambers, and two unchambered control sites, selected at random from those in the main experiment. Fifteen pots were placed in each location, providing a sample size of 30 for each of the experimental treatments (ambient CO_2, elevated CO_2, and the unchambered controls).

The experimental site at Bailey Field experienced full sun throughout the day. The plants were watered as needed, usually twice daily. There was no fertilizer supplementation. Pure CO_2, mixed with ambient air by ventilation fans, was supplied 24 hours per day to the elevated chambers. Ambient air was circulated within the ambient chambers by the same type of fan. CO_2 levels were monitored continuously and levels were recorded on a computer at three-minute intervals (Curtis and Teeri, 1992). Mean daytime (0700-1900 hours) CO_2 partial pressure inside the elevated chambers was 72 ± 6 Pa (± s.d.), with the mean daytime CO_2 partial pressure inside the ambient chambers being 36 ± 3 Pa. Quantum sensors and shaded thermocouples attached to a LI-1000 datalogger (LICOR Inc., Lincoln NB, USA) recorded irradiance levels and temperature. Daytime temperatures were 1.7 ± 0.6 °C higher inside the chambers than in the unchambered control sites, with no significant difference in temperature between ambient and elevated chambers. Three weeks into the experiment the young plants were exhibiting symptoms of light stress (prostrate growth and red pigmentation of the leaf bases) and all chambers and control sites were covered with shade cloth. The shade cloth reduced ambient light by 68%, and the plants recovered their normal phenotype.

Leaf number, the number of vegetative shoots (shoot number), and the basal diameter of the plant (shoot diameter) taken just above the soil surface, were measured during three censuses for the growth experiment (Day 13, Day 27, and Day 49) and during four censuses for the main experiment (Day 10, Day 59, Day 78, and Day 127). Physiological measurements were taken immediately prior to the final census for the plants in the growth experiment (methodology for physiological measurement described in Chapter 2). The most recently fully expanded leaf of each plant in the growth experiment was measured physiologically, then removed from the plant and pressed. After drying fully, the area and mass of each leaf were measured and specific leaf area was calculated. The growth experiment plants were harvested on Day 49. The roots and shoots were separated at the soil surface, bagged, and dried at 60 °C. Aboveground biomass, belowground biomass, and root: shoot ratio were determined from the dried plant material. Plants in the main experiment were not harvested until the end of the growing season on Day 127.

Statistical Analysis

The census data for the main experiment and the growth experiment were analyzed using a repeated measures profile analysis, as recommended by von Ende (1993) and Potvin *et al.* (1990). The repeated measures analysis is analogous to a split-plot analysis in which the repeated measure is considered to be a within-subject effect, and the experimental effects are considered between-subjects effects (Potvin *et al.*, 1990; von Ende, 1993). I performed two separate repeated measures analyses. The census data were analyzed for the repeated measure of time, and the physiological data gathered from the growth experiment were analyzed for the repeated measure of CO_2 measurement level.

The census data were first compared using a multivariate model; then each variable was analyzed individually in a separate repeated measures analysis to determine its influence on the multivariate analysis. In this experiment, time (i.e., the repeated census dates) was the repeated measure. Significance levels for the main effects in the multivariate analysis were interpreted using Roy's Greatest Root, recommended because of its statistical power and the fact that it is applicable to post hoc statistical comparisons (Scheiner, 1993). Greenhouse-Geisser F statistics were used to determine the significance of the treatment effects for the individual variables. Greenhouse-Geisser F values are adjusted to account for inequalities of variance in the univariate analysis of repeated measures, and are considered to be conservative with respect to Type I errors (von Ende, 1993). The repeated measures analysis produces two kinds of output: a "between-subjects" analysis, and a "within-subjects" analysis. The between-subjects analysis tested the significance of each main effect and interaction in the overall experimental design summed over the repeated censuses. The first line of the within-subjects analysis showed the significance of time, summed over all of the main effects. The following lines of the within-subjects analysis showed the significance of the interaction of time and each main effect of the experimental model. For the main experiment, the main effects were Block, Treatment (including the ambient and elevated CO_2 chambers and the unchambered control sites), Population, and Family nested within population. The growth experiment did not include the effect of individual families nested within the populations.

Because the measurement of growth traits took place more than twice, follow-up contrasts were performed to determine the significance of each census interval and its interaction with the main effects. For the main experiment there were three follow-up contrasts: Day 10 with Day 59, Day 59 with Day 78, and Day 78 with Day 127. The growth experiment included two contrasts: Day 13 with Day 27, and Day 27 with Day 49.

The physiological data for the main experiment were analyzed using a profile analysis similar to the census analysis, but with CO_2 measurement level as the repeated measure. Since there were only two repeated measures in the physiological analysis, ambient CO_2 and elevated CO_2, follow-up contrasts were not required.

The final biomass allocation data for the growth experiment were analyzed using analysis of variance to compare treatment means. Treatment means for the chambers and unchambered control sites were individually tested for treatment differences, using Bonferroni corrections for multiple tests of means (Scheiner, 1993). Sample size for each treatment in the growth experiment was 17 - 29. Sample size for each treatment in the main experiment was 87 - 92. Sample size for each family in each CO_2 environment for the main experiment was $n \leq 6$. Of the 24 families used in the main experiment, 6 families were excluded from analysis because 2 or fewer individuals germinated in one or both CO_2 environments.

RESULTS

The mean values for leaf number, shoot number, and shoot diameter varied considerably over the course of the growing season for both the main experiment (Figures 6- 8, Table 7. A) and the growth experiment (Figures 6 - 8, insets; Table 7. B). Although the plants in the ambient CO_2 chambers, the elevated CO_2 chambers, and the unchambered control sites were indistinguishable in size for each of the variables at the beginning of the experiment, they assumed different growth trajectories early in the experiment. In some cases the early differences were maintained until the end of the experiment; in other cases, early differences among the main treatments in aboveground growth traits disappeared by the end of the experiment. Leaf number increased more for the plants in the elevated CO_2 chambers during the first census interval than for the other two treatments (Figure 6).

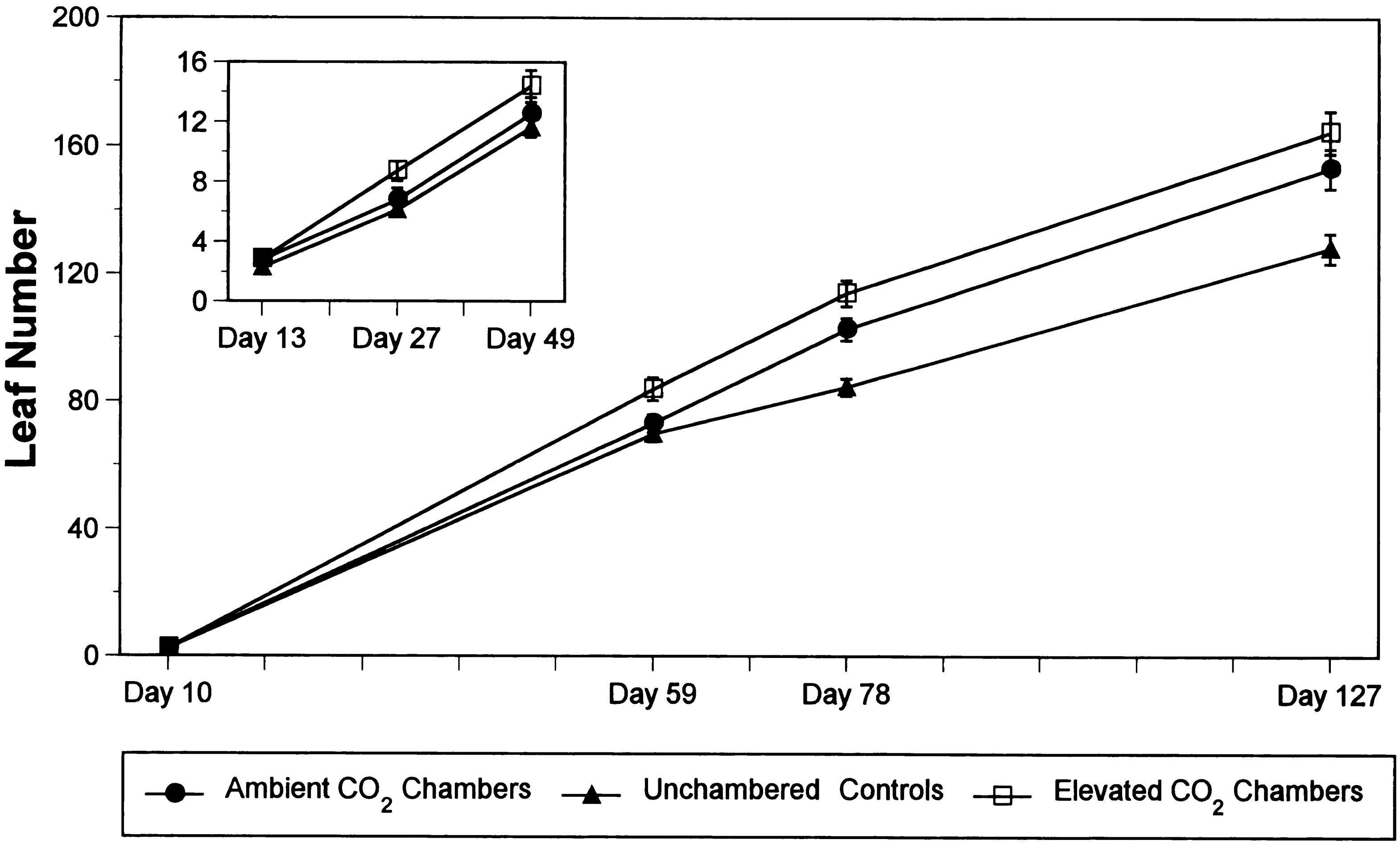


Figure 6. Overall means for leaf number at each census date for main experiment and growth experiment (inset). Vertical bars indicate one s.e.

