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Reproductive response to elevated CO_2: the roles of vegetative carbon storage, nitrogen and seed traits

Leanne M. Jablonski

Department of Biology

McGill University

1205 Ave. Docteur Penfield

Montréal, Québec, Canada H3A 1B1

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A thesis submitted to the Faculty of Graduate Studies and Research

in partial fulfillment of the requirements of the degree of

Doctor of Philosophy

DEDICATION

This thesis is dedicated to my parents,

INA NETTIE ANDRUSKOW (6. February 8, 1930)
&
TED MARION JOSEPH JABLONSKI

(February 11, 1933 - March 13, 1994)

who did not have the opportunity of a University education.

They taught me essential tools for research: wisdom, perseverance and 'common sense'

& nurtured my love of nature and science

& to my

MARIANIST FAMILY

who prepared me so well over these past twenty years for the discipline and love that I would need to make this journey of head and heart integration.

'Be patient with all that is unsolved...
Try to love the questions themselves
Do not search for the answers that cannot be given to you now
because you would not be able to live them

and the point is, to live everything.

Live the questions now
perhaps then,
someday far in the future
you will gradually, without even noticing it,
live your way into the answer.'

- Rainer Maria Rilke

ABSTRACT

This study focused on the reproductive response to elevated CO_2 of plants possessing below-ground storage. I tested the hypotheses that under elevated CO_2: 1) Plants with greater non-foliar storage capacity will show more reproductive response and 2) The altered foliar physiology of carbon (C) and nitrogen (N) use will cause increases in seed number and quality. Carbon dioxide treatments of High (650 $\mu L\ L^{-1}$) and Ambient (360 $\mu L\ L^{-1}$) were used in a controlled environment, simulated growing season, and in a natural pasture community. Hypothesis 1 was tested experimentally using four *Raphanus* varieties that differed in hypocotyl and leaf sizes. N fertilization and harvest times were used to obtain a range of root:shoot ratios. Enhancements in vegetative leaf area rather than the hypocotyl predicted reproductive responsiveness to CO_2. However, after three years of CO_2 exposure in the pasture, hypocotyl-storing *Taraxacum officinale* responded strongly in vegetative biomass which correlated with inflorescence size and number. Fitness was enhanced four-fold, while the leaf-storing *Plantago major* produced more ramets and had only a two-fold fitness increase. Hypothesis 2 was tested by examining the C and N physiology underlying the vegetative organs and seeds of the pasture plants. Under elevated CO_2, photosynthesis increased two-fold and senescence was delayed. Total plant C:N ratio did not differ, suggesting N acquisition increased. N similarly limited seed number in all cases suggesting an unchanged physiology of N use in reproduction. While morphology constrained total biomass response, provisioning to seeds increased as shown by higher seed mass and number and decreased variability in number and mass. In all cases, leaf mass increase under high CO_2 corresponded with fitness increase. Phenology constrained response to CO_2 as there was no plasticity in flowering day in *Raphanus* and *Plantago*, while there were flowering delays but greater seed maturation rate in *Taraxacum*. Direct effects of CO_2 on phenology and seed mass may counteract effects

of higher temperatures with global change. Elevated CO_2 effects on below-ground traits, total N, and resource flux to seeds suggest that storage plants will impact future community and ecosystem processes.

RÉSUMÉ

Cette étude porte sur l'effet des teneurs élevées en CO_2 sur la reproduction des plantes. J'ai vérifié deux hypothèses selon lequelles sous une concentration élevée de CO_2: 1) Les plantes qui ont une capacité de réserve non-foliaire auront un potentiel reproducteur plus élevé, et 2) La modification de la physiologie des feuilles quant à l'utilisation du carbone (C) et de l'azote (N) produira une augmentation de la quantité et de la qualité des graines. Des teneurs en CO_2 élevées (650 $\mu l\ L^{-1}$) et ambiantes (360 $\mu l\ L^{-1}$) ont été utilisées à la fois dans un environnement contrôlé pour simuler la saison de croissance et dans un paturage naturel. L'hypothèse 1 fut vérifiée expérimentalement en utilisant d'une part quatre variétés de *Raphanus* qui différaient quant à la taille de l'hypocotyle et des feuilles, et d'autre part, deux dicots ayant un système racinaire différent. La fertilisation en N et le temps de récolte ont été utilisés pour produire une grande amplitude de rapport racine à tige. La réaction de reproduction au CO_2 semblait reliée à l'accroissement de la surface des feuilles plutôt qu'à l'hypocotyle. Cependant, trois ans après une exposition au CO_2 dans le paturage, la biomasse végétative de *Taraxacum officinale* a augmenté et variait de concert avec le nombre et la taille des inflorescences. Le potentiel reproducteur de *Taraxacum* a quadruplé. D'autre part, *Plantago major* s'est reproduit de façon végétative. Son potentiel reproducteur n'a que doublé. L'hypothèse 2 a été évaluée par l'examination de la physiologie du C et N au niveau des organes végétatifs et des graines des plantes du paturage. Sous des teneurs élevées de CO_2, la photosynthèse a doublé et la sénescence a été retardée. Le rapport C:N de la plante entière ne différait pas entre les teneurs en CO_2 atmosphérique, ce qui suggère que l'acquisition du N a augmenté. Le N a limité le nombre de graines de façon similaire dans tous les cas, ce qui suggère que la physiologie de l'utilisation du N pour la reproduction n'a pas changé. Bien que la morphologie limite la réponse de la biomasse totale, l'approvisionnement des resources aux graines a augmenté.

Cette augmentation est due à la fois à l'augmentation de la masse et du nombre de graines et à la diminution de la variabilité de leur nombre et masse. Dans tous les cas, l'augmentation du potentiel reproducteur a été positivement relié à la masse des feuilles exposés aux teneurs élevées en CO_2. La phénologie a contraint la réponse au CO_2 car il n'y a pas eu de plasticité dans la date de floraison chez *Raphanus* et *Plantago*. Cependant, il y a eu un délai dans la floraison ainsi qu'un taux de maturation des graines plus élevé chez *Taraxacum*. Les effets directs du CO_2 sur la phénologie et la masse des graines ont contrebalancé les effets d'une température plus élevée associée au changement global. Les effets du CO_2 sur les caractéristiques sous-terraines des plantes, le N total, et le flux des resources aux graines suggèrent que les réserves des plantes auront un impact futur sur les communautés et les processus écosystémiques.

ACKNOWLEDGMENTS

The specific contributions to the research are acknowledged within each chapter. Here, I want to acknowledge all those who have helped with the entire thesis process.

This thesis was entirely conceived and written by me, but only as a product of all those who have influenced me by their example, ideas, hands, and listening minds and hearts as I struggled to give birth to the questions from the darkness of the unknown to the light of day. So, I first must thank those who nurtured my questioning spirit and love of nature at a young age, particularly all those involved in Science Fairs and Symposia in Winnipeg, Manitoba; and for all at U of Manitoba Botany who fostered my initial University research experiences, beginning with a high school dish-washing job!

During my first year at McGill, a friend conveyed a theory that we choose as the subject of our doctoral dissertation, an issue that we are working upon in our lives. I will only understand in retrospect all the whys and wherefores of my own choice, but I do already know that the allocation of energies and reserves, short-term and long-term choices and gains, quality vs. quantity, looking within or beneath appearances to carefully assess, and the individual performance within the intact community context, are all issues of importance to me both personally and scientifically.

The environment of McGill department of Biology, has fostered my growth as an independent researcher and has helped me to discover the sacredness of the research process. I am deeply grateful to my thesis advisor, Catherine Potvin, for her support and encouragement, her calling me to excellence, her challenges to reach my potential, and her enthusiastic sharing in the thrills of exciting data. Her expertise in experimental design and

statistics and skills in focusing on "the essence" have been invaluable aids. My supervisory committee of Rajinder Dhindsa, Daniel Gagnon and Joe Rasmussen provided encouragement and wisdom along the way, such that I was able to "find my spirituality in my work". The post-docs and faculty taught me much about the rewards of dedication to one's work, and the administrative staff and research technicians provided help and kept me smiling through many intense and tedious moments. I thank all the undergraduate and graduate students and post-Docs who assisted along the way, particularly the Potvin, Lechowicz and Schoen plant ecology lab groups, our plant discussion groups, Matt Sclafani my office mate, and the Price lab. A special thanks to Marc Trudel who assisted with translation of the abstract, and to Richard Preziosi for statistical conversations. I am particularly grateful for my sixth floor companions who provided support for the journey of being a woman in science: for Andrée Nault, Danielle Cantin, Denise Tousignant, Irene Wisheu, Jessica Meeuwig, Liette Vasseur and Maite Maldonado.

I am grateful for the McConnell family foundation who synchronously provided me with both the major fellowship money to support the research and the house of Unitas, which was my spirituality center of support throughout the thesis. Association with the McGill Center for Climate and Global Change Research provided a broad perspective and financially supported the completion of the thesis.

Several influences beyond McGill were important to sustaining my life during these years in Montreal. I thank the people of Montréal and Québec with whom I shared the anguish of this challenging and painful time in history. I will miss this city with her cultural beauty, bike trails, festivals, street performers and all the enrichment provided by a bi-lingual and multi-cultural world. Over the years, the Olympic Athletes and Figure Skaters engendered my own sacrifice, dedication and striving for excellence. I bow to

Mont Royal on whom I wrote my thesis, her beautiful energies of strength and her nurturing ski trails; and also to all participants at Unitas who gave expression to the beauties of the earth and the traditions of her indigenous peoples.

I am deeply grateful for the communities who gave me a place of support amid the lonely days of the journey: my Marianist Sisters empowered and enabled me to "be away" to do this degree; my foster sisters of the CND shared their living space with me for several years; my Wellsprings community weekly meetings throughout my time in Montreal; Newman Centre and Unitas, my worship and spirituality expression spaces. Many e-mail connections sustained me on the weekends, and I am especially grateful for Kim, Gary and Laura. I particularly cherish special friends that accompanied me through all the joys and pains, especially Teri Moss, and the family of Steve & Angela Goodale-McCanny.

I am grateful for the gentle spirits that accompanied me. The quiet joyful gifts of Ray Rabbit, my companion of 10 years (d. January 10, 1997) who saw me through to the last analysis of data essential for the thesis. Hildegard the barn cat adopted us out at Farnham and birthed kittens of whom (Tri)Nity remains. In the final year, dwarf rabbits Char and Bandit, discovered on the mountain, eased long days with their antics.

Finally, I am deeply grateful for that Faithful Love, the Energy of the Universe that beckons us all to us to know Connections, and for the fidelity of my Beloved, who empowered completion. May I be worthy of the responsibilities conferred on me by my training, and ever serve the communities of the earth reverently with integrated knowledge of both head and heart.

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PREFACE

For the purpose of informing the examiners of Faculty regulations, the Faculty of Graduate Studies and research requires the following passage to be reprinted in every thesis:

> "Candidates have the option of including, as part of the thesis, the text of one or more papers submitted or to be submitted for publication, or the clearly-duplicated text of one or more published papers. These texts must be bound as an integral part of the thesis.
>
> If this option is chosen, **connecting texts that provide logical bridges between the different papers are mandatory**. The thesis must be written in such a way that it is more than a mere collection of manuscripts; in other words, results of a series of papers must be integrated.
>
> The thesis must still conform to all other requirements of the "Guidelines Concerning Thesis Preparation". The thesis must include: A Table of Contents, an abstract in English and French, an introduction which clearly states the rationale and objectives of the study, a review of the literature, a final conclusion and summary, and a thorough bibliography or reference list.
>
> Additional material must be provided where appropriate (e.g. in appendices) and in sufficient detail to allow a clear and precise judgment to be made of the importance and originality of the research reported in the thesis.
>
> In the case of manuscripts co-authored by the candidate and others, **the candidate is required to make an explicit statement in the thesis as to who**

contributed to such work and to what extent. Supervisors must attest to the accuracy of such claims at the doctoral oral defense. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make perfectly clear the responsibilities of all the authors of co-authored papers.

In accordance with these instructions, I include the following explanations. All three chapters of this thesis are in the format of manuscripts. The ideas and writing are my own. I conducted all the work reported in this thesis for Chapters 1 and 3, including ideas, conception, design, execution, analysis and writing. The design of the field site open-top chambers on an intact community was conceived by Catherine Potvin. In Chapter 2, work on *Plantago major* in 1992 and 1993 had been carried out in collaboration with Liette Vasseur who is a co-author on this paper. I am grateful for her protocol and advice. My reproductive focus, the data analysis and 1994 work on *Plantago*, and entire work on *Taraxacum* was carried out on my own. Data collection assistance was provided for each chapter and these are acknowledged within each chapter. Catherine Potvin contributed advice during all stages of the research, and editing comments on the resulting manuscripts. Chapter 1 has been published in the journal, Canadian Journal of Botany 75: 533-545 (Jablonski, L. M. 1997). Chapter 2 is in review with the journal, Ecology (Jablonski L.M., L. Vasseur and C. Potvin) and Chapter 3 has been submitted to the journal, Oecologia (Jablonski L. M. and C. Potvin). Individual chapters have been prepared for different journals, so formats differ among the chapters. In Chapters 2 and 3, the first person plural is used, as this is the form used in the journal submissions. The chapters are very similar to the papers published in, or submitted to the respective journals. Because of the manuscript format chosen, the literature reviews are incorporated into each chapter, and a general review of the topic is given as part of the introduction.

ORIGINAL CONTRIBUTIONS TO KNOWLEDGE

1. The main contribution of this work is that it is the first integrated look at whole plant response to elevated CO_2 throughout the life history, including traits of morphology and physiology. It examines the reproductive response of the functional group (those with storage organs) that has shown greatest vegetative response to elevated CO_2, in order to ascertain the importance of this trait. Evidence of a four-fold relative fitness enhancement in *Taraxacum officinale*, and two-fold enhancement in *Plantago major* suggests that performance of perennial plants with hypocotyl storage organs may be enhanced under elevated CO_2.

2. Physiological work under elevated CO_2 work has focused predominantly upon the vegetative phase. This thesis provides the first examination of relationships between traits of the vegetative phase and the reproductive response in order to identify those that may have predictive value (Chapters 1 and 3). Enhancement of leaf area in response to elevated CO_2 was the greatest vegetative predictor of reproductive mass response. Vegetative biomass response did not predict reproductive response in *Raphanus* varieties. Response to CO_2 and N fertilization depended upon allocation patterns which changed with ontogeny.

3. This study is the first resource-based approach to examining seed trait responses under elevated CO_2 in a natural community setting. Previous reproductive studies have been almost totally on annuals grown in pots or artificial soil mixes. This is the first long-term and comprehensive look at reproductive response to CO_2 in perennial forbs (Chapter 2). While N has been shown to be an important factor in vegetative leaf responses to elevated CO_2, this is the first study that links vegetative and reproductive phases and examines the implications of altered N use for reproductive traits (Chapter 3). Taken at a whole plant level and over the entire life history, there was no no evidence of a change in C:N ratio and no evidence that N use was altered in seed production in *Plantago* or

Taraxacum, as total vegetative N predicted seed number. Resource responses detected at the inflorescence level of increases in seed mass and seed number, and decreases in variability of these traits, suggest that maternal effects may have an important role under future global regimes.

4. This thesis was also the first to examine long-term phenological responses to CO_2 under natural daily and seasonal rhythms in the field (Chapter 2), and the second in a controlled environment (Chapter 1). Lack of response to CO_2 or N in flowering day in *Raphanus* likely constrained reproductive response. The initiation of all reproductive events in *Taraxacum* was delayed over all years of the study and maturation time of inflorescences was reduced, while no response was seen in *Plantago*. This provides evidence that the direct effect of elevated CO_2 may counteract earlier flowering onsets that are predicted to occur with temperature increases.

5. Below-ground responses to elevated CO_2 have been little examined. This is the first report of strong enhancement in hypocotyl mass, and of CO_2 effects on root allocation patterns in non-arable species (Chapter 2), and shifts in biomass and nutrient allocation (Chapter 3). That responses in phenology, allocation, biomass and seed traits were species-specific, suggest these will contribute to shifts in community and ecosystem dynamics under an elevated CO_2 regime.

GENERAL INTRODUCTION

Predicting the response of wild plant species to anticipated future global atmospheric regimes requires an understanding of the effect of elevated CO_2 and other environmental factors on plant performance throughout the life history. The relatively few studies that have investigated reproductive traits under high CO_2 have focused on annuals and have shown that responses are both less in quantity and more variable than observed in the vegetative phase. Well-documented changes in carbon (C) and nitrogen (N) in foliar physiology and below-ground storage under high CO_2 could impact on the resources available to seed formation. My goal was to investigate the attributes of C and N underlying the response to CO_2 in order to elucidate the key factors determining reproductive response. The role of storage capacity, N availability and phenology on constraining response were examined. To assess potential impacts on fitness, seed traits of number, mass and N content were measured. I focused on the functional group of plants with storage organs because of the inherent potential to manifest long-term resource response, and because of the predominant impact this type has on ecosystem processes in north-temperate regions.

Effect of Global Climate Change on Plant Resources for Reproduction

The relatively rapid increase in atmospheric CO_2 in the next century will impact on plant growth and processes at individual, population, community and ecosystem levels. CO_2 is the main input of photosynthesis, the process converting atmospheric C to organic forms. CO_2 is predicted to more than double the pre-industrial level of 280 ppmv to reach 650 ppm by midway through the next century (IPCC 1995). At the plant organismal level, this change in atmospheric composition is potentially a source of increased C stores,

structural materials and energy to drive metabolism. In addition to the direct effect of CO_2 on plants, the CO_2 increase will cause climate changes of an estimated increase in temperature of 2 to 4 degrees at a latitude of 45^0 N and decreases in precipitation (IPCC 1995). All of these climate changes can affect phenology (the timing of events) and the overall growth and reproductive response.

At the same time, mineral nutrient availability will likely change due to direct and indirect effects of atmospheric changes on soil processes and through the effects of gaseous pollutants such as nitrous oxides (Environment Canada 1991, IPCC 1995). Availability of N under high CO_2 is of particular importance since N is the mineral nutrient that plants require in greatest quantities and N most frequently limits growth and productivity in both agricultural and natural ecosystems (Chapin et al. 1987, Tilman 1988). Though N levels may increase near urban areas and due to elevated CO_2 effects on soil processes (e.g.Hungate et al. 1996), it will be much less in magnitude than the C increase.

Consideration of N and C together is important for evaluating plant responses to CO_2 since the allocation of C and N are the major resources that govern growth, maintenance, defense, reproduction and seed formation (Bazzaz et al. 1987, Chapin et al. 1987). N interacts with C in nearly all plant processes. It is a major constituent of plant proteins, chlorophyll and nucleic acids and is thus needed for all physiological functions. Photosynthetic C acquisition is dependent on the N invested in RUBISCO and chlorophyll (Evans 1989, Field 1983) and the effect N has on promoting leaf area production (Terry et al. 1983, Koch et al. 1989). About 20-50% of the plant C budget is spent on total N acquistion processes including 10% on the root growth necessary for absorption of soil deposits and the remainder on the energy for uptake, nitrate reduction in the root or shoot and assimilation into organic forms (Chapin et al. 1987, Robinson et al. 1991, Oaks and Hirel 1985, Oaks 1992).

At the time of reproduction, all of the plant N including leaf and stem proteins and storage in stems, hypocotyls or roots can be recycled into seeds (Chapin et al. 1990, Millard 1988) and additional N can also be acquired directly from the soil. Seed accumulation of N can be in excess of the apparent needs for germination (Benner and Bazzaz 1988, Fenner 1986a). Seed N quantity has important ecological consequences as it is a determinant of germinability, seedling size, and competitive success (McGinley and Charnov 1988, Parrish and Bazzaz 1985, Charest and Potvin 1993). Within seeds, C stores in carbohydrates and lipids provide energy, while proteins and mineral N, determine germinability and initial seedling growth (Willson 1983).

Because reproductive response is a coordinated system which is sensitive to environmental factors (Roach and Wulff 1987), changes in C and N availability can alter plant processes that lead to reproductive success. Resource abundance and the ability of a plant to assimilate and allocate resources can increase overall growth, reduce the time to flowering, influence the aspects of phenological pattern such as the duration and variance of flowering, fruiting and seed maturation and increase seed number or weight (Lloyd 1980, Chiarello and Gulmon 1991, Rathcke and Lacey 1985). Resource limitation is one of the major factors limiting seed set, through its effect on selective abscission of flowers and fruits (Stephenson 1981). Within individual seeds, adequate storage reserves of C (carbohydrates or lipids) and N (predominantly in the form of protein) are needed for germination and initial growth until the seedling can establish itself as independent of the maternal reserves (Willson 1983).

Because in recent evolutionary time global CO_2 has been relatively constant (Vostok core records showing levels between 200 and 300 ppm in the past 220,000 years, IPCC 1995) it is likely that plants possess patterns of C synthesis and allocation that optimize resource use under the low CO_2 regime. Because some of these patterns will be less responsive under increased CO_2 while others may be enhanced, the relative performance

of individuals possessing these may change. To assist predictions at the community or ecosystem level or about evolutionary responses at the population level it is important to investigate how the reproductive allocation of C and N will be altered under increased CO_2. N amount may limit response to the novel environment of global CO_2 change if plants are not able to acquire enough to meet their needs.

Plants are likely well adapted to a low CO_2 regime, and it is difficult to predict the direction of evolutionary response to a novel environment. Also, the relatively rapid increase in CO_2 to a doubling in 100 years, will leave relatively little evolutionary time to adapt, particularly for plants that are clonal or long-lived. However, plants often show adaptive plasticity in response to difference resource levels, that is, a flexibility to respond in morphology or physiology in ways that optimize use of the resource to ultimately maximize their fitness, the number of high-performing offspring that themselves survive to adulthood. Individuals that already exhibit a certain level of physiological plasticity or morphological traits allowing resource storage may be better able to optimize reproductive output for a given environmental resource availability and should be favoured.

Past research has shown that increased CO_2 has many influences on vegetative plant physiology and morphology that may affect resource allocation to reproduction. In general, C accumulates in excess of N. In most species examined, total plant biomass increased while total leaf area and shoot number are increased or unchanged (reviewed in Bazzaz (1990), Newton (1991), Woodward et al. (1991)). Net photosynthesis usually shows an initial increase though it is not always maintained (Farrar and Williams 1991) Foliar starch often increases in C_3-plants, suggesting that C is in excess of the other metabolic and growth demands of the plant and is being stored in this reserve, though an excess can feed-back to limit photosynthesis (DeLucia et al. 1985). This reserve is potentially able to be remobilized to provide for the needs of the reproductive phase, but appears not to be always utilized (Havelka et al. 1984a).

Under increased CO_2, foliar N levels show varying responses. The N amount is often lower when expressed on a dry weight basis likely due to increased starch production, but is sometimes slightly increased or unchanged when expressed per unit leaf area. Where N level is lower, the major component is a decreased total quantity or amount of the active form of RUBISCO (Sage et al. 1989), the enzyme which normally comprises around 50% of total leaf protein (Millard 1988). Only a few studies have examined the distribution of whole plant N accumulation in response to increased CO_2. The decreased N sometimes found in the leaves is not a trend found throughout the entire plant, as total plant N acumulation was not different (Garbutt et al. 1990, Coleman and Bazzaz 1992) or slightly increased (Hocking and Meyer 1991a,b). If the root or stem tissue can serve as a major store for N under high CO_2 in some species, as found in wheat root and tassel (Hocking and Meyer 1991a), then it is possible that this may be remobilized during the reproductive phase.

A body of literature has developed around the issue of whether N availability or acquisition is limiting the vegetative response of plants to CO_2. One physiological explanation is that less N is found in leaves because less is needed. Photosynthesis is more efficient and less RUBISCO (protein-N) is required to produce the same amount of dry matter (Warrick et al. 1986, Wong 1979). A second approach focuses on whether the soil volumes or pot sizes used in studies have restricted root growth. Any root constraints imposed can reduce sink strength, which feeds back to inhibit photosynthesis, and the overall growth response (Thomas and Strain 1991). Since increased CO_2 appears to have no effect on the rate of N uptake by roots (Yelle 1987, Chu et al. 1992, Coleman and Bazzaz 1992, Curtis et al. 1994), increased root area would be needed for plant increase in N. The root-restriction argument was supported by Arp (1991) who surveyed the literature and showed that root:shoot ratio only increased under elevated CO_2 in pots with over 3.5 L soil volume. Both increased soil volume or nutrient level can increase root growth under

increased CO_2 with the degree of response dependent upon root type (McConnaughay et al. 1993, Berntson and Bazzaz 1997). Hilbert et al. (1991) integrated considerations of both root growth and photosynthesis and showed through cost-benefit modeling that the C costs necessary for growth and maintenance of a larger root area in order to acquire higher N would reduce the relative growth rate. They suggest a lower leaf N is more optimal even if photosynthetic capacity is less. Work in natural systems using rhizotron technology is beginning to show root growth and morphology enhancements under elevated CO_2 (e.g. Curtis et al. 1994), and direct and indirect effects of CO_2 on soil processes (e.g. Hungate et al. 1996, Berntson and Bazzaz 1997), but little is known of these impacts on whole plant growth.

In summary, under an elevated CO_2 regime plants generally acquire more carbon relative to nitrogen. The relatively reduced N need for carboxylation could mean that more is available for seeds in the reproductive phase. Alternatively, if seed-filling is tightly coupled to foliar physiology, the N supply may be reduced. Additional carbon of the elevated CO_2 regime could provide for the metabolic needs of the reproductive phase provided there are no other constraints. Root growth may be important to allowing CO_2 response.

Search for Functional Groups

Physiological constraints of C and N use may provide insights regarding how types or groups of species will respond throughout their life history. Such knowledge is critical for predicting responses to CO_2 at the community and ecosystem level. Because of the impossibility of following all individual species and traits a functional group approach has sometimes been taken in an attempt to classify responses. A functional group is "elements that bear a certain set of common structural and/or process features" (Korner 1993). Groups based on classical physiological traits of the vegetative phase have been tested,

including N-fixers, and C_3 vs C_4 plants. Beyond short-term, single species pot experiments, these have failed to yield consistent responses to CO_2, and responses appear to be limited by other factors, including community components. Ecosystem responses require an understanding of long-term responses, yet entire life history has rarely been investigated in wild species.

For predicting reproductive response to CO_2, a new way of thinking about functional groups is needed, an approach based on resource storage. I propose that since plants have evolved to maximize storage and reproductive output under a low CO_2 regime, they may not have the plasticity to respond under the novel condition of high CO_2. The functional group that already contains storage that is not likely to be inhibitory of photosynthesis (those with a below-ground storage organ), may have the inherent ability for greater response. I decided to focus my thesis work on the growth form of below-ground storage, the group that has shown the most responsiveness to CO_2 in the vegetative stage. This type which is found in root crops, biennials and perennials, has shown a vegetative biomass response of 1.5-fold on average compared with the 1.3-fold average over all types (Poorter 1993, Poorter et al. 1996). This storage organ may also allow for an increase in N acquisition. Because of the lack of negative downregulation in the leaves that might occur with this storage regime, I proposed that more C would be available too for the metabolic processes of N acquisition and root growth. I focused on plants possessing storage capacity in the hypocotyls, the region of the stem that lies between the cotyledons and the radicle. Because these organs are adapted as reserves for later reproduction, I expected that total reproductive output would be enhanced.

Hypothesis 1: Plants with greater non-foliar (hypocotyl) storage capacity will show increased reproductive output under elevated CO_2.

Because this growth form is dominant in north temperate systems and common in disturbed areas, understanding the response has critical implications for resource use and phenology

at the community and ecosystem levels.

Impact of Elevated CO_2 on Reproductive Traits

When I began my doctoral work, nearly all increased CO_2 studies had focused on the vegetative phase of growth and had not considered the potential impact of the altered vegetative C and N allocation pattern on the reproductive characters necessary to assess fitness. Existing patterns of vegetative allocation are also expected to be optimal for the reproductive phase since natural selection should favor allocational patterns that maximize fitness of the individual (Cody 1966, Willson 1983). Fitness is difficult to measure, but can be estimated by examining seed quantity and components of offspring quality that are indicative of performance of a genotype in the next generation (Primack and Kang 1989). Examining the quality parameters indicative of seedling performance can help assess if the reduced N requirement for foliar photosynthesis is contributing to a decreased reproductive performance. In the few studies which have recorded the allocation of C and N to reproduction under increased CO_2 in wild species, the distribution to both seed number and seed quality has not been examined (Garbutt et al. 1990, Curtis et al. 1989) or replicates were too few to demonstrate that trends were significant (Larigauderie et al. 1988). In this thesis, I studied the impact of elevated CO_2 on the supply of vegetative resources to reproduction, and evaluated potential fitness consequences by examining a complete suite of reproductive traits. Because of the role of maternal effects in provisioning increased resources during the time of seed-filling, I examined seed traits related to resource use; seed mass, number and the variability of these traits.

Hypothesis 2: The altered foliar physiology of increased C and decreased N under elevated CO_2 will cause increased seed number and quality.

Besides testing the two major hypotheses, I had two other objectives. The first was to examine which traits in the vegetative phase could best predict reproductive performance

under elevated CO_2. Experimenters are spending considerable effort examining the response to CO_2 of traits in the early vegetative phase (e.g. relative growth rate (Poorter 1996), physiological traits (Roumet and Roy 1996)) that may not be relevant to responses throughout the entire life history. If reliable diagnostic traits could be identified that would best predict reproductive performance, this could allow for earlier screening for CO_2 responsiveness.

A second major objective was to examine the role that shifts or lack thereof in the phenology could have on enhancing or constraining the reproductive response to elevated CO_2. Because plant development is under the control of signals that respond to environmental cues such as daylength and temperature, plasticity may not be present in the developmental timing to respond to the increased resource of an elevated CO_2 regime. Throughout my work I followed the timing of reproductive and physiological events including the induction and completion of life history phases as well as the responses through time in leaf physiological traits. Within individual structures, I followed the rates of maturation and senescence.

Experimental Approach

I chose environmental conditions that were as natural as possible and implemented a protocol that was rather novel for a reproductive study. Because of the known impact of diurnal and seasonal rhythms on reproduction, I used a controlled environment program that simulated light, temperature and humidity throughout the growing season. I also used a field component in which I examined the reproductive response of perennials growing within the intact pasture community, with the natural soil and background vegetation conditions. Thus, I minimized the artifacts introduced by the experimenter, and had a system that was as realistic as possible to estimate the "true" response of these species within their natural community and ecosystem settings.

Over the time period of my observations within the pasture study plots (1991 to 1996), there were CO_2 effects on community composition, and the pattern and rate of succession (Potvin and Vasseur 1997, Vasseur and Potvin, 1997). In this pasture community, elevated CO_2 was shown to alter competitive interactions between species (Stewart and Potvin 1996) and responsiveness to elevated CO_2 appeared to be influenced by the disturbance regime (Taylor and Potvin 1997). I recognize that all of these factors could enhance or limit the performance of my focus species within this particular intact community, as compared with their performance in individual pots or mono-cultures, or within another community. I chose to follow the species within the intact, natural environment, since this is their natural background. I considered the biological components of the background species and competitive environment as part of the chamber environment.

I used two main approaches for my work. The first was a controlled environment study of the influence of levels of CO_2 and N on both vegetative and reproductive traits (Chapter 1). I used a system of *Raphanus* (radish) varieties that differed in hypocotyl storage organ size and thus tested my first hypothesis within a biologically similar system. My second major approach was to follow the reproductive responses to long-term exposure to CO_2 in an intact pasture soil community. In Chapter 2, I tested the first hypothesis using two rosette perennials (*Taraxacum officinale* and *Plantago major*) that differ in hypocotyl storage size. I report the phenology responses over the five-year study, the growth during three years and examine the biomass and allocation patterns after three years exposure to elevated CO_2. In Chapter 3, I examined the physiology underlying the reproductive response, and tested the second hypothesis. Here I focus upon the second and third years of exposure to elevated CO_2. In the conclusions and summary section I highlight the implications of this work for physiological ecology, and community and ecosystem level studies.