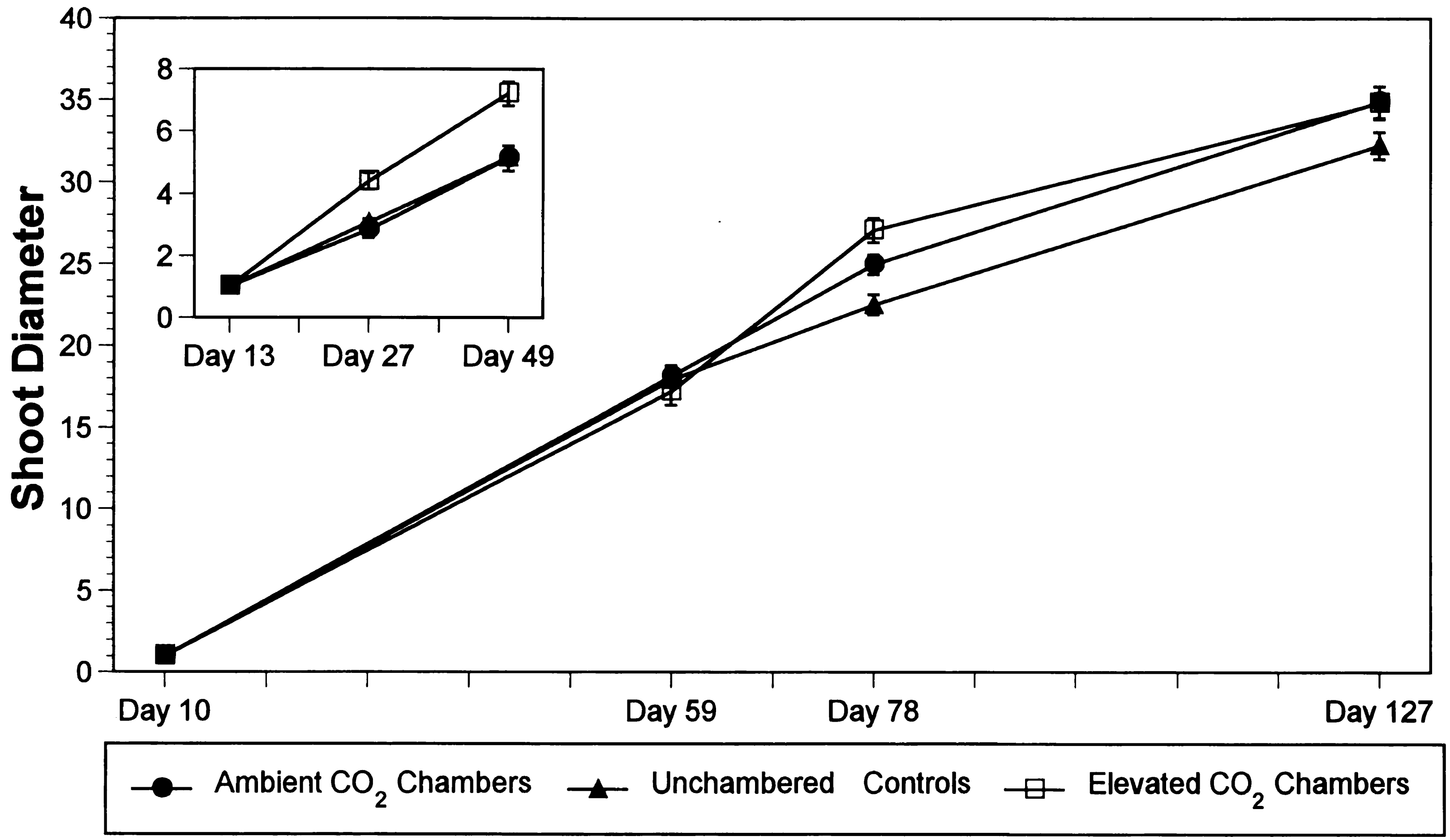


Figure 7. Overall means for basal shoot diameter at each census date for main experiment and growth experiment (inset). Vertical bars indicate one s.e.

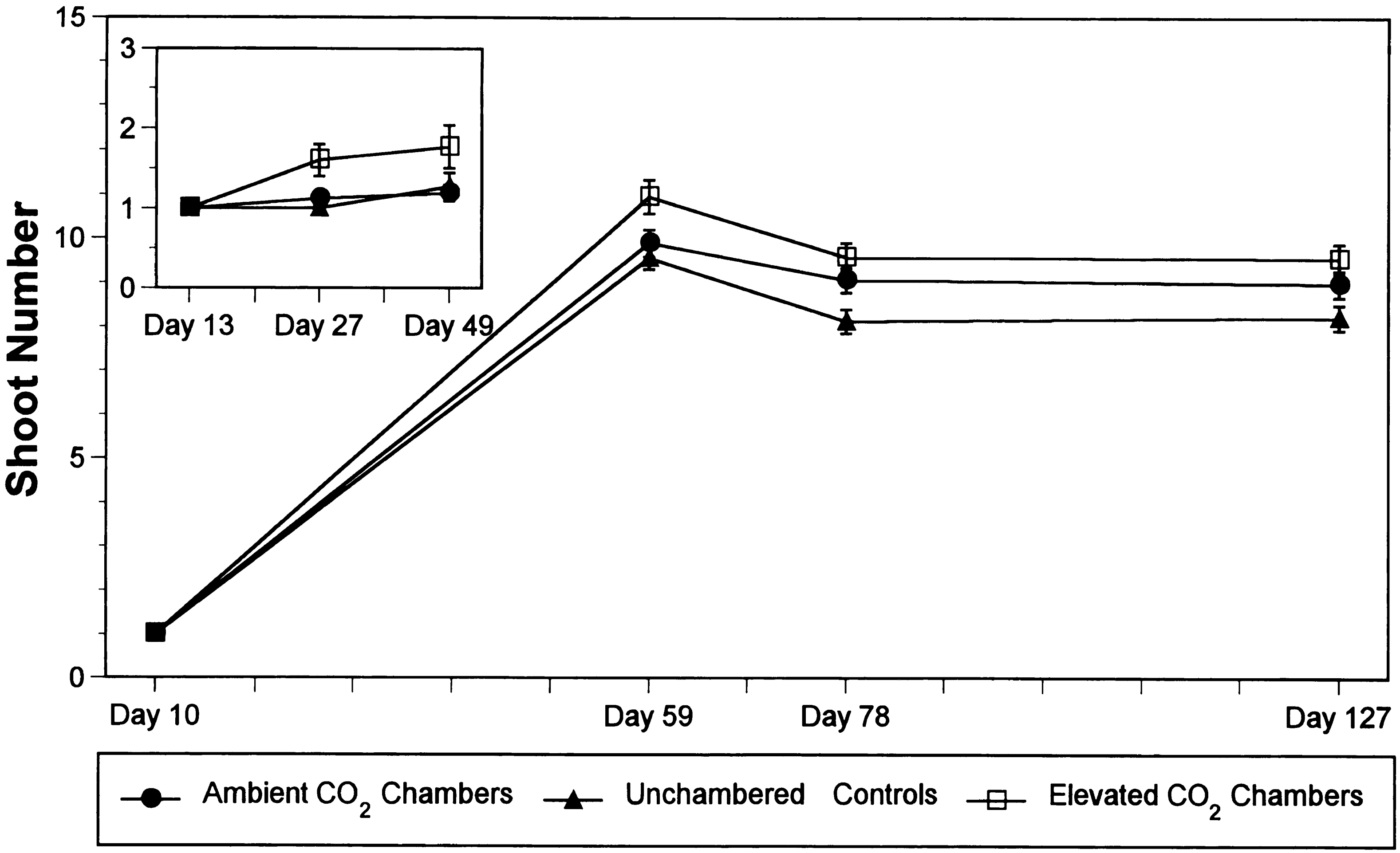


Figure 8. Overall means for vegetative shoot number at each census date for main experiment and growth experiment (inset). Vertical bars indicate one s.e.

Table 7. A. Individual tests of treatment means for main experiment. Comparison of ambient CO_2 chambers, elevated CO_2 chambers, and unchambered controls. Shoot number and shoot diameter for Day 10 were not compared since each plant had only one shoot which was too small to be measured on that date. Means were compared using pairwise contrasts in an analysis of variance. Different letters indicate that means differ by $p < 0.017$ (Bonferroni adjustment for multiple contrasts).

Growth Variables:	**Ambient CO_2 Chamber Mean (s.e.)** n = 87 - 92	**Unchambered Control Site Mean (s.e.)** n = 79 - 82	**Elevated CO_2 Chamber Mean (s.e.)** n = 91 - 99
Leaf Number, Day 10	2.38 (0.07)[a]	2.38 (0.06)[a]	2.38 (0.08)[a]
Leaf Number, Day 59	73.04 (2.80)[b]	69.72 (2.49)[b]	83.80 (3.56)[a]
Leaf Number, Day 78	102.59 (3.57)[a]	84.44 (2.67)[b]	113.91 (4.13)[a]
Leaf Number, Day 127	153.00 (6.14)[a]	128.09 (4.71)[b]	164.28 (6.56)[a]
Shoot Diameter, Day 59	18.14 (0.66)[a]	17.90 (0.44)[a]	17.19 (0.83)[a]
Shoot Diameter, Day 78	24.98 (0.59)[a]	22.52 (0.62)[b]	27.07 (0.74)[a]
Shoot Diameter, Day 127	34.89 (0.95)[a]	32.24 (0.83)[a]	34.84 (0.99)[a]
Shoot Number, Day 59	9.89 (0.30)[ab]	9.55 (0.25)[b]	10.94 (0.38)[a]
Shoot Number, Day 78	9.05 (0.28)[ab]	8.12 (0.27)[b]	9.57 (0.32)[a]
Shoot Number, Day 127	8.96 (0.30)[ab]	8.20 (0.29)[b]	9.53 (0.34)[a]

Table 7. B. Individual tests of treatment means for growth experiment. Comparison of ambient CO_2 chambers, elevated CO_2 chambers, and unchambered controls. Shoot number and shoot diameter for Day 13 were not compared since each plant had only one shoot which was too small to be measured on that date. Means were compared using pairwise contrasts in an analysis of variance. Different letters indicate that means differ by $p < 0.017$ (Bonferroni adjustment for multiple contrasts).

Growth Variables:	**Ambient CO_2 Chamber Mean (s.e.)** n = 23 - 26	**Unchambered Control Site Mean (s.e.)** n = 29 - 30	**Elevated CO_2 Chamber Mean (s.e.)** n = 29 - 30
Leaf Number, Day 13	$2.78\ (0.33)^{a}$	$2.24\ (0.15)^{a}$	$2.83\ (0.19)^{a}$
Leaf Number, Day 27	$6.77\ (0.79)^{ab}$	$6.10\ (0.32)^{b}$	$8.70\ (0.65)^{a}$
Leaf Number, Day 49	$12.50\ (1.10)^{a}$	$11.60\ (0.62)^{a}$	$14.37\ (1.07)^{a}$
Shoot Diameter, Day 27 (mm)	$2.83\ (0.26)^{b}$	$3.06\ (0.13)^{b}$	$4.39\ (0.27)^{a}$
Shoot Diameter, Day 49 (mm)	$5.13\ (0.41)^{b}$	$5.15\ (0.21)^{b}$	$7.21\ (0.38)^{a}$
Shoot Number, Day 27	$1.12\ (0.08)^{b}$	$1.0\ (0.0)^{b}$	$1.60\ (0.20)^{a}$
Shoot Number, Day 49	$1.19\ (0.10)^{a}$	$1.23\ (0.17)^{a}$	$1.77\ (0.27)^{a}$
Biomass Variables:			
Aboveground Biomass (g)	$0.37\ (0.04)^{b}$	$0.50\ (0.03)^{ab}$	$0.62\ (0.05)^{a}$
Belowground Biomass (g)	$0.19\ (0.03)^{b}$	$0.38\ (0.03)^{a}$	$0.45\ (0.05)^{a}$
Root: Shoot Ratio	$0.44\ (0.03)^{b}$	$0.76\ (0.05)^{a}$	$0.69\ (0.04)^{a}$
Specific Leaf Area ($cm^2\ g^{-1}$)	$348.16\ (9.18)^{a}$	$288.49\ (11.73)^{b}$	$315.42\ (12.91)^{b}$

This was true for both the main experiment and the growth experiment. For the growth experiment, all three treatments increased in leaf number in parallel after Day 27. Leaf number was indistinguishable for the three treatments in the growth experiment at Day 49 (Table 7. B). In the main experiment, leaf number increased in the ambient CO_2 chambers during the second census interval to run parallel to the elevated chambers. After Day 78, leaf number in the ambient CO_2 and elevated CO_2 chambers was indistinguishable (Table 7. A).

Shoot diameter diverged very early on for the plants in the three treatments in the growth experiment (Figure 7, inset). The plants in the elevated CO_2 treatment maintained a higher shoot diameter than either of the other two treatments through Day 49 (Table 7. B). In the growth experiment, shoot diameter did not diverge for the three treatments until after Day 59, when the increase in shoot diameter for the elevated CO_2 grown plants was more rapid than for the other two treatments (Figure 7). After Day 78, the ambient CO_2 chamber plants and the unchambered control plants continued to increase in shoot diameter to the same degree, while the increase in shoot diameter for the elevated CO_2 grown plants tapered off . However, by the end of the experiment, the three treatments could not be distinguished from each other for shoot diameter (Table 7. A.).

The increase in shoot number for the plants in the experiment was quite different than that for the other two size variables (Figure 8). Again, shoot number diverged very early on for the elevated CO_2 plants in the growth experiment (Figure 8, inset), as it did for the plants in the main experiment. Shoot number declined for the plants in the main experiment between Day 59 and Day 78; thereafter, the ranking of the three treatments with respect to shoot number remained consistent until the end of the experiment.