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CHAPTER 1

Responses of vegetative and reproductive traits to elevated CO_2 and Nitrogen in *Raphanus* varieties

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ABSTRACT

The relationships between the responses to elevated CO_2 of the vegetative and reproductive phase were investigated in radish, used as a test system. The hypothesis that an increase in non-foliar vegetative storage capacity promotes reproductive output was tested. Three cultivars of *Raphanus sativus* and the wild, *R. raphanistrum*, differing in root:shoot ratios, were grown under two levels of CO_2 and two levels of Nitrogen fertilization. Varieties possessed different strategies of carbon storage, and showed distinct responses to CO_2 at each vegetative harvest time. Vegetative sinks of hypocotyls, petioles, and young blades were enhanced by CO_2. Nitrogen promoted vegetative shoot growth, but did not enhance the reproductive response to CO_2. By the end of the reproductive phase, varieties did not differ in total biomass. Reproductive response to CO_2 may have been limited by the lack of an effect on the timing of flowering. Correlations in CO_2 enhancement ratios were examined in 12 traits of each phase. Only vegetative total leaf area correlated with reproductive mass. Foliar starch correlated with decreased abortion. Enhancements in vegetative biomass did not correlate with any reproductive response. Detailed studies of the reproductive phase are needed in order to understand the whole-plant response to elevated CO_2.

Abbreviations

AFP, aborted flowers and pods; CER, carbon enhancement ratio; CHL, chlorophyll per leaf area; LAR, leaf area ratio; RA, reproductive allocation; RSR, root to shoot ratio; SLM, specific leaf mass; EXP I, experiment I; EXP II, Experiment II; Varieties: V, Variety; CB, Cherry Belle; FB, French Breakfast; IC, Icicle; W, Wild; Treatments:
CN, 650 $\mu l\ L^{-1}$ (high) CO_2, high Nitrogen (N); Cn, high CO_2, low N;
cN, 350 $\mu l\ L^{-1}$ (low) CO_2, high N; cn, low CO_2, low N.

RÉSUMÉ

L'auteure a étudié les relations entre les réactions aux teneurs élevées en CO_2 de la phase vegetative et de la phase reproductive, en utilisant le radis comme modèle. Elle a vérifié l'hypothèse qu'une augmentation de la capacité d'accumulation de réserves dans un organe végétatif non-foliaire promeut l'effort de reproduction. Elle a cultivé en présence de deux teneurs en CO_2 et de deux niveaux de fertilisation azotée, trois cultivars de *Raphanus sativus* ainsi que le radis sauvage *Raphanus raphanistrum*, différents par leurs rapports racine à tige. Les variétés possèdent différentes stratégies pour accumuler le carbone et montrent des réactions différentes au CO_2 à chaque étape du développement végétatif. Les puits végétatifs des hypocotyles, des pétioles et des jeunes limbes augmentent sous l'influence du CO_2. L'azote stimule le développement végétatif de la tige, mais n'augmente pas la réaction de la reproduction sous l'influence de CO_2 accru. Vers la fin de la phase reproductive, les variétés ne montrent pas de différence dans leur biomasse totale. La réaction de la reproduction au CO_2 pourrait avoir été limitée par le manque d'un effet sur le moment de la floraison. L'auteure a examiné les corrélations entre les rapports d'augmentation du CO_2 selon 12 caractères pour chaque phase. Seule la surface foliaire végétative totale montre une corrélation avec la masse reproductrice. L'amidon foliaire est relié avec une diminution de l'avortement. L'augmentation de la biomasse végétative n'est pas corrélée avec la réaction reproductive. Pour comprendre la réaction de la plante entière au CO_2 élevé, il faudra entreprendre des études détaillées de la phase de reproduction.

INTRODUCTION

Most research on elevated CO_2 effects in plants has been on crop species and the early vegetative phase of non-arable species. Effects of CO_2 include increased photosynthesis, foliar starch and growth rate, and delayed senescence (Lawlor and Mitchell 1991, Rogers and Dahlman 1993, Woodward et al. 1991). Vegetative phase biomass was enhanced 33% by doubling CO_2 (Kimball 1983, Poorter 1993) and consisted of non-structural stores such as starch as well as structural materials.

These responses to CO_2 may be limited by a lack of sufficient developmental or metabolic sinks (Arp 1991, Bowes 1993). Carbon stored as starch in the mature (source) leaves causes a feed back that lowers photosynthesis, so enhancement in sinks such as developing leaves, roots, hypocotyls and petioles (Geiger 1986, Ho 1988) will increase the response to CO_2 (Reekie 1996). The biomass response to CO_2-doubling is greater (over 50%) in plants that have non-foliar starch storage organs, such as biennials, perennials and root crops (Kimball 1983, Hunt et al. 1991, Poorter 1993).

Much less is known about the effects of high CO_2 on the reproductive phase. Since reproductive output is influenced by the vegetative phase stores , plants with greater storage capacity may show greater response to high CO_2 in the reproductive phase. Reproductive responses are less pronounced and more variable than vegetative responses (Newton 1991). The enhancement of biomass with CO_2 doubling averages only 22% for flower, grain and fruit crops (Kimball 1983) and 26% for non-arable C_3 species (Ackerly and Bazzaz 1996). High CO_2 also affects the balance between vegetative and reproductive phases, and responses vary with species, experimental conditions and population (e.g. Garbutt and Bazzaz 1984). Reproductive allocation is often decreased (Ackerly and Bazzaz 1996) and vegetative starch stores are not fully utilized (Havelka et al. 1984).

Phenological responses vary, and do not correspond with vegetative growth responses (Reekie and Bazazz 1991). Individual seed weight may be enhanced but seed numbers vary.

Nitrogen supply also influences the biomass and growth response to high CO_2 (Bazzaz 1990, Field et al. 1992, Rogers and Dahlman 1993). Foliar starch is lower under high N (Wong 1990). Both photosynthesis and N utilization efficiencies are enhanced at elevated CO_2 such that 24 - 38% less foliar N is required to support maximum productivity (Conroy and Hocking 1993). Storage organs typically have higher nutrient concentrations than leaves (Chapin et al. 1990), so plants having these may acquire more N and increase reproductive output under high CO_2. Available N and CO_2 have an interactive effect on seed number (Curtis et al. 1994), individual seed weight (Larigauderie et al.1988) and total C/N (Conroy and Hocking 1993).

The altered allocation, partitioning and metabolism of C and N under high CO_2 in the vegetative phase may strongly influence the reproductive response, but multiple vegetative and reproductive traits have been examined in the same study only a few times, all in annuals without storage organs (e.g. Farnsworth and Bazzaz 1995, Garbutt et al. 1990, Tousignant and Potvin 1995). My goal in this study was to investigate the relationship between the vegetative and reproductive responses to increased CO_2 and N fertilization. I hypothesized that reproductive response to CO_2 would be enhanced by a greater non-foliar storage capacity. I also examined which growth, biomass and physiological traits in the vegetative phase have the strongest relationships with phenology, partitioning, and pod output in the reproductive phase. Varieties of *Raphanus* L. were chosen because each had a distinct carbon storage strategy and had different morphological constraints on the partitioning of C and N stores between leaf and hypocotyl.

METHODS

Plant Material

Four varieties (V) of *Raphanus* L. (radish) were selected that differed in hypocotyl shape and root:shoot ratio (RSR). Three cultivars of *Raphanus sativus* L. were used: Cherry-Belle (CB) (spherical, high RSR), French Breakfast (FB) (short cylindrical, intermediate RSR), and Icicle (IC) (elongate, low RSR). All were spring or summer varieties whose hypocotyls matured in 24 to 27 days. Seeds of wild radish (W), *Raphanus raphanistrum* L. (very low RSR) were pooled from two populations collected by Agriculture Canada (Ottawa, ON). One population was from a wheat field in St. Robert, QC (45^{o} 58'N, 73^{o} 00' W) and the other was from a corn field in Ste. Victoire de Sorel, QC (45^{o} 57'N, 73^{o} 05' W). Plants grow as a basal rosette in the vegetative phase. The reproductive phase commences by a bolt emerging from the storage hypocotyl, and flowers open a week or so later.

Growth Conditions

Several seeds were planted in 3.5 L pots containing 3 parts peat humus: 1 part pure sand (Fafard & Freres, St. Guillaume, QC). Lime (Stonelite $CaCO_3$*$MgCO_3$) was added at the rate of 1.4 g /L soil to adjust the pH to 6 for optimum nutrient availability. Plants were grown under two levels of CO_2: 350 $\mu L\ L^{-1}$ (ambient, low) and 650 $\mu L\ L^{-1}$ (high) in Conviron PGW36 growth chambers (Conviron, Winnipeg, MB, Canada) at the McGill University phytotron. Radish is a cold-hardy, day-length neutral plant (Kosta-Rick and Manning 1993). The protocol used simulated diurnal and seasonal conditions (Wang et al. 1994) for the north temperate regime of Quebec. Optimal root development was achieved by simulating conditions in the period from June 1 (Day 1) until October 18 (Day 140). Plants were given distilled water as needed. Seedlings emerged four to six days after

planting. Germination was 80% in W and 97% in the cultivars. Plants were randomly thinned to one plant per pot on Day 11.

Two experiments were carried out that complemented each other while providing partial replication. In both experiments, plants were fertilized with two levels of nitrogen (N). Beginning in the third week, all plants received 100 ml of 20-20-20 N:P:K All Purpose Fertilizer (Plant Products, Brampton, ON) at the rate of 1.5 g/L. Plants receiving a high N treatment, received an additional N in the form of $Ca(NO_3)_2 * 4 H_2O$ (rate of 2.5 g/l). In Experiment I (EXP I) , plants were fertilized every five days in the third and fourth weeks. Because mild foliar symptoms of nutrient deficiency were evident, the fertilization application was increased to twice weekly from the fifth week. In Experiment II (EXP II), the fertilization interval was twice weekly from the third week. Thus, the plants of EXP II received two more fertilization treatments than in EXP I. After 140 days, when growth was reduced, plants in both experiments were fertilized once per week.

Plants were placed in a split-plot design, with one CO_2 level in each chamber. To reduce the possibility of pollination between varieties, varieties were assigned randomly to subplots within each chamber. Within each variety(V) subplot, N treatments were arranged at random. The four growth environments are represented in the text as: CN=high CO_2 (650 $\mu L\ L^{-1}$) and high N; Cn=high CO_2 (650 $\mu L\ L^{-1}$) and low N; cN=low CO_2 (350 $\mu L\ L^{-1}$) and high N; cn=low CO_2 (350 $\mu L\ L^{-1}$) and low N.

Flowers were mass-pollinated within each variety twice each week. A small paintbrush was utilized, and hand pollination proceeded by movement in random order between all open flowers. New flowers were initiated in each variety throughout the growing season. Seed pods were defined as mature when the pedicel senesced and the pod detached easily. CB and FB did not form mature pods prior to the final harvest. Any mature seed pods in W and IC were collected at approximately one to two-week intervals.

The pedicel was clipped midway to mark the location of mature pods for later abortion counts.

Destructive harvests occurred at three developmental times: seedling, vegetative growth prior to bolting, and reproductive. Sample sizes were five plants for each variety x CO_2 x N combination of the vegetative and reproductive harvests. The seedling harvest was 11 days after planting. In EXP I, the vegetative harvest for all four varieties was chronologically equivalent, at 32 days. In EXP II, the vegetative harvest occurred when varieties were at the physiologically equivalent stage. This was several days prior to the first observation of bolt emergence in EXP I (V-time in days): W-28, IC-37, CB-56, FB-60. In both experiments, the reproductive harvest was spread over the last week of the growing season, so as to allow time to obtain reproductive data and process each individual. Harvests were in the order of flowering (V- time in days): W-132, IC-134, CB-137, FB-140.

Biomass allocation and growth analysis

For the first harvest, seedlings were left intact. In all other harvests, above-ground vegetation was detached at the top of the hypocotyl, and was divided into leaf blades, petioles, reproductive shoot and pods, where present. Total leaf blade area was determined using a Li-3000 (Li-cor, Lincoln, Nebraska) leaf area meter. By the reproductive harvest, the few leaves present were included with the shoot. Below-ground parts were hand-washed to remove soil. The storage hypocotyl was detached from the fibrous root. In the vegetative harvests, the hypocotyl was cut in two longitudinally. The fresh weights of both portions were taken, and one was frozen in liquid N for later analysis. The other portion of the hypocotyl and the fibrous root, stem, blade and petiole were dried at 70^{o}C for three days and then weighed. Total hypocotyl biomass was then calculated using the dry weight: fresh weight: ratio. Root includes fibrous roots and hypocotyl. Pods collected previously

and all immature and mature pods at harvest were dried at 30 - 35°C for three days to complete senescence, but not limit viability for future germination trials. Differences in biomass partitioning were examined by calculating RSR, leaf area ratio (LAR, total leaf area/total plant mass), and reproductive allocation (RA, total pod mass/ (root mass + shoot mass).

Reproductive characteristics

The frequency of pod and flower abortion (AFP) was estimated. Counts were made on three branches: the central axis, an old lateral and a young lateral. Pods collected at the final harvest were opened and separated into those mature (formed seeds) and those immature (no developed seeds). Mature seeds were not formed in all the cultivars. The effects on pod development rate were calculated on days (from first flowering) to mature the first pod, and pod maturation % (% of total mature pod mass that was mature on the first pod collection day in the cultivars and on Day 87 in W, which was the average first day for the cultivars). For W, the frequency and abundance of pod production allowed for more detailed analysis of mature pods than possible in the cultivars. Seed numbers in W were estimated by counts of the bead-like protrusions on the pod exterior, and pod mass allocated per seed was calculated.

Photosynthesis measurements

Photosynthesis was measured during the week prior to the vegetative harvest, during the middle of the day, under uniform light conditions (PAR: 600 - 650 $\mu mol \cdot m^{-2} \cdot s^{-1}$) on two source leaves per plant. Gas exchange measurements for all of EXP I and for W and IC of EXP II were made using a portable open-system infra-red gas analyzer (Model LCA-2 by ADC, Analytical Development Co. Ltd., Hoddesdon, England). Air humidity in the growth chamber was determined from readings with the leaf chamber open

and empty between every photosynthesis measurement, and then CO_2 assimilation rate (A) was recomputed according to von Caemmerer and Farquhar (1981). Because of LCA-2 machine malfunction, gas exchange of CB and FB varieties of EXP II were measured using a Portable LI-6200 Photosynthesis System (LI-COR, Inc., Lincoln, Nebraska).

Metabolite measurements

Metabolite analyses were done in the morning of the harvest day from the same plants on which photosynthesis measurements were made. Assessments of leaf metabolites were made on the interveinal lamina of the two most recently-expanded source leaves of each plant. Chlorophyll concentration (CHL) was then measured on four leaf quadrants using a Chlorophyll meter SPAD-502 (Minolta Corp., Ramsey, New Jersey) and determined as: (Monje and Bugbee 1992, and Bugbee, personal communication):

[1] CHL (mg / m^2) = 1.034 + 3.08 * [SPAD] + 0.110 * [SPAD]^2

where SPAD is the SPAD-502 instrument value.

Within the first three hours of the harvest day, two 1.07 cm^2 disks from opposite leaf quadrats were removed with a sharp punch and immediately frozen in liquid N for later determination of basal starch. Two similar disks were removed and dried at 65°C for 48h for determination of the specific leaf mass (SLM, dry weight / area).

Starch analysis

For each leaf sample, sugars were extracted from two frozen disks (2.14 cm^2 total area) and the disks macerated and assayed for starch following the method of Fox and Geiger (1984), with the following exceptions. After sugar extraction and incubation in KOH, tissue was homogenized for 6 minutes and rinsed with a total of 600 µl 0.2 M KOH. The brei was boiled for 15 minutes, and the volume was brought to 1 ml with

water. Starch was assayed as glucose released after adding 2 units of amyloglucosidase (Sigma Chemical Co., St. Louis, Missouri) in 1 ml of 200 mM potassium acetate, pH 4.8 with 0.02% w/v thymol, and incubating for 4 h at 55°C. Glucose was determined with an enzymic assay based on the direct spectrophotometric measure of NADPH. A sample of 10 µl - 40 µl was added to a reaction mixture to make up a total volume of 1 ml, containing 100 mM imidoazole (pH 6.9 with HCl), 5 mM $MgCl_2$, 0.02 %w/v BSA, 1 mM ATP, 0.4 mM $NADP^+$, 0.5 mM DTT and 0.2 µg glucose-6-P dehydrogenase (Boehringer-Mannheim Corp., Laval, Quebec). Reaction was initiated by adding 2 µg of hexokinase (Boehringer-Mannheim Corp). Prior to use, $(NH4)_2SO_4$ was removed from enzymes by centrifugation, redissolved in 50mM Tris and 0.1% BSA and centrifuged again. The ∂A340 was determined on a Philips PU 8800 spectrophotometer (PyeUnicam Ltd., Cambridge, England). Each leaf sample was assayed twice.

Statistical analyses

Data were analyzed by ANOVA (Type III) in a fixed split-plot design with CO_2 in the main plot, variety in the sub-plot and N level (SAS 1988 v. 6.0.4). Analyses were performed on untransformed data, after testing for normal distributions of the measured variables. Tukey HSD post hoc tests were performed on significant interactions and main effects ($p < 0.05$). Possible effects of using a different machine for the photosynthesis measurements in EXP II were eliminated by ANOVA for pooled photosynthesis values from all varieties.

The two experiments were analyzed separately due to the slight differences in fertilization treatment. Because of the physiological differences between growth stages, each harvest was also analyzed separately. My main focus was the interactive effect of CO_2 with varieties and N treatments during ontogeny, so I did not replicate the CO_2 chambers within each experiment. Different chambers were used in each experiment. The

effect of CO_2 was assessed using a carbon enhancement ratio (CER) defined as the value of the trait under high CO_2 : value of the trait under low CO_2 for each N level.

Pearson correlation analyses were performed on the CER set for each trait to assess how source leaf physiology and whole plant growth and mass traits were related to mass accumulation and reproductive traits under CO_2 enhancement. Because the CER is based on paired comparisons, the two experiments could be pooled, and variety size differences and harvest times could be standardized. The means for each of the 32 variety x CO_2 x N treatment combinations for each trait were used to construct 16 CERs, one for the high N and one for the low N of each variety at each harvest. Each CER set was tested for normality, and the log-transformed values of starch, vegetative RSR, vegetative root mass, AFP, and pod number were utilized. Correlations examined among traits in both the vegetative and reproductive phases used mean values that were calculated on the same individuals. To examine which traits in the vegetative phase best correlated with the response to CO_2 in the reproductive phase, means of the same treatment group were used.

RESULTS

Correlations between traits in carbon dioxide enhancement effects

In the vegetative phase, there were many significant correlations between source leaf physiological and plant mass traits in the response to high CO_2 (Table 1). Leaf area and thickness responded similarly, as total leaf area correlated positively with the mass of blade, petiole, and total plant. LAR was correlated positively with photosynthesis, but negatively with CHL. Starch enhancement was positively correlated with all leaf growth and storage characteristics. Photosynthesis rate response was negatively correlated with total mass. Leaf CHL positively correlated with all mass traits.

Correlations among traits within the reproductive phase show trade-offs in timing and allocation patterns (Table 2). Later flowering initiation correlated with enhancement in shoot mass and mass per mature pod, and with a reduced time from flowering to mature the first pod. The time for pod maturation correlated with total pod mass and RA. As expected from resource allocation shifts, pod maturation correlated with abortion. The correlations between mass components in the reproductive phase (Table 2) were not as frequent nor as strong as in the vegetative phase (Table 1).

Only a few leaf characteristics of the vegetative phase showed significant relationships with reproductive phase traits, suggesting leaf traits may be the most important determinant of reproductive response to CO_2. Vegetative total leaf area was a predictor of total pod mass ($r = 0.47$, $p < 0.10$) and the related traits of RA ($r = 0.42$, $p < 0.10$) and total mass ($r = 0.55$, $p < 0.05$) (Figure 1). Increased allocation to the shoot in the vegetative phase (decreased RSR) correlated with enhancement in mass per pod ($r = 0.43$, $p < 0.10$). Leaf starch enhancement correlated with a reduced flower and pod abortion ($r = 0.53$, $p < 0.05$). No traits of vegetative mass correlated with any reproductive responses.

TABLE 1. Correlations among vegetative phase traits in response to high CO2.

Values are Pearson correlations shown for carbon enhancement ratios (CER) of each pair of traits.

Values are multiplied by 100, and those in boldface are significant at $p < 0.05$.

* $p < 0.10$; ** $p < 0.05$; *** $p < 0.01$; ****$p < 0.001$. Data for traits in Tables 3 and 4 and Figures 2A and 2C.

For each trait, the set of CER was calculated using the mean values of CN/cN and Cn/cn of each variety.

Experiments I and II are combined. n = 16 CER per trait. LAR, leaf area ratio; SLM , specific leaf mass;

RSR, root to shoot ratio. Log-transformed values were used for starch, RSR, and root mass.

	Leaf Number	Total Leaf Area	Chloro-phyll	Photo-synthesis	Starch	SLM	Blade mass	Petiole mass	Root mass	Total mass	RSR
Total Leaf Area	-39										
Chlorophyll	24	-1									
Photosynthesis	-47 *	-14	-43 *								
Starch	-36	**66** ***	6	-05							
SLM	-13	48 *	**68** ***	-21	**57** **						
Blade mass	-15	**77** ****	**51** **	-42	**70** ***	**86** ****					
Petiole mass	-28	**52** **	29	-35	**58** **	**51** **	**68** ***				
Root mass	-19	41	**58** **	**-47** *	40	**77** ****	**80** ****	**63** ***			
Total mass	-8	**68** ***	**56** **	**-56** **	**57** **	**82** ****	**97** ****	**72** ***	**89** ****		
RSR	-10	-24	33	-29	-24	26	10	9	**66** ***	36	
LAR	-20	-24	**-75** ****	**55** **	-30	**-74** ****	**-75** ****	**-52** **	**-82** ****	**-84** ****	-40

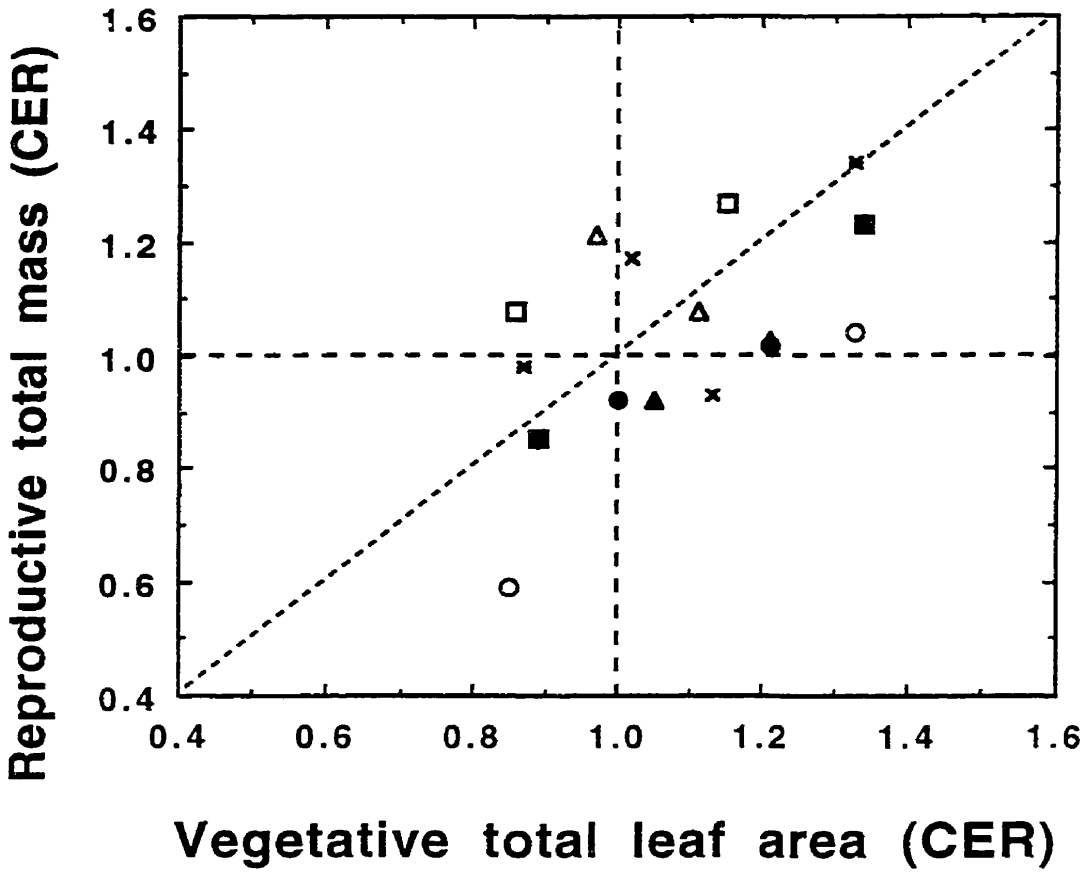
TABLE 2. Correlations among reproductive phase traits in response to high CO2.

Pearson correlation values, carbon enhancement ratios (CER), significance levels, symbols and CER calculations as in Table 1. Data for traits in Table 5 and Figures 2B and 2D. Experiments I and II are combined. n = 16 CER per trait, except for those involving mature pods, where n=15. AFP, % aborted flowers and pods; RA, reproductive allocation. RSR=Root:shoot ratio. Log-transformed values were used for pod number, and AFP.

	Day of first flower	Days to maturity of first pod	Pod maturation (%)	Pod number	Mass per mature pod	Total pod mass	Root mass	Shoot mass	Total mass	RSR	RA
Days to maturity of first pod	**-63** ***										
Pod maturation (%)	-37	26									
Pod number	-4	25	-30								
Mass per mature pod	**52** **	-21	-13	-30							
Total pod mass	-25	**54** **	-27	**83** ***	-23						
Root mass	14	-11	-15	0	-4	16					
Shoot mass	**57** **	-15	10	10	50 *	18	29				
Total mass	0	32	21	41	-23	**69** ***	**50** **	**59** **			
RSR	-38	10	-28	-5	-37	5	**50** **	**-63** ***	-5		
RA	-38	**65** ***	30	**70** ***	-27	**95** ****	7	1	**60** ***	13	
AFP	-29	0	**68** ***	-14	-3	1	-15	0	-2	-15	5

Figure 1

Carbon enhancement ratios of vegetative total leaf area and reproductive total mass. Values are the enhancement for each experiment under high N (closed symbols) and low N (open symbols). Varieties: CB-Cherry Belle (squares), FB-French Breakfast (circles), IC-Icicle (triangles), W-Wild (x). The CER was calculated using the mean values of CN/cN and Cn/cn for each variety in Experiments I and II. $y=0.5975x + 0.3945$ ($r=0.56$, $p=0.026$). Diagonal dotted line denotes line of equality.


Reproductive total mass (CER)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Vegetative total leaf area (CER)

Responses of varieties to CO_2 and N for individual traits and harvest times

Seedling harvest

At 11 days, W seedlings had less mass (mg) than the three other varieties: W(15.8) < CB (29.5), IC (30.3), FB (33.1) ($F= 15.3$, $p<0.01$, data not shown). There was no effect of CO_2 on mass ($p=0.63$).

Experiment I

Vegetative harvest at 32 days

There were strong V differences after 32 days (Figure 2A, Table 3A). Partitioning of biomass in CB and FB was largely to the roots, while W and IC showed greater leaf growth. There were significant V effects on all mass and growth traits (F values not shown, all $p < 0.05$). The RSR was distinct (CB 2.7 > FB 1.4 > IC 0.65 > W 0.17) while reverse order was found for total mass and leaf number (W 9.1, IC 8.3 > FB 6.4, CB, 6.6; data not shown). On the extremes, root mass was 65% less in W than in the cultivars, while CB had a total leaf area and petiole mass of less than 25% that of W.

Under high CO_2, enhancements were 50% in total mass, 20% in leaf area, 46% in petiole mass, 70% in root mass, and 10% in RSR (Table 3A). The LAR was reduced by 23%. Varieties showed different responses to CO_2 only in blade mass ($F=15.7$, $p<0.05$) which was doubled in W and increased by 20% in the cultivars. High N affected all leaf traits, increasing blade mass by 20% ($F= 7.2$, $p<0.01$), petioles by 30%($F= 4.7$, $p<0.05$), total plant by 50% ($F= 5.36$, $p<0.05$) and LAR by 14% ($F= 7.3$, $p<0.01$). Varieties responded differently to N in blade mass ($F= 3.3$, $p<0.05$) total mass ($F= 2.9$, $p<0.05$) and LAR ($F= 3.6$, $p<0.05$). Greatest enhancement responses to N were shown in those with larger shoots (lowest RSR), the IC and W. There were no significant interactions of CO_2 and N.

Figure 2

Biomass allocation for harvests. (A) EXP I - Vegetative harvest (at 32 days). Root (white), petiole (solid), blade (hatched), reproductive (grey). (B) EXP I - Reproductive harvest. Root (white), shoot (hatched), pod (grey). (C) EXP II - Vegetative harvest (Pre-Reproductive stage, prior to bolting). Shading as in 2A. (D) EXP II - Reproductive harvest. Shading as in 2B. Error bars are standard deviations for total mass. Reproductive includes the stem material that is part of the reproductive bolt. Root includes the storage hypocotyl and fibrous root. Variety symbols as in Figure 1. CN: 650 $\mu l\ L^{-1}$ (high) CO_2, high N; Cn: high CO_2, low n; cN: 350 $\mu l\ L^{-1}$ (low) CO_2, high N; cn: low CO_2, low N. Pre-reproductive harvest days for (C): W-28, IC-37, CB-56, FB-60. Several values of W hypocotyl mass were extrapolated from the fresh weight, using the fresh weight: dry weight ratio of the treatment group. Reproductive harvest days for (B) and (D): W-132, IC-134, CB-137, FB-140.