Repeated Measures Analysis of Census Variables

The overall multivariate profile analysis of the census variables revealed a significant main effect of block and an interaction of block with family effects (main experiment) (Table 8. A) or population effects (growth experiment) (Table 9. A). These block interactions were due to unequal representation of the families or the populations in the blocks, depending upon the experiment. The biologically relevant effects in the experimental model resulted from the treatments and the interactions of the treatments with the populations, families, or time of measurement. Overall, the treatment and time of measurement effects were significant for both the main experiment and the growth experiment (Table 8. A; Table 9. A). In addition, there were significant two- and three-way interactions involving treatments, populations, families, and time in the main experiment; only the two-way interaction of time with treatment was significant overall for the growth experiment.

Repeated measures analysis of the main experiment

The main effect of time was significant for each of the three comparisons of census dates for the main experiment, except for shoot number during the third census interval (Table 8. B). Leaf number (Figure 6) and shoot diameter (Figure 7) increased over time for all three census intervals, while shoot number (Figure 8) did not significantly increase after the third census. The significant time by treatment interaction terms during the first and second intervals indicated that the plants in the chambers and the unchambered control sites did not respond to the same degree for leaf number and shoot number (Figure 6, Figure 8). Both the ambient and elevated CO_2 chambers had similar leaf numbers during the second and third census intervals; plants inside both types of chambers had greater leaf

Table 8. A. Repeated measures profile analysis of main experiment variables. Comparisons include ambient CO_2 chambers, elevated CO_2 chambers, and unchambered control sites. Between subjects tests detect differences among main effects and interactions, summed over the repeated measure. Within subjects tests detect main effect of the repeated variable and interaction of the repeated variable with the main experimental effects.

Experimental Effects					**Growth Variables**	
Between Subjects Effects:	Numdf, Den df	Overall (Roy's Greatest Root)	df	**Leaf Number**	**Shoot Number**	**Shoot Diameter**
Block	3, 129	***	3	**	NS	***
Treatment (Trt)	3, 128	*	2	NS	NS	NS
Pop	3, 127	NS	1	NS	NS	NS
Fam (Pop)	26, 129	***	26	**	*1	**
Block*Trt	6, 129	*	6	NS	NS	NS
Block*Pop	3, 129	NS	3	NS	NS	NS
Block*Fam (Pop)	60, 129	**	63	*	*	*
Trt*Pop	3, 128	*	2	*	*	NS
Trt*Fam(Pop)	17, 129	**	17	NS	NS	*
Error	-----	-----	140	-----	-----	-----
Within Subjects Effects:						
Time	3, 127	***	3	***	***	***
Time*Block	3, 129	***	9	***	**	***
Time*Trt	3, 128	*	6	NS	NS	*
Time*Pop	3, 127	NS	3	NS	NS	NS
Time*Fam(Pop)	26, 129	***	78	***	*1	**
Time*Block*Trt	6, 129	*	18	NS	NS	**
Time*Block*Pop	3, 129	NS	9	NS	NS	NS
Time*Block*Fam(Pop)	60, 129	**	180	**	*1	**
Time*Trt*Pop	3, 128	*	6	**	*	NS
Time*Trt*Fam(Pop)	17, 129	**	51	NS	NS	*
Error (Time)	-----	-----	387	-----	-----	-----

Table 8. B. Comparisons of census dates for growth experiment. Tests for interactions between main effects and successive census dates.

Comparison:		**Growth Variables:**		
Day 10-Day 59	df	**Leaf Number**	**Shoot Number**	**Shoot Diameter**
Time	1	***	***	***
Time*Block	3	***	NS	***
Time*Trt	2	*	*1	NS
Time*Pop	1	NS	NS	NS
Time*Fam(Pop)	26	*	NS	*
Time*Block*Trt	6	NS	NS	**
Time*Block*Pop	3	NS	NS	NS
Time*Block*Fam(Pop)	60	*1	*1	*
Time*Trt*Pop	2	NS	NS	NS
Time*Trt*Fam(Pop)	17	NS	NS	*1
Error	129	-----	-----	-----
Day 59-Day 78	df	**Leaf Number**	**Shoot Number**	**Shoot Diameter**
Time	1	***	***	***
Time*Block	3	**	**	***
Time*Trt	2	*	NS	***
Time*Pop	1	NS	NS	NS
Time*Fam(Pop)	26	NS	NS	NS
Time*Block*Trt	6	*	NS	***
Time*Block*Pop	3	NS	NS	NS
Time*Block*Fam(Pop)	62	NS	NS	*
Time*Trt*Pop	2	NS	NS	NS
Time*Trt*Fam(Pop)	17	*1	NS	*1
Error	149	-----	-----	-----

Table 8. B. (cont.)

Day 78-Day 127	**df**	**Leaf Number**	**Shoot Number**	**Shoot Diameter**
Time	1	***	NS	***
Time*Block	3	*	NS	*
Time*Trt	2	NS	NS	NS
Time*Pop	1	NS	NS	NS
Time*Fam(Pop)	26	**	NS	NS
Time*Block*Trt	6	NS	NS	NS
Time*Block*Pop	3	NS	NS	NS
Time*Block*Fam(Pop)	63	NS	NS	*1
Time*Trt*Pop	2	*1	NS	NS
Time*Trt*Fam(Pop)	17	NS	NS	NS
Error	149	-----	-----	-----

Table 9. A. Repeated measures profile analysis of growth experiment variables. Comparisons of ambient CO_2 chambers, elevated CO_2 chambers, and unchambered control sites. Between subjects tests detect differences among main effects and interactions, summed over the repeated measure. Within subjects tests detect main effect of the repeated variable and interaction of the repeated variable with the main experimental effects.

Experimental Effects				**Growth Variables**		
Between Subjects Effects:	Num df, Den df	Overall (Roy's Greatest Root)	df	**Leaf Number**	**Shoot Number**	**Shoot Diameter**
Block	3, 69	NS	2	NS	NS	NS
Treatment (Trt)	3, 69	***	2	NS	NS	**
Pop	3, 68	NS	1	NS	NS	NS
Block*Trt	3, 68	NS	1	NS	NS	NS
Block*Pop	3, 69	*1	2	*1	NS	NS
Trt*Pop	3, 69	NS	2	NS	NS	NS
Error	70	-----	70	-----	-----	-----
Within Subjects Effects:						
Time	3, 68	***	2	***	***	***
Time*Block	3, 69	NS	4	NS	NS	NS
Time*Trt	3, 69	**	4	NS	NS	**
Time*Pop	3, 68	NS	2	NS	NS	NS
Time*Block*Trt	3, 68	NS	2	NS	NS	NS
Time*Block*Pop	3, 69	NS	4	NS	NS	NS
Time*Trt*Pop	3, 69	NS	4	NS	NS	NS
Error (Time)	70	-----	140	-----	-----	-----

Table 9. B. Comparisons of census dates for growth experiment. Tests for interactions between main effects and successive census dates.

Comparison:			**Growth Variables:**	
Day 13-Day 27	df	**Leaf Number**	**Shoot Number**	**Shoot Diameter**
Time	1	***	**	***
Time*Block	2	NS	NS	NS
Time*Trt	2	*1	*1	**
Time*Pop	1	NS	NS	NS
Time*Block*Trt	1	NS	NS	NS
Time*Block*Pop	2	*1	*1	*1
Time*Trt*Pop	2	NS	NS	NS
Error	70	-----	-----	-----
Day 27-Day 49	df	**Leaf Number**	**Shoot Number**	**Shoot Diameter**
Time	1	***	*	***
Time*Block	2	NS	NS	*1
Time*Trt	2	NS	NS	NS
Time*Pop	1	NS	NS	NS
Time*Block*Trt	1	NS	NS	NS
Time*Block*Pop	2	NS	NS	NS
Time*Trt*Pop	2	NS	NS	NS
Error	70	-----	-----	-----

numbers than the unchambered control sites (Table 7. A). Plants in the elevated CO_2 chambers had greater shoot numbers than the unchambered control sites also, but the ambient CO_2 chambers could not be distinguished from the either the elevated CO_2 chambers or the unchambered control sites (Table 7. A).

Repeated measures analysis of the growth experiment

In the growth experiment, the time effect was significant for both census intervals for all three census variables (Table 9. B). All three variables showed increases in size throughout the experiment (Figures 6 - 8, insets). The interaction of time with treatment, however, was only significant for the first census interval. The chambers and unchambered control sites increased in leaf number, shoot number and shoot diameter at different rates during the first census interval, but after that point the responses increased in parallel. By the time the plants in the growth experiment were harvested, the plants in the chambers and the unchambered control sites could not be distinguished from each other for leaf number or shoot number, but the plants in the elevated CO_2 chambers had a greater shoot diameter than the plants in either the ambient CO_2 chambers or the unchambered control sites (Table 7. B).

Biomass Allocation in the Growth Experiment

After the growth experiment was harvested, biomass allocation patterns were compared for the three treatments. Plants in the ambient CO_2 chambers were smaller in terms of overall biomass and root: shoot ratio than in either of the other two treatments (Figure 9), and they had greater specific leaf area than the elevated CO_2 plants or the unchambered control plants (Table 7. B). Plants in the elevated CO_2 chambers and the unchambered control sites could not be distinguished from one another for aboveground

Figure 9. Comparison of overall means for aboveground biomass, belowground biomass, and root: shoot ratio for chambers and unchambered control sites. Vertical bars indicate one s.e. Scale for root: shoot ratio appears to the right. Means were tested by pairwise contrasts in an analysis of variance. Different letters indicate that means differ $p < 0.017$ (Bonferroni adjustment for multiple contrasts).

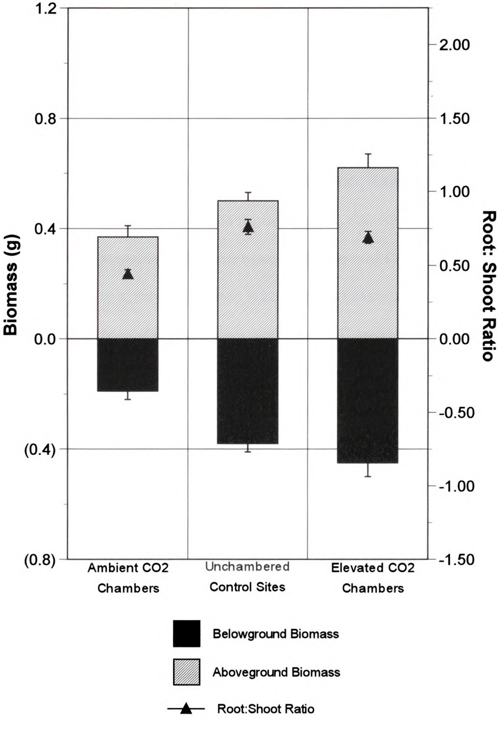

1.2
0.8
0.4
0.0
(0.4)
(0.8)
Biomass (g)
2.00
1.50
1.00
0.50
0.00
-0.50
-1.00
-1.50
Root: Shoot Ratio
Ambient CO2 Chambers
Unchambered Control Sites
Elevated CO2 Chambers
Belowground Biomass
Aboveground Biomass
Root:Shoot Ratio

Figure 9

biomass, belowground biomass, root: shoot ratio, or specific leaf area (Figure 9; Table 7. B). Therefore, the plants in the ambient CO_2 chambers had less biomass overall and produced leaves of smaller mass per unit area than the plants in either of the other treatments.