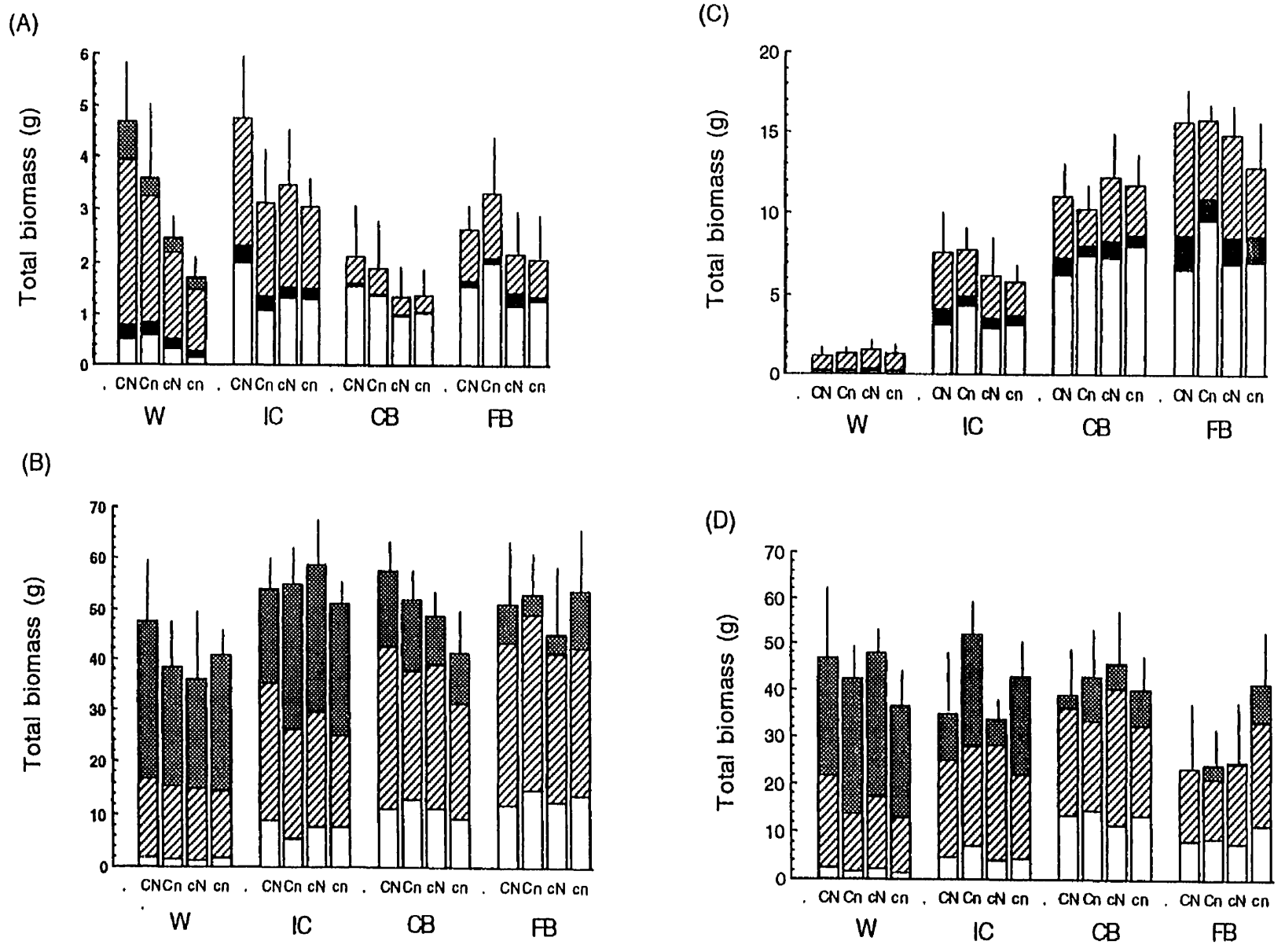

(A)
Total biomass (g)
0
1
2
3
4
5
6
. CN Cn cN cn . CN Cn cN cn . CN Cn cN cn . CN Cn cN cn
W
IC
CB
FB
(C)
Total biomass (g)
0
5
10
15
20
. CN Cn cN cn . CN Cn cN cn . CN Cn cN cn . CN Cn cN cn
W
IC
CB
FB
(B)
Total biomass (g)
0
10
20
30
40
50
60
70
. CN Cn cN cn . CN Cn cN cn . CN Cn cN cn . CN Cn cN cn
W
IC
CB
FB
(D)
Total biomass (g)
0
10
20
30
40
50
60
70
. CN Cn cN cn . CN Cn cN cn . CN Cn cN cn . CN Cn cN cn
W
IC
CB
FB

TABLE 3. Summary of vegetative phase leaf and growth characteristics.

(A) Experiment I. (B) Experiment II. Values are means ± SD. Sample size, n=5 for each V x CO2 x N combination. Treatments: CN, high (650 µl L-1) CO2, high N; Cn, high CO2, low N; cN, ambient (350 µl L-1) CO2, low N; cn, ambient CO2, low N. LAR - leaf area ratio; RSR - root to shoot ratio; V - variety, symbols as in Figure 1. Experiment I harvest at 32 days. Experiment II harvest days: W, 28; IC, 37; CB, 56; FB, 60.

V	Treatment	Total leaf area (cm2)	LAR (cm2/g)	RSR
	A. Experiment I			
W	CN	572 ± 81	125.9 ± 32.8	0.15 ± .07
	Cn	438 ± 72	130.7 ± 47.4	0.26 ± .17
	cN	431 ± 84	157.1 ± 22.6	0.18 ± .04
	cn	387 ± 54	224.2 ± 18.6	0.12 ± .03
IC	CN	458 ±106	101.4 ± 29.1	0.73 ± .15
	Cn	451 ± 79	152.0 ± 36.6	0.51 ± .26
	cN	437 ± 93	127.7 ± 27.2	0.63 ± .36
	cn	406 ± 51	132.3 ± 25.2	0.73 ± .15
CB	CN	142 ± 51	64.8 ± 7.7	2.73 ± .24
	Cn	124 ± 46	63.9 ± 9.8	2.60 ± .42
	cN	107 ± 28	80.4 ± 12.2	2.64 ± .62
	cn	107 ± 34	75.9 ± 4.8	2.99 ± .62
FB	CN	263 ± 28	98.9 ± 6.5	1.43 ± .28
	Cn	289 ± 58	88.2 ± 12.8	1.48 ± .32
	cN	218 ± 86	100.0 ± 26.7	1.16 ± .37
	cn	217 ± 64	104.6 ± 13.7	1.59 ± .26

TABLE 3. Continued.

V	Treatment	Total leaf area (cm2)	LAR (cm2/g)	RSR
	B. Experiment II			
W	CN	268 ± 88	240.0 ± 67.7	0.17 ± .02
	Cn	248 ± 44	180.9 ± 16.0	0.13 ± .03
	cN	309 ± 64	193.7 ± 18.4	0.13 ± .03
	cn	243 ± 52	194.8 ± 14.5	0.12 ± .02
IC	CN	800 ± 125	113.7 ± 39.3	0.71 ± .21
	Cn	570 ± 77	78.4 ± 9.2	1.28 ± .32
	cN	660 ± 119	119.5 ± 40.7	0.84 ± .39
	cn	586 ± 114	103.5 ± 22.8	1.24 ± .35
CB	CN	839 ± 133	79.4 ± 29.8	1.29 ± .27
	Cn	534 ± 94	52.2 ± 8.3	2.77 ± .54
	cN	947 ± 280	77.1 ± 8.9	1.55 ± .25
	cn	623 ± 60	53.2 ± 4.9	2.14 ± .19
FB	CN	1228 ± 139	78.3 ± 5.9	0.72 ± .15
	Cn	776 ± 135	49.0 ± 9.0	1.59 ± .20
	cN	1227 ± 209	84.5 ± 25.0	0.87 ± .14
	cn	913 ± 161	76.6 ± 36.0	1.22 ± .30

Vegetative source leaf metabolites and physiology

Varieties differed significantly in CHL ($F=187$, $p<0.01$), SLM ($F=13.6$, $p<0.05$) and photosynthesis ($F= 19.3$, $p<0.05$) (Table 4A). CHL was 17% lower in FB than in W and CB. The varieties that showed less partitioning to the root (lower RSR) had a higher SLM, with W being significantly greater than CB and FB, while IC was intermediate. Photosynthesis rates were higher in the slower-growing varieties that had larger hypocotyls, with FB being significantly greater than IC and W, while CB was intermediate.

Under high CO_2, starch was enhanced six-fold and the SLM by 30%. Varieties responded differently to CO_2 in CHL ($F= 29.1$, $p<0.05$) and photosynthesis ($F= 11.8$, $p<0.05$). CHL was increased 5% in CB and 10% in W. Photosynthesis was enhanced by 20 - 40% in the cultivars, but was reduced by 7% in W. The only significant effect of N was on promoting CHL by 10% ($F=7.8$, $p<0.05$), though there was a trend of lower starch at high N ($F=3.5$, $p=0.07$).

Reproductive mass & growth

Differences between varieties in total mass which were apparent prior to flowering were not obvious at the end of the growing season (Figure 2B). However, varieties differed in partitioning. Those flowering latest, CB and FB, had mass predominantly in the root and shoot, while the majority of W and IC mass was allocated to pods. Hence, significant V main effects were found on RA ($F= 20.4$, $p< 0.05$), shoot mass ($F= 206$, $p< 0.001$), root mass ($F= 37.7$, $p< 0.01$) and RSR ($F= 17.2$, $p< 0.05$). The RSR was similar for the three cultivars (CB,FB 0.45; IC 0.36) and was significantly less in W (0.12) (Table 5A). High CO_2 enhanced pod mass, total mass and RSR by 10%, but reduced RA by 6%. There were no significant V x CO_2 or N effects for any mass trait. Reproductive phase leaves were small. At harvest, CB had twice as many as W, while few remained on IC (CB 53, FB 32, W 26, IC 6) (data not shown, $F=30.1$, $p < 0.01$).

TABLE 4. Vegetative source leaf physiology and metabolites. (A) Experiment I (B) Experiment II. Values are means ± SD and (n), when n different than colur n, sample size for each V x CO2 x N combination. Treatments as in Table 3. V, variety; SLM, specific leaf mass.

V	Treatment	Chlorophyll (mg cm-2)	Starch (µg C cm-2)	SLM (mg cm-2)	Photosynthesis (µmol CO2 m-2 s-1)
A. Experiment I					
		n = 5	n = 3	n=3	n=5
W	CN	447 ± 60	135 ± 65	5.28 ± 0.42	30.0 ± 5.3
	Cn	391 ± 28	307 ± 278	6.36 ± 2.65	27.4 ± 3.9
	cN	426 ± 18	23 ± 15	4.03 ± 0.07	34.2 ± 2.0
	cn	332 ± 45	43 ± 10	3.66 ± 0.20 (4)	26.0 ± 5.9
IC	CN	381 ± 69	82 ± 61	4.12 ± 0.64	33.5 ± 5.1
	Cn	361 ± 53	119 ± 99	4.44 ± 1.02	35.1 ± 5.0
	cN	410 ± 51	24 ± 12	3.93 ± 0.26	26.4 ± 2.6
	cn	371 ± 60	33 ± 37	3.90 ± 0.35	26.5 ± 5.6
CB	CN	411 ± 14	48 ± 13	4.00 ± 0.09 (4)	36.8 ± 3.8
	Cn	410 ± 51	113 ± 64	4.35 ± 0.17 (4)	38.6 ± 3.6
	cN	405 ± 72	10 ± 10	3.46 ± 0.71	32.3 ± 4.9
	cn	380 ± 31	9 ± 3	3.21 ± 0.16	32.3 ± 2.8
FB	CN	325 ± 20	64 ± 31	4.15 ± 0.16	44.1 ± 8.6
	Cn	340 ± 42	118 ± 21	4.45 ± 0.33	39.4 ± 5.8 (4)
	cN	343 ± 52	27 ± 11	3.22 ± 0.16	32.0 ± 6.3
	cn	323 ± 24	19 ± 12	3.29 ± 0.42 (4)	29.3 ± 3.7

TABLE 4. Continued.

B. Experiment II

V	Treatment	Chlorophyll (mg cm-2)	Starch (µg C cm-2)	SLM (mg cm-2)	Photosynthesis (µmol CO2 m-2 s-1)
		n=5	n=3	n=5	n=4
W	CN	303 ± 63	47 ± 5	3.57 ± 0.39	20.4 ± 1.7 (8)
	Cn	362 ± 39	80 ± 4	3.96 ± 0.49	21.6 ± 0.8 (8)
	cN	332 ± 40	26 ± 12	3.88 ± 0.43	16.7 ± 2.4 (8)
	cn	364 ± 74	72 ± 54	4.10 ± 0.39	16.4 ± 1.7 (8)
IC	CN	414 ± 76	175 ± 55	4.70 ± 1.35	21.9 ± 1.1 (3)
	Cn	389 ± 40	224 ± 11	5.36 ± 0.38	21.4 ± 0.4 (3)
	cN	453 ± 50	8 ± 3	3.77 ± 0.45	16.2 ± 1.7 (8)
	cn	351 ± 28	68 ± 57	3.68 ± 0.50	15.8 ± 1.4 (8)
CB	CN	456 ± 66	22 ± 22	3.95 ± 0.49	20.2 ± 5.4
	Cn	432 ± 39	17 ± 4	3.74 ± 0.36	22.4 ± 2.5
	cN	417 ± 67	17 ± 6	3.66 ± 0.26	15.6 ± 4.1
	cn	431 ± 29	96 ± 111	3.80 ± 0.42	18.7 ± 2.6
FB	CN	375 ± 67	163 ± 51	4.44 ± 0.71	23.8 ± 11.3
	Cn	389 ± 40	309 ± 37	5.28 ± 0.42	22.2 ± 2.5
	cN	405 ± 44	48 ± 16	4.20 ± 0.45	18.4 ± 6.8 (3)
	cn	352 ± 52	177 ± 19	4.59 ± 0.83	20.8 ± 6.0

TABLE 5. Summary of reproductive growth, phenology and pod characteristics. (A) Experiment I (B) Experiment II.

Values are means ± SD and (n). Where not indicated, n = 5. V, variety. Treatments as in Table 3.

AFP, aborted flowers and pods (%); RA, reproductive allocation; RSR, root to shoot ratio.

Time to mature first pod determined from first flower day. Data was not measured for seeds CB, FB and IC.

V	Treat-ment	RSR	Time to maturity of first pod (days)	Total number of pods	Mass per mature pod (mg)	Pod maturation (%)	AFP (%)	RA	Total seed number	Number of seeds per mature pod	Pod mass per seed (mg)
	A. Experiment I.										
W	CN	0.12 ± .02	46.4 ± 4.6	302 ± 87	106 ± 18	51.6 ± 22.6	46.8 ± 7.0	1.87 ± .62	1093 ± 457	3.55 ± 0.54	29.6 ± 7.0
	Cn	0.11 ± .03	51.0 ± 4.9	253 ± 30	94 ± 9	50.2 ± 23.0	50.8 ± 10.0	1.51 ± .38	881 ± 132	3.50 ± 0.54	26.7 ± 2.9
	cN	0.10 ± .05	52.6 ± 3.8	215 ± 118	102 ± 40	49.2 ± 14.2	44.9 ± 10.0	1.41 ± .89	883 ± 331	4.56 ± 1.09	22.6 ± 8.8
	cn	0.14 ± .04	50.6 ± 3.8	233 ± 46	124 ± 24	30.2 ± 14.3	42.9 ± 6.2	1.84 ± .21	972 ± 244	4.20 ± 0.77	28.6 ± 7.1
IC	CN	0.35 ± .14	59.6 ± 3.3	256 ± 96	161 ± 20 (4)	25.0 ± 23.1	49.8 ± 7.7	0.53 ± .29			
	Cn	0.26 ± .07	70.0 ± 4.4	189 ± 51	180 ± 27	51.4 ± 21.7	51.2 ± 7.7	1.09 ± .15			
	cN	0.39 ± .18	71.0 ± 4.2	296 ± 149	132 ± 52	37.6 ± 30.0	48.0 ± 2.9	1.05 ± .52			
	cn	0.45 ± .08	69.2 ± 2.8	199 ± 33	165 ± 24	36.8 ± 21.2	52.9 ± 5.3	1.04 ± .15			
CB	CN	0.36 ± .06	52.6 ± 5.0	441 ± 142	122 ± 64	23.0 ± 12.7	37.9 ± 5.5	0.38 ± .12			
	Cn	0.56 ± .19	47.0 ± 4.8	233 ± 51	117 ± 26	42.2 ± 36.2	41.4 ± 9.0	0.43 ± .35			
	cN	0.44 ± .12	50.0 ± 5.7 (2)	204 ± 148	97 ± 14 (2)	25.0 ± 29.8 (4)	45.0 ± 19.6	0.30 ± .33			
	cn	0.43 ± .14	56.0 ± 2.6 (3)	199 ± 116	104 ± 6 (3)	26.4 ± 26.8	48.0 ± 13.6	0.40 ± .37			
FB	CN	0.41 ± .16	51.5 ± 2.1 (2)	274 ± 176	103 ± 6 (2)	29.5 ± 12.0 (2)	62.6 ± 4.2	0.16 ± .04			
	Cn	0.44 ± .18	52.0 ± 5.7 (2)	53 ± 65	136 ± 64 (2)	27.0 ± 37.3	62.2 ± 2.4	0.10 ± .19			
	cN	0.44 ± .08	54.3 ± 7.6 (3)	58 ± 45	130 ± 32 (3)	27.6 ± 26.7	58.1 ± 9.0	0.11 ± .17			
	cn	0.50 ± .14	59.5 ± 8.2 (4)	138 ± 55	101 ± 6 (4)	56.8 ± 34.1 (4)	48.0 ± 13.6	0.31 ± .27			

TABLE 5. Continued.

V	Treat-ment	RSR	Time to maturity of first pod (days)	Total number of pods	Mass per mature pod (mg)	Pod maturation (%)	AFP (%)	RA	Total seed number	Number of seeds per mature pod	Pod mass per seed (mg)
B. Experiment II.											
W	CN	0.12 ± .05	46.4 ± 4.3	296 ± 107	83 ± 28	31.4 ± 15.9	48.4 ± 6.4	1.12 ± .57	854 ± 360	2.85 ± 0.58	27.5 ± 6.8
	Cn	0.13 ± .03	52.2 ± 8.9	240 ± 56	128 ± 12	32.6 ± 15.0	49.9 ± 9.0	2.2 ± .35	1073 ± 182	4.56 ± 0.68	30.1 ± 6.0
	cN	0.14 ± .05	50.4 ± 8.3	273 ± 55	114 ± 15	30.6 ± 15.7	55.3 ± 12.5	1.81 ± .46	1197 ± 461	4.28 ± 1.04	28.0 ± 9.0
	cn	0.12 ± .01	47.4 ± 4.1	233 ± 38	116 ± 34	37.2 ± 12.5	57.4 ± 3.2	1.91 ± .62	938 ± 236	4.15 ± 1.3	27.4 ± 3.6
IC	CN	0.24 ± .10	72.6 ± 22.0	170 ± 128	129 ± 88 (3)	54.8 ± 44.7	54.0 ± 11.9	0.40 ± .34			
	Cn	0.34 ± .08	52.6 ± 3.7	273 ± 113	134 ± 24	25.8 ± 13.8	61.0 ± 7.7	0.89 ± .25			
	cN	0.16 ± .08	62.4 ± 13.8	124 ± 112	180 ± 9 (3)	66.6 ± 35.0	60.8 ± 25.6	0.21 ± .24			
	cn	0.26 ± .08	55.6 ± 1.9	216 ± 32	112 ± 22	24.2 ± 10.4	61.0 ± 11.9	1.02 ± .46			
CB	CN	0.61 ± .25	66.0 ± 6.6 (3)	274 ± 151	100 ± 45 (4)	2.4 ± 2.9	48.8 ± 11.8	0.076 ± .039			
	Cn	0.80 ± .18	65.7 ± 8.1 (3)	179 ± 142	121 ± 32 (2)	44.0 ± 44.0	56.6 ± 11.7	0.35 ± .48			
	cN	0.41 ± .12	69.7 ± 5.5 (3)	398 ± 290	102 ± 37 (3)	23.8 ± 43.2	52.8 ± 10.6	0.15 ± .14			
	cn	0.78 ± .24	61.3 ± 9.0 (4)	283 ± 68	111 ± 16 (3)	18.6 ± 21.7	34.7 ± 8.6	0.29 ± .26			
FB	CN	0.58 ± .18	-	8 ± 0	-	0 (2)	86.1 ± 1.9 (2)	0.005 ± .07			
	Cn	0.70 ± .16	67.0 ± 4.2 (4)	69 ± 43	137 ± 64 (3)	34.8 ± 43.9	65.9 ± 10.6	0.14 ± .18			
	cN	0.49 ± .12	-	21 ± 11	-	0 (4)	75.4 ± 9.3	0.012 ± .016			
	cn	0.56 ± .19	65.3 ± 6.6 (4)	74 ± 78	177 ± 68 (3)	29.8 ± 29.4	73.3 ± 15.9	0.27 ± .27			

Phenology

The day of first flower nearly followed RSR (W 37 < IC 55 < CB 86,FB 85) (data not shown, F= 151, $p < 0.001$) and was unaltered by both CO_2 and N. All plants produced a bolt, but flowering did not occur in several of CB and FB under high N. There was a V main effect on the time to mature the first pod ($F=10.8$, $p < 0.05$), with IC showing delay (Table 5A). Under high CO_2, pod maturation took 6% fewer days, and 8% more pods matured. In W, high CO_2 reduced the time taken to mature 50% of the pods by 3.5 days. There were no V x CO_2 interactions or N effects on phenology traits.

Pod characteristics

The abortion percentage was distinctly higher in FB (FB 0.63 > IC 0.51, W 0.47, CB 0.43) ($F= 17.6$, $p < 0.05$ Table 5A). There was no effect of CO_2 or N. There were no variety main effects or interactions on pod mass or number. High CO_2 enhanced mass per mature pod by 17%. In W, CO_2 enhanced seed number by 6%. The seeds per pod of W were reduced by 20% but the pod mass per seed was enhanced by 7%. There was a significant interaction of CO_2 and N on pod number ($F=10.2$, $p < 0.01$). The CN treatment produced 60% more pods. High N alone enhanced pod number by 37% ($F= 9.6$, $p < 0.01$).

Experiment II

Vegetative harvest (Pre-reproductive)

The vegetative harvest of EXP II allowed for comparisons of varieties just prior to reproduction. Variety differences were more pronounced than in the earlier harvest of EXP I (Figure 2C). There were significant V effects on all mass and growth traits (F values not shown, all $p<0.01$). The W was smallest and had only 11% of the mass of FB prior to bolting. Leaf numbers were distinct (12.0 CB > 10.4 FB > 8.8 IC > 6.0 W) (data not shown). Leaves of CB were small, so CB did not differ from IC in total leaf area, petiole

mass and blade mass. Root mass was similar in FB and CB. In comparison to the values of EXP I (Table 3A), the RSR was lower for CB, FB and W, due to an increased partitioning to the shoot prior to reproduction, while IC showed an opposite shift (Table 3B).

High CO_2 enhanced total mass by only 5% (Figure 2C). Blade mass was increased 5% and root mass by 8%, but total leaf area decreased by 4% (Table 5B). At this stage, varieties showed no significant differences in their response to CO_2 in mass and growth. Nitrogen increased partitioning to the shoot and decreased partitioning to the root, decreasing the RSR by 22% ($F= 83$, $p<0.001$). Under high N, leaf number was enhanced by 10 % ($F= 13.0$, $p<0.001$) and root mass was reduced by 20% ($F= 8.4$, $p<0.01$). Variety x N interactions were seen on total leaf area ($F= 6.8$, $p<0.001$), blade mass ($F=11.8$, $p<0.001$), petiole mass ($F= 3.2$, $p<0.05$) and RSR ($F=14.3$, $p<0.001$). Under high N, total leaf area and blade mass were significantly increased by 50% in CB and FB and by 20% in IC. Also, petiole mass increased by 70% in CB, 50% in IC, and 30% in FB. W showed little response to N at this stage. Under high N, the RSR was reduced by about 40% in the three cultivars, but was unaltered in W. There were CO_2 x N interactions on petiole mass ($F= 8.6$, $p<0.01$), LAR($F=4.4$, $p<0.05$) and RSR ($F=11.5$, $p<0.01$). Petiole mass (g) was enhanced more under high CO_2 with high N, CN(1.1) > cN(0.83) > Cn (0.61), while cn (0.72) was intermediate. The LAR showed the same trends, with CN (128), cN (119) > Cn (91) and the cn (107) was intermediate. Partitioning to the root was enhanced under both low N and high CO_2, resulting in the RSR of Cn(1.4) > cn(1.2) > cN(0.85), CN(0.72).

Vegetative source leaf metabolites and physiology

Varieties differed in SLM ($F=12.2$, $p<0.05$) and starch ($F=34.2$, $p<0.01$) (Table 4B). The SLM of FB was significantly greater than CB. High CO_2 enhanced

photosynthesis rates by 20%, SLM by 10% and had little effect on CHL. Varieties responded differently to CO_2 in starch ($F=18.8$, $p<0.05$). Under high CO_2, FB and IC had four-fold greater starch than W and CB which showed little response.

Effects of N were more pronounced at this stage of development. Under high N, there was a lowering of SLM by 7% ($F= 5.0$, $p<0.05$) and of starch. Varieties responded differently to N in starch ($F=4.0$, $p<0.05$), with FB showing over a two-fold lower level. There was a V x N interaction on CHL ($F=3.4$, $p<0.05$), with CB being highest. There was a significant CO_2 x N interaction on starch ($\mu g\ C\ cm^{-2}$) with Cn (157) > cn (103), CN (102) > cN (25) ($F=9.2$, $p<0.01$).

Reproductive mass & growth

As in EXP I, there were no differences in the total mass at the final harvest between varieties (Figure 2D). Varieties differed in partioning as there were significant V main effects on pod mass ($F= 36.5$, $p< 0.01$), root mass ($F=90.5$, $p< 0.01$), RSR ($F=95.7$, $p< 0.01$) and RA ($F=27.2$, $p< 0.05$). W produced significantly higher pod mass. Root mass differences were distinct, and RSR followed the same order (CB 0.65, FB 0.58 > IC 0.25, W 0.13) (Table 3B). There were no CO_2 enhancement effects on total mass. The RA was reduced by 10% under high CO_2. As in EXP I, there was no CO_2 x V interaction on any mass trait.

There were many significant effects of N. Vegetative traits of shoot mass ($F=10.2$, $p<0.01$). and leaf number ($F=10.2$, $p<0.01$, data not shown) were enhanced, while RA declined ($F=24.7$, $p<0.001$). There were variety x N interactions in total pod mass ($F=4.9$, $p<0.01$), RSR ($F=3.6$, $p<0.05$) and total mass ($F=5.8$, $p<0.01$). Under high N, IC had over 30% less pod and total mass, while W had 20% more total mass. In CB under high N, partitioning shifted to the shoot and RSR was reduced by 35%. There were no CO_2 x N interactions.

Phenology

Effects on flowering were nearly identical to EXP I (data not shown). Varieties did not differ in pod maturation (Table 5B). High CO_2 enhanced the maturation rate by 8% and there were no significant CO_2 interactions Under high N, the time to mature the first pod was increased by five days ($F= 4.2$, $p<0.01$). There was a variety x N interaction in pod maturation(%) ($F= 5.1$, $p<0.01$). At low N, varieties averaged 31%. At high N, the cultivars differed.

Pod characteristics

There were no V, CO_2 or N effects on abortion. Abortion was 5 - 12% higher than in EXP I. There were no V differences in mass per mature pod. High CO_2 enhanced the average pod mass by 8 - 11% in all but FBn. In W, CO_2 increased the pod mass allocated per seed by 7%, but total seed number was reduced by 10%. Pod number showed significant V interactions with CO_2 ($F=90.6$, $p< 0.01$) and N ($F=3.1$, $p < 0.05$). Under high CO_2, pod number was promoted by 30% in IC, reduced by 50% in CB and was not affected in W and FB. Under high N, CB produced over double the pods of IC, while FB had significantly less pods. There was a significant CO_2 x N interaction on seeds/pod in W ($F= 4.7$, $p< 0.05$). Under CN, there were 30% less .

DISCUSSION

Relationships between vegetative and reproductive responses to CO_2

The time of reproductive phase onset was the greatest determinant of reproductive output (pod mass) in this study. The lack of a CO_2 or N effect on flowering day may be characteristic of radishes because paternal families of wild radish responded similarly (Curtis et al. 1994). On a broader scale, flowering responses to CO_2 appear to be species-

specific. Earlier flowering in many crop species has been attributed to enhanced development rate (Lawlor and Mitchell 1991, Tousignant and Potvin 1996). Several other wild species have been unaffected or delayed in their flowering response to elevated CO_2 (Garbutt and Bazzaz 1984, Garbutt et al. 1990, Reekie et al. 1994). The realization of a reproductive response may be limited by a lack of developmental plasticity. This study shows that the reproductive response to CO_2 cannot be inferred from increases in total vegetative mass. Although enhanced mass is the predominant response to CO_2 in the vegetative stage, it did not correlate with responses over the entire life history. A similar lack of relationship between vegetative and reproductive mass due to allocation changes under elevated CO_2 was detected in an assemblage of data (Ackerly and Bazzaz 1996) and in *Brassica juncea* (Tousignant and Potvin 1996). Because biomass response to elevated CO_2 changes with ontogeny and allocation, the suite of traits contributing to reproductive output must be examined, particularly leaf traits.

Much increased vegetative mass under high CO_2 may be structural materials that are unavailable for reproductive growth and seed-filling. Typically, in starch-enhancing conditions, only 25% (basal, pre-dawn) to 40% (day-end) of leaf mass is "non-structural ", in wild radish (Chu et al. 1992) and sugar beet (Jablonski and Geiger 1987). In young radishes, about 30% of the hypocotyl is non-structural (Pell et al. 1990). Under high CO_2, structural materials may also be enhanced and partitioning altered as found in sweet potato (Lawlor and Mitchell 1991) and cotton (Wong 1990).

The few correlations between vegetative and reproductive traits indicate that the coordination of C and N storage, allocation and translocation between the phases is disrupted under high CO_2. Production of seeds relies on the translocation of vegetative stores as well as reproductive physiology (Schulze et al. 1994). Traits that are specific to the reproductive phase (e.g. pollen production, fertilization, development of structures) may be adversely or differentially affected by CO_2. The strength of correlations between

vegetative and reproductive responses may have been limited by random variation. Genetic variability for traits of reproductive response to high CO_2 has been shown in wild radish (Curtis et al. 1994) and rice (Ziska and Teramura 1992).

The trend to increased rate of pod maturation under high CO_2 suggests that metabolism during seed production is more efficient. Enhancements of development and maturation have been previously observed (St. Omer and Horvath 1983, Lawlor and Mitchell 1991). These effects could have been masked (such as in the high N of EXP II) by the delay in senescence that is often observed under elevated CO_2 (Frick et al. 1994).

The lower number of seeds per pod in the W variety under CN suggests that allocation of resources to seed-filling may shift under high CO_2. It is unlikely that the lower seed number arose from trade-offs with seed weight and pod number as all pod and seed characteristics showed nearly parallel trends in the response to CO_2 across the C and N treatments. Similar reductions in seed number per unit under high CO_2 have been previously observed (Lawlor and Mitchell 1991, Garbutt and Bazzaz 1984). It is likely, as found for leaves (Conroy and Hocking 1993), that less N is required for pod photosynthesis under high CO_2. Thus, a smaller N pool may be available for seed development, since the majority of seed N is recycled from other tissues while the remainder is obtained from concurrent uptake (Chapin et al. 1990). A lower N pool is also suggested by decreased grain N and protein of wheat (Conroy 1992) and in N/C ratios of cereal grains (Lawlor and Mitchell 1991).

Source-sink relationships in the CO_2-response

One goal of this study was to examine if the capacity for non-foliar carbon storage would be advantageous for reproductive output in an elevated CO_2 environment. Contrary to the hypothesis, hypocotyl enhancement alone did not promote reproductive response. Varieties possessed different storage strategies which showed distinct responses to CO_2

with vegetative ontogeny. However, each coordinated strategy of sink storage, growth and reproductive timing yielded a similar total reproductive mass. Multiple sinks, including the hypocotyl, petioles, and the blades of developing leaves, were involved in the response to CO_2. Varieties differed in sink strategies. W maintained sink strength by rapid growth and production of new leaves with a high N content. The early shift into the reproductive phase counteracted the potential limitations of leaf mass build up. In addition to rapid leaf growth, IC had a second sink enhancement mechanism of the hypocotyl. But in the reproductive phase of IC, the high partitioning to structural support of the shoot relative to pods (lower harvest index), and the lower leaf production compared with W, likely constrained reproductive output. CB appeared to be more efficient than FB, with higher CHL, lower SLM and greater partitioning to petioles and hypocotyl. Enhancement of FB may have been prevented by the higher abortion rate, lower pod number and greater sensitivity to mineral fertilization.

Leaf production under elevated CO_2 may be the most efficient way to promote growth and to provide photosynthate for reproductive growth and the remobilization of stores. Leaves recycle mineral nutrient and C stores for the seeds, and provide the majority of photosynthate required for reproductive growth and seed formation (Chapin et al. 1990, Schulze et al. 1994). Since 10% (W) to 32% (FB) of total productivity occurred in the reproductive phase, maintenance of photosynthetic area was imperative. Despite the variability in responses between varieties, and within varieties between harvests, leaf area response to CO_2 was an indicator of the physiological well-being of the plant, and reproductive performance could be assessed at the vegetative level using this trait.

Petioles are a functionally important sink in radish, as under high CO_2 they were enhanced more than blades The storage capacity of the petiole may have allowed leaf production to be beneficial without an inhibitory buildup of carbon in the blades. Similar CO_2 enhancement of petioles has been found in sugar beets (Wyse 1980). Partitioning to

petioles creates an important N store since they can accumulate twice the nitrate concentration than in blades (Schulze et al. 1985). The lack of a correlation between hypocotyl (root) enhancement in the vegetative phase and reproductive traits may be because of constraints imposed by the growing season. In the late-flowering cultivars, the shorter daylengths and cooler temperatures found at the end of the growing season may have limited flower and pod development. This would lower the sink activity level drawing reserves from the hypocotyl stores (Gifford et al. 1984). Low temperatures can limit CO_2 responses if sink strength is not maintained (Potvin 1994).

This study provides evidence that vegetative starch stores promote reproductive parameters. Starch measured was the basal level, the supply that exceeds the daily sink demand. The many CER correlations of starch with parameters of vegetative growth and reproductive output suggests that starch buildup was a reserve store that was utilized in both growth phases. Starch increases under high CO_2 should not be considered as an indicator of limitation, without examining long-term effects. Some studies have shown SLM increase to be correlated with starch buildup which inhibits photosynthesis (Cave et al.1981, DeLucia et al. 1985). However, Vu et al. (1989) found increases in leaf thickness and palisade cell length, which enhance source leaf sink capacity. In cotton, the sinks of stem, taproot, and leaf all were enhanced in non-structural carbohydrates and supplied boll development (Hendrix et al.1994). Measurements of starch and reproductive output on the same individuals are needed to ascertain the predictive importance of this trait.

Nitrogen fertilization effects

At the whole plant level, N enhanced leaf area for carbon storage, reducing individual source leaf SLM. The chlorophyll and photosynthesis results suggest that the N pool did not decline on a per area basis under high CO_2, since these parameters usually correlate with total leaf N and the enzymes invested in photosynthetic machinery (Evans

1989, Kuppers et al. 1988). Chlorophyll and protein were maintained per unit area under high CO_2 in other long-term studies (Delgado et al. 1994, Reeves et al. 1994).

Contrary to expectations, N did not promote reproductive total mass. This might be explained by the interactive effect of N and CO_2 in enhancing the RSR under low N and high CO_2, and an optimal hypocotyl store not developing under high N. The strong effects of N on plant performance often observed in elevated CO_2 may be attributable to near-limiting and moderate levels being chosen as the "low" and "high", whereas I chose moderate and enriched levels. In this study, N fertilization did not increase hypocotyl yield, as is typical for radishes under low fertility (Pell et al. 1990, Kostka-Rick and Manning 1993). Under high N of this study, W had four to 15-fold more seed for the low and high CO_2 respectively than in the high fertility regime of Curtis et al. (1994).

In EXP II, two additional applications of fertilizer were given, yet total mass of all cultivars at the final harvest was less than in EXP I. Chemical properties of the fertilizer may have inadvertently caused root damage or altered the soil chemistry and reduced nutrient availability. It is unlikely that nitrate was detrimental as radishes can concentrate excess levels (Schulze et al. 1985). The calcium carrier of nitrate used is required by radishes (Barker et al. 1983) and is known to promote nitrate uptake. However, it may have contributed to a salt imbalance which limited the uptake of other ions (Tisdale et al. 1985). It is also possible that other nutrients may have been inadequate to match N availability.

This study provides evidence that fertilization practises may need to be altered under an elevated CO_2 regime, due to differences in plant growth, partitioning and sensitivity. Because the effect of the N fertilizer was more pronounced under high CO_2, the response may be related to differential mineral acquisition. Because of the typical decreased transpiration rates under high CO_2 (Conroy and Hocking 1993) and the reduced root:shoot partitioning found at high N, roots may have been unable to acquire all the

available N. Additionally, the reduced acquisition may have resulted in a salt buildup in the soil solution. The sensitivity of radish roots to watering (Kosta-Rick and Manning 1993) would be expected to be pronounced in larger plants, (CN treatment) and those of greatest leaf area (FB,IC), which were the ones most affected. As suggested for other species, a reassessment in critical nutritional diagnosis, N and other fertilization amount is needed under high CO_2 (Hocking and Meyer 1991, Conroy 1992).

In conclusion, vegetative responses to elevated CO_2 and N differed with ontogeny, and with variety hypocotyl to shoot ratios. Vegetative mass enhancements showed no relationship with reproductive traits. Reproductive responses to elevated CO_2 were correlated only with vegetative leaf traits of total area and starch per area. Effects of N on promoting vegetative responses to elevated CO_2 were not sustained in the reproductive phase. Reproductive phenology, development and physiology may constrain the response to CO_2.

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CHAPTER 2

Long-term reproductive responses to elevated CO_2 of two perennial forbs under natural pasture conditions

ABSTRACT

Vegetative growth, phenology and allocation to reproduction were examined in two rosette perennials that differ in vegetative carbon storage organs and flowering seasonality. We hypothesized that the below-ground storage capacity of *Taraxacum officinale* versus the predominant leaf storage of *Plantago major* would contribute to greater reproductive response to CO_2. We also predicted that the increased carbon resource of a high CO_2 regime would enhance reproductive maturation rates and total reproductive output. Open-top chambers were placed on an intact pasture community and were maintained at CO_2 levels of 650 $\mu l\ L^{-1}$ and 350 $\mu l\ L^{-1}$ during five growing seasons. Under elevated CO_2, *T. officinale* showed significant enhancement in both hypocotyl and root biomass, number of rosettes and allocation to leaves. Allocation to reproduction significantly increased and capitulum areas were enhanced by 12%. Initiation of flowering was delayed by three days. However, inflorescence maturation rate was faster, such that the average duration of the reproductive period was not altered. The combined effect was a four-fold enhancement in relative fitness. In contrast, *P. major* showed little response to elevated CO_2 in vegetative or reproductive traits in a single season. Ramet production was marginally enhanced during the second year, and this corresponded with a relative enhancement in reproductive spike production by the third year. The flowering season showed a trend of being extended under elevated CO_2 and inflorescence senescence was delayed. A chamber wall effect in enhancing onset of flowering was detected. In both species, phenological responses were consistent in all years. Results suggest that hypocotyl storage of *Taraxacum* contributed to a strong enhancement in reproductive output under elevated CO_2 while *Plantago* showed little response. The direct effect of CO_2 on phenology may counteract earlier flowering onsets predicted by temperature increases.

INTRODUCTION

Predicting the response of plants to future increases in CO_2 and temperature requires an understanding of impacts on reproduction within the natural environment. Under elevated CO_2, an increased vegetative production along with a coordinated reproductive response could increase reproductive output. Conversely, hastening development in the reproductive stage without increasing the reproductive duration can limit the yield (Pinter et al. 1996). Internal constraints such as fixed phenological traits could limit responsiveness to the external increased carbon resource of an elevated CO_2 regime (Reich 1994). Over the long-term, differential responses of species in growth and reproductive output could result in shifts in community dynamics via seed bank contributions (Bazzaz and McConnaughy 1992). Knowledge of phenological responses to CO_2 and the relationship between vegetative growth, storage and the allocation to reproduction over the long-term in natural environments will contribute to our predictions of long-term community and ecosystem responses to elevated CO_2.

Plants in natural systems have evolved an integrated sensitivity to both photoperiod and temperature cues which might not be adapted to future environments (Lechowicz 1995, Kramer 1995). With global change, we can anticipate phenological changes such as advancements (or sometimes delays) in flowering days shown in response to temperature (Schwartz 1994, Fitter et al. 1995). These changes together with shifts in leaf senescence (Kramer 1995) could change the growing season and ecosystem productivity (Lieth 1974). However, the direct effects of elevated CO_2 may enhance (e.g.Curtis et al. 1989) or counteract the temperature effect. Effects of high CO_2 on development and phenology have been shown in day-length sensitive species grown in controlled environments (Reekie 1996, Reekie et al.1994, Ellis et al. 1995) and microcosms (Roy et al. 1996). Within a natural community, such shifts in phenology, can impact critical factors affecting

fitness such as yield and seed quality, as well as interactions with competitors, herbivores and pollinators (reviewed in Newton 1991, Rathcke and Lacey 1985, Ayres 1993).

This study examines the impact of increased CO_2 on the phenology, vegetative growth and reproduction of two perennial weeds in their natural community. Since vegetative responses to elevated CO_2 have been greatest in C_3 plants possessing large sinks (Reekie 1996), especially below-ground storage organs (Poorter et al. 1996, Poorter 1993), we hypothesized that plants possessing a larger below-ground sink capacity would both be more enhanced in storage under elevated CO_2 and show greater reproductive output. Responses were surveyed over a five-year period and examined in detail during a third growing season of CO_2 exposure to ensure responses were not merely due to acclimation. Open-top chambers were used for CO_2 enrichment and also to provide an increased temperature regime that is predicted to occur with future global change (Manabe 1983). Natural systems are advantageous for studies of perennials, as the limitations of transplant shock and a long establishment phase are eliminated, while soil processes are maintained naturally. In addition, roots are given the natural constraints of their environment, which allows manifestation of increases in below-ground storage or root growth that are often observed under CO_2 (Norby 1994). By this system we could examine the plasticity of response to CO_2 *in situ* with the natural environmental cues such as diurnal and seasonal rhythms of light and temperature which can have a large impact on growth and phenology.

The focal species chosen were the long-lived rosettes, *Taraxacum officinale* and *Plantago major*, which are the dominant forbs in our long-term elevated CO_2 pasture community (Potvin and Vasseur 1997). Both allocate a large proportion of resources to seed production (Solbrig 1971, Hawthorn and Cavers 1978) and they represent contrasting seasonal strategies (*sensu* Gentry 1974 in Rathcke and Lacey 1985). *Taraxacum* has a large storage hypocotyl and reproduction is a spring "mass flowering" which likely relies

predominantly on the remobilized stores of the previous year (Solbrig 1971). *Plantago* possesses a small below-ground storage caudex, and shows a "steady state flowering" throughout summer that is likely more coupled to the leaves and current photosynthate (Kuiper and Bos 1992). Due to the reduction in photosynthetic capacity that can occur when sink capacity is limited or leaf storage builds up under high CO_2 (Arp 1991), we expected the relative degree of reproductive response to CO_2 to be greater in *Taraxacum*.

Both species have previously shown responsiveness to CO_2. In CO_2-doubling controlled environment studies of vegetative growth in pots, the fast-growing *Taraxacum* has shown a weight-ratio enhancement of 1.7, while the intermediate-growing *Plantago* has shown one of 1.48 (Poorter 1993, Poorter et al. 1996). After three years of exposure to elevated CO_2 in our competitive grassland, leaf level photosynthesis showed a two-fold enhancement (Jablonski and Potvin, unpublished data) and species presence in the community was maintained (Potvin and Vasseur 1997). We wanted to examine the mechanisms underlying this responsiveness, particularly if below-ground storage and vegetative spread traits were enhanced, and the impact of this on reproduction. We also investigated if the increased carbon resource contributed to shifts in timing, rates, or allocation of resources to reproduction. These were expected since perennials can typically show more flexibility in allocation of resources than annuals (Chiarello and Gulmon 1991). We predicted that the increased carbon resource of the high CO_2 environment could be used in reproduction to enhance reproductive maturation rates, and that total reproductive output would be enhanced.

METHODS

Study site

Our site was on an intact grassland pasture community located in Farnham, Quebec (45°17'N, 72°59°W), 45 km southeast of Montreal, Canada. It had been grazed, but not ploughed for ten years prior to the commencement of our study. In the fall of 1991, the cows were removed from the pasture, and three experimental treatment areas were designated for study. Four open-top chambers of 3m diameter and 2.4 m height were set up. Two chambers were maintained at 625 ± 50 uL*L-1 CO_2 (High CO_2), and two at ambient air flow (approximately 350 ul L-1 CO_2) (Ambient CO_2). Two unenclosed circles of the same area served as chamber control plots (Control). The high CO_2 gas was administered each year from 1992 to 1996 during the frost-free season from mid-May to October. Details of the site and chamber design have been previously described (Stewart and Potvin 1996, Potvin and Vasseur 1997).

Mean air and soil temperature were recorded daily throughout the growing season (Datapod recorder, Model DP 212, Omnidata, Logan, UT). The temperature pattern over a typical growing season has been previously reported (Potvin and Vasseur 1997). The average temperatures for the summer months (June to August) ranged from 17 - 20°C. For the growing season of May through September, the chambers were warmer than the control plots by on average 1°C, 0.8°C and 3°C for the daily average, minimum and maximum temperatures respectively. Soil temperatures differed on average less than 0.5 °C between the chambers and control plots. Rainfall was collected in each plot by two pluviometers. Readings were made following each rainfall and the plot average was calculated. Precipitation was abundant during the years of the study, and totaled 69 cm in the open plots of 1994. The impact of the chamber walls on environmental parameters was examined by comparing the control plots with the two chamber treatments of Ambient CO_2 and High CO_2. Chamber walls reduced rainfall by 15 to 20% (data not shown).

Light intensity was reduced by the chamber walls depending on the time of day and plant location. Average reduction was to about 75% of full sunlight, though intermittently the reduction at any one location reached 50% (data not shown). There were no differences between the High CO_2 and Ambient CO_2 treatments in any climate parameter examined nor were there any differences among the replicate chambers within each treatment.

Plant Material

Two perennial weed species were chosen, *Plantago major* L. (common plantain) and *Taraxacum officinale* Weber (common dandelion). These were the dominant forbs, occupying about 25 % of the pasture vegetation. External pollination was not required as *Taraxacum* is apomictic (Mogie and Ford 1988) and *Plantago* is self-compatible and wind-pollinated (Hawthorne 1974). Both are rosettes and have contrasting storage organ sizes. *Taraxacum* possesses a large hypocotyl while *Plantago* has a small caudex plug and thick fibrous roots. Both species represent contrasting reproductive and seasonal strategies. *Taraxacum* is among the first species to emerge in our pasture community. Flowering heads (capitula) containing ray flowers emerge in a flush in May and June and reproduction is complete in a month. *Plantago* emerges as a small rosette with 6-12 leaves in early June. Additional leaves are produced sequentially. Axillary buds, can produce reproductive stems or can initiate clonal propagation by production of a ramet (Kuiper and Bos 1992). Flower stalks emerge sequentially throughout the summer. These inflorescences are spikes born on scapes, which contain numerous capsules that contain the flowers and subsequently the seeds.

Study plants were chosen at random from the intact population within the treatment areas. New plants were chosen to replace any plants that died. Observations of *Taraxacum* began in May of 1993 and continued over two full growing seasons. In the early spring of 1994, rodents excavated several plants. We controlled the population by

intensive trapping from May to July. The study of *Plantago* commenced in June of 1992, just after CO_2 treatment began in the chambers, and continued to the end of the October 1994. In *Plantago*, sample size was five plants per chamber (10 per environment) in 1992 and 1993. In 1994, sample size was increased to census nearly all individuals (n= 19 High CO_2, 10 Ambient CO_2, 17 Control). For *Taraxacum*, sample sizes of about 10 per chamber were chosen in 1993 (n= 25 High CO_2, 20 Ambient CO_2 and Control), and individuals were also followed in 1994.

Reproductive Phenology & Structures

At a whole plant level, the first and last days of reproductive stages were recorded. *Taraxacum* plants were examined daily in 1994 during flowering and seed production. Reproductive buds appear at the centre of the rosette associated with the hypocotyl. After several days, stalks elongate. The yellow capitulum opens gradually over a couple of days, then closes again several days later as seeds mature. Once the seeds are mature, the capitulum re-opens. Each reproductive stalk was followed over its lifetime from emergence to production of mature seeds. For each, the day of reaching each stage was recorded: bud presence (buds), bud emergence from base of rosette (pre-open), capitulum opening (open), capitulum closing (closed), presence of mature seeds (mature). On the day of capitulum re-opening, all mature seeds were collected. Diameter of the capitulum and the length of the scape were measured. Capitula were then clipped from the top of the scape, so as to allow usual recycling of nutrients back to the plant. Phenology was also surveyed in two other years. The plants followed in detail in 1994 were also followed in 1993, the second season of CO_2 exposure. In 1996, the fifth season of CO_2 treatment, plants that were established from the seedbank since 1994 were observed. For each survey year, the number of plants in each stage were recorded during the third week of May.

Plantago plants were observed throughout the growing season. During 1994, plants were observed in detail every second day. General surveys were conducted in 1992 where plants were assessed weekly and in 1993 where plants were observed every three weeks. Flowering occurs gradually acropetally, about 1/3 of the spike per day. Fruiting then commences, and mature seeds are formed after several weeks. At each observation time, the developmental stage of each reproductive structure was observed: emergence of the spike from the petiole, length > 2 cm (initiation), first anthesis (flowering), anthesis complete and seed-filling begun (fruiting). Spikes were termed mature when capsules were brown, and senescence had progressed through the spike to the scape. At maturity, spikes were cut from the top of the scape, to allow nutrient recirculation back to the plant. In both species, flowering duration was determined as the days from day of first flowering to the last day that flowering was observed. Reproductive duration was calculated in *Taraxacum* as the average time from first flowering to last mature seed.

Leaf production, phenology and longevity

To examine leaf phenology and production, leaves were observed at regular intervals. In *Taraxacum*, monthly counts were made of the total number of leaves. For *Plantago*, leaves were observed weekly in 1992 and 1994 and every three weeks in 1993. For each leaf, the time of initiation (leaf unfolded) and complete senescence was recorded, to determine leaf longevity. Ramet production was followed by observing the appearance of new small plants (rosettes) beside the mother plant and finding the links between them.

Biomass and allocation measurements

At the conclusion of the study, in October 1994, plants that were not near permanent study quadrats were excavated and harvested. For *Taraxacum*, n= 12 High CO_2, 14 Ambient CO_2, 13 Control. For *Plantago*, n= 14 High CO_2; 10 Ambient CO_2, 16

Control. Total number of leaves was counted, and blade area of *Plantago* was determined on a leaf area meter (LICOR LI-3000, Lincoln, NE). The length of immature reproductive spikes was measured. To estimate storage capacity, diameter and length of the hypocotyl and caudex were measured. To estimate foraging capacity, extent of the storage and fibrous roots were estimated at the widest extent using calipers. All reproductive structures present at harvest and collected during the season were dried at 35°C to allow for maintenance of seed viability for further analysis. Remaining parts were dried for 72 h at 65°C, prior to weighing.

Sample size available for destructive harvest was limited for the treatments. To increase the sample size for examination of allometric relationships across the range of sizes, in order to establish reliable covariates, size and allocation relationships were examined in a sample of both species. Transects were laid outside the plots, on the outer perimeter and area between the study plots, such that the entire area of the surrounding pasture population was sampled (n=60 *Plantago*, n=80 *Taraxacum*). Plants were chosen at random within every 1.5 m interval and the identical measurement and drying protocol followed as for the study plants. Total leaf number in both species showed strong and significant relationships with other parameters of plant size (data not shown).

Statistical Analysis

Data for 1994 in both species and 1993 in *Taraxacum* were analyzed by ANOVA (SAS 1988 v. 6.0.4). After testing for normal distributions of measured variables, appropriate log transformations were made. Type III sums of squares were used to account for missing data. The two species were analyzed separately. Our model was a nested design with Treatment as a fixed factor and Chambers considered as random. Chamber(CO_2), replicated twice for each treatment, was used as the error term to test the main effect of the three Treatments (High CO_2, Ambient CO_2 and Control). During the

course of 1994, all of the *Plantago* plants died in one of the Ambient CO_2 chambers because of the taller grass competition in that chamber (Potvin and Vasseur 1997). Because significant Chamber (CO_2) effects were rare for parameters tested in this study or others from our study site (Stewart and Potvin 1996, Potvin and Vasseur 1997) chamber replicates were pooled for *Plantago* analysis in 1994, and the general error term used. Because the sampling intensity and traits measured differed between years for *Plantago*, each year of data was analyzed separately. In both species, to account for size differences, leaf number at the beginning of each growing season was used as a covariate in the analysis of growth, biomass and allocation traits. To correct for seasonal effects, day of first reproductive initiation was used as a covariate for all phenology and maturation traits. Planned comparisons were performed to examine differences between means to test for a CO_2 environment effect: (High CO_2 vs Ambient CO_2) and Chamber wall environment effect (Ambient CO_2 vs Control). Repeated measures ANOVA (ANOVAR) was used to examine leaf number changes through time in *Taraxacum*. To examine CO_2 treatment and chamber wall effects in the phenology surveys and mortality data in both species, and for ramet propagation between years in *Plantago*, contingency chi-square analyses were performed. For each treatment, replicate chamber values were pooled prior to analysis.

RESULTS

Reproductive phenology & structures

Under elevated CO_2, *Taraxacum* plants were significantly delayed in the onset of flowering in all years (Table 1a). A delay of three days was detected in the detailed study of 1994 (Figure 1). During the single-day survey of 1993, the second season of CO_2 exposure, a greater proportion of plants flowered later in High CO_2 compared with ambient ($X^2 = 9.21$, $p < 0.01$) (Figure 2a). Similarly, in 1996, the fifth year of the study, High CO_2 plants were delayed in both first bud elongation ($X^2=15.97$, $p < 0.005$) (data not shown) and flowering stages (Figure 2b, $X^2 = 11.30$, $p < 0.025$). Overall, flowering phenologies of *Taraxacum* in control and ambient CO_2 were generally similar and both earlier than found in High CO_2. All other phenological events were shifted similarly from the first flowering delay, so that when the day of first flowering was included as a covariate to correct for differences in initiation times, treatments showed no difference in the timing of any first or last reproductive events (Table 1a). There was a seasonal effect on maturation time (Figure 3). On an entire plant basis, the average inflorescence maturation day was significantly later under elevated CO_2 (mean $\pm$ standard deviation, 30.1 ± 2.7) compared with ambient CO_2 (25.6 ± 2.8). Corresponding with the later maturation day, inflorescences took significantly less time on average to mature seeds under elevated CO_2 (10.4 ± 0.56 days) than in ambient CO_2 (11.0 ± 1.0 days). During the time intervals where inflorescences matured in both high CO_2 and ambient CO_2, there were no differences in the time to mature seed at either individual or per plant levels. All CO_2 effects were independent of plant size, as leaf number was not a significant covariate for day of first flowering, nor any other phenological or reproductive trait (Table 1a). A significant effect of the chamber wall was detected in *Taraxacum* only in 1994 (Table 1a).

Table 1. F-values and significance levels from the analysis of variance of phenology and reproductive structure data in 1994.

(A) Taraxacum officinale (B) Plantago major. Significance levels * $p < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns $p > 0.10$.

The main effects are Treatment (High CO2, Ambient CO2 and Control) and Chamber replicate (Treatment) for Taraxacum.

For Plantago, Chamber replicates were pooled. Beneath Treatment, the results of planned comparisons are shown

for the CO2 effect (High CO2 vs Ambient CO2) and the Chamber wall effect (Ambient CO2 vs Control).

Effects F values are for analyses using leaf number as the covariate for first flowering (A) and first spike initiation (B)

and the day of first flowering (A) or first initiation (B) for all other traits.

For (A), sample size, n= 21 High CO2, 26 Ambient CO2, except for inflorescence weight and capitulum diameter where n=24; and 23 C;

For (B), sample size, n=19 High CO2, 10 Ambient CO2, 17 Control, except for % maturation of spikes where C=14.

Traits

A. Taraxacum officinale

	day of first flowering	day of last flowering	average day of flowering	time to mature first seed	average time to mature seed	flowering duration	reproductive duration	average capitulum diameter	average inflorescence weight
Treatment	25.08 *	2.41 ns	21.24 *	7.03 (0.07)	0.26 ns	0.64 ns	3.26 ns	114.51 **	1.85 ns
High CO2 vs Ambient CO2	**	ns	*	ns	ns	ns	0.1	***	ns
Ambient CO2 vs Control	ns	ns	**	*	ns	ns	ns	ns	ns
Chamber (Treatment)	0.40 ns	0.91 ns	0.81 ns	1.11 ns	1.92 ns	3.19 *	1.9 ns	0.05 ns	1.14 ns
Covariate (leaf number)	0.95 ns	ns	ns	ns	ns	ns	ns	ns	ns
Covariate (day of first flowering)	-	16.3 ***	90.5 ***	31.59 ***	11.99 ***	12.33 ***	84.82 ***	1.43 ns	0.03 ns

TABLE 1. Continued.

B. Plantago major

	day of first first spike initiation	day of first flowering	day of first nature seed	time from flowering to mature first seed	duration of flowering (first to last flowering day)	% maturation of spikes by end of season
Treatment	6.16 **	3.28 *	10.82 ***	4.44 *	1.82 ns	3.96 *
High CO2 vs Ambient CO2	ns	ns	ns	ns	0.08	**
Ambient CO2 vs Control	**	*	***	*	ns	ns
Covariate (number of leaves)	3.54 (0.07)	ns	ns	ns	ns	ns
Covariate (day of first spike)	-	184.90 ***	144.97 ***	10.82 **	10.16 **	0.92 ns

Figure 1

Reproductive phenology in *Taraxacum officinale* during the third season of CO_2 treatment (1994). For each treatment set, days of first and last flowering (below,left) and first and last mature seed production (above, right) (Mean ± 1 standard deviation). High CO_2 (n=21), closed squares; Ambient CO_2 (n=26), open squares; Control (n=23), open circles. Letters denote significant differences in mean day of first flowering.

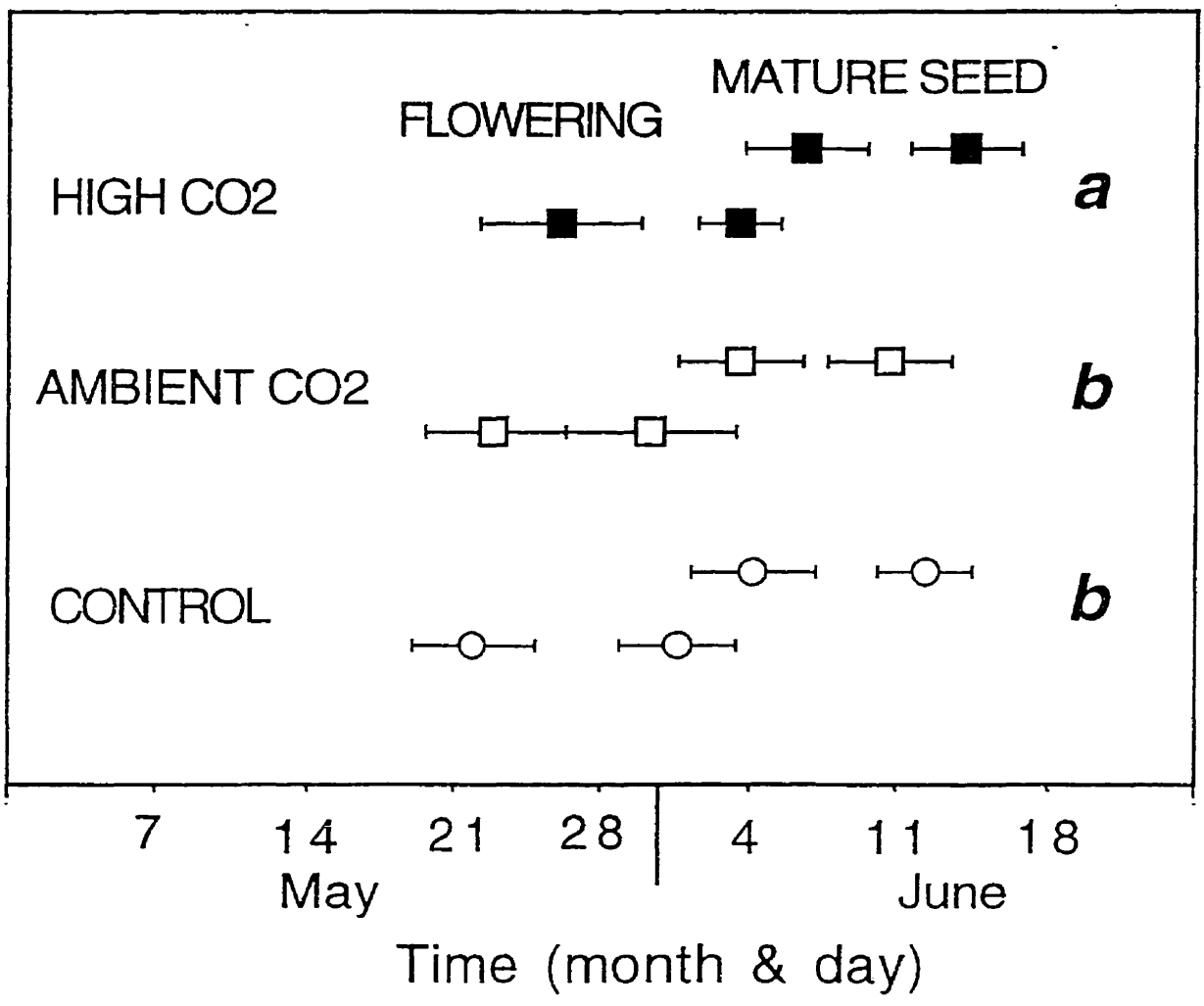
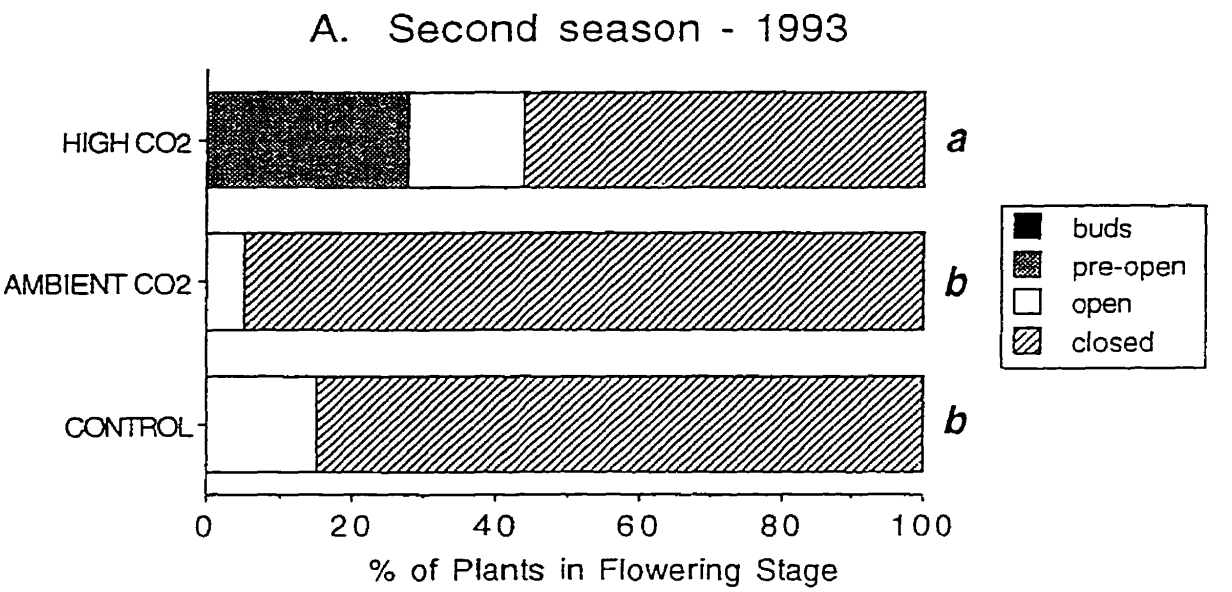

MATURE SEED
FLOWERING
HIGH CO2
a
AMBIENT CO2
b
CONTROL
b
7
14
21
28
4
11
18
May
June
Time (month & day)

Figure 2

Phenology surveys showing relative differences in reproductive stages in *Taraxacum officinale*. (A) Second season of CO_2 treatment, surveyed on May 22, 1993. (B) Fifth season of CO_2 treatment, surveyed on May 24, 1996. Bars for each treatment show the relative proportion of all individuals in the treatment that are in each stage of reproduction: basal buds (buds - solid); buds raised before inflorescence opens (pre-open - stippled); open inflorescence, (open - white); closed inflorescence during seed formation (closed - striped). Letters denote significant differences between treatments based on two-way chi-square comparisons of High CO_2 vs Ambient CO_2 and Ambient CO_2 vs. Control. Sample sizes: (A) 1993 (n = 25 H; 20 A,C); (B) 1996 (n = 16 H; 11 A, 14 C).