Because the plants in the ambient CO_2 chambers showed increases in leaf number and shoot number similar to the plants in the elevated CO_2 chambers, but were smaller in terms of overall biomass, the plants in the ambient CO_2 chambers were increasing in size without similarly increasing in biomass compared to elevated CO_2 treatment. Therefore, at the time of harvest, the plants grown in both types of chambers were the same size, but the plants in the elevated CO_2 chambers were heavier. Conversely, the unchambered control plants were smaller than the elevated CO_2 chamber plants in terms of leaf number and shoot number at the final harvest, but were the same as the elevated CO_2 plants in terms of final biomass and biomass allocation.

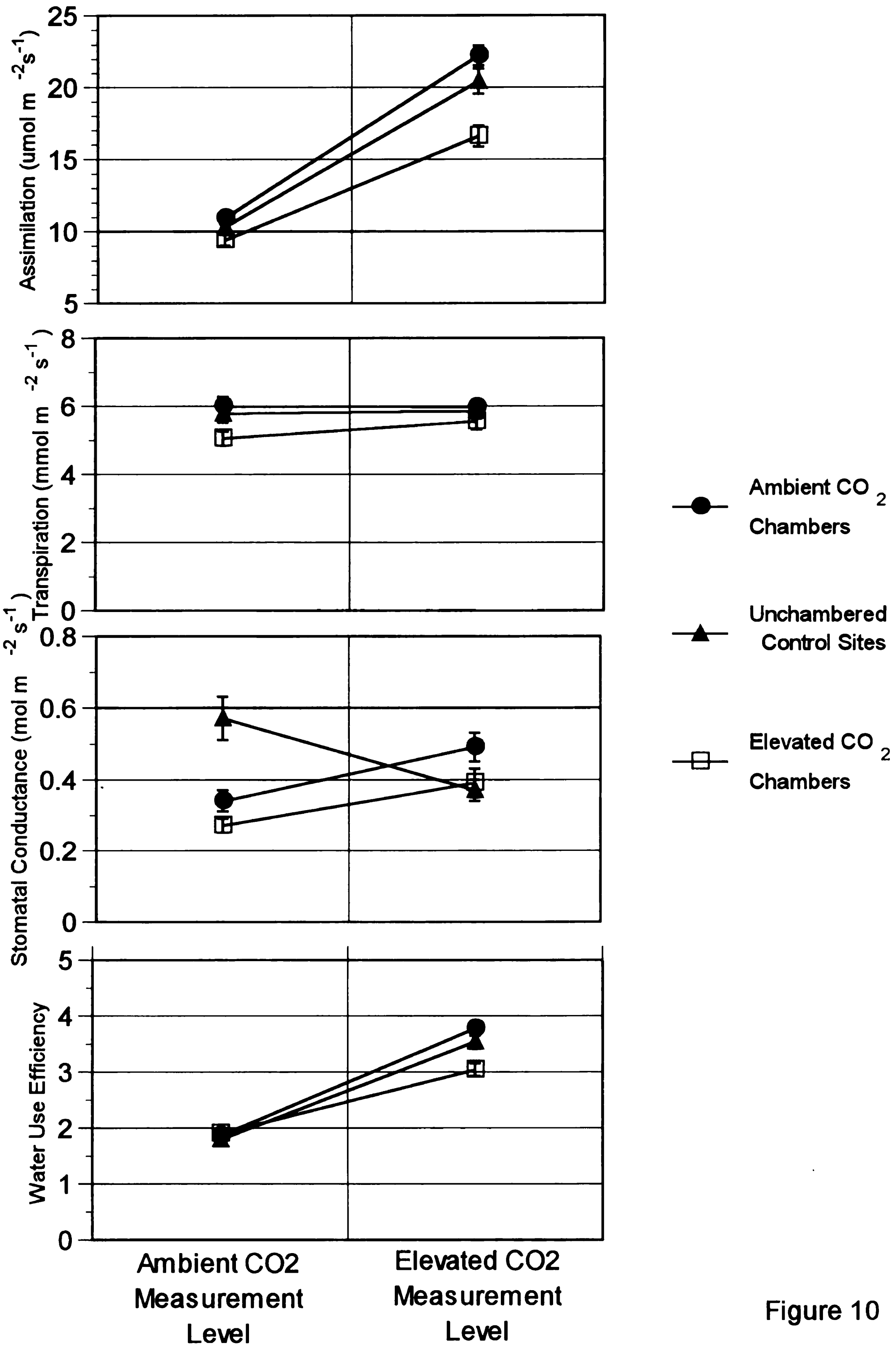
Physiology in the Growth Experiment

A repeated measures analysis was used to determine the response of the plants in the growth experiment to CO_2 measurement level. Plants from the three treatments could not be distinguished from each other for assimilation rates and water use efficiency at the ambient CO_2 measurement level (Table 10); however, the plants responded in different degrees to the elevated CO_2 measurement level (Figure 10). While the plants from all three treatments had higher assimilation rates and instantaneous water use efficiency in the elevated CO_2 measurement level than in ambient CO_2, both the ambient CO_2 grown plants and the unchambered control plants had greater photosynthetic rates and water use efficiency in elevated CO_2 than the elevated CO_2 grown plants (Figure 10; Table 11). A

Table 10. Individual tests of treatment means for physiological variables in growth experiment. Comparison of ambient CO_2 chambers, elevated CO_2 chambers, and unchambered controls. Means were compared using pairwise contrasts in an analysis of variance. Letters indicate significant differences, $p < 0.017$ (Bonferroni adjustment for multiple contrasts).

Variable	**Ambient CO_2 Chamber Mean (s.e.)** n = 17 - 18	**Unchambered Control Site Mean (s.e.)** n = 29	**Elevated CO_2 Chamber Mean (s.e.)** n = 29
Assimilation Rate, Ambient CO_2 Level	10.94 (0.45)[a]	10.33 (0.55)[a]	9.41 (0.39)[a]
Assimilation Rate, Elevated CO_2 Level	22.22 (0.68)[a]	20.43 (0.88)[a]	16.59 (0.73)[b]
Transpiration Rate, Ambient CO_2 Level	5.99 (0.28)[a]	5.78 (0.26)[ab]	5.05 (0.21)[b]
Transpiration Rate, Elevated CO_2 Level	5.96 (0.21)[a]	5.85 (0.22)[a]	5.54 (0.24)[a]
Water Use Efficiency, Ambient CO_2 Level	1.86 (0.07)[a]	1.79 (0.06)[a]	1.90 (0.08)[a]
Water Use Efficiency, Elevated CO_2 Level	3.77 (0.13)[a]	3.54 (0.13)[a]	3.04 (0.11)[b]
Stomatal Conductance, Ambient CO_2 Level	0.34 (0.03)[b]	0.57 (0.06)[a]	0.27 (0.02)[b]
Stomatal Conductance, Elevated CO_2 Level	0.49 (0.04)[a]	0.37 (0.03)[a]	0.39 (0.04)[a]
Intercellular CO_2, Ambient CO_2 Level	218.06 (3.59)[a]	245.97 (3.18)[a]	236.70 (3.92)[a]
Intercellular CO_2, Elevated CO_2 Level	520.63 (6.65)[a]	519.78 (8.38)[a]	541.05 (6.00)[a]

Figure 10. Comparison of overall growth experiment means (± s.e.) for assimilation, transpiration, stomatal conductance, and water use efficiency at both ambient and elevated CO_2 measurement levels.



25
20
15
10
5
Assimilation (umol m -2 s-1)
8
6
4
2
0
Transpiration (mmol m -2 s-1)
0.8
0.6
0.4
0.2
0
Stomatal Conductance (mol m -2 s-1)
5
4
3
2
1
0
Water Use Efficiency
Ambient CO2 Measurement Level
Elevated CO2 Measurement Level
Ambient CO 2 Chambers
Unchambered Control Sites
Elevated CO 2 Chambers

Figure 10

Table 11. Repeated measures profile analysis of physiological variables in growth experiment. Comparisons of ambient CO_2 chambers, elevated CO_2 chambers, and unchambered control sites. Between subjects tests detect differences among main effects and interactions, summed over the repeated measure. Within subjects tests detect main effect of the repeated variable and interaction of the repeated variable with the main experimental effects.

Experimental Effects:				**Physiological Variables:**			
Between Subjects Effects:	Num df, Den df	Overall (Roy's Greatest Root)	df	**A**	**E**	**G_s**	**C_i**
CO_2 Trt	3, 68	***	2	***	NS	*	**
Pop	3, 67	*1	1	NS	*1	NS	NS
CO_2 Trt*Pop	3, 68	NS	2	NS	NS	*1	NS
Error	-----	-----	69	----	-----	-----	-----
Within Subjects Effects:							
CO_2 Level	3, 67	***	1	***	*	NS	***
Level*CO_2 Trt	3, 68	***	2	**	*1	***	***
Level*Pop	3, 67	NS	1	NS	NS	NS	NS
Level*CO_2 Trt*Pop	3, 68	NS	2	NS	NS	NS	NS
Error (Level)	-----	-----	69	-----	-----	-----	-----

Figure 11. Assimilation versus intercellular leaf CO_2 concentration at both ambient and elevated CO_2 measurement levels for growth experiment. At the ambient CO_2 measurement level, intercellular CO_2 concentration was close to 200 μbar for all treatments. At the elevated CO_2 measurement level, intercellular CO_2 concentration was close to 500 μbar for all treatments. Vertical bars indicate ± s.e. for intercellular CO_2 means.

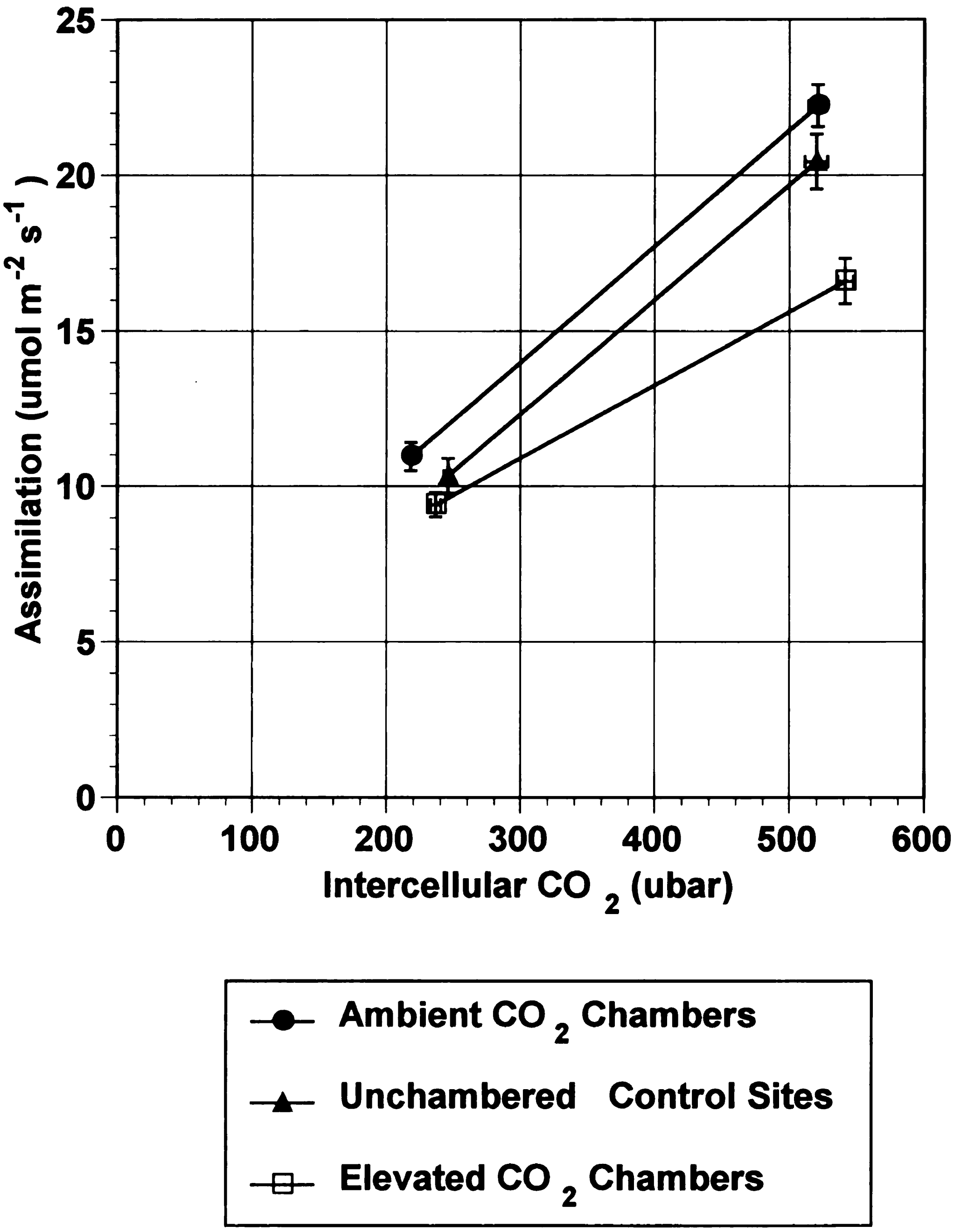

25
20
15
10
5
0
0
100
200
300
400
500
600
Assimilation (umol m-2 s-1)
Intercellular CO 2 (ubar)
Ambient CO 2 Chambers
Unchambered Control Sites
Elevated CO 2 Chambers