A. Second season - 1993
HIGH CO2
AMBIENT CO2
CONTROL
a
b
b
buds
pre-open
open
closed
0
20
40
60
80
100
% of Plants in Flowering Stage

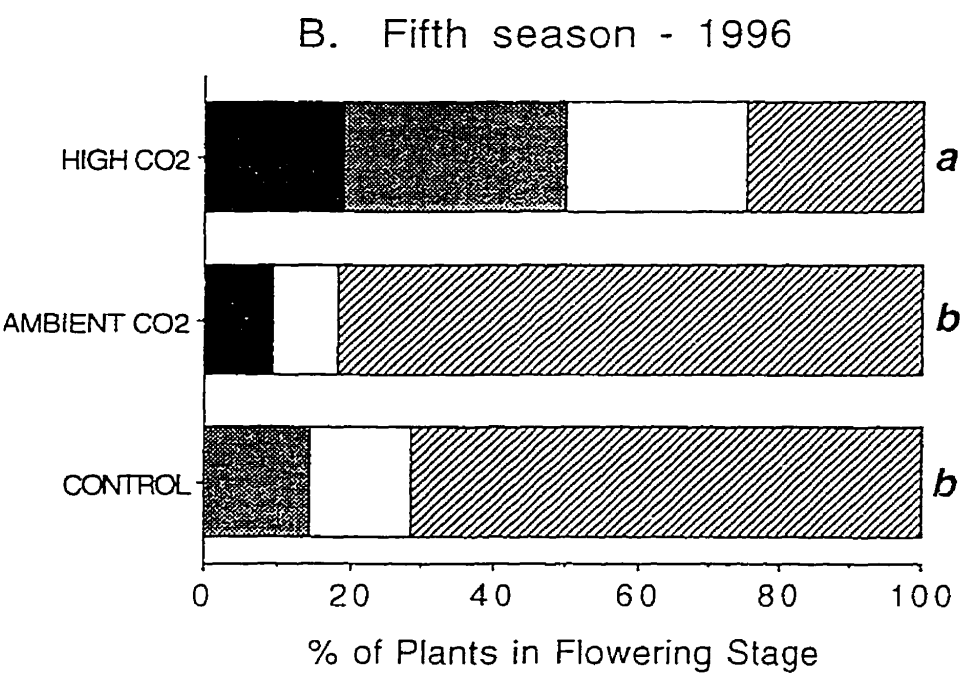
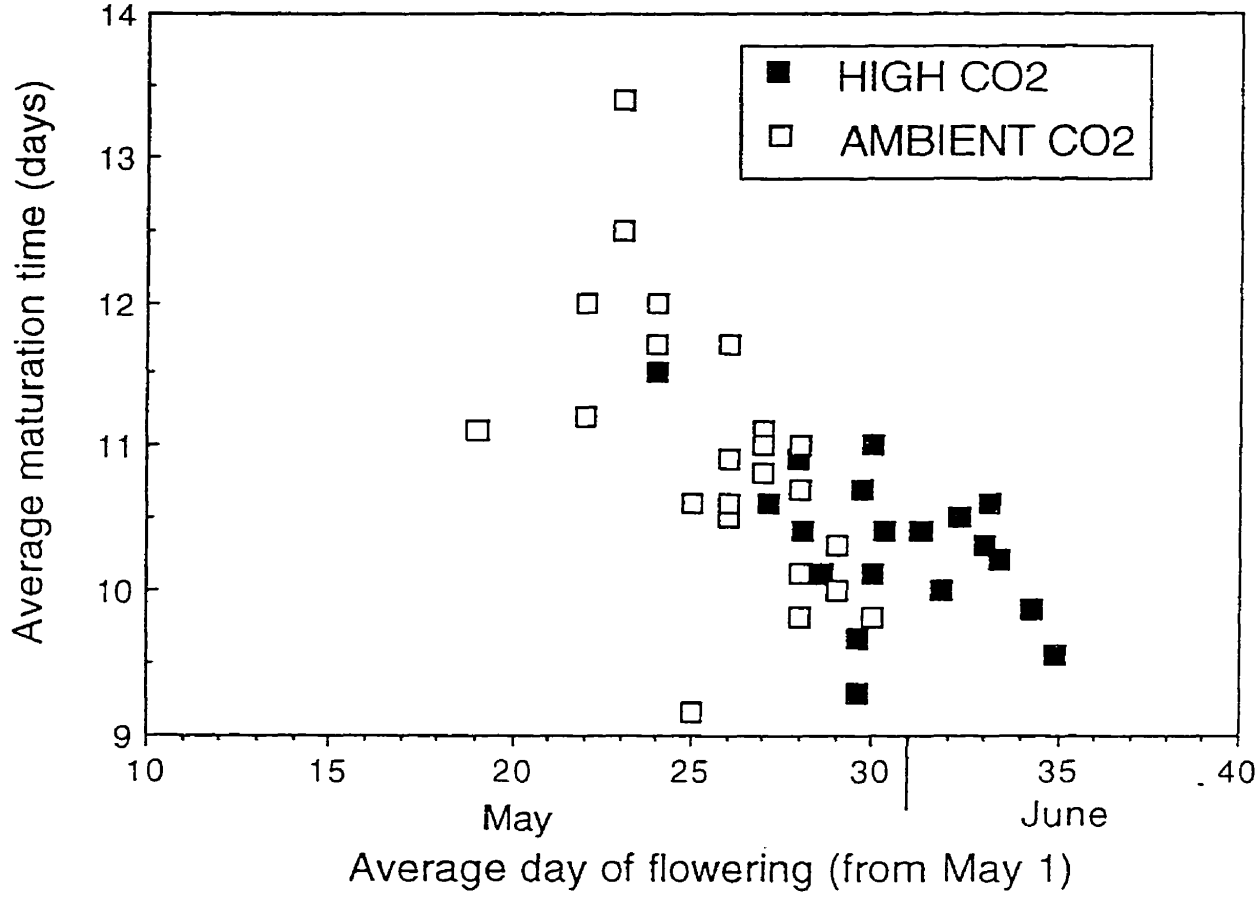

B. Fifth season - 1996
HIGH CO2
AMBIENT CO2
CONTROL
a
b
b
0
20
40
60
80
100
% of Plants in Flowering Stage

Figure 3

Effect of time in growing season on time taken to mature inflorescences in *Taraxacum officinale*. Data are per plant averages of all inflorescences produced in the 1994 growing season. Maturation time determined as days from first inflorescence opening. Symbols as in Figure 1. (Sample sizes, n = 20 High CO_2 ; 23 Ambient CO_2).


HIGH CO2
AMBIENT CO2
Average maturation time (days)
14
13
12
11
10
9
10
15
20
25
30
35
40
May
June
Average day of flowering (from May 1)

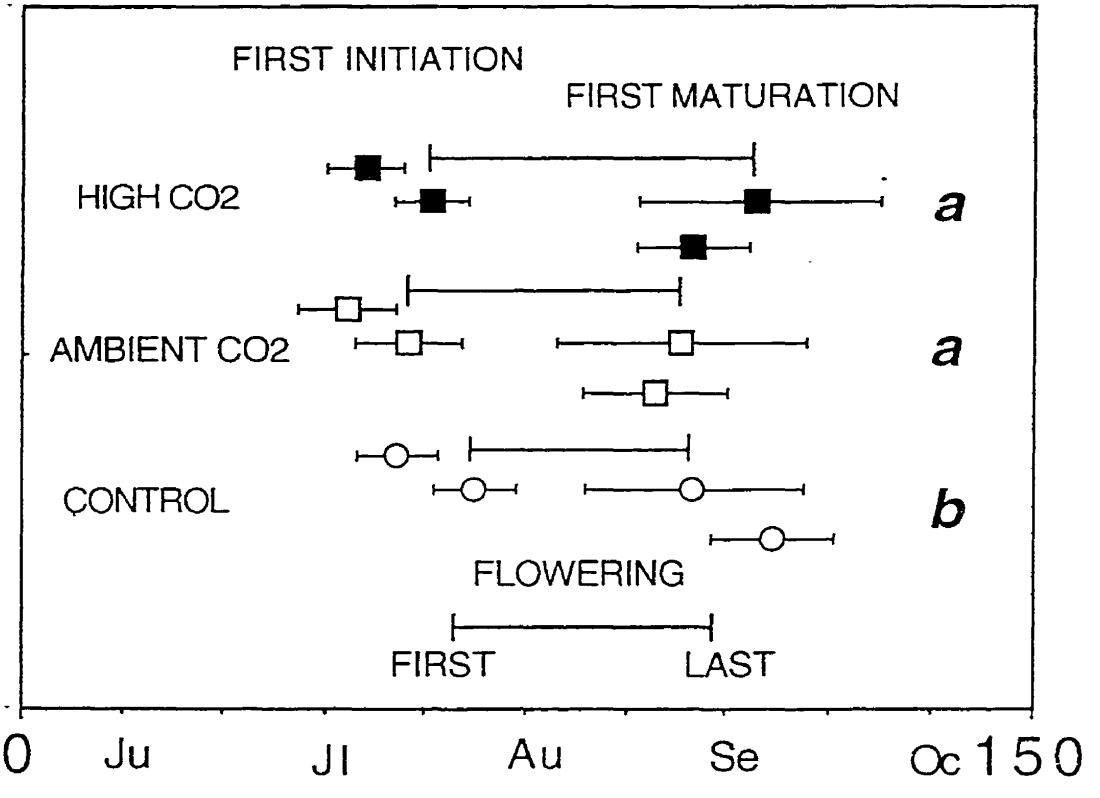
The peak of the reproductive period was two days later in the control environments than in ambient CO_2. As well, maturation took on average two days longer in control plots (13.3 ± 1.6) compared with ambient CO_2 (11.4 ± 1.6). Plants in replicate chambers within treatments showed consistent responses as a significant chamber (treatment) effect was only found once.

Plantago major differed from *Taraxacum*, in that little response to CO_2 was found. There was no CO_2 effect detected on the initiation of reproductive events in the detailed study of 1994 (Figure 4, Table 1b) or in the flowering proportions surveyed in 1992 ($x^2 = 2.5$, $p > 0.10$) (Figure 5a) or 1993 ($x^2 = 2.5$, $p > 0.10$) (Figure 5b). However, under elevated CO_2, flowering in *Plantago* continued until later in the season, such that the duration of the flowering period was longer in 1994 (days; High CO_2: 58.6 ± 19.3, Ambient CO_2: 49.9 ± 22.3, Control: 44.2 ± 17.5). The adjusted means showed a marginal trend of being longer in High CO_2 (56.9 ± 17.7) then in Ambient CO_2 (44.6 ± 18.3) (Table 1b). At the end of the growing season, a greater proportion of spikes had not yet reached full maturity under elevated CO_2, as judged by completed senescence (%, High CO_2: 79.1 ± 12.8., Ambient CO_2: 92.6 ± 12.7, Control: 87.3 ± 12.9). As found in *Taraxacum*, there was no CO_2 effect on the duration between successive first events in *Plantago*. In both High CO_2 and Ambient CO_2, the time from first initiation to first flowering was around 10 days, and from first flowering to first mature seed about 38 days. On larger plants, spikes tended to initiate earlier, as shown by the marginally significant size covariate (Table 1b). All subsequent reproductive events were affected similarly by size. As in *Taraxacum*, the time of first inflorescence initation was a significant covariate for all other phenology events.

Plantago showed a consistent effect of the chamber wall on several phenological traits (Table 1b). During 1994, all first initiation events were over six days later in the control environments (Figure 4). After adjusting for initiation day, the chamber wall

Figure 4

Reproductive phenology in *Plantago major* during third season of elevated CO_2 treatment, 1994. From upper left to lower right for each treatment: days of first initiation, first and last flowering, and first maturation. Mean ± standard deviation. Abbreviations indicate beginning of each month from Ju (June) to Oc (October). Letters denote significant differences in mean day of all first events. Bars indicate average duration of flowering phase (from first to last day of inflorescence anthesis). Symbols as in Figure 1. Sample sizes: n= 19 High CO_2; 10 Ambient CO_2, 17 Control.

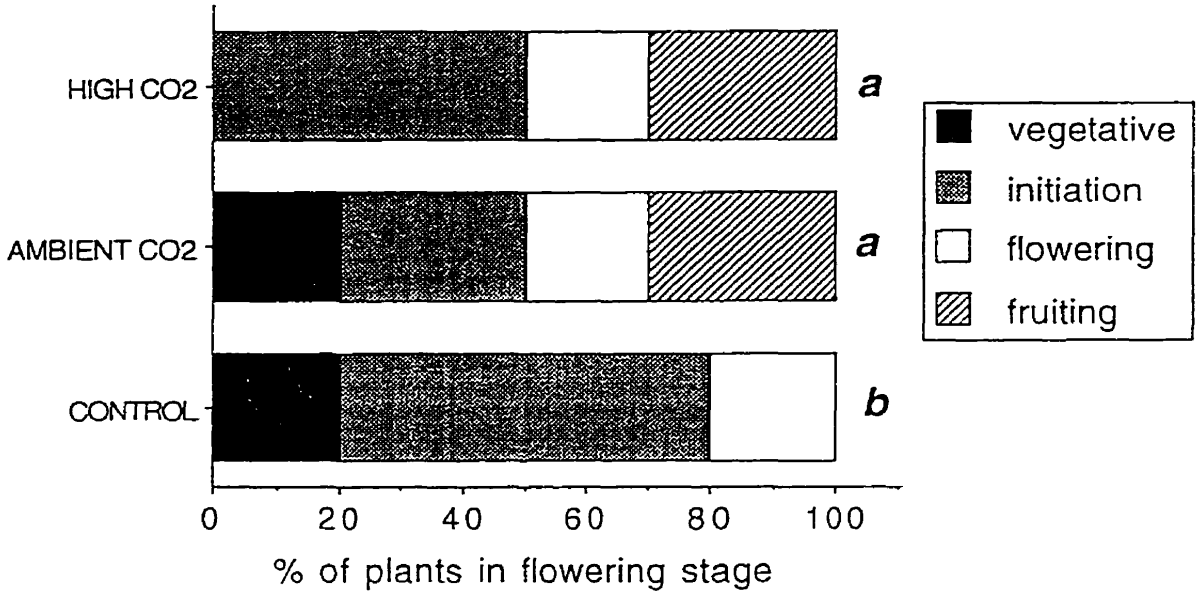


Time (days from May 15)

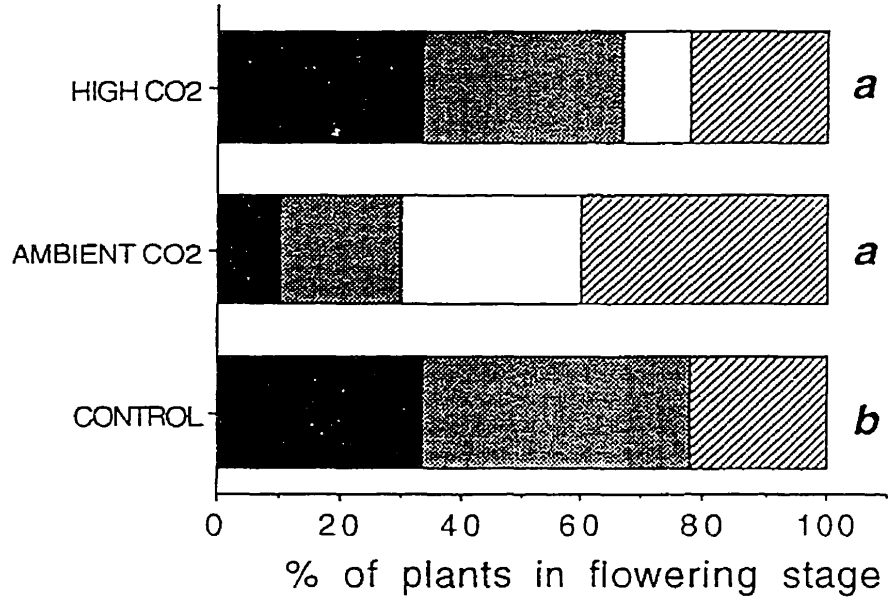
Figure 5

Phenology surveys showing relative differences in reproductive stages in *Plantago major*. (A) First season of CO_2 treatment, 1992 (surveyed on July 15). (B) Second season of CO_2 treatment, 1993 (surveyed on July 26). Bars for each treatment show the relative proportion of all individuals in the treatment in which their most advanced stage of reproduction is: no reproductive spikes present (vegetative - solid), spike present (initiation - stippled), spike in anthesis (flowering - white), spike during seed formation (fruiting - striped). Letters denote significant differences between treatments based on two-way chi-square comparisons of proportions in pre-flowering (vegetative and initiation) vs. post-flowering (flowering and fruiting) in High CO_2 vs Ambient CO_2 and Ambient CO_2 vs. Control. For both years, n=10 in each treatment group.

A. First season - 1992



B. Second season - 1993



significantly enhanced developmental time from initiation to flowering by two days, and from flowering to maturation by eight days (Table 1b). Delays were also observed in the surveys of the other years (Figures 5 a,b 1992, $x^2 = 3.5$, $p < 0.10$; 1993, $x^2 = 4.5$, $p < 0.05$).

Inflorescences were examined in detail in *Taraxacum* in 1994. Under elevated CO_2, capitulum diameters were significantly greater than in ambient CO_2 (cm); (High CO_2: 1.11 ± 0.07 a, Ambient CO_2: 1.03 ± 0.09 b, Control: 1.02 ± 0.13 b) (Table 1a). This conferred a 12% increase in inflorescence area which could result in an increased seed production under elevated CO_2 (Jablonski and Potvin, in preparation). Capitulum size response was independent of plant size. In *Plantago*, no effects of CO_2 or chamber wall were observed on inflorescence size relative to vegetative plant size.

Biomass Allocation

There was a strong and significant elevated CO_2 effect on *Taraxacum* size and allocation patterns observed at the end of the third growing season (1994) (Table 2a). Adjusting for the initial size, plants were significantly enhanced two-fold or more in all biomass traits (Figure 6). The relative allocation to leaf biomass increased under elevated CO_2, resulting in a significantly lower root:shoot ratio (Table 3). Leaves were both more numerous and were larger, being 35% greater in average mass per entire leaf (Table 3) and occupying more area (below). The allocation allometry across treatments was examined in *Taraxacum* to ensure that the non-destructive growth measure of leaf number could be used to estimate storage. In all cases there was a strong relationship between total number of leaves and the mass of the hypocotyl and root (Figure 7). The allocation allometry did not differ with plant size nor with treatment in the experimental plots or the surrounding pasture plants. Plants in elevated CO_2 were clearly larger than in the other experimental environments and the surrounding pasture. Plants in the surrounding pasture showed a

Table 2. F-values and significance levels from the analysis of end-of-season harvest data in 1994 in (A) Taraxacum officinale and (B) Plantago major.

Effects and symbols as in Table 1. The number of leaves on each individual in May (Taraxacum) and early June (Plantago) was included as a covariate.

Sample sizes, (A), n= 12 H, 14 A, 13 C, except for number of inflorescences, where n as in Table 1. (B), n=14 H, 10 A, 16 C.

Relative allocation to reproduction was grams biomass at harvest per inflorescence produced in 1994.

Traits

A. Taraxacum officinale

	number of leaves	total leaf mass	average mass per leaf	number of rosettes	root mass	total mass	root:shoot ratio	diameter of storage hypocotyl	extension of roots from hypocotyl	number of inflorescences	relative allocation to reproduction
Treatment	9.35 *	23.57 **	17.16 *	40.93 **	243.99 ***	139.61 ***	9.73 *	23.38 *	8.54 (0.06)	5.3 (0.10)	30.19 **
High CO2 vs Ambient CO2	*	*	*	*	***	**	*	0.053	0.1	*	*
Ambient CO2 vs Control	ns	ns	ns	ns	*	*	ns	*	ns	0.1	*
Chamber(Treatment)	0.38 ns	0.32 ns	0.80 ns	0.32 ns	0.02 ns	0.05 ns	0.55 ns	0.12 ns	0.36 ns	0.54 ns	0.12 ns
Covariate	0.14 ns	2.16 ns	0.93 ns	36.65 ***	13.71 ***	11.3 **	1.63 ns	18.28 ***	39.38 ***	54.25 ***	0.02 ns

TABLE 2. Continued.

B. Plantago major

	total leaf area	total blade mass	specific leaf mass (g/cm2)	total petiole mass	petiole: blade ratio	total root mass	caudex: total root ratio	total vegetative mass	total reproductive mass	plant total mass	reproductive allocation
Treatment	7.12 **	9.12 ***	3.01 (.06)	7.37 **	1.76 ns	1.69 ns	3.53 *	5.66 **	5.46 **	5.45 **	0.17 ns
High CO2 vs Ambient CO2	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
Ambient CO2 vs Control	**	**	*	*	*	ns	0.09	0.053	*	*	ns
Covariate	11.12 **	6.07 *	0.01 ns	8.21 **	.17 ns	13.8 ***	2.63 ns	10.61 **	12.44 ***	14.54 ***	0.62 ns

Figure 6

Total biomass and vegetative allocation in *Taraxacum officinale* at end of third growing season, October 1994. Bar symbols are leaves (solid) and hypocotyl and root (open). Letters denote significant differences in total mass. Error bars are 1 standard deviation. Sample size: n=12, High CO_2; 14, Ambient CO_2, 12, Control.

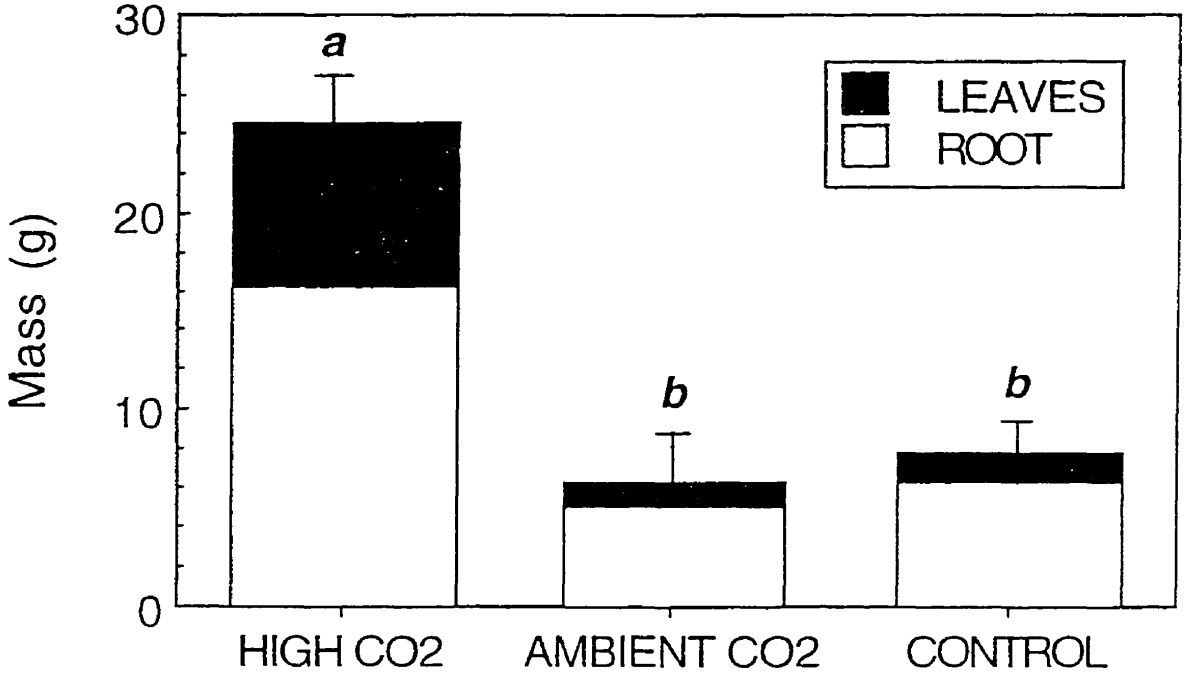

30
20
10
0
Mass (g)
a
b
b
LEAVES
ROOT
HIGH CO2
AMBIENT CO2
CONTROL

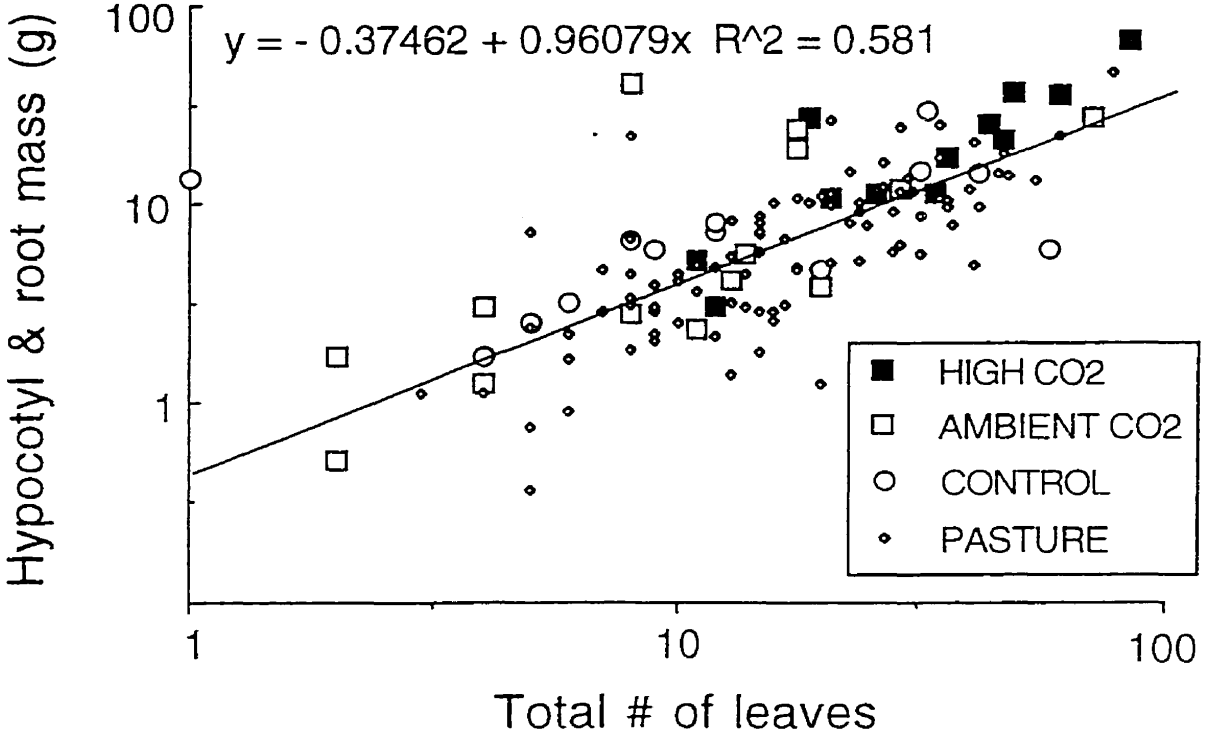
Table 3. The effects of elevated CO_2 on hypocotyl, root and leaf characteristics in Taraxacum officinale.

Data from harvest in October 1994. Mean ± sd. Letters denote significant differences between means in planned comparisons between High CO_2 vs Ambient CO_2 (a,b) and Ambient CO_2 vs. Control (c,d).

	Trait					
Treatment	diameter of storage hypocotyl (cm)	extension of roots from hypocotyl (cm)	number of rosettes	number of leaves	mass per leaf (g)	root:shoot ratio
High CO_2	3.28 ± 1.29 a	53.6 ± 16.1 a	4.72 ± 3.15 a	31.3 ± 21.5 a	0.24 ± 0.13 a	2.33 ± 1.17 a
Ambient CO_2	2.02 ± 1.67 bc	32.2 ± 23.6 bc	2.63 ± 2.10 bc	9.7 ± 17.7 bc	0.098 ± 0.061 bc	8.05 ± 2.34 bc
Control	2.33 ± 1.44 c	42.8 ± 22.3 c	2.36 ± 1.59 c	10.1 ± 17.5 c	0.067 ± 0.048 c	9.25 ± 2.44 c

Figure 7

The relationship between leaf number and combined mass of hypocotyl and root in *Taraxacum officinale* harvested in October 1994. Sample size as in Figure 6 and N=80, pasture. The allometry did not differ significantly with treatment group or with plant size.


y = - 0.37462 + 0.96079x R^2 = 0.581
Hypocotyl & root mass (g)
100
10
1
1
10
100
Total # of leaves
HIGH CO2
AMBIENT CO2
CONTROL
PASTURE

similar total mass (10.04 ± 1.67) and root:shoot allocation patterns to both the ambient CO_2 chambers and control plots.

In *Plantago*, there was no effect of elevated CO_2 on any growth or biomass trait (Figure 8, Table 2b). However, plants in High CO_2 allocated relatively more mass to the fibrous root rather than the storage caudex which resulted in a significantly lower caudex:total root ratio (adjusted means, High CO_2: 0.59 ± 0.20 a, Ambient CO_2 0.77 ± 0.13 b, Control: 0.66 ± 0.18). The chamber wall of the Ambient CO_2 environment enhanced all above-ground traits and total mass compared to the Control (Table 2b). As expected, initial plant size at the beginning of the season significantly affected biomass and growth traits, but none of the allocation traits. The surrounding pasture was intermediate to the ambient CO_2 and control treatments in total vegetative mass (6.13 ± 1.33 g).

Growth, Leaf production and display

In *Taraxacum*, elevated CO_2 significantly enhanced the display of the leaf canopy and the basal area occupied by the plant (Table 4). For the equivalent leaf number in September 1993, the leaf canopy of plants under elevated CO_2 occupied 1.7-fold more area (cm^2) (High CO_2: 598 ± 202, Ambient CO_2: 360 ± 124, Control 473 ± 165). Similarly, under elevated CO_2, the basal area cover (cm^2) of the hypocotyl at the soil surface were significantly greater by two-fold (High CO_2: 11.4 ± 6.1, Ambient CO_2: 5.7 ± 3.4, Control: 8.6 ± 4.7). Elevated CO_2 also enhanced plant size in early spring of 1994, relative to the size in 1993. Basal area (cm^2) in May of 1994 was 1.3-fold greater (High CO_2: 24.3 ± 6.2, Ambient CO_2: 18.7 ± 4.8, Control: 25.1 ± 6.3). Leaf number in May of 1994 covaried with that of September 1993 similarly in all treatments.

The timing of the elevated CO_2 effect on leaf production in *Taraxacum* was examined by following the variation in leaf number during 1993 and 1994 (Figure 9). A similar seasonality of leaf production was observed in all treatments, so treatments were compared

Figure 8

Total biomass and vegetative allocation in *Plantago major* at end of third growing season, October 1994. Bar symbols are reproductive (striped), blade (open), petiole (solid) and root (stippled). Letters denote significant differences in total mass. Error bars are 1 standard deviation. Sample sizes: n= 14 High CO_2; 10 Ambient CO_2, 16 Control.

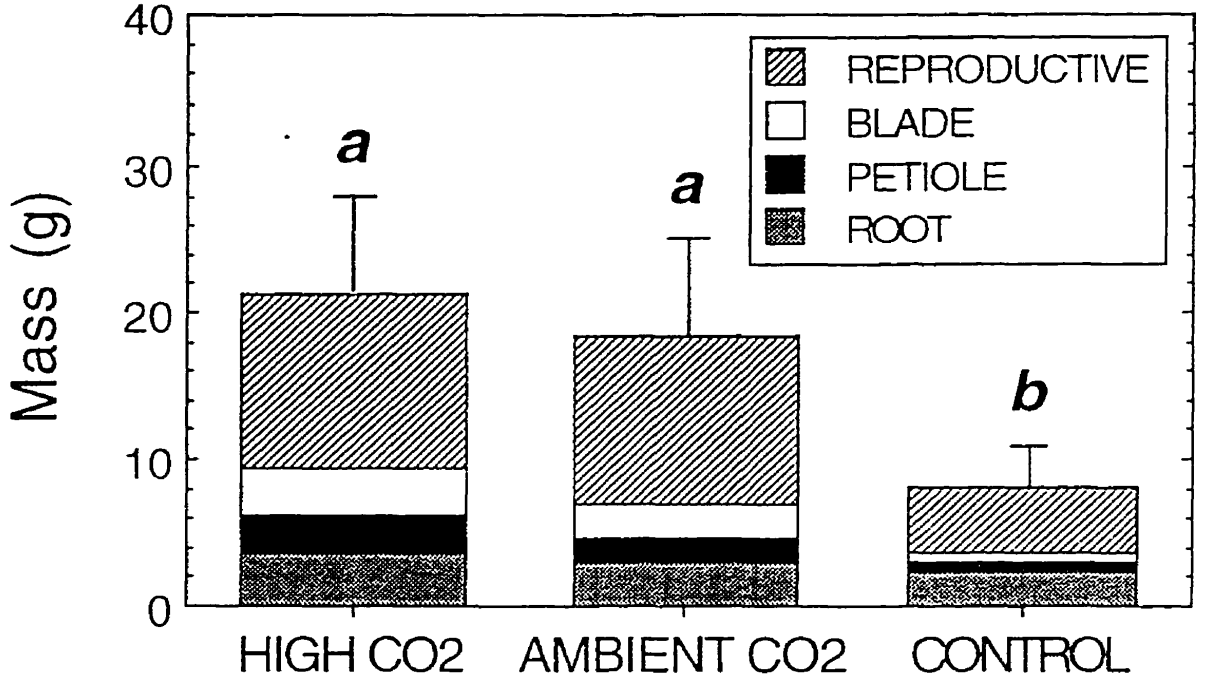
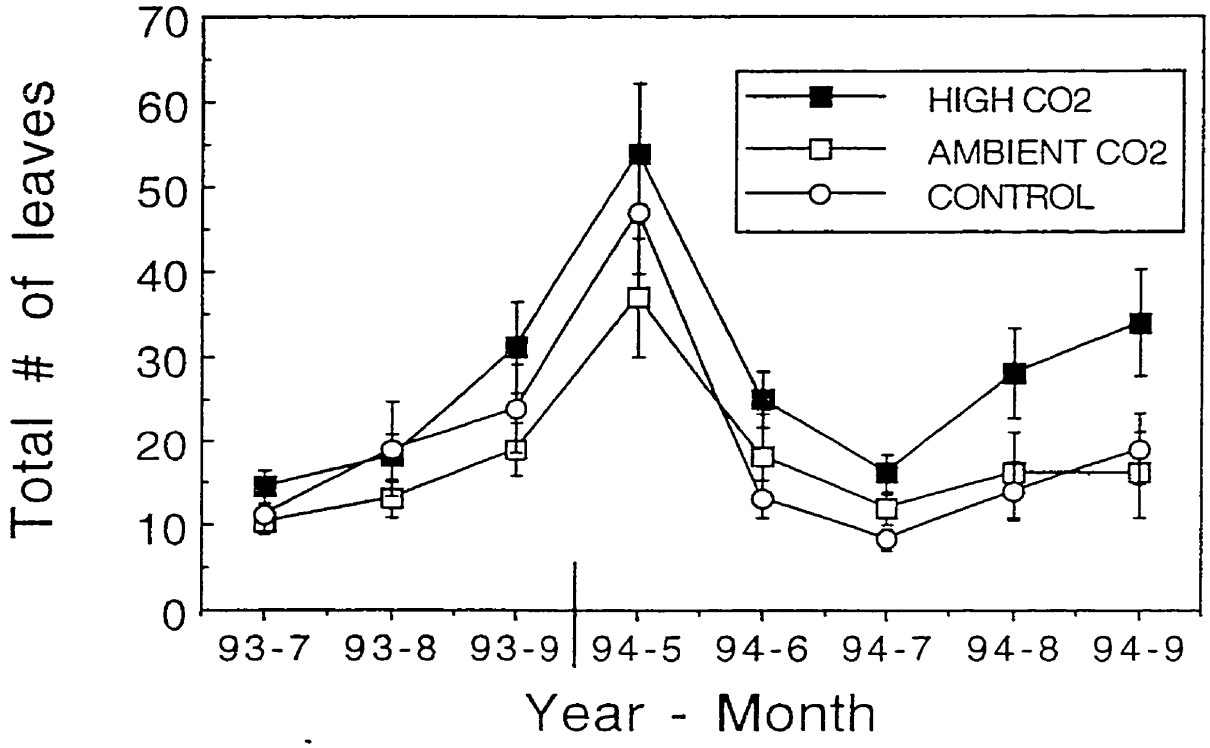

40
30
20
10
0
Mass (g)
REPRODUCTIVE
BLADE
PETIOLE
ROOT
a
a
b
HIGH CO2
AMBIENT CO2
CONTROL

Table 4. F-values and significance level from the analysis of leaf and mass allometry of Taraxacum officinale. The main effects are Treatment (High CO2, n=27-1993,22-1994); Ambient CO2, n=26; and Control, n= 25-1993, 23-1994) and Chamber replicate (Treatment), n=2). The number of leaves on each individual in September 1993, was included as a covariate. Effects and symbols as in Table 1. The log transformed values of all traits were used for analysis.

	September 1993		May 1994		
	basal area	leaf area cover	number of inflorescences	number of leaves	basal area
Treatment	8.85 (0.06)	12.7 *	15.34 *	6.01 *	9.32 *
High CO2 vs Ambient CO2	*	**	*	*	*
Ambient CO2 vs Control	0.1	0.1	**	ns	*
Chamber(Treatment)	1.5 ns	0.30 ns	0.46 ns	0.40 ns	0.19 ns
Covariate	169.97 ***	117.64 ***	26.89 ***	68.34 ***	34.36 ***

Figure 9

Leaf production in *Taraxacum officinale* over the duration of the study from July, 1993 to September, 1994. Mean ± sem. Only plants that were alive throughout the study were used in this analysis (Sample sizes: n=16 High CO_2; 18 Ambient CO_2, 13 Control).


Total # of leaves
70
60
50
40
30
20
10
0
HIGH CO2
AMBIENT CO2
CONTROL
93-7
93-8
93-9
94-5
94-6
94-7
94-8
94-9
Year - Month

during the period of leaf number increase (July to September) in each year. Plants within treatment groups did not differ significantly at the first observation time (July 1993, $F=1.23$, $p > 0.10$) and there was no effect of CO_2 detected in 1993. In 1994, there was a marginally significant treatment x time interaction (ANOVAR, $F=2.19$, $p < 0.08$). By September, plants in high CO_2 had 2.5 times the leaves of ambient CO_2 ($F=3.33$, $p < 0.05$).

Plantago plants within CO_2 treatment groups did not differ significantly in the size-related trait of leaf number at the beginning of the study (June 10, 1992): High CO_2, 5.9 ± 1.6; Ambient CO_2, 6.2 ± 1.8. In all years, there was no effect of CO_2 on the total number of leaves produced in a growing season (Table 5). Similarly, leaf phenology which included timing of initiation and leaf longevity did not differ significantly between treatments (data not shown). A chamber wall effect was seen in 1994, where the control plots produced less leaves (data not shown). The CO_2 treatment did not alter the strong relationship observed between the day of leaf initiation and the time to day of flowering of the corresponding spike, which was 23 days on average.

Vegetative propagation

Both species showed an increase in vegetative propagation in response to elevated CO_2. In *Taraxacum* under high CO_2, the hypocotyl occupied more basal surface, extended a greater distance and was able to support more clusters of leaves (Table 3). Under high CO_2, there was significant enhancement of 1.6-fold in hypocotyl diameter and in the extension of the roots from the hypocotyl through the soil (Table 2a). Corresponding with the increase in hypocotyl mass and dimensions, the number of rosettes where leaf clusters emerge was significantly greater by nearly two-fold under elevated CO_2. All of these CO_2 effects were still apparent after correcting for size differences. For all these traits, the ambient CO_2 and the control plants did not differ significantly.

Table 5. F-values and significance level from the analysis of leaf and reproductive spike production in Plantago major for each season. The main effects are Treatment (High CO2, Ambient CO2 and Control), and Chamber replicate (Treatment) for 1992 and 1993. Chambers were pooled under Treatment in 1994. Sample size n=5 in 1992 and 1993; 1994 as in Table 1. Effects and Symbols as in Table 1. The number of leaves on each individual in early June of each year was included as a covariate.

Year	1992			1993			1994		
Trait	no. of leaves	no. of spikes	total length of spikes	no. of leaves	no. of spikes	total length of spikes	no. of leaves	no. of spikes	total length of spikes
Treatment	2.32 ns	5.47 (.10)	33.98 **	0.37 ns	0.38 ns	0.71 ns	7.09 **	3.65 *	5.24 **
High CO2 vs Ambient CO2	ns	ns	ns	ns	ns	ns	ns	ns	ns
Ambient CO2 vs Control	ns	0.1	**	ns	ns	ns	**	*	*
Chamber (Treatment)	1.67 ns	0.22 ns	0.10 ns	0.75 ns	2.04 ns	1.26 ns	-	-	-
Covariate	15.84 ***	2.44 ns	3.62 (0.07)	25.74 ***	2.81 ns	0.41 ns	27.55 ***	7.39 **	15.89 ***

In *Plantago,* an elevated CO_2 effect was detected on the onset of ramet initiation and the frequency of ramet production (Figure 10a). There was no effect of CO_2 on ramet production in 1992, the first season of the pasture not being grazed. Elevated CO_2 enhanced ramet production by two-fold in the second growing season (1993). The proportion of plants forming ramets differed significantly between the three treatments ($x^2 = 4.0$, $p < 0.05$). Production under High CO_2 was marginally higher than under the treatments not receiving CO_2 (pooled ambient CO_2 chambers and control plots) ($x^2 = 3.75$, $p = 0.054$). There was a chamber wall effect on ramet production. The control plants did not produce ramets until the third season (1994). This contributed to the proportions differing marginally in the three treatment groups between 1993 and 1994 ($x^2 = 4.27$, $p = 0.12$). Control plot levels in 1994 were similar to the Ambient CO_2, while the High CO_2 was 10% higher ($x^2 = 2.76$, $p = 0.10$). Ramet production in the surrounding pasture plants was similar to both the ambient CO_2 and control environments.

Allocation to Reproduction

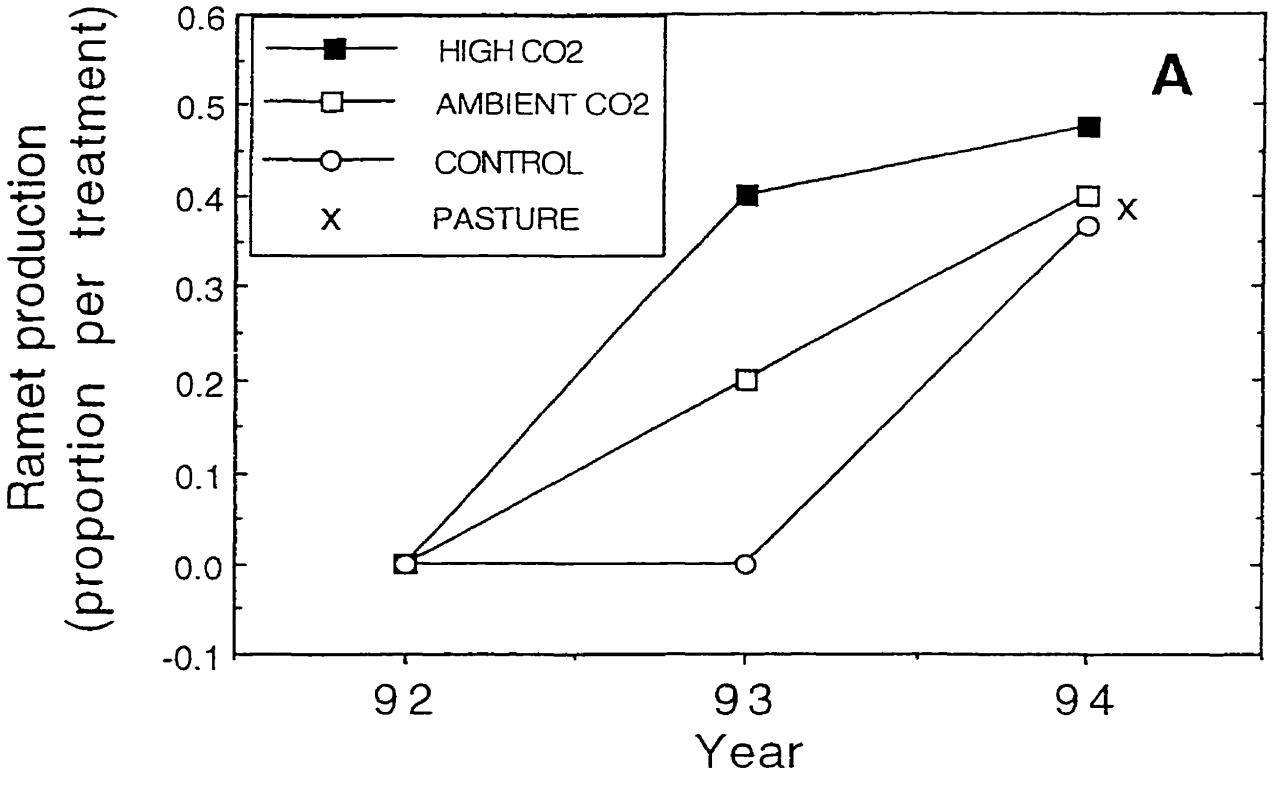
There was a strong elevated CO_2 effect on the allocation to reproductive traits in *Taraxacum* (Table 2a). Overall, twice as many inflorescences were produced in 1994 under High CO_2 (20.4 ± 11.9 a) compared with Ambient CO_2 (10.5 ± 4.1 bc), while the Control had 19.1 ± 10.6 c. There was a significant CO_2 effect on reproductive output per unit size in the 1994 growing season. For each inflorescence produced in the spring, twice the vegetative biomass (g) was found under elevated CO_2 than in the ambient CO_2 chambers (High CO_2: 1.12 ± 2.60, Ambient CO_2: 0.57 ± 1.35, Control: 0.32 ± 0.46).

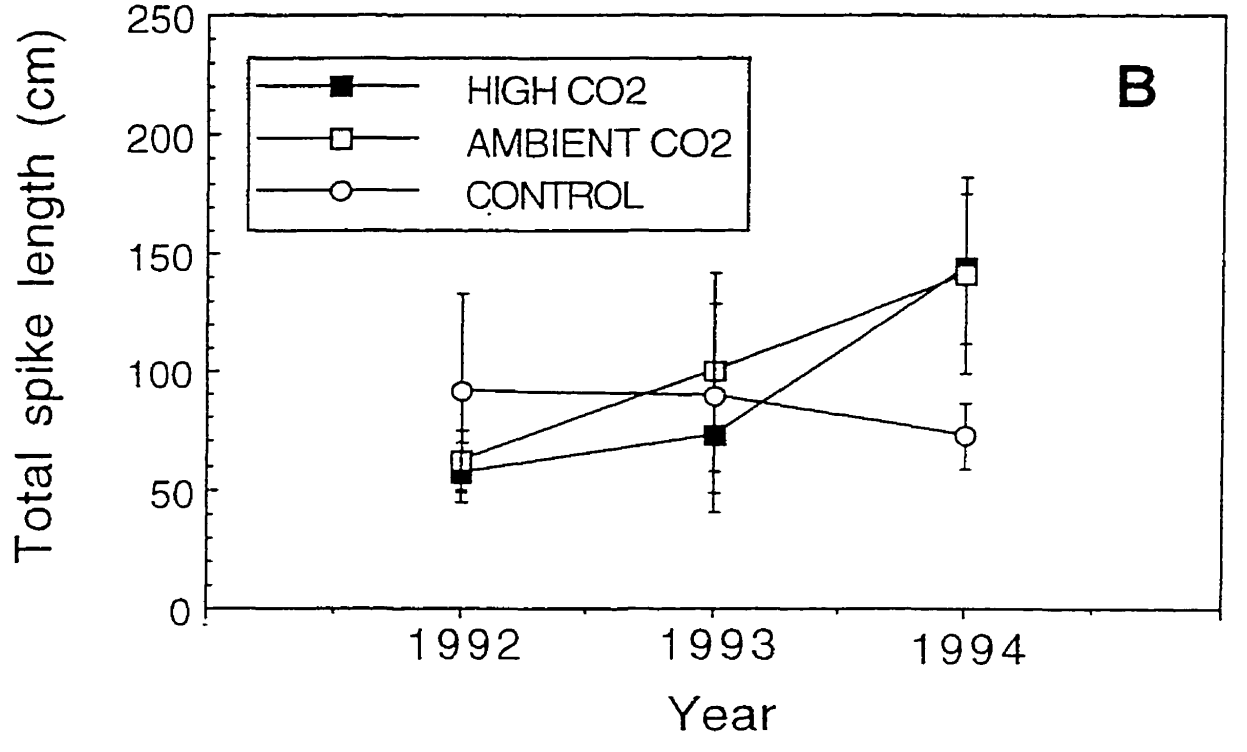
There was also a significant effect of CO_2 on enhancing inflorescence production per below-ground size of the previous year (Table 4). This measure allows for comparisons of allocation based on an estimate of storage acquisition the previous season. Because of the strong relationship between leaf number and the below-ground mass of

Figure 10

Clonal propagation and sexual reproduction in *Plantago major* over three years of elevated CO_2 treatment. (A) Ramet production. (B) Spike production. Ramet production determined as proportion of all plants in treatment that showed production of new ramets. Total spike length is sum of all spikes on the plant. Symbols as in Figure 1, and pasture, denoted by " X ". Mean ± 1 standard deviation. Sample sizes as in Tables 1b and 5 and for pasture, n = 60.

Table 4


Ramet production
(proportion per treatment)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
HIGH CO2
AMBIENT CO2
CONTROL
X PASTURE
A
X
92
93
94
Year

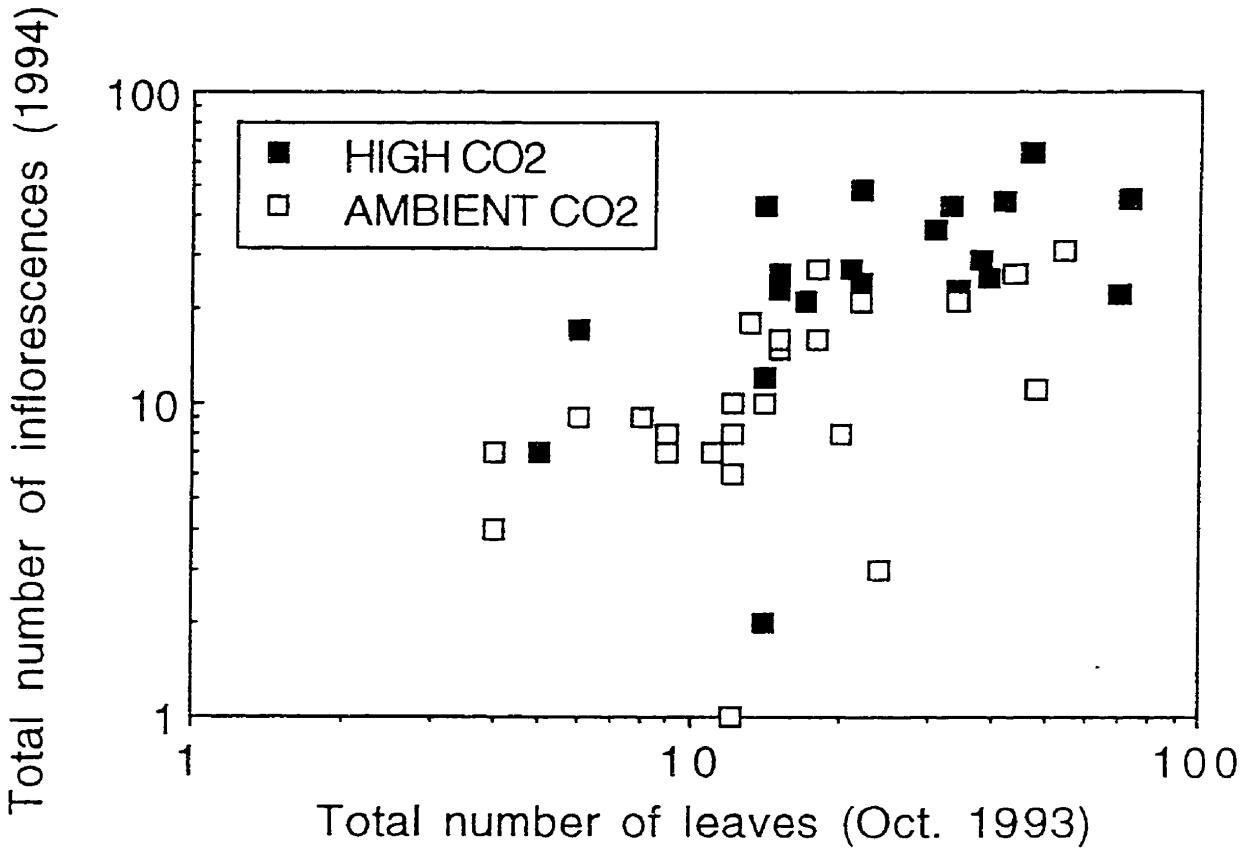

Total spike length (cm)
250
200
150
100
50
0
HIGH CO2
AMBIENT CO2
CONTROL
B
1992
1993
1994
Year

hypocotyl and root (Figure 7), leaf number at the end of the 1993 growing season was used as an indicator of relative storage size for reproduction the following spring. Under elevated CO_2, over twice as many inflorescences were produced in 1994 for plants of equivalent size at the end of the 1993 season (Figure 11). The allocation to reproduction was also examined using leaf area cover in September 1993 as a covariate in order to compare inflorescence production by above-ground cover size. A significant effect of elevated CO_2 was still found ($F = 4.47$, $p < 0.05$) though the magnitude of enhancement was reduced to 1.6-fold.

Unlike the strong response found in *Taraxacum*, there was no elevated CO_2 effect on reproductive allocation in *Plantago*, expressed relative to biomass at the end of study (Table 2b). Total spike length was used as an estimate of reproduction over the three growing seasons (1992-1994) (Figure 10b). Within each year there was no effect of CO_2 on either the number of spikes per plant or the total spike length (Table 5). However, between years, CO_2 enhanced the relative increase in reproduction. Between the first (1992) and second (1993) seasons, High CO_2 showed only a 27 % increase in spike production. This was the year that ramet production was high. But, from 1993 to 1994, High CO_2 showed an increase of 114%. The ambient CO_2 chambers showed a constant 40% increase each year. The effect of chamber wall appeared to differ with time. In 1992, the first year of the study, the two CO_2 chamber treatments produced only 50% the spikelength of the controls, perhaps caused by the disturbance of chamber installation the previous fall. In 1994, the two CO_2 chamber treatments produced twice the spikelength of the control plots. The control plots differed little in spike production from the plants of the surrounding pasture (data not shown).

Figure 11

Allocation to reproduction in *Taraxacum officinale*, 1994. Vegetative storage estimated by total leaf number in October of 1993, and reproductive output by total number of inflorescences produced throughout the flowering season. Symbols as in Figure 1. Sample size, n=18, High CO_2; 23, Ambient CO_2.


Total number of inflorescences (1994)
100
10
1
HIGH CO2
AMBIENT CO2
1
10
100
Total number of leaves (Oct. 1993)

Plant longevity and mortality

Under elevated CO_2, there was a trend of lower mortality in both species. In *Taraxacum* examined over 1993 and 1994, only 4 % of studied plants died in the High CO_2 chambers as compared with the ambient CO_2 and control plots where 14 % died ($x^2 = 2.13$, $p > 0.10$). In *Plantago* during the same period, a trend of a 15 % lower mortality was observed in High CO_2 compared to Ambient CO_2 ($x^2 = 2.25$, $p > 0.10$). Where minimum plant age was known, there were no differences detected in longevity.

DISCUSSION

Long-term Responses to Elevated CO_2 in Natural Systems

We examined the reproductive response to CO_2 *in situ,* in the natural community, since the responses of plants grown in multi-species stands usually differ from those of individuals grown alone (Bazzaz and McConnaughy 1992, Korner et al. 1996a). Our study is among the first to examine reproductive responses to elevated CO_2 within intact natural populations of perennial forbs and to our knowledge, the first in a pasture community. Long-term examinations over the entire perennial life history are particularly important (Loehle 1995). Since growth responses to CO_2 can change with ontogeny (Poorter 1993, Poorter et al. 1996), the large vegetative phase response may not be maintained through the reproductive phase. Our system allowed the natural environmental variability and numerous interactions with the abiotic as well as biotic components of the community. Such natural study systems have been recently advocated as essential (Korner 1995) as they provide a realistic sense of response, relative to the natural conditions. In a community exposed to elevated CO_2, over time there will be changes in species composition (Potvin and Vasseur 1997), plant competitive interactions (Stewart and Potvin 1996) and interactions with environmental variables (Vasseur and Potvin (in press)) including soil nutrients (e.g. Hungate et al. 1996, Berntson and Bazzaz 1997, Zak et al. 1993). All these cannot be simulated adequately in short-term studies in controlled environments.

In our study, *Taraxacum* showed an even greater enhancement than observed previously with plants grown singly in pots (Poorter 1996). This suggests the true growth potential was more manifest within the natural competition and soil system background than within the space restriction of the pots. Pots have limitations for studying CO_2 effects on reproduction as their growth conditions may over- or under- estimate responses

(McConnaughay et al. 1993). The strong enhancements in hypocotyl traits in *Taraxacum* and fibrous root mass enhancement in *Plantago* suggests shifts in the contribution of these species to below-ground processes under elevated CO_2 (Norby 1994, Rogers et al. 1994). These below-ground enhancements support claims that fibrous weed management will be more challenging in the future (Patterson 1995). It is likely that the strong responsiveness of Taraxacum was facilitated by the high soil fertility of a recently abandoned pasture ecosystem, as resource-rich ecosystems are expected to produce larger short-term productivity increases to elevated CO_2 (Curtis et al. 1989, Koch and Mooney 1995).

Predictions of long-term response of species and communities to elevated CO_2 requires examining impacts on relative fitness. Such changes can indicate shifts in the future role of the species in the ecosystem (Carpenter et al. 1993, Chapin et al. 1996, Shaver et al. in press). For *Taraxacum*, considering together the components of a two-fold increase in mass (size), and two-fold increase in number of inflorescences per size class, no change in mortality, and assuming no decrease in seed number, the overall fitness enhancement after three years was estimated to be at least four-fold under elevated CO_2. The wider capitulum diameters found in plants grown in elevated CO_2 may allow space for a higher seed mass or number (Jablonski and Potvin, in preparation). In *Plantago*, the relative increase in spike production suggests that the relative fitness enhancement was 1.8-fold during the three years of our study. In both species, there could be seed weight, number and quality differences that would affect the fitness estimate (Jablonski and Potvin, in preparation). Possibly, the higher relative growth rate in *Taraxacum* (Poorter 1993) contributed to it being more responsive than *Plantago*. If the increased growth accompanied a decreased life-span under elevated CO_2, a lifetime fitness benefit might not be incurred. However, because there was no evidence of mortality differences with CO_2 treatment over three years, our study does provides evidence that increased lifetime

reproductive output (and thus fitness) would be greater under elevated CO_2 to a strong extent in *Taraxacum* and to a lesser degree in *Plantago*.

Responses of these two species to elevated CO_2, examined over the entire life history, were greater in magnitude and relative difference than detected in previous short-term, controlled-environment studies. To ascertain the importance of storage perennials as a carbon sink under future global change, other species should be assessed over the long-term and within their native ecosystem.

Reproductive Functional Groups Based on Source-Sink Relations

We hypothesized that reproductive response under elevated CO_2 would be greater in plants possessing an increased non-foliar storage capacity. In controlled environment studies, below-ground responses to CO_2 have been commonly found, and increased root:shoot ratios less frequently (Rogers 1994, Norby 1994). Only a few studies have examined storage root or organ responses to CO_2 in natural species, and enhancement has been shown (e.g. Curtis et. al. 1990, reviewed in Norby 1994, Farnsworth et al. 1996). These below-ground stores can maintain perennial longevity via growth, can support re-growth after herbivory or grazing, and provide resources for reproductive metabolism and seeds (Chapin et al. 1990). The impact of this storage enhancement under elevated CO_2 on reproduction has been investigated in controlled environments (Jablonski 1997). However, little is known about CO_2 effects on the allocation of resources from these plant storage sinks in natural environments (e.g. Korner et al. 1996a).

Maximal responsiveness to a carbon resource is maintained when source or sink strength are enhanced by either increased activity or size (Ho 1988). Supporting our hypothesis, hypoctyls were enhanced under elevated CO_2, and the larger hypocotyl size of *Taraxacum* corresponded with an overall increase in reproduction. The strong responsiveness of *Taraxacum* may be due to both the morphology of a large hypocotyl

storage which was not restricted in size, and the likely dependence of reproduction predominantly with that storage, both in an indeterminate formation of buds and in resource provisioning. The trend of reproductive response to CO_2 in *Plantago* appeared only through promotion of clonal growth. *Plantago* may be more constrained, since caudex storage is relatively small. Additionally, spike number is limited by the leaf number (Kuiper and Bos 1992) which was unresponsive to CO_2. The reliance on leaf storage in *Plantago* may limit CO_2 responsiveness due to photosynthetic acclimation or downregulation (Arp 1991). This occurs when there are insufficient sinks such that there is a feedback with leaf carbon build-up and lowering of photosynthesis rate (Bowes 1993).

We suggest that functional group characterization based on morphology of source-sink relations may be most helpful in elucidating responsiveness to elevated CO_2 in the reproductive phase. Detecting functional group responsiveness to elevated CO_2 work is an important aid for future predictions of species responses (Korner 1993b, Korner 1996b). Previous functional group categories in elevated CO_2 studies have been predominantly based on traits of vegetative phase physiology. These have failed to predict reproductive responsiveness, which has been more variable (Ackerly and Bazzaz 1995). Our results suggest that the trait of large vegetative phase sink strength, such as the possession of a below-ground storage organ, may best indicate reproductive responsiveness to elevated CO_2. The greatest reproductive responsiveness of annuals with indeterminate growth form (Ackerly and Bazzaz 1995) supports the sink strength criteria.

Our study provides information pertaining to how storage-mediated enhancement of reproduction may occur under elevated CO_2. The combined traits in *Taraxacum* of increased storage hypocotyl diameter, number of rosettes and leaves, and overall root spread likely allowed for greater extension, foraging for light and soil resources, and decreased vulnerability to predation. Hypocotyl growth likely enhanced longevity, since any fragment of the hypocotyl is capable of regenerating the clone (Solbrig 1971).

Enhanced leaf area likely increased the source strength and reproductive output as leaf area enhancement is the vegetative trait that has correlated strongest with reproductive response under elevated CO_2 in hypocotyl-storing plants (Jablonski 1997). Source-sink relations would be enhanced by the continued growth evident by *Taraxacum* size. The predominance of the elevated CO_2 plants in the largest size class in *Taraxacum* (Figure 7) supports the suggestion that a larger maximal size may be reached under elevated CO_2 (Loehle 1995), but a longer period than three years is needed to verify this. At a community level, it is likely that size increase conferred a competitive advantage. It is possible that other species-specific traits other than storage may have contributed to the strong response of *Taraxacum* relative to *Plantago*. The importance of this trait could be verified by using genetically related individuals that differ in storage traits (e.g. Jablonski 1997). Our reproductive phase results support the role of developmental pattern and plasticity of source and sink structures in determining responses to elevated CO_2 (Mousseau et al. 1996, Reekie 1996).