Figure 11

graph of the relationship between assimilation and intercellular CO_2 showed that the elevated CO_2 grown plants had lower assimilation rates at elevated CO_2 than the other two groups of plants for an equivalent intercellular CO_2 concentration (Figure 11). The relationship between assimilation rate and water use efficiency at the two CO_2 measurement levels in the growth experiment was similar to that in the main experiment (Klus, Ch. 2).

Surprisingly, both the ambient CO_2 grown plants and the elevated CO_2 grown plants had higher stomatal conductance in elevated CO_2 than they did in ambient CO_2 (Figure 10), opposite the response of the plants to CO_2 level in the main experiment (Ch. 2). Only the unchambered control plants had lower stomatal conductance in elevated CO_2 than in ambient CO_2. Therefore, stomatal conductance had a significant treatment by level interaction term in the analysis (Table 11). Transpiration was the least variable of the physiological traits measured at both ambient and elevated CO_2 (Table 10; Figure 11). Transpiration rates were similar for all three groups of plants at both measurement levels.

DISCUSSION

The effects of elevated CO_2 on the aboveground size components of leaf number, shoot number and shoot diameter appeared to be transitory in this experiment. The stimulatory effects of the elevated CO_2 treatment occurred within the first census intervals, after which the plants growing in the ambient CO_2 chambers grew to match the plants in the elevated CO_2 with respect to the size traits. These results are consistent with previous experiments investigating growth responses to elevated CO_2, in which the positive growth influence of elevated CO_2 was short-lived (Wulff and Strain, 1982; Poorter, 1993). In fact, in this experiment, the effect of the open-topped chambers seemed to be at least as important an influence on the aboveground size traits as was the elevated CO_2. The plants in both chambers, with or without added CO_2, were more similar to each other in terms of aboveground size characteristics than they were to the unchambered control plants.

Even though the aboveground size traits often ended up to similar for the treatments, some differences between the elevated CO_2 grown plants and the ambient CO_2 grown plants were maintained over the extent of the experiment. The allocation of biomass was affected by the presence of elevated CO_2. For both the main experiment and the growth experiment, specific leaf area was larger for the plants in the ambient CO_2 chambers (probably a chamber effect), but the plants in the elevated CO_2 chambers had decreased specific leaf area, probably due to the presence of elevated CO_2 counteracting the chamber effect (Klus, Ch. 2). The plants in the main experiment differed with respect to final biomass allocation after 127 days of growth, primarily by increasing root: shoot ratios in the elevated CO_2 environment (Klus, Ch. 1). The biomass allocation patterns of the plants harvested in the growth experiment after 49 days followed this general pattern.

That the pattern of biomass allocation response was similar in the growth experiment compared to the main experiment occurred despite differences in the growth conditions of the two sets of plants. The plants in the growth experiment were planted in four-inch square pots; to maintain air flow within the chambers, the growth experiment pots were placed on the ground between the taller cylindrical pots used for the main experiment. For part of the day the smaller growth experiment pots were shaded by the pots in the main experiment. This condition worsened as the main experiment plants grew larger. Perhaps as a consequence of the shading, the growth experiment plants were much

smaller at the time of their harvest (Day 49) than the main experiment plants were only ten days later at their second census, but an examination of the growth response variables indicated that the growth experiment plants had not plateaued in response to elevated CO_2 under the shaded conditions (Figures 6 - 8, insets).

Despite the difference in growth conditions between the plants in the growth experiment and the plants in the main experiment, the plants in both experiments responded in similar ways to CO_2 treatment. The allocation of biomass to belowground tissues in the growth experiment was greater in the elevated CO_2 grown plants than it was in the plants in the ambient CO_2 chambers; these results matched the results of the main experiment (Klus, Ch. 1) Physiologically, the plants grown and measured in elevated CO_2 in the growth experiment were photosynthesizing at approximately twice the rate of the plants grown and measured in ambient CO_2 (Figure 10), similar to the plants measured in the main experiment (Klus, Ch. 2). Previous studies have determined that the physiological response to elevated CO_2 is not hindered by a shade treatment (Ehret and Joliffe, 1985). Yet the decline in maximum photosynthetic potential described for the plants in the main experiment (Klus, Ch. 2) had also occurred in the growth experiment plants by Day 49 of the experiment. The plants grown and measured in elevated CO_2 had lower assimilation rates than the plants grown in ambient CO_2 and measured in elevated CO_2, both those in the ambient CO_2 chambers and in the unchambered control sites (Table 10; Figure 10). The relationship between assimilation rates and intercellular CO_2 concentration (Figure 11) indicates that either the quantity or the activation state of Rubisco had declined during the period of exposure to elevated CO_2 for the plants in the elevated CO_2 chambers (von Caemmerer and Farquhar, 1981; Klus, Ch. 2).

The response of stomatal conductance in the plants at the elevated CO_2 measurement level was atypical. Only one other study, conducted on an arctic alpine tundra ecosystem, has found increased stomatal conductance in plants exposed to elevated CO_2 (Oberbauer *et al.*, 1986). In the tundra study, the increase in stomatal conductance was associated with increased temperatures, up to 4 °C. The chambers in this study were only 1.7°C warmer than the unchambered control sites; moreover, the stomatal conductance results from the growth experiment did not match the results from the main experiment (Klus, Ch. 2), even though temperature conditions were the same for both experiments. Therefore, some other environmental condition must have been interacting with the increased intercellular CO_2 to increase the stomatal conductance. The mechanics of stomatal dynamics are complicated and not well understood (Zeiger *et al.*, 1987). Other than CO_2, water relations play a role in controlling stomatal aperture (Morison, 1987; Mott, 1990); perhaps the water relations of the plants in the growth experiment which were growing in at least partial shade affected the relationship between stomatal aperture and intercellular CO_2. Given the positive relationship between stomatal conductance and assimilation rate (Pereira, 1994), any environmental condition which would have enabled the growth experiment plants to keep their stomates open despite the increase in intercellular CO_2 would have helped them to maximize their photosynthetic response to elevated CO_2.

In this experiment, the overall effect of elevated CO_2 did not appear to affect plant size traits as much as the allocation of photosynthate due to increased assimilation rates in the elevated CO_2 environment. Root: shoot ratios increased for the elevated CO_2 grown plants, while specific leaf areas declined. These results were the same for both the growth experiment and the main experiment. Since assimilation rates did decrease somewhat from their maximum potential in the elevated CO_2 plants, the question arises concerning the fate of nitrogen that may have been reallocated from Rubisco. For a small subset of plants from the main experiment that were analyzed for nitrogen concentration, nitrogen concentration was the same aboveground for the plants grown in ambient CO_2 and elevated CO_2. The plants appeared to mobilize nitrogen from their belowground tissues, because carbon: nitrogen ratios increased belowground and decreased aboveground, resulting in no net change in overall C:N ratio (Klus, Ch. 1). Since the types of nitrogen compounds the plants manufactured was not analyzed, it was not possible to determine the nature of the reallocation of nitrogen.

The additional carbon compounds that resulted in decreased specific leaf area in elevated CO_2 were not being stored either as starch or soluble sugars (Klus, Ch. 1); it is possible that the plants were manufacturing additional quantities of cellulose or secondary metabolites (Dijkstra and Lambers, 1989; but see Fajer *et al.*, 1992). It was not possible to determine the specific nature of the carbon compounds made in response to elevated CO_2 in this experiment. Overall, however, it appeared that *Plantago lanceolata* was flexible in terms of integrating its physiological processes with patterns of nutrient and biomass allocation within the plant.

Even though I examined physiology, growth and allocation patterns in response to elevated CO_2 in this experiment, I was not able to determine how elevated CO_2 would ultimately affect this species' ability to interact in its community. Because the response of the populations and the families of *P. lanceolata* to elevated CO_2 was not uniform, some or all of the changes in physiology and allocation patterns may ultimately help certain

families of *Plantago lanceolata* to survive, compete, and reproduce with greater success in an elevated CO_2 world. Thus, the genotypic variation in response to elevated CO_2 that was documented in this study suggests that elevated CO_2 may act as an agent of natural selection. The potential for ecological and evolutionary change in response to elevated CO_2 should be addressed by a longer-term study that follows *Plantago lanceolata* across several growing seasons and several generations in an elevated CO_2 environment. Only in this way will it be possible to explore the ultimate effects of changes in physiology and allocation patterns in response to elevated CO_2 on the fate of *Plantago lanceolata* in its community.

APPENDIX

APPENDIX

METHODS

In the summer of 1991, I conducted a pilot experiment to investigate the nature of genetic variation in response to elevated CO_2 for physiological and biomass allocation traits in two populations of *Plantago lanceolata*. The Kellogg Field (KF) population (Kalamazoo County, Michigan) was located in partial shade on the edge of a mown field. The Ray Boom (RB) population was located in suburban Chicago, Illinois. A total of 50 randomly selected families, 25 from each population, were used in the experiment. In early July, 1991, all seeds from each family were sown in flats and placed in a greenhouse at W. K. Kellogg Biological Station, Hickory Corners, Michigan. Germination took place over 7 - 10 days. After germination, the seedlings were transported to the Duke University Phytotron in Durham, North Carolina. Ten seedlings from each family were transplanted into separate 30-cm high pots made from 10-cm diameter PVC pipe with mesh screen bottoms. The pots were filled with a soil-less composition of hardened clay particles to provide a uniform substrate. Five seedlings from each family were placed into either an ambient (35.5 Pa) or twice-ambient (71.0 Pa, hereafter referred to as "elevated") greenhouse in the Phytotron.

The plants were watered *ad libitum* in the morning with half-strength Hoagland's solution. If the plants showed signs of wilting in the afternoon, they were watered again with deionized water. Daytime temperature was 28 °C; nighttime temperature was 22 °C. 55 days into the experiment, physiological measurements of assimilation and transpiration were made using three Model LCA-2 and one Model LCA-3 portable infra-red gas analyzers (Analytical Development Corporation, Hoddesdon, UK) and narrow Parkinson Leaf Chambers (Analytical Development Corporation, Hoddesdon, UK) at both ambient and elevated CO_2. Elevated CO_2 was provided from the elevated CO_2 greenhouse. The CO_2 source (ambient or elevated CO_2) was rotated to a different machine each day. Measurements of physiological traits were made on an intact, most recently fully expanded leaf of each plant. Calculations of CO_2 assimilation (μmol CO_2 m^{-2} s^{-1}), transpiration (mmol H_2O m^{-2} s^{-1}), stomatal conductance (mol H_2O m^{-2} s^{-1}) and intercellular CO_2 concentration (μbar) were made using equations from Analytical Development Corporation (1992) and von Caemmerer and Farquhar (1981). At the end of 60 days the plants were harvested. The roots and leaves (shoots) were separated at the soil line, dried at 60°C for at least five days, and weighed.