Functional group considerations should also be given to traits unique to the reproductive phase. Capitulum diameter enhancement in *Taraxacum* appears to be a direct effect of CO_2 on the reproductive phase, at it was independent of both the size and time of maturation. Although high CO_2 has long been used as a horticultural short-term treatment to enhance flowering and bud formation (Wittwer 1983), little is known about the response of reproductive structures in wild species during long-term exposure. Inflorescence heads of white clover were also enhanced in size by CO_2 in model eco-systems (Overdieck and Lieth 1986). These results suggest that capitulum size increase may be a consequence of increased reserves available to floral structures. Reproductive responsiveness may be greater in species that have such trait plasticity. In a grassland microcosm study, Composites, the family to which *Taraxacum* also belongs, showed more positive reproductive responsiveness to CO_2 than the other families (Roy et al. 1996).

Plantago showed a relatively smaller reproductive responsiveness to CO_2. The increased ramet production and relative increase in spike length the following year, demonstrates the fitness advantage of splitting in *Plantago*. There is more seed output from additional ramets than from maintaining them on the same mother plant (Hawthorne 1974). Vegetative phase studies of *P. major* under elevated CO_2 have shown that though responsive initially, the self-shading caused by increased light area capture limits the responsiveness (Poorter et al. 1988). Over a longer term, the increased ramet production in *P. major* would likely counteract the shading, by allowing for an increased spread and area cover. These new ramets may become a predominant sink, thus limiting allocation to the root (Fonseca et al. 1996). Reproductive allocation may be relatively fixed in *Plantago,* as the lack of a change observed in this study is similar to previous responses of *P. major* under a varying carbon regime utilizing light (Hawthorne and Cavers 1978). *P. lanceolata* showed a decreased response in reproductive mass and allocation under elevated CO_2 (Fajer *et al.* 1991). Vegetative allocation as indicated by constant root:leaf and petiole:blade ratios in our study, and by a constant growth pattern in the CO_2 study of Fonseca *et al.* (1996), also appears to be fixed and may have constrained response. Inflorescence structure type and plasticity may be important morphological traits for functional group characterization.

Phenological Shifts with Global Change

There were distinct, consistent delays in the onset of *Taraxacum* phenological stages under elevated CO_2. This could be explained by the delay on the initiation of reproduction and the shifting of all other events similarly. That the delays were not dependent on plant size or years of exposure to CO_2 suggests they are a direct and immediate response to elevated CO_2. The delay may be related to developmental effects mediated via phytochrome which have been shown under elevated CO_2 (Reekie 1996).

Although the spring-flowering *Taraxacum* is functionally day-neutral, it showed the delayed onset of flowering that has been observed in short-day plant response to CO_2 (Reekie et al.1994, Roy et al.1996). However, lack of flowering response to CO_2 observed in the long-day *Plantago* differs from the earlier response often found in long-day annuals (Reekie et al.1994). The delays in *Taraxacum* could also be caused by changes in resource storage or utilization under elevated CO_2. It is plausible that the increased reserve C stores that may accumulate under high CO_2, could shift the series of metabolic changes that *Taraxacum* undergoes during the transition from overwintering to spring growth (Cyr et al. 1990) and affect the metabolism of reproductive initiation. Delays in tree budbreak under high CO_2 have been by as much as two to three weeks (Mousseau et al. 1996, Murray et al. 1994, Korner 1995) and could involve changes in carbon relations. Further examination of storage and shifts in overwintering processes in perennials under elevated CO_2 is needed.

While most global change studies assume an overall temperature enhancement that would advance flowering day (though in some cases it is delayed) (eg. Fitter et al. 1995, Lechowicz 1995, Kramer 1995) our results suggest that the direct effect of elevated CO_2 may sometimes be the opposite. Any delay in onset caused by CO_2 could potentially counteract any benefit. However, in our community situation, the delay in *Taraxacum* flowering did not appear to disadvantage the overall seed production. On a whole plant level, the rate of inflorescence production was nearly doubled under elevated CO_2, as the high CO_2 plants were able on average to mature twice the number of inflorescences as in ambient CO_2 during a similar reproductive duration. This was likely an advantageous response as the relative time that a reproductive structure was exposed to a risk of damage such as by predators or inclement weather was reduced (Hendrix 1988). The shift in onset of flowering and seed maturation events could confer a potential disadvantage in a community situation where other members were enhanced in growth or flowering, and

could thus outcompete *Taraxacum*. It could also be a disadvantage in a community if it disrupted the synchrony with predator avoidance. The seasonal decline that occurred in average inflorescence maturation time in *Taraxacum* was likely due to the warmer temperatures and increased daylength of the days approaching the solstice.

The increased carbon resource under elevated CO_2 may have contributed to extending the flowering season in *Plantago*. That a higher percentage of the reproductive spikes had not fully senesced by the end of the growing season, suggests a potential for an increased reproductive output that could be realized in warmer climates or in the longer growing seasons which will be likely under an increased CO_2 regime. Since after-ripening of green structures can occur, it is possible that these seeds had adequate resources even if not fully mature.

The differential responses of the species in our study of growth, flowering initiation and duration suggest that the timing and synchrony of events in this pasture community will change under a future global regime. A similar differential response to CO_2 has been shown in flowering phenology for co-occurring annuals (Reekie and Bazzaz 1991, Roy et al. 1996) and in growth phenology for grassland species (Campbell et al. 1995). Changes in reproductive phenology and output in storage perennials could affect ecosystem level processes by altering resource flow, since the onset of the reproductive phase causes a dramatic shift of nutrient resources from below-ground storage organs to above-ground seeds. Any shifts in species responses under elevated CO_2 could have a strong impact on community and ecosystem dynamics, such as synchrony with herbivores (Ayres 1993) and cycles of nutrient use (Korner et al. 1996b, Curtis et al. 1994).

CONCLUSION

Long-term studies of global change on natural ecosytems should include a focus on the reproductive phase of plant responses, particularly in perennial forbs. The strong enhancement of both hypocotyl storage and relative fitness in *Taraxacum*, suggests storage organ capacity may be a functional indicator of reproductive responsiveness to elevated CO_2. It is imperative that new criteria for functional groups be developed for the reproductive phase, considering morphology of storage and inflorescence structure. The direct effects of CO_2 on phenology may counteract earlier flowering onsets predicted by temperature increases. Some effects of elevated CO_2, such as on flowering onset were observed in a single season, but most other responses were only manifested after several seasons of exposure. Species-specific responses of perennials in vegetative growth, phenology and reproductive responses could contribute over the long-term to shifts in community and ecosystem dynamics under elevated CO_2.

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CHAPTER 3

Carbon and nitrogen physiology underlying reproductive responses to elevated CO_2 in two forbs in a natural pasture

ABSTRACT

We examined whether the physiological and increased C:N typically observed in the vegetative phase under elevated CO_2 altered seed traits. We hypothesized that plant N acquisition would be greater under high CO_2 and correlate with increased seed number. We also hypothesized that reproductive photosynthesis would be strongly enhanced in plants with storage organs and that the additional C would contribute to seed metabolism and seed mass. The rosette perennials, *Plantago major* and *Taraxacum officinale*, growing in an intact pasture community under open-top chambers at CO_2 levels of 650 $\mu L\ L^{-1}$ and 350 $\mu L\ L^{-1}$ were examined at the end of the second, and during the third enrichment seasons. Under elevated CO_2, leaf photosynthesis was enhanced over two-fold. Time-courses of photosynthesis and chlorophyll suggest senescence was delayed. On a whole plant basis, C:N ratio did not increase, indicating that N acquisition was enhanced with biomass increases under elevated CO_2. In *Plantago,* allocation shifted to increased N in the storage caudex and increased C in the fibrous root. Vegetative total N predicted seed number in all cases, suggesting a similar N limitation. An increase in seed number and decrease in variability of weight and number in *Taraxacum,* and an increase in seed weight in *Plantago*, with no changes in the seed C:N ratio, suggests an enhancement in fitness and the role of maternal effects under elevated CO_2. This study emphasizes the importance of studying physiological traits over the entire life history and within the natural ecosystem to best predict future responses to global change.

INTRODUCTION

An increase in C relative to N is a common vegetative phase response to elevated CO_2 (Conroy and Hocking 1993, Wong 1979, Drake et al. 1996, Koch and Mooney 1996). Little is known about the impact of shifts in C and N physiology on seed production, a process that is influenced by accumulation of C and N (Willson 1983, Fenner 1986 a,b) and by reproductive phase photosynthesis (Potvin and Charest 1991). In seeds, the C stores provide the energy for germination and initial growth while the N stores provide the enzymes of metabolism (Willson 1983), so any changes in C:N contents can affect performance. In leaves under elevated CO_2, typically storage C accumulates, while N declines because there is a decreased physiological need due to greater photosynthetic efficiency. Depending on how tightly coupled seed production is with leaf resources, this may change the availability of C and N to seed production and may, in part, account for the range of reproductive responses observed. Reproductive biomass enhancements to CO_2 doubling in natural species have been less in magnitude than in the vegetative phase, on average 26% (Ackerly and Bazzaz 1996) compared with 33% (Kimball 1983, Poorter et al. 1996). Reproductive responses have also been more variable (Newton 1991) and have not been predicted from vegetative biomass responses (Ackerly and Bazzaz 1996, Jablonski 1997, Farnsworth and Bazzaz 1995). Where seed traits have been examined, CO_2 has been sometimes shown to increase seed parameters of total number and mass (Jackson et al.1994, Garbutt and Bazzaz 1984), performance (Wulff and Alexander 1985) seed N (Jackson et al. 1994) and total N amount in reproductive output (Garbutt et al. 1990, Zangerl and Bazzaz 1984). Since seed mass is generally determined by resource amount (Fenner 1986b), and seed number by N (Fenner 1986a, Benner and Bazzaz 1988), CO_2 effects on seed traits suggest that altered C and N physiology may impact the reproductive phase. Seed performance measures of quality

(e.g. germination, seedling growth) have not always correlated with traditional fitness measures of seed mass (Wulff and Alexander 1985) or seed number (Farnsworth and Bazzaz 1995), suggesting that seed nutrient differences may underly the response. Decreases have also been found in traits related to fitness, depending upon genera and species (Farnsworth and Bazzaz 1995) and genotypes (Curtis et al. 1994b), suggesting that response to CO_2 may require particular functional traits.

The functional group most responsive to CO_2 in the vegetative phase (enhanced 50% in biomass on average) is plants with below-ground storage organs (Poorter et al. 1996). Because these organs store nutrients that are not coupled with current photosynthesis, these plants may have a greater capacity to increase N acquisition under a CO_2-rich regime. As well, the capacity for increased storage may effect reproductive traits. We hypothesized that under elevated CO_2, the altered vegetative physiology of C and N use would increase seed mass and number. To assess physiological use of N we examined the relationship between plant total N and seed number which we expected to be strong (Fenner 1986a,b). Shifts in this relationship might indicate physiological changes underlying seed production under elevated CO_2. We expected that reproductive photosynthesis would be strongly enhanced under elevated CO_2 because of the large storage organ sink capacity, and that the additional C would provide for seed metabolism. We examined resource provisioning to seeds within the inflorescence to assess the role of maternal effects under elevated CO_2. Since resource supply is typically related to seed mass and can promote seed filling, we predicted that there would be an increase in mass per seed and a decrease in the variability of seed mass and number under elevated CO_2.

Our focus species were the two dominant rosette perennial forbs of an intact pasture community (Potvin and Vasseur 1997). After three years of exposure to elevated CO_2, *Taraxacum officinale* which possesses a large hypocotyl storage, showed a strong biomass enhancement, allocation to reproduction and enhancement in inflorescence size

(Jablonski et al. unpublished). In that same study, the less responsive *Plantago major,* which has more storage in the leaves, produced more ramets and showed only a trend of increased reproductive structures. In the present study, we examined traits of both vegetative and reproductive parts together, to see if differential physiological responses of the two species accounted for the biomass response. We expected that since these species have adapted to intense grazing pressures, they would have more N in storage, rather than in direct physiological use (Chapin et al. 1990). We expected *Taraxacum* to be relatively more responsive to CO_2 than *Plantago* because of the larger non-photosynthetic storage organ of the hypocotyl. To adequately assess resource use and reproductive response, differences in C:N quality of the organs underlying the biomass responses were examined. To assess relative fitness, reproductive structures and the number, mass and packaging of seeds were examined. Such measures of both quantity and quality of seeds produced (traits related to fitness) are necessary to predict the impact of elevated CO_2 on reproductive performance of individuals in a natural community. This has been rarely done in the same study (Garbutt and Bazzaz 1984, Tousignant and Potvin 1996, Farnsworth and Bazzaz 1995), and only with annuals in controlled environments.

Only a few prior physiological studies have examined reproductive traits over the long-term or in natural systems (e.g. Jackson et al. 1994). Our use of a natural community system eliminated pre-determined choices on fertility levels or unnatural constraints on root growth which can affect source-sink relations (Jablonski 1997). This allowed as to assess performance within natural community processes, where elevated CO_2 may indirectly or directly alter soil N positively (Hungate et al. 1996) or negatively by its impact on the microbial community. Use of a natural system is particularly important for a realistic study of reproduction, which can be influenced by natural environmental fluctuations (Jablonski et al. unpublished). This study provides important information regarding resource use for seed production in storage perennials, the

dominant vegetation type of north temperate systems. Shifts in C:N content of the vegetative and reproductive tissues are important to examine, as these could have impact on ecosystem processes..

METHODS

Study site

Our research site was on an intact pasture located in Farnham, Quebec, CA (45°17'N, 72°59°W), 45 km southeast of Montreal, Canada. It had been grazed, but not ploughed for ten years prior to the commencement of our study in the fall of 1991. Four open-top chambers of 3m diameter and 2.4 m height were set up. Two chambers were maintained at 650 ± 50 μL L^{-1} CO_2 (High CO_2), and two at ambient air flow (approximately 355 μL L^{-1} CO_2) (Ambient CO_2) during the frost-free season. Two circles of the same area but without chamber walls, served as control plots (Control). Details of the site, chamber design and environmental parameters measured have been previously described (Stewart and Potvin 1996). The chamber wall environment contributed to slight reductions in total precipitation and increases in the temperature of the air but not the soil. Light intensity was also reduced depending upon the time of day and plant location. There were no differences between the High CO_2 and Ambient CO_2 treatments in any climate parameter nor were there any differences among the replicate chambers within each treatment. The present study reports physiological measurements made during the second (1993) and third (1994) seasons of CO_2 exposure.

Plant Material

Our focus species were the perennial rosette weeds, *Plantago major* L. (common plantain) and *Taraxacum officinale* Weber (common dandelion). These were the dominant forbs in the community which had maintained their presence under elevated CO_2 after three years, while other forbs had declined (Potvin and Vasseur 1997). Both species are polycarpic rosette perennials that are long-lived and they produce inflorescences that are

many-seeded (Hawthorne 1974, Solbrig 1971). They are both adapted to disturbed, trampled areas and represent contrasting vegetative growth and reproductive strategies. *Taraxacum* possesses a large hypocotyl and a springtime mass flowering born on capitula. *Plantago* has a small caudex and spikes are continually produced from mid-June onwards possessing inflorescences and then seeds. *Taraxacum* is apomictic, while *Plantago* is self-compatible and wind-pollinated. Thus, we assumed that influences on seed production are primarily from vegetative resource availability.

Growth, biomass and phenology responses over the long-term study have been reported (Jablonski et al. 1997). Here we report reproductive characteristics and underlying physiological measurements made in the late summer 1993 to fall 1994 in *Taraxacum*, and throughout 1994, the third growing season, in *Plantago*. Plants were chosen at random among the healthy individuals. For *Plantago*, plants that produced at least 4 inflorescences were used. In *Plantago*, sample size was five plants per chamber (10 per environment). In 1994, sample size was increased to census nearly all individuals (n= 19 High CO_2, 10 Ambient CO_2, 17 Control). For *Taraxacum*, sample sizes of about 10 per chamber were chosen in 1993 (n= 25 High CO_2, 20 Ambient CO_2, Control), and individuals were also followed in 1994.

Photosynthesis measurements

Source leaf photosynthesis was measured during the peak of the flowering and seed production period in each species using a portable open-system infrared gas analyzer (Model LCA-2 by ADC, Analytical Development Co. Ltd., Hoddesdon, England). Air humidity in the growth chamber was determined from readings with the leaf chamber open and empty between every photosynthesis measurement, and then CO_2 assimilation rate (A) was recomputed according to von Caemmerer and Farquhar (1981). All measurements were taken *in situ* during the mid-day and only on days of full sunlight.

In *Taraxacum,* four sets of measurements were made from May 23 - June 15, 1994 on eight plants per chamber. Plants were chosen at random from among those that possessed inflorescences and were sampled in random order without replacement as follows: Each measurement period was divided over several days and a subset of half the plants in one chamber of each treatment was first assessed in random chamber order over one or several days if necessary. For each plant measurement time, two mature, healthy, and non-senescing leaves were chosen and the values averaged. Initiation of flowering occurred during the last two weeks of May and first week of June; seed maturation occurred during the first two weeks of June (Jablonski et al. unpublished).

In *Plantago*, three sets of photosynthesis readings were made over a two-week period from August 11 to 26, 1994. For photosynthesis and other physiological traits, six plants from each chamber (except C1, n=5) were measured that each had at least four reproductive structures. At the start of the measurement period, leaf and reproductive stalk pairs were chosen and marked on each plant of two stages: a) older leaves associated with stalks "seed-filling" (full anthesis had occurred), and b) younger leaves that were associated with reproductive stalks that were initiating and flowering, "flowering". On average, spikes take nine days from initiating to first flowering, and at least 35 days from flowering to mature seeds (Jablonski et al. unpublished). Thus, repeated measurements were made on the same leaf three times over the two-week period.

Leaf metabolites

Leaf metabolites of specific leaf mass (mass per area), chlorophyll and C:N ratio were assessed for both species. In *Taraxacum,* measurements were taken on the same plants three times: during the last week of August, 1993, at the time of completion of photosynthesis measurements in mid-June 1994, and during the last week of September, 1994. At each measurement time, chlorophyll and leaf mass was assessed on two leaves,

and the values averaged. Chlorophyll concentration was measured using a chlorophyll meter SPAD-502 (Minolta Corp., Ramsey, N.J.) and determined as (Monje and Bugbee 1992; and B. Bugbee, personal communication):

$$[1]\quad CHL\ (mg/m^2) = 1.034 + 3.08 * [SPAD] + 0.110 * [SPAD]^2$$

where SPAD = the machine units. Measurements were taken as the mean of the four leaf quadrants. For the reproductive period, the measure was taken just prior to the photosynthesis measurement, on the same leaf.

Once photosynthesis measurements were complete for the set in June 1994, and after the chlorophyll measurements at the other times, specific leaf mass was determined. Depending upon leaf size, six to ten interveinal disks of 0.59 cm^2 area were removed from each of two leaves per plant and dried at 65°C for 72 h, then weighed on a microbalance. In *Plantago*, leaf metabolite measurements were made separately for the younger flowering and older seed-filling leaves. To assess leaf chlorophyll and extent of decline with leaf aging, chlorophyll was measured at two times on the identical leaf, first mid-way through the time of photosynthesis measurements (August 18), and second when the corresponding reproductive heads were maturing seed (September 1st). Leaf metabolite disks were taken as for *Taraxacum*, but only at one time, with disk area per leaf totalling 0.817 cm^2. To partially account for the differences in leaf age, the older "seed-filling" leaves were sampled on August 28, and the younger leaves on September 3.

Seed mass and number

In both species, reproductive structures were checked daily for maturation. On the maturation day, size was measured (spike length in *Plantago* and capitulum diameter in *Taraxacum*). Inflorescence structures were then cut where they joined the stalk and any seed loss through wind or handling was estimated. Structures with their seeds were dried

at 30 - 35°C to allow for ripening, without altering the viability of the seeds for future studies.

To estimate seed production for the whole plant, the relationship between reproductive structure size and actual seed number was examined on a subset of inflorescences on a subset of plants, all chosen randomly. Only reproductive structures that had not lost any seeds (that had 95% or more of seeds) were used. In *Taraxacum*, six capitula were examined in detail for each of six plants per chamber (12 plants per environment). Seeds were removed from the capitulum and weighed (including the pappus, which was less than 10% of biomass - data not shown). The capitulum head without the seeds was also weighed. A random subsample of 100 seeds was weighed to determine average seed mass (hundred-seed mass). Hundred-seed mass was then used with the total seed mass per inflorescence to estimate the total seed number for the capitulum. Total seed number per plant was then estimated as the product of the total number of inflorescences and the average seed number per inflorescence.

In *Plantago*, potential differences in seed production per spike were examined by determining the density of capsules per spike length (the capsule abundance) as well as the packing of seeds within the capsule (seeds per capsule). A set of five spikes per plant were chosen for analysis, each matured within one 10-day period (from August 15 to October 13). On each, six intact capsules from throughout the length were randomly chosen, opened, and the number of seeds counted. Seeds from these capsules were combined with additional seeds to make up 100 seeds, and the hundred-seed mass determined. A subset of these spikes was used to examine capsule-packing relationship to spike length. The capsule number was counted in detail on a set of five spikes in one plant per environment. As well, the spike that matured on each plant in the middle of the series (first two weeks of September) on each of five other plants in that environment were counted. The relationship between spike length and capsule number were examined

by regression, and was found to be highly significant and did not differ significantly with sequence of initiation, or between plants. Thus total seed number production was estimated using the relationship between total seed mass per spike, spike length and hundred-seed mass.

Allometry of leaves and reproductive structures size

In *Plantago*, the relationship between leaf size and reproductive structures were examined to investigate if there were any allometric differences that could indicate carbon allocation differences. A series of the four leaves (two each of the flowering and seed-filing age) were examined. Prior to senescence, blade length, width, and petiole length were measured, and then leaves were traced on transparent plastic. The leaf area was analyzed on a DIAS II Image Analyzer (Decagon Devices, Pullman, WA, USA). There were highly significant relationships that did not differ with treatment.

Carbon: Nitrogen Analysis

A subset of plants, three from each chamber for each species were randomly chosen from those that had a complete suite of mass and physiology measurements. Prior to analysis, root and petiole tissues were ground on a Wiley mill. Portions of several interveinal blade disks from two leaves per plant were combined. Seeds were taken from one inflorescence per plant that was randomly chosen from those produced midway during the reproductive period. For each tissue, a 3 - 4 mg sample was analyzed on a Fisons EA 1108 Elemental Analyzer (City, Italy), and tissue %C and %N and determined using the K-factor method, with acetanilide used as the standard. Total plant values were then calculated using estimates of contents and biomass previously determined from plants harvested at the end of October, 1994 (Jablonski et al. submitted).

Statistical Analysis

Data was analyzed using SAS (SAS 1988 v. 6.0.4, Sas Institute, Cary, North Carolina, USA). After testing for normal distributions of measured variables, appropriate log transformations were made. The two species were analyzed separately. Type III sums of squares were used to account for missing data. Our model was a nested design with Treatment as a fixed factor and Chambers considered as random. Chamber(CO_2) replicated twice for each treatment, was used as the error term to test the main effect of the three Treatments (High CO_2, Ambient CO_2 and Control) in *Taraxacum.* In *Plantago*, all of the plants under ambient CO_2 treatment were taken from one chamber as all plants died in the other replicate because of the taller grass competition in that chamber (Potvin and Vasseur 1997). Because there was no significant Chamber (Treatment) effect for any parameter tested in this study or in previous studies of these species (Jablonski et al. unpublished, Potvin and Vasseur 1997, Stewart and Potvin 1996) chamber replicates were pooled for analysis of *Plantago*. Data for photosynthesis, and leaf metabolites was analyzed by ANOVAR to detect if there were differences over time (the weeks of photosynthetic measurements in both species, and chlorophyll measurement in *Plantago,* and the three seasonal measurements of leaf mass, chlorophyll, %C, %N and C:N in *Taraxacum).* Our Model accounted for variance due to Treatment (High CO_2, Ambient CO_2, Control), Time, and the interactions of Treatment and Time. All other traits were analyzed using ANOVA Proc GLM, testing for the effect of Treatment. For *Plantago,* an additional effect of Leaf(Plant) was used to test for differences in the leaves associated with spikes in the flowering or seed-filling age stages. To account for initial differences in size in the analysis of seed traits, two covariates, leaf number and maturation day were used. In all analyses, planned contrasts were used to test for the Treatment effect of CO_2 (High CO_2 vs Ambient CO_2) and the chamber wall (Ambient CO_2 vs Control). Relationships between *Plantago* leaf parameters of blade length, width and area; and

petiole and spike lengths; and *Taraxacum* capitulum diameter and seed mass and number were examined by regression analysis (Systat 5.2, Systat 1992, Evanston, IL, USA). The variability of inflorescence traits of seed number and mass in both species were examined. For each of six plants, the coefficient of variation (CV) values were calculated from six inflorescences per plant in *Taraxacum* and five in *Plantago*, and then analyzed for the High CO_2 and Ambient CO_2 treatments using the Kruskal-Wallis non-parametric analysis of variance test (Systat 5.2).

RESULTS

Photosynthesis

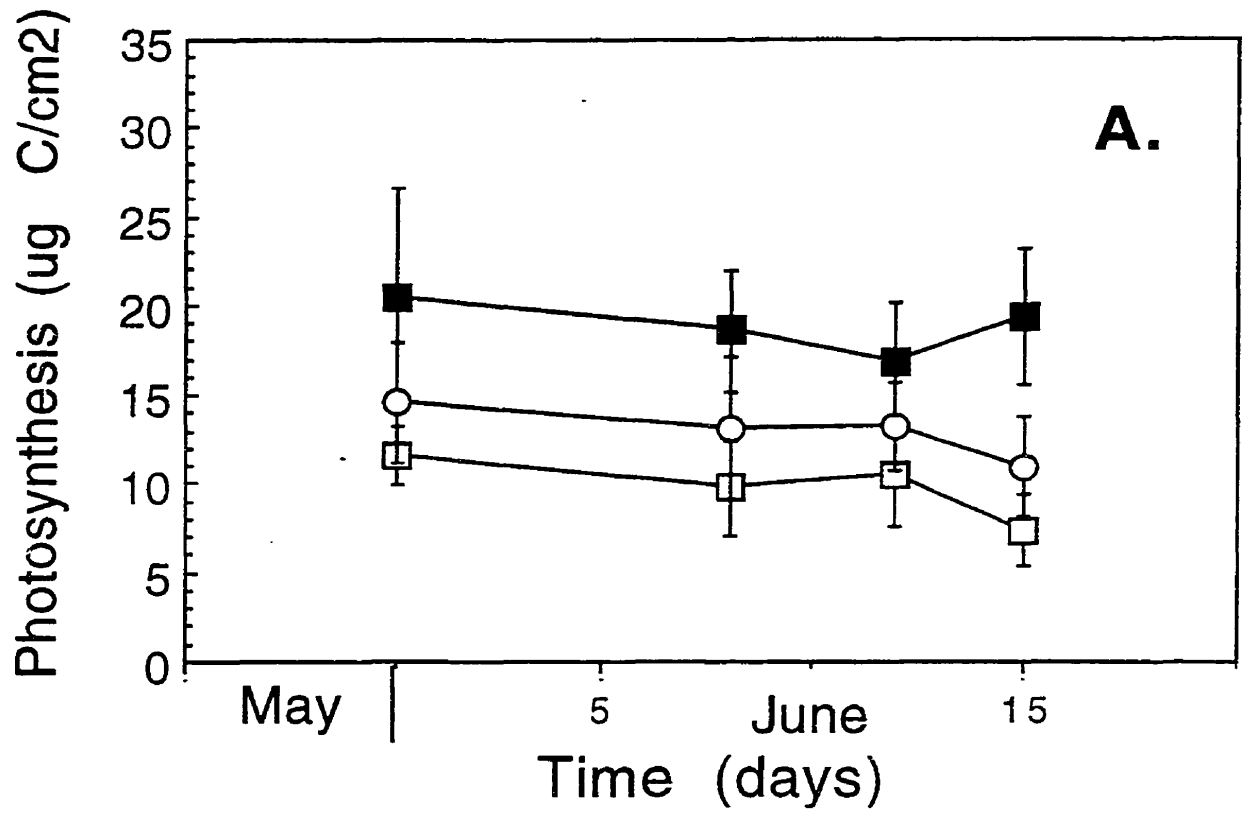
Photosynthesis rates were measured during the peak of the reproductive period in 1994 (Figure 1 A,B). Under High CO_2, rates were significantly higher than in Ambient CO_2 by 1.9-fold in *Taraxacum* and 2.0-fold in *Plantago* (Table 1). There was a significant effect of the chamber wall in reducing photosynthesis in *Taraxacum* (Table 1) as Control rates were higher than in Ambient CO_2 (Figure 1A). Photosynthesis differed significantly through time in both species. In *Taraxacum*, there was a significant CO_2 x time interaction. Under High CO_2, there was evidence of a longer photosynthetic duration, as higher rates were maintained through the fourth measurement period while Ambient CO_2 and Control plots showed over a 25% decline (Figure 1). In *Plantago*, CO_2 and chamber wall had no effect on the pattern with leaf age. Younger leaves associated with spikes in the flowering stage had higher photosynthetic rates (High CO_2: 44.2 ± 6.9, Ambient CO_2: 22.0 ± 1.4, Control: 24.2) compared with leaves associated with spikes in the seed-filling stage (High CO_2: 37.8 ± 4.3, Ambient CO_2: 20.2 ± 2.1, Control: 20.8 ± 2.8) but they were affected similarly by CO_2.

Source leaf metabolites

Source leaf metabolites were examined during the growing season to assess changes related to C and N contents. In *Taraxacum*, values were assessed from late summer 1993 to Fall 1994 (Figure 2). Chlorophyll was used as a measure of leaf photosynthetic N use (Figure 2E) and specific leaf mass served as an indicator of carbon accumulation (Figure 2D). There was no effect of high CO_2 on any leaf metabolite in *Taraxacum* including chlorophyll per area, specific leaf mass, %C, %N and C:N ratio (Table 2). There was also no significant effect of CO_2 on leaf chlorophyll expressed on a dry mass basis ($p > 0.10$). There was also an overall significant chamber wall effect on

Figure 1

Reproductive phase photosynthesis during the third season of exposure to CO_2, 1994. A. *Taraxacum officinale*. B. *Plantago major*. Mean ± sd. High CO_2 (solid squares), Ambient CO_2 (open squares), Control (circles). Sample sizes as in Table 1.