Statistical Analysis

Preliminary analysis indicated that the four gas exchange variables (assimilation, transpiration, stomatal conductance, and internal leaf CO_2 concentration) differed according to machine used for measurement and date of measurement. The variables were adjusted for these differences by subtracting the mean for each machine and date from each individual measurement and adding back the grand mean summed over all machines and dates (Tonsor and Goodnight, 1996). This procedure removed deviations due to intrinsic differences between the infra-red gas analyzers, and variation in environmental conditions on different days, while retaining the differences due to CO_2 treatment and other main effects.

The gas exchange variables assimilation, transpiration, and stomatal conductance were initially analyzed using a multivariate analysis of variance (MANOVA) to determine overall main and interaction effects. The multivariate analysis was followed by appropriate univariate mixed model analyses for these physiological variables plus instantaneous water use efficiency (the ratio of assimilation to transpiration) and intercellular CO_2 concentration. The overall model for the analysis of the physiological variables included the fixed effects of CO_2 treatment, and population, one random effect (family nested within population), and interactions for all main effects. Interaction terms containing the random effect were also treated as random effects. Expected mean squares for the main effects in the mixed model ANOVA were divided by the appropriate interaction terms to produce the F values for significance tests. Significance levels for the main effects in the multivariate analysis were interpreted using Roy's Greatest Root, recommended because of its statistical power and the fact that it is applicable to *post hoc* statistical comparisons (Scheiner, 1993). Sample size for each family in each CO_2 environment was $n = 3$-5. Of the 50 families used in the main experiment, 6 families were excluded from the analyses because 2 or fewer individuals germinated.

A separate multivariate analysis was conducted for aboveground and belowground biomass, also using Roy's Greatest Root. Mixed model univariate analyses were subsequently conducted for aboveground biomass, belowground biomass, whole plant (total) biomass, and root: shoot ratio.

RESULTS

CO_2 treatment means differed significantly for all of the physiological and biomass allocation variables (Table 12). Multivariate analysis of the physiological variables revealed that the main effects of CO_2 treatment and family were highly significant (Table 13. A). In addition, the interaction of CO_2 treatment with family was also highly significant, indicating that the families responded differentially to the elevated CO_2 treatment (Figures 12 and 13). The multivariate analysis of the biomass variables showed that all of the main effects were highly significant (Table 14. B). No interaction terms were statistically significant for the biomass variables.

Each of the physiological variables used in the multivariate analysis was also tested using a univariate mixed model analysis of variance (Table 14. A); water use efficiency and intercellular CO_2 concentration were analyzed univariately as well. Each of the physiological variables showed a significant response to CO_2 treatment. Assimilation rates, water use efficiency, and intercellular CO_2 concentration were higher in the elevated CO_2 treatment; transpiration and stomatal conductance were lower in the elevated CO_2 treatment than in the ambient CO_2 treatment. Univariate mixed model analysis of the biomass variables showed significant main effects of CO_2 treatment, population, and family for aboveground, belowground and total biomass, and a significant CO_2 treatment effect for root: shoot ratio (Table 14. B). Aboveground, belowground, whole plant biomass, and root: shoot ratio were all greater in the elevated CO_2 treatment than in ambient CO_2. Population KF was significantly larger than Population RB for each biomass variable ($p < .05$), yet the two populations were similar physiologically. The families showed considerable variation in magnitude of response to elevated CO_2 for each biomass variable

(Figure 14).

The CO_2 treatment x family interactions for the physiological traits and the considerable variation in magnitude and degree of response for the biomass traits for the different families clearly indicated that intraspecific genotypic variation in response to elevated CO_2 exists for these two populations of *Plantago lanceolata*. Genotypic variation in response to elevated CO_2 occurred for both instantaneous physiological traits and biomass allocation traits which might influence long-term survival and reproduction in this species. Moreover, the significant differences in response at the population level suggested that a population's habitat of origin might affect its capacity to respond to elevated CO_2.

Yet, the design of the 1991 experiment did not allow me to determine unequivocally either the time of onset of the CO_2 response or its duration past 60 days. Additionally, pseudoreplication was a problem, because cost restricted the experiment to only one elevated and one ambient CO_2 greenhouse. Therefore, I decided to conduct follow-up experiments in 1992 at the W. K. Kellogg Biological Station to explore more fully the nature of genotypic variation in response to elevated CO_2 under more natural conditions. Setting up the experiment in open-topped chambers in an old field allowed me to investigate whether the variation uncovered in controlled, high nutrient greenhouse conditions would manifest itself over the course of an entire growing season under less productive conditions. The design of the 1992 experiment also allowed me to measure early growth traits to determine the time of onset of CO_2 response, and to investigate possible changes in patterns of physiological and biomass allocation response to elevated CO_2 over the course of an entire growing season.

Table 12. Overall and population means (s.e.) for CO_2 treatments for ambient and elevated CO_2 measurements. CO_2 treatment means were compared using univariate one-way ANOVAs. Letters represent significant differences for each variable between the two CO_2 treatments. Differences were considered to be significant at the $p < 0.025$ level (Bonferroni adjustment for multiple comparisons of means).

Physiological Variables:		**Overall**	**Population KF**	**Population RB**
Assimilation:	Ambient CO_2	29.9 (0.5)^A	29.4 (0.7)^A	30.6 (0.8)
	Elevated CO_2	32.8 (0.9)^B	32.9 (1.2)^B	32.6 (1.2)
Transpiration:	Ambient CO_2	10.0 (0.2)^A	9.9 (0.2)^A	10.2 (0.2)^A
	Elevated CO_2	8.5 (0.2)^B	8.2 (0.3)^B	8.8 (0.3)^B
Water Use Efficiency:	Ambient CO_2	3.0 (0.04)^A	3.0 (0.1)^A	3.0 (0.1)^A
	Elevated CO_2	4.3 (0.3)^B	4.6 (0.5)^B	3.9 (0.2)^B
Stomatal Conductance:	Ambient CO_2	1.41 (0.08)^A	1.35 (0.10)^A	1.52 (0.14)^A
	Elevated CO_2	0.89 (0.06)^B	0.94 (0.09)^B	0.82 (0.08)^B
Intercellular CO_2:	Ambient CO_2	345.0 (2.0)^A	343.9 (3.0)^A	345.6 (3.0)^A
	Elevated CO_2	523.5 (5.5)^B	523.0 (8.2)^B	524.1 (7.4)^B
Biomass Variables:				
Aboveground Biomass:	Ambient CO_2	10.9 (0.4)^A	13.7 (0.5)^A	7.6 (0.5)^A
	Elevated CO_2	14.9 (0.4)^B	17.6 (0.6)^B	12.0 (0.5)^B
Belowground Biomass:	Ambient CO_2	5.9 (0.3)^A	7.6 (0.5)^A	3.8 (0.4)^A
	Elevated CO_2	10.2 (0.6)^B	12.1 (0.9)^B	8.0 (0.7)^B
Whole Plant Biomass:	Ambient CO_2	16.8 (0.7)^A	21.3 (0.8)^A	11.4 (0.8)^A
	Elevated CO_2	25.1 (0.9)^B	29.7 (1.3)^B	20.0 (1.1)^B
Root: Shoot Ratio:	Ambient CO_2	0.53 (0.10)^A	0.56 (0.11)^A	0.48 (0.09)^A
	Elevated CO_2	0.66 (0.16)^B	0.66 (0.04)^B	0.66 (0.04)^B

Table 13. Multivariate analysis of variance for the main effects of CO_2 treatment, population, and family nested within population, and their one-way interactions. Part A. Overall MANOVA for three physiological variables: assimilation, transpiration, and stomatal conductance. Part B. Overall MANOVA for two biomass variables: aboveground biomass, belowground biomass.

Table 13. A. Overall multivariate analysis for physiological variables.

Source:	**Numerator df**	**Denominator df**	**Roy's Greatest Root**	**F value**	**p > F**
CO_2 Treatment (**CO_2**)	3	211	0.7765	54.61	0.0001***
Population (**Pop**)	3	211	0.0096	0.67	0.5689
Family (Population) (**Fam(Pop)**)	42	213	0.3324	1.69	0.0091**
CO_2 * Pop	3	211	0.0108	0.76	0.5165
CO_2 * Fam(Pop)	40	213	0.2772	1.48	0.0428*

Table 13. B. Overall multivariate analysis for biomass variables.

Source:	**Numerator df**	**Denominator df**	**Roy's Greatest Root**	**F value**	**p > F**
CO_2 Treatment (**CO_2**)	2	330	0.2339	38.60	0.0001***
Population (**Pop**)	2	330	0.4696	77.48	0.0001***
Family (Population) (**Fam(Pop)**)	42	331	0.5027	3.96	0.0001***
CO_2 * Pop	2	330	0.0026	0.43	0.6501
CO_2 * Fam(Pop)	42	331	0.1024	0.81	0.7989

Table 14. Summary of probabilities from univariate mixed model ANOVAs. Part A. Physiological variables: A = assimilation rate. E= transpiration rate. GS = stomatal conductance. C_i = intercellular CO_2 concentration. WUE = water use efficiency. Part B. Biomass variables: Aboveground biomass, belowground biomass, total biomass, root:shoot ratio.

Table 14. A. Physiological variables.

Source:	df	A	E	G_s	C_i	WUE
CO_2 Treatment (**CO_2**)	1	0.0152*	0.0002***	0.0001***	0.0001***	0.0001***
Population (**Pop**)	1	0.8301	0.7997	0.5345	0.8210	0.2288
Family (Population) (**Fam(Pop)**)	42	0.4252	0.7744	0.2577	0.7524	0.6196
CO_2 * Pop	1	0.6492	0.6019	0.5582	0.9996	0.1866
CO_2 * Fam(Pop)	40	0.0703	0.0484	0.2473	0.1039	0.5280

Table 14. B. Biomass variables.

Source:	df	Aboveground	Belowground	Total Biomass	Root:shoot Ratio
CO_2 Treatment (**CO_2**)	1	0.0001***	0.0001***	0.0001***	0.0001***
Population (**Pop**)	1	0.0001***	0.0001***	0.0001***	0.2951
Family (Population) (**Fam(Pop)**)	42	0.0001***	0.0127*	0.0001***	0.0909
CO_2 * Pop	1	0.6300	0.6271	0.9335	0.2435
CO_2 * Fam(Pop)	42	0.8020	0.8582	0.8342	0.8600

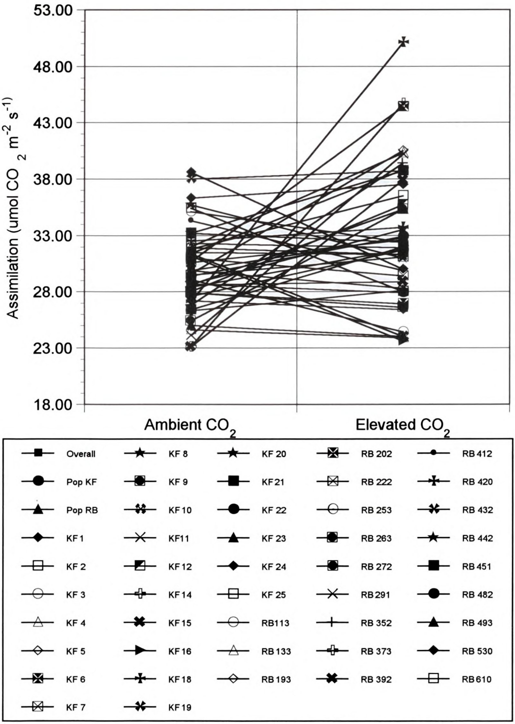


Figure 12. Comparison of overall, population and family means for assimilation for ambient and elevated CO2 treatments for 1991 experiment.