A.
Photosynthesis (ug C/cm2)
35
30
25
20
15
10
5
0
May
5
June
15
Time (days)

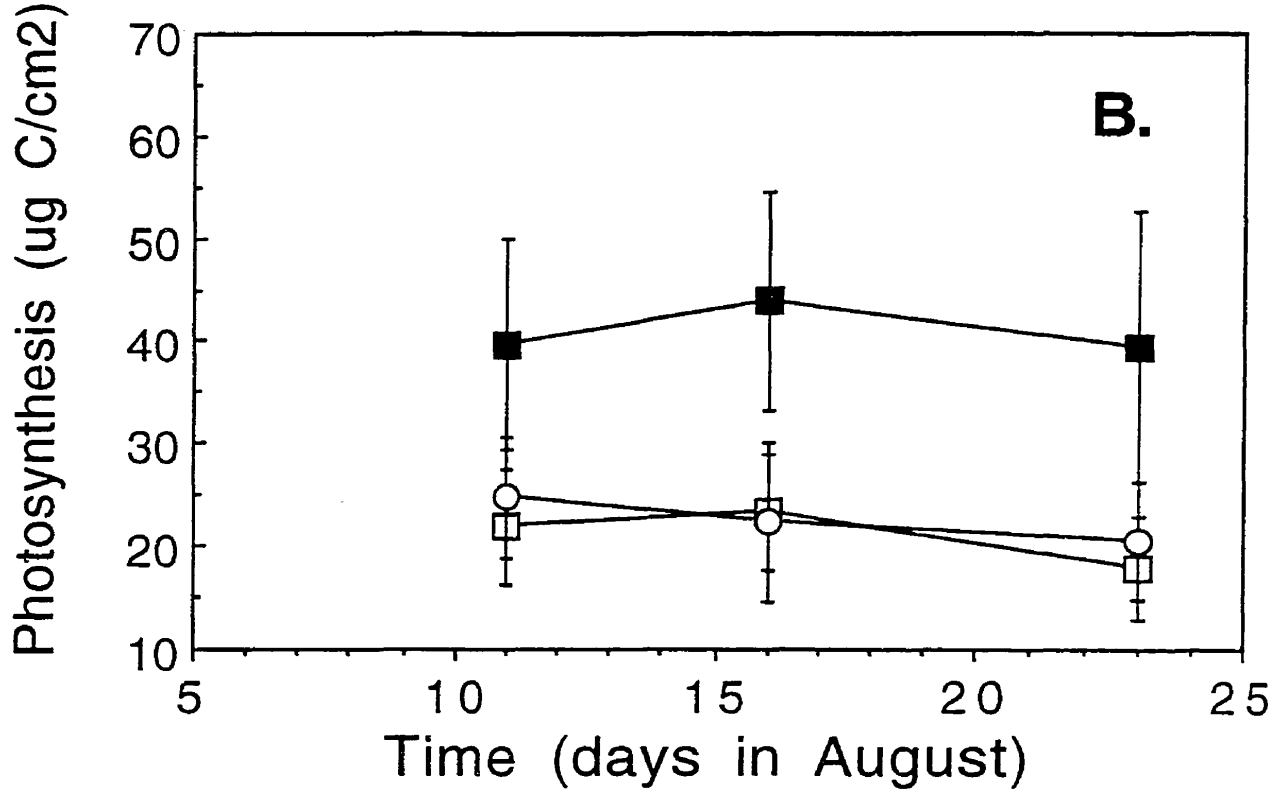
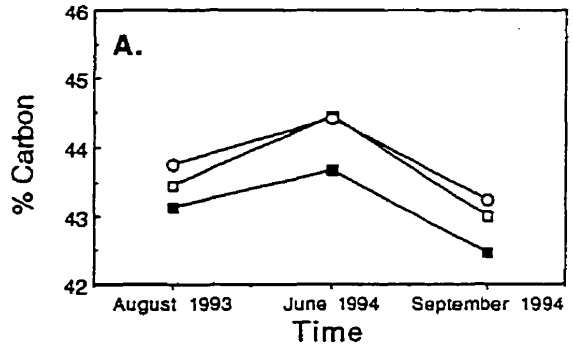

B.
Photosynthesis (ug C/cm2)
70
60
50
40
30
20
10
5
10
15
20
25
Time (days in August)

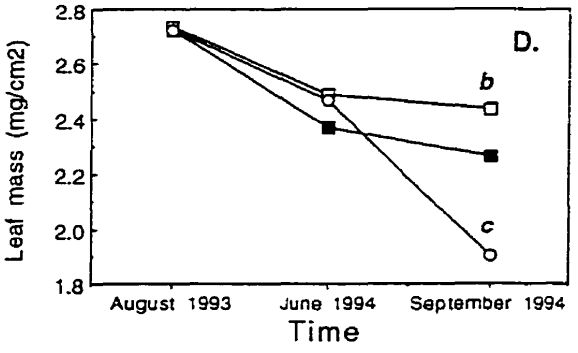
Table 1. Results of repeated measures analysis of variance for leaf physiology in Taraxacum officinale and Plantago major during the reproductive phase, 1994. F values and significance levels. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns= not significant, $p > 0.10$. Results of planned comparisons for CO2 effect (High CO2 vs Ambient CO2) and Chamber wall effect (Ambient CO2 vs Control) are shown beneath Effects. For Taraxacum photosynthesis, 16 plants were measured in each treatment at four times. In Plantago, the same four leaves were measured for photosynthesis at three times, and for chlorophyll, the same two leaves were measured twice. Sample sizes: 12 High CO2, 6 Ambient CO2, 11 Control, except for chlorophyll, where control was 9.

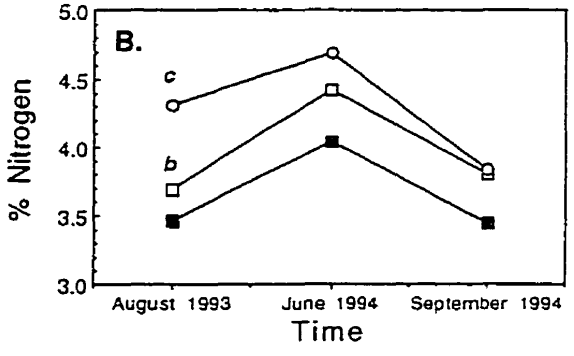
Effect	Taraxacum photosynthesis	Plantago photosynthesis	Plantago chlorophyll
Treatment	72.57 ***	150.26 ***	2.02 ns
High CO2 vs Ambient CO2	140.65 ***	193.26 ***	2.43 ns
Ambient CO2 vs Control	16.78 ***	0.98 ns	3.93 *
Time	2.76 (0.10)	5.68 **	52.4 ***
Treatment x Time	9.92 ***	1.80 ns	6.0 **
High CO2 vs Ambient CO2 x Time	16.87 ***	0.82 ns	11.99 ***
Ambient CO2 vs Control x Time	0.31 ns	0.92 ns	5.66 *

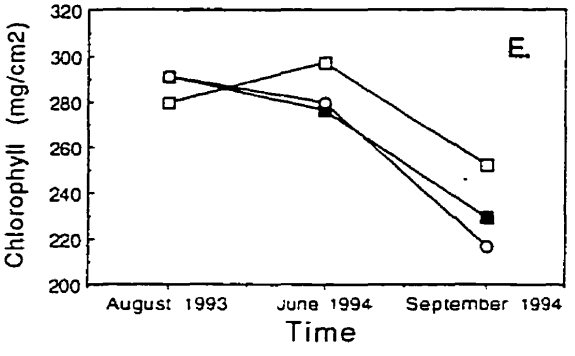
Figure 2

Seasonal changes in source leaf blade metabolites in *Taraxacum officinale* (A) % Carbon, (B) % Nitrogen, (C) C:N Ratio, (D) Interveinal Leaf mass, (E) Chlorophyll. Symbols as in Figure 1. Sample sizes as in Table 2. Letters mark significant differences for one-way anova comparisons at the time interval for planned comparisons for the effects of CO_2 (a, b) and for the chamber wall (b,c).


A.
% Carbon
46
45
44
43
42
August 1993
June 1994
September 1994
Time


D.
Leaf mass (mg/cm2)
2.8
2.6
2.4
2.2
2.0
1.8
b
c
August 1993
June 1994
September 1994
Time


B.
% Nitrogen
5.0
4.5
4.0
3.5
3.0
c
b
August 1993
June 1994
September 1994
Time


E.
Chlorophyll (mg/cm2)
320
300
280
260
240
220
200
August 1993
June 1994
September 1994
Time

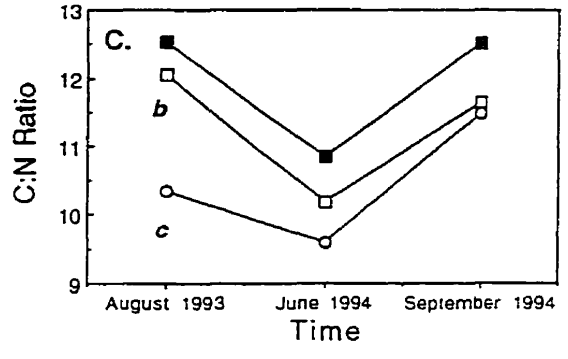

C.
C:N Ratio
13
12
11
10
9
b
c
August 1993
June 1994
September 1994
Time

Table 2. ANOVAR results for source leaf traits in Taraxacum officinale from Fall 1993 to Fall 1994. F values and significance levels. *, $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$, ns= not significant, $p > 0.10$. Results of planned comparisons for CO2 effect (High CO2 vs Ambient CO2) and Chamber effect (Ambient CO2 vs Control) are shown beneath effects. Sample sizes, n= 12 for chloropyll and 11 for leaf mass in High CO2, 10 in Ambient CO2 and 11 for Control in both parameters. For %C, %N and C:N ratio, n=6 for each.

Effect	chlorophyll	specific leaf mass	% C	%N	C:N ratio
Treatment	0.27 ns	1.06 ns	2.38 ns	6.29 *	6.48 *
High CO2 vs Ambient CO2	0.32 ns	0.60 ns	2.05 ns	0.93 ns	0.30 ns
Ambient CO2 vs Control	0.50 ns	2.11 ns	0.17 ns	4.36(0.06)	6.07 *
Time	15.51 ***	20.68 **	7.48 **	6.21 **	6.13 **
Treatment x Time	0.87 ns	2.57 *	0.30 ns	0.73 ns	0.85 ns
High CO2 vs Ambient CO2 x Time	1.01 ns	0.33 ns	0.27 ns	0.63 ns	0.68 ns
Ambient CO2 vs Control x Time	1.46 ns	4.35 *	0.59 ns	1.00 ns	1.53 ns

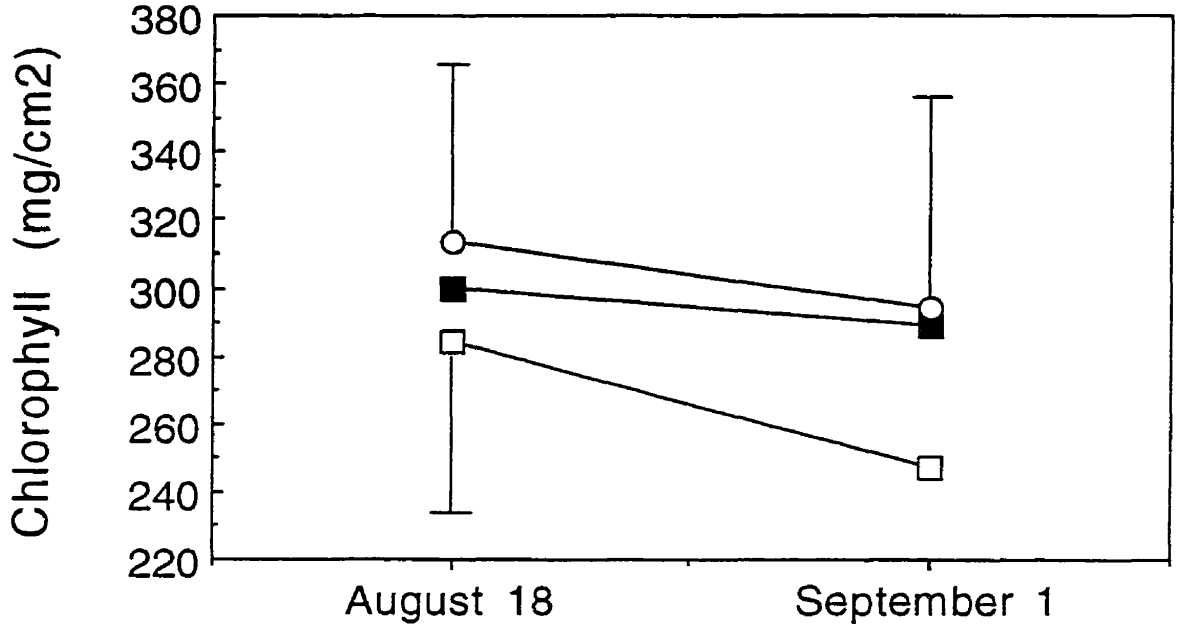
the C:N ratio (Table 2). Plants in the control plots had a significantly lower C:N ratio than in ambient CO_2, predominantly due to the lower %N content.

All parameters differed significantly with time (Table 2). During the reproductive season (June 1994), the %N was relatively higher than the %C, resulting in a lower C:N ratio (Figures 2 A-C). There was a chamber wall effect detected in September, where plants in the ambient CO_2 chamber showed greater chlorophyll per area (Figure 2 E) and higher specific leaf mass (Figure 2D) than in the control plants. Specific leaf mass was lower during the peak of the reproductive period (June 1994), than at the end of summer (August 1993) (Figure 2D). As expected, chlorophyll declined in the autumn portion of the growing season (September 1994)(Figure 2E).

In *Plantago*, there were significant effects of elevated CO_2 in enhancing interveinal leaf mass ($F=12.17$, $p < 0.001$) and in delaying senescence, measured as chlorophyll decline (Table 1). Leaves were significantly heavier in elevated CO_2 (4.80 ± 0.66 mg/cm^2) compared to ambient CO_2 (4.13 ± 0.42) ($F=24.34$, $p < 0.001$). Measurements of chlorophyll on the same leaf allowed assessment of chlorophyll decline over time (Figure 3). Average chlorophyll per leaf did not differ significantly with treatment (Table 1). Under ambient CO_2, leaves showed a nearly four-fold greater decline in chlorophyll than those under high CO_2 for the same two-week period. The chamber wall had a significant effect on leaf mass as the control leaves had a mass of 4.60 ± 0.40 mg/cm^2 ($F=11.62$, $p < 0.001$) (Table 1). Senescence was hastened in the chambers, as ambient CO_2 plants were significantly greater than the control plots in chlorophyll decline through time. Leaves of the older seed-filling stage had 8% more mass per area in both CO_2 treatments (data not shown).

Figure 3

Reproductive phase source leaf chlorophyll in *Plantago major*. Values are average of four leaves, measured at each time period (two leaves each associated with spikes in the stages of (A) flowering and (B) seed-filling at the start of measurements). Symbols as in Figure 1.
Sample sizes as in Table 1.


Chlorophyll (mg/cm2)
380
360
340
320
300
280
260
240
220
August 18
September 1

C:N contents at harvest

Despite the large biomass enhancement shown in *Taraxacum* with elevated CO_2 (Jablonski et al.unpublished), there was no evidence of any CO_2 effect on C or N content of any of the tissues (Table 3 B). In *Plantago*, there was a significant effect of elevated CO_2 only in shifting the root allocation patterns of C and N (Table 3A). At a whole plant level there was no effect of CO_2 on C:N ratio estimated from biomass and C:N contents of each vegetative part in either *Plantago* (High CO_2: 19.2 ± 7.7, Ambient CO_2: 19.6 ± 3.6, Control 10.7 ± 6.7) or *Taraxacum* (High CO_2: 17.5 ± 2.9, Ambient CO_2: 17.2 ± 2.7, Control 14.9 ± 2.5). Previously, it was shown that there was no CO_2 effect on total biomass, but a significantly lower caudex:total root ratio (Jablonski et al. submitted). In the present study we found that in the fibrous root under elevated CO_2 compared with ambient CO_2, the %N was much lower ($F= 4.93$, $p =0.40$) and the %C slightly lower ($F= 4.64$, $p < 0.05$) resulting in an increased C:N ratio ($F= 3.09$, $p = 0.10$). The storage caudex showed opposite trends. The %N was significantly greater in high CO_2 ($F= 7.11$, $p < 0.18$), resulting in a significantly lower C:N ratio ($F= 7.37$, $p = 0.016$). In both species, elevated CO_2 and the chamber wall had no effect on seed contents of %C, %N or C:N ratio. The species differed in distribution of contents. *Taraxacum* had a higher %N content in seeds, and lower C:N ratios, while *Plantago* storage caudex was high in %N.

Seed traits

Characteristics related to differences in the use of C or N within reproductive structures were analyzed by examining the seed packaging, mass and reproductive structure size. In *Plantago*, spike length influenced seed production as it was a significant covariate for seeds per capsule, and seed number per spike length (Table 4). However, there was no significant difference in seed packaging (about 12 seeds per capsule) detected between any of the treatment groups. There was also no difference between treatments in

Table 3. Percent carbon and nitrogen contents and C:N ratio of plant parts at time of harvest in 1994.

A) Plantago major and B) Taraxacum officinale. Mean ± sd. Sample size, n=6 per treatment. Symbols following means indicate significance differences at $p < 0.05$ for planned contrasts of CO2 effect (High CO2 vs Ambient CO2), *, and chamber wall (Ambient CO2 vs Controls), w. Vegetative parts were harvested in October. Seeds analyzed were harvested at maturity during the midpoint of the reproductive period.

Plant part	% Carbon			% Nitrogen			C:N Ratio		
	High CO2	Ambient CO2	Control	High CO2	Ambient CO2	Control	High CO2	Ambient CO2	Control
A. Plantago major									
Fibrous root	39.1 ± 1.4 *	40.8 ± 1.7	39.9 ± 0.9	0.84 ± 0.05 *	0.99 ± 0.16	0.99 ± 0.16	46.9 ± 3.1 *	41.0 ± 7.1	41.2 ± 6.3
Storage caudex	41.1 ± 0.5	41.0 ± 1.7	41.9 ± 1.5	3.25 ± 0.24 *	2.38 ± 0.53 w	3.17 ± 0.78	12.7 ± 0.9 *	18.1 ± 5.1 w	13.8 ± 3.1
Petiole	37.2 ± 0.3	37.7 ± 1.2	37.2 ± 0.6	0.79 ± 0.07	0.78 ± 0.06	0.92 ± 0.24	47.8 ± 5.0	48.4 ± 4.4	42.6 ± 9.7
Blade	38.1 ± 0.6	37.7 ± 1.2 w	39.5 ± 0.8	2.07 ± 0.23	2.10 ± 0.25	2.22 ± 0.16	18.6 ± 2.0	18.1 ± 1.9	17.9 ± 1.5
Seeds	50.1 ± 1.2	51.0 ± 2.5	50.2 ± 1.3	3.59 ± 0.17	3.64 ± 0.26	3.59 ± 0.32	14.0 + 0.9	14.0 ± 0.8	14.1 ± 1.4
B. Taraxacum officinale									
Hypocotyl and root	39.2 ± 1.2	39.0 ± 3.1	37.4 ± 6.7	1.53 ± 0.42	1.60 ± 0.47	2.03 ± 0.47	27.0 ± 6.3	26.2 ± 7.4	19.3 ± 6.4
Leaves	42.5 ± 0.7	43.0 ± 0.6	43.2 ± 1.1	3.44 ± 0.45	3.80 ± 0.71	3.83 ± 0.60	12.5 ± 1.5	11.6 ± 2.2	11.5 ± 1.5
Seeds	55.3 ± 3.3	54.9 ± 2.9	55.8 ± 3.3	4.97 ± 0.31	4.84 ± 0.32	5.03 ± 0.48	11.1 ± 0.1	11.3 ± 0.3	11.2 ± 0.7

Table 4. Results of analysis of variance for reproductive and seed traits in 1994. A. Taraxacum officinale B. Plantago major. F values and significance levels. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ ns= not significant, $p > 0.10$. Results of planned comparisons for CO2 effect (High CO2 vs Ambient CO2) and Chamber effect (Ambient CO2 vs Control) are shown beneath effects. For Taraxacum, analysis was done using average inflorescence maturation day (per plant) as a covariate. Number of leaves (plant size) was not a significant covariate for any analysis. For Plantago, mean reproductive spike length was used as a size covariate. Sample sizes, for Taraxacum: n= 6 for each of two chamber replicates per Treatment. For Plantago, n=6 for Control and High CO2; and n=5 for Ambient CO2. Values are the mean of five spikes per plant.

A. Taraxacum officinale

Effect	number of seeds per inflorescence	total seed mass per inflorescence	hundred-seed mass	inflorescence mass (minus seeds)
Treatment	8.14 (0.06)	1.25 ns	0.88 ns	10.00 *
High CO2 vs Ambient CO2	15.13 *	2.37 ns	0.01 ns	8.4 (0.06)
Ambient CO2 vs Control	0.81 ns	1.10 ns	1.24 ns	19.12 *
Chamber (Treatment)	0.40 ns	0.87 ns	0.84 ns	0.29 ns
Covariate (maturation day)	4.05 *	1.43 ns	0.02 ns	0.63 ns

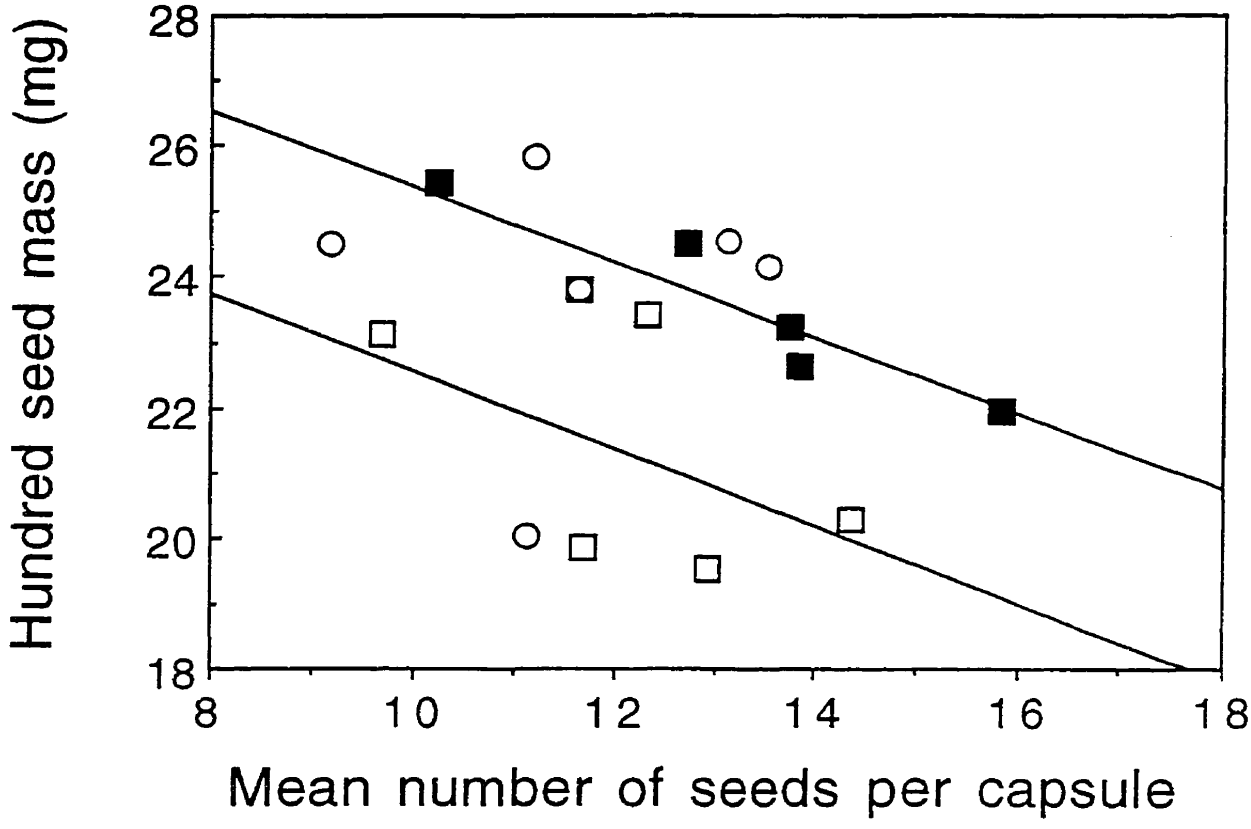
Table 4. Continued

B. Plantago major

	seeds per capsule	hundred seed mass	seed number per cm	seed mass per cm	stalk mass per cm
Treatment	1.08 ns	3.69 *	6.37 **	6.11 *	0.61 ns
High CO2 vs Ambient CO2	0.05 ns	6.56 *	0.24 ns	0.80 ns	0.80 ns
Ambient CO2 vs Control	1.8 ns	4.59 *	10.49 **	5.58 *	0.02 ns
Covariate (average stalk length)	6.24 *	2.55 ns	5.89 *	3.43 (.09)	3.69 (.08)

Figure 4

Relationship between capsule packing of seeds and seed mass in *Plantago major*. Values are the average assessed from five spikes per plant, and six capsules per spike. Significant relationships were found for High CO_2: y=31.47 - 0.06055x, R^2 = 0.852 and Ambient CO_2: y=28.613 - 0.06044, R^2 = 0.306. There was no significant relationship for the Control plot, analyzed with or without the outlier (seed mass =20 mg).


Hundred seed mass (mg)
28
26
24
22
20
18
8
10
12
14
16
18
Mean number of seeds per capsule

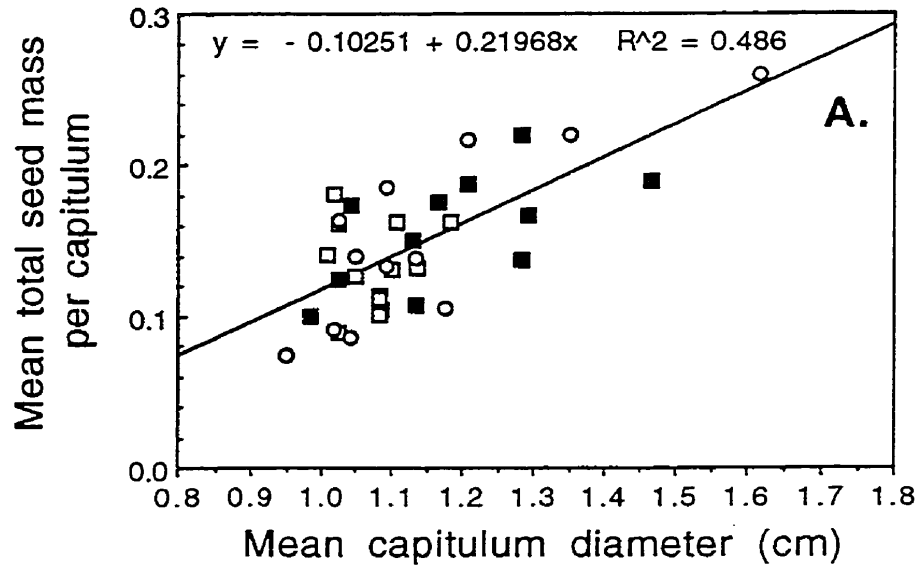
the density of capsules along the stalk length. However, a trade-off between seed mass and packaging was found (Figure 4) that was significant and similar in both high and ambient CO_2. Across the size classes, hundred-seed mass (mg) was significantly greater under elevated CO_2 (24.89 ± 3.5) than in ambient CO_2 (21.24 ± 1.87) ($F= 4.92$, $p < 0.04$). There was also a significant chamber wall effect ($F= 4.59$, $p < 0.05$) as seeds in control plants weighed 23.79 ± 1.98 mg.

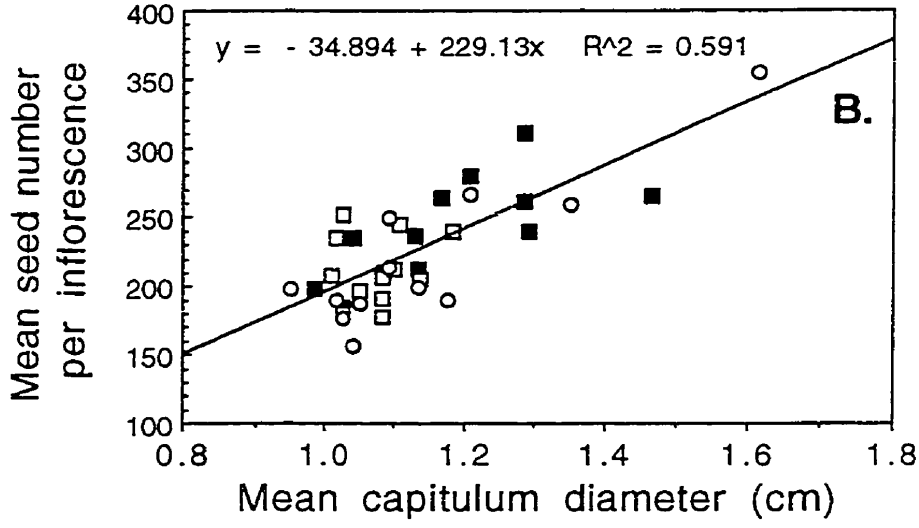
In *Taraxacum*, the effect of CO_2 was on increasing seed number rather than seed mass (Table 4). There was a significantly higher seed number produced per inflorescence on average in High CO_2 (241 ± 37) compared with Ambient CO_2 (212 ± 25), and the control plants (220 ± 54). Both the total seed mass per inflorescence and the hundred-seed mass did not show any treatment differences (Table 4). Previously, we have found that inflorescences (measured as capitulum diameters) were significantly larger under elevated CO_2, and that maturation time differed with growing season (Jablonski et al. unpublished). Corresponding with the larger size, the mass of the inflorescence head (minus seeds) was significantly greater (adjusted means, mg) under high CO_2 (57.6 $\pm$7.31) compared with ambient CO_2 (48.8 ± 6.96) while mass in control plants was (60.9 ± 6.89). To examine potential fitness consequences, the relationship with other seed traits was explored. Capitulum diameter predicted both total seed mass per capitulum (Figure 5A), and seed number per capitulum (Figure 5B). Unlike *Plantago*, there was no evidence of a trade-off between seed mass and seed number, as no relationship was found (Figure 5C).

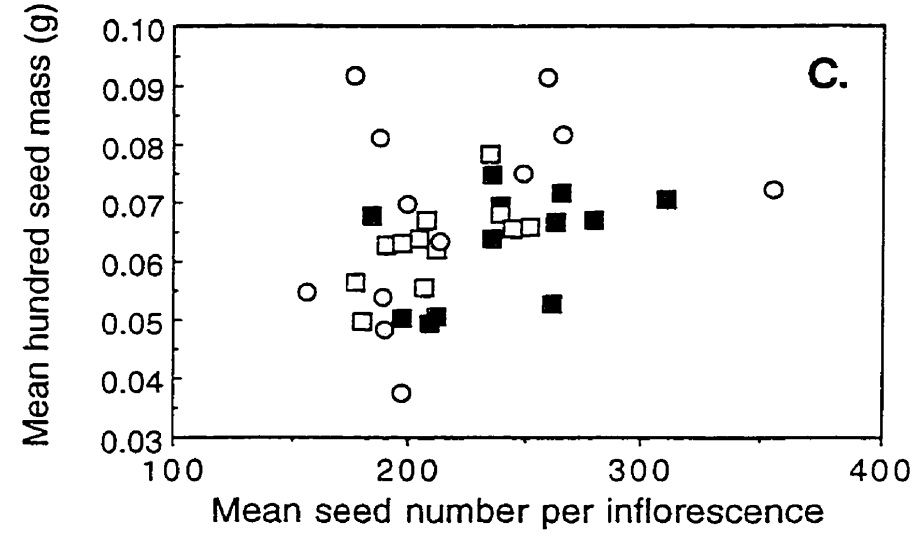
To examine if there was evidence that the increased carbon resource of the CO_2 regime resulted in a decrease in the variability of seed traits under High CO_2, the coefficient of variation (CV) was examined among inflorescences gathered from the same plant. In *Taraxacum*, CO_2 decreased the variability of hundred-seed mass (CV, High CO_2: 0.0877 ± 0.040, Ambient CO_2: 0.1216 ± 0.036; Mann-Whitney $U=105$,

Figure 5

Relationships between inflorescence size and seed traits for plants of *Taraxacum officinale*. (A) Seed weight and capitulum size, (B) Seed number and capitulum size, and (C) Seed weight and seed number per inflorescence. Sample size, n=12 per treatment. Symbols as in Figure 1. Values are the average capitulum diameter for the entire plant, and the average seed mass and seed number calculated from six inflorescences per plant. Regressions are plotted for the pooled treatments. Ambient