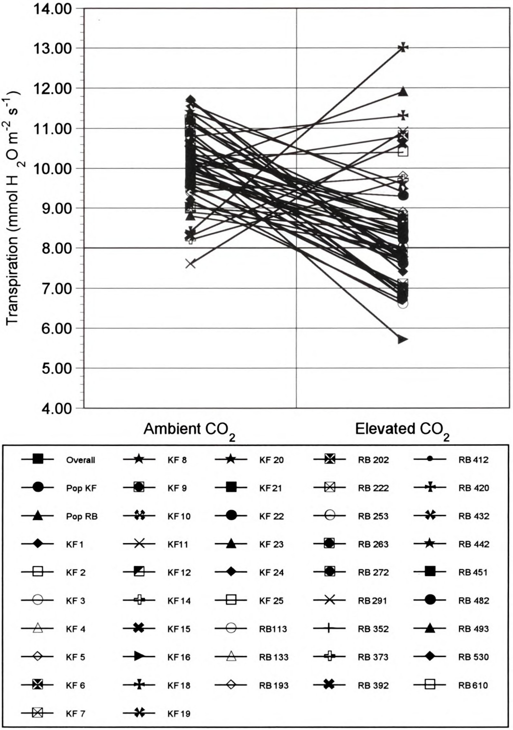
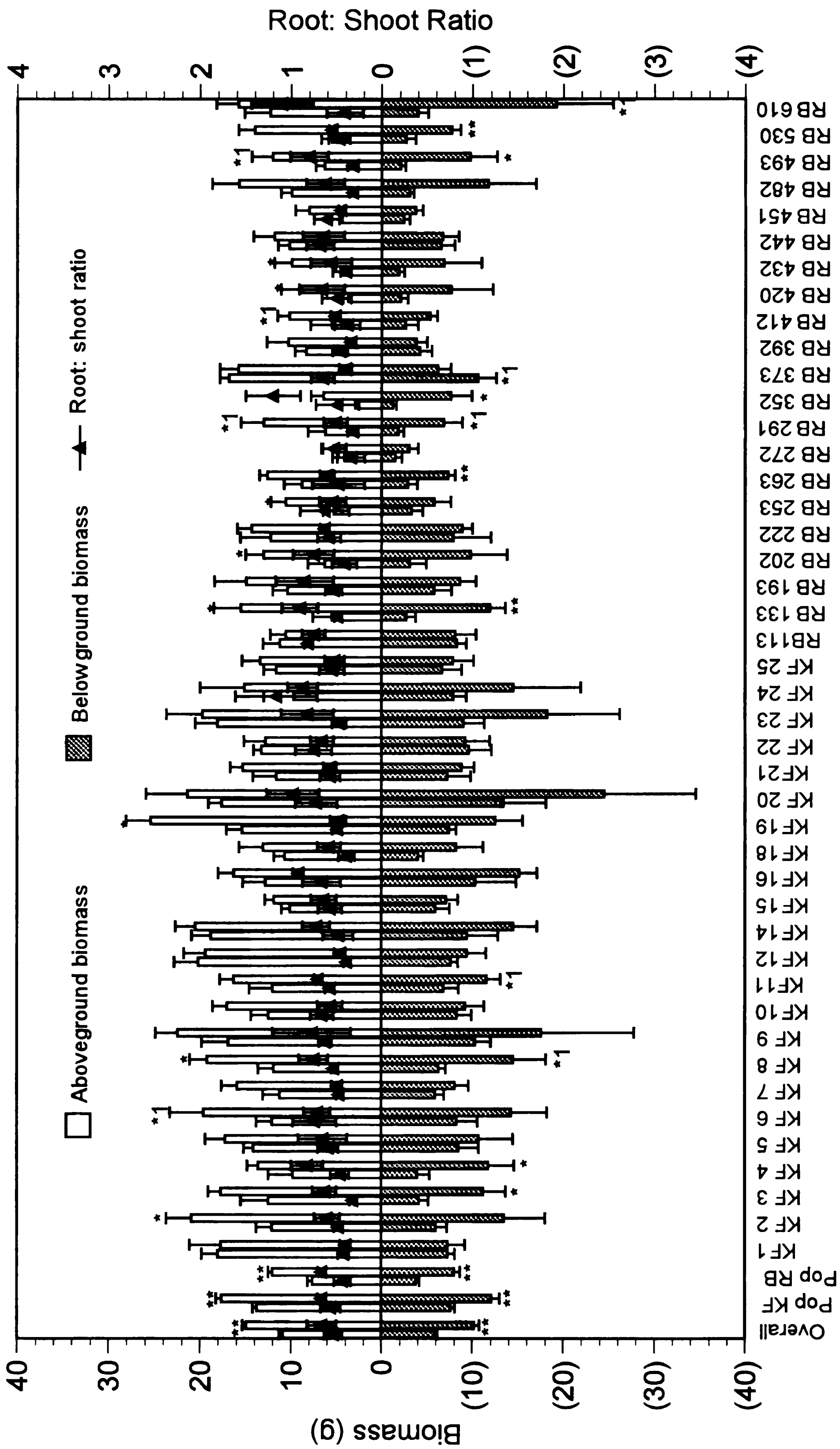


Table 13. Comparison of overall, population and family means for transpiration for ambient and elevated CO2 treatments for 1991 experiment.

Figure 14. Comparison of overall, population, and family means for aboveground biomass, belowground biomass, and root: shoot ratio by CO_2 treatment for 1991 experiment. For each category (overall, population, family) means for ambient CO_2 treatment appear to the left of means for elevated CO_2 treatment. Vertical bars indicate one s.e. Scale for root: shoot ratio appears to the right. Significance levels for individual tests of means appear above bars for aboveground biomass and below bars for belowground biomass. Significance levels: *1 $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.



Root: Shoot Ratio
Biomass (g)
Aboveground biomass
Belowground biomass
Root: shoot ratio
Overall
Pop KF
Pop RB
KF 1
KF 2
KF 3
KF 4
KF 5
KF 6
KF 7
KF 8
KF 9
KF10
KF11
KF12
KF14
KF15
KF16
KF18
KF19
KF 20
KF21
KF 22
KF 23
KF 24
KF 25
RB113
RB 133
RB 193
RB 202
RB 222
RB 253
RB 263
RB 272
RB 291
RB 352
RB 373
RB 392
RB 412
RB 420
RB 432
RB 442
RB 451
RB 482
RB 493
RB 530
RB 610

Figure 14

LITERATURE CITED

LITERATURE CITED

Analytical Development Corporation (1992) LCA-3 Operations Manual. Hoddesdon, UK.

Antonovics J, Primack RB (1982) Experimental ecological genetics in *Plantago*. VI. The demography of seedling transplants of *P. lanceolata*. J Ecol 70:55-75

Arnold SJ, Wade MJ (1984) On the measurement of natural and sexual selection: theory. Evolution 38:709-719

Arp WJ (1991) Effects of source-sink relations on photosynthetic acclimation to elevated CO_2. Plant Cell Environ 14:869-875

Arp WJ, Drake BG (1991) Increased photosynthetic capacity of *Scirpus olneyi* after 4 years of exposure to elevated CO_2. Plant Cell Environ 14:1003-1006

Bazzaz FA (1990) The response of natural ecosystems to the rising global CO_2 level. Annu Rev Ecol Syst 21:167-196

Bhattacharya NC, Biswas PK, Bhattacharya Ch , Sionit N, Strain BR (1985) Growth and yield response of sweet potato to atmospheric CO_2 enrichment. Crop Sci 25:975-981

Bowes G (1991) Growth at elevated CO_2: photosynthetic responses mediated through Rubisco. Plant Cell Environ 14:795-806

Bowes G (1993) Facing the inevitable: plants and increasing atmospheric CO_2. Annu Rev Plant Physiol Plant Mol Biol 44:309-332

Bradshaw AD, McNeilly T (1991) Evolutionary response to global climatic change. Ann Bot 67 (Suppl 1):5-14

Carter DR, Peterson KM (1983) Effects of a carbon dioxide-enriched atmosphere on the growth and competitive interaction of a C_3 and a C_4 grass. Oecologia 58:188-193

Cure JD, Acock B (1986) Crop responses to carbon dioxide doubling: a literature survey. Agric For Meteor 38:127-145

Curtis PS, Drake BG, Whigham DF (1989) Nitrogen and carbon dynamics in C_3 and C_4 estuarine marsh plants grown under elevated CO_2 in situ. Oecologia 78:297-301

Curtis PS, Snow AA, Miller AS (1994) Genotype-specific effects of elevated CO_2 on fecundity in wild radish (*Raphanus raphanistrum*). Oecologia 97:100-105

Curtis PS, Teeri JA (1992) Seasonal responses of leaf gas exchange to elevated carbon dioxide in *Populus grandidentata*. Can J For Res 22:1320-1325

Davidson RL (1969) Effect of root/leaf temperature differentials on root/shoot ratios in some pasture grasses and clover. Ann Bot 33:561-569

DeLucia EH, Sasek TW, Strain BR (1985) Photosynthetic inhibition after long-term exposure to elevated levels of atmospheric carbon dioxide. Photosyn Res 7:175-184.

den Hertog J, Stulen I, Lambers H (1993) Assimilation, respiration and allocation of carbon in *Plantago major* as affected by atmospheric CO_2 levels. Vegetatio 104/105:369-378

Dijkstra P, Lambers H (1989) A physiological analysis of genetic variation in relative growth rate within *Plantago major* L. Func Ecol 3:577-587

Ehret DL, Joliffe PA (1985) Photosynthetic carbon dioxide exchange of bean plants grown at elevated carbon dioxide concentrations. Can J Bot 63:2026-2030

Evans LT (1975) The physiological basis of crop yield. In: Evans LT (ed) Crop physiology: some case histories. Cambridge Univ Press, Cambridge UK, pp 327-355

Fajer ED, Bowers MD, Bazzaz FA (1992) The effect of nutrients and enriched CO_2 environments on production of carbon-based allelochemicals in *Plantago*: a test of the carbon/nutrient balance hypothesis. Am Nat 140:707-723

Fichtner K, Koch GW, Mooney HA (1994) Photosynthesis, storage, and allocation. In: Schulze E-D, Caldwell MM (eds) Ecophysiology of photosynthesis. Springer-Verlag, Berlin, pp 133-146

Garbutt K, Bazzaz FA (1984) The effects of elevated CO_2 on plants. III. Flower, fruit and seed production and abortion. New Phytol 98:433-446

Garnier E (1992) Growth analysis of congeneric annual and perennial grass species. J Ecol 80:665-675

Geber MA, Dawson TE (1990) Genetic variation in and covariation between leaf gas exchange, morphology, and development in *Polygonum arenastrum*, an annual plant. Oecologia 85:153-158

Geber MA, Dawson TE (1993) Evolutionary responses of plants to global change. In: Kareiva PM, Kingsolver JG, Huey RB (eds) Biotic interactions and global change. Sinauer Associates Inc, Sunderland MA USA, pp 179-197

Gill JL (1978) Design and analysis of experiments in the animal and medical sciences. Iowa State University Press, Ames IA USA, 409 pp

Goldberg DE, Landa K (1991) Competitive effect and response: hierarchies and correlated traits in the early stages of competition. J Ecol 79:1013-1030

Goldberg DE, Miller TE (1990) Effects of different resource additions on species diversity in an annual plant community. Ecology 71:213-225

Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am Nat 111:1169-1194

Hollinger DY (1987) Gas exchange and dry matter allocation responses to elevation of atmospheric CO_2 concentration in seedlings of three tree species. Tree Physiol 3:193-202

Idso SB, Kimball BA (1989) Growth response of carrot and radish to atmospheric CO_2 enrichment. Environ Exper Bot 29:135-139

Idso SB, Kimball BA, Mauney JR (1988) Effects of atmospheric CO_2 enrichment on root:shoot ratios of carrot, radish, cotton and soybean. Agric Ecosys Envt 21:293-299

Johnson IR (1985) A model of the partitioning of growth between the shoots and roots of vegetative plants. Ann Bot 55:421-431

Jones MGK, Outlaw WH Jr, Lowry OH (1977) Procedure for the assay of sucrose in the range of 10^{-7} - 10^{-14} moles. Plant Phys 60:379-383

Kalisz, S (1986) Variable selection on the timing of germination in *Collinsia verna* (Scrophulariaceae) Evolution 40:479-491

Keeling CD (1986) Atmospheric CO_2 concentrations. Mauna Loa Observatory, Hawaii 1958-1986. NDP-001/R1. Carbon dioxide information analysis center. Oak Ridge Natl Lab USA, Oak Ridge TN USA

Kimball BA (1983) Carbon Dioxide and agricultural yield: An assemblage and analysis of 430 prior observations. Agron J 75:779-788

Kimball BA, Mauney JR, Nakayama FS, Idso SB (1993) Effects of increasing atmospheric CO_2 on vegetation. Vegetatio 104/105:65-75

Konings H (1989) Physiological and morphological differences between plants with a high NAR or a high LAR as related to environmental conditions. In: Lambers H, Cambridge ML, Konings H, Pons TL (eds) Causes and consequences of variation in growth rate and productivity of higher plants. SPB Academic Publishing, The Hague, pp 101-123

Kuiper PJC, Bos M (eds) (1992) Plantago: a multidisciplinary study. Springer-Verlag, Berlin

Lambers H (1987) Does variation in photosynthetic rate explain variation in growth rate and yield? Neth J Agric Sci 35:505-519

Larigauderie A, Hilbert DW, Oechel WC (1988) Effect of CO_2 enrichment and nitrogen availability on resource acquisition and resource allocation in a grass, *Bromus mollis*. Oecologia 77:544-549

Leadley PW, Drake BG (1993) Open top chambers for exposing plant canopies to elevated CO_2 concentrations and for measuring net gas exchange. Vegetatio 104/105:3-15

McGraw J B, Wulff RD (1983) The study of plant growth: a link between the physiological ecology and population biology of plants. J Theor Biol 103:21-28

Morison JIL (1987) Intercellular CO_2 concentration and stomatal response to CO_2. In: Zeiger E, Farquhar GD, Cowan IR (eds) Stomatal function. Stanford Univ Press, Stanford CA USA, pp 229-251

Mott KA (1990) Sensing of atmospheric CO_2 by plants. Plant Cell Environ 13:731-737

Neftel A, Moor E, Oeschger H, Stauffer B (1985) The increase of atmospheric CO_2 in the last two centuries. Nature 315:45-47

Norby RJ, O'Neill EG, Luxmoore RJ (1986) Effects of atmospheric CO_2 enrichment on the growth and mineral nutrition of *Quercus alba* seedlings in nutrient-poor soil. Plant Physiol 82:83-89

Norby RJ, O'Neill EG, Hood WG, Luxmoore RJ (1987) Carbon allocation, root exudation and mycorrhizal colonization of *Pinus echinata* seedlings grown under CO_2 enrichment. Tree Physiol 3:203-210

Oberbauer SF, Oechel WC, Riechers GH (1986) Soil respiration of Alaskan tundra at elevated atmospheric carbon dioxide concentrations. Plant Soil 96:145-148

Oechel WC, Strain BR (1985) Native species responses to increased carbon dioxide concentration. In: Strain BR, Cure JD (eds) Direct effects of increasing carbon dioxide on vegetation, DOE/ER-0238, US Dept of Energy, Washington DC USA, pp 117-154

O'Neill EG, Luxmoore RJ, Norby RJ (1987) Increases in mycorrhizal colonization and seedling growth in *Pinus echinata* and *Quercus alba* in an enriched CO_2 atmosphere. Can J For Res 17:878-883

Patterson DT, Flint EP (1982) Interacting effects of CO_2 and nutrient concentration. Weed Sci 30:389-394

Patterson DT, Flint EP, Beyers JL (1984) Effects of carbon dioxide enrichment on competition between a C_4 weed and C_3 crop. Weed Sci 32:101-105

Pereira JS (1994) Gas exchange and growth. In: Schulze E-D, Caldwell MM (eds) Ecophysiology of photosynthesis. Springer-Verlag, Berlin, pp 147-181

Poorter H (1989) Interspecific variation in relative growth rate: on ecological causes and physiological consequences. In: Lambers H, Cambridge ML, Konings H, Pons TL (eds) Causes and consequences of variation in growth rate and productivity of higher plants. SPB Academic Publishing, The Hague, pp 45-68

Poorter H (1993) Interspecific variation in the growth response of plants to an elevated ambient CO_2 concentration. Vegetatio 104/105:77-97

Poorter H, Pot S, Lambers H (1988) The effect of an elevated atmospheric CO_2 concentration on growth, photosynthesis and respiration of *Plantago major*. Phys Plant 73: 553-559

Potvin C, Lechowicz MJ, Tardif S (1990) The statistical analysis of ecophysiological response curves obtained from experiments involving repeated measures. Ecology 71:1389-1400

Primack RB (1979) Reproductive effort in annual and perennial species of *Plantago* (Plantaginaceae). Am Nat 114:51-62

Primack RB, Antonovics J (1982) Experimental ecological genetics in *Plantago*. VII. Reproductive effort in populations of *P. lanceolata* L. Evolution 36:742-752

Radin JW, Lu Z, Percy RG, Zeiger E (1994) Genetic variability for stomatal conductance in Pima cotton and its relation to improvements of heat adaptation. Proc Natl Acad Sci 91:7217-7221

Sage RF, Sharkey TD, Seemann JR (1989) Acclimation of photosynthesis to elevated CO_2 in five C_3 species. Plant Physiol 89:590-596

SAS Institute (1988) SAS/STAT User's Guide, Release 6.03 Edition. SAS Institute Inc, Cary NC USA, 1028 pp

Scheiner SM (1993) MANOVA: Multiple response variables and multispecies interactions. In: Scheiner SM, Gurevitch J (eds) Design and analysis of ecological experiments. Chapman and Hall, New York NY USA, pp 94-112

Scheiner SM, Gurevitch J (eds) (1993) Design and analysis of ecological experiments. Chapman and Hall, New York NY USA, 445 pp

Scheiner SM, Gurevitch J, Teeri JA (1984) A genetic analysis of the photosynthetic properties of populations of *Danthonia spicata* that have different growth responses to light level. Oecologia 64: 74-77

Sharkey TD (1985) Photosynthesis in intact leaves of C_3 plants: physics, physiology and rate limitations. Bot Rev 51:53-105

Shipley B (1995) Structured interspecific determinants of specific leaf area in 34 species of herbaceous angiosperms. Funct Ecol 9:312-319

Smith SD, Strain BR, Sharkey TD (1987) Effects of CO_2 enrichment on four Great Basin grasses. Funct Ecol 1:139-143

Solbrig OT (1981) Studies on the population biology of the genus *Viola*. II. The effect of plant size on fitness in *Viola sororia*. Evolution 35:1080-1093

Strain BR (1987) Direct effects of increasing atmospheric CO_2 on plants and ecosystems. TREE 2:18-21

Strain BR, Cure JD (eds) (1985) Direct effects of increasing carbon dioxide on vegetation. DOE/ER-0238, US Dept of Energy, Washington DC USA, 286 pp

Stulen I, den Hartog J (1993) Root growth and functioning under atmospheric CO_2 enrichment. Vegetatio 104/105 99-115

Teramura AH (1983) Experimental ecological genetics in *Plantago*. IX. Differences in growth and vegetative reproduction in *Plantago lanceolata* L. (Plantaginaceae) from adjacent habitats. Amer J Bot 70:53-58

Teramura AH, Strain BR (1979) Localized populational differences in the photosynthetic response to temperature and irradiance in *Plantago lanceolata.* Can J Bot 57:2559-2563

Thornley JHM (1972) A balanced quantitative model for root: shoot ratios in vegetative plants. Ann Bot 36: 431-441

Tilman D (1993) Carbon dioxide limitation and potential direct effects of its accumulation on plant communities. In: Kareiva PM, Kingsolver JG, Huey RB (eds) Biotic interactions and global change. Sinauer Associates Inc, Sunderland MA USA, pp 333-346

Tonsor SJ (1985) Intrapopulational variation in pollen-mediated gene flow in *Plantago lanceolata* L. Evolution 39:775-782

Tonsor SJ (1990) Spatial patterns of differentiation for gene flow in *Plantago lanceolata.* Evolution 44:1373-1378

Tonsor SJ, Kalisz S, Fisher J, Holtsford TP (1993) A life-history based study of population genetic structure: seed bank to adults in *Plantago lanceolata.* Evolution 47:833-843

Tonsor SJ, Goodnight CJ (1996) Evolutionary predictability in natural populations: Do mating system and epistasis interact to affect heritabilities in *Plantago lanceolata* L.? Evolution: in press

Van der Aart PJM (1985) Demographic, genetic and ecophysiological variation in *Plantago major* and *P. lanceolata* in relation to vegetation type. In: White J (ed) The population structure of vegetation. Handbook of vegetation science, Part III. W Junk, Boston MA USA

Via S, Lande R (1983) Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution 39:505-522

von Caemmerer S, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153: 376-387

von Ende CN (1993) Repeated-measures analysis: growth and other time-dependent measures. In: Scheiner SM and Gurevitch J (eds) Design and analysis of ecological experiments. Chapman and Hall, New York NY USA, pp 113-137

Wade MJ, Kalisz S (1990) The causes of natural selection. Evolution 44:1947-1955

Williams WE, Garbutt K, Bazzaz FA (1988) The response of plants to elevated CO_2. V. Performance of an assemblage of serpentine grassland herbs. Envt Expt Bot 28:123-130

Wolff K, van Delden W (1989) Genetic analysis of ecological relevant morphological variability in *Plantago lanceolata* L. IV. Response and correlated response to bidirectional selection for leaf angle. Heredity 62:153-160

Woodward FI, Thompson GB, McKee IF (1991) The effects of elevated concentrations of carbon dioxide on individual plants, populations, communities and ecosystems. Ann Bot 67 (S1):23-38

Wright S (1969) Evolution and the Genetics of Populations. . Univ Chicago Press, Chicago IL USA

Wu L, Antonovics J (1976) Experimental ecological genetics in Plantago. II. Lead tolerance in *Plantago lanceolata* and *Cynodon dactylon* from a roadside. Ecology 57:205-208

Wulff RD, Alexander HM (1985) Intraspecific variation in the response to CO_2 enrichment in seeds and seedlings of *Plantago lanceolata* L. Oecologia 66:458-460

Wulff RD, Strain BR (1982) Effects of CO_2 enrichment on growth and photosynthesis in *Desmodium paniculatum*. Can J Bot 60:1084-1091

Yelle S, Beeson RC Jr, Trudel MJ, Gosselin A (1989) Acclimation of two tomato species to high atmospheric CO_2. Plant Physiol 90:1465-1472

Zeiger E, Farquhar GD, Cowan IR (eds) Stomatal function. Stanford Univ Press, Stanford CA USA, 503 pp

Ziska LH, Teramura AH (1992) Intraspecific variation in the response of rice (*Oryza sativa*) to increased CO_2 - photosynthetic, biomass and reproductive characteristics. Physiol Plant 84:269-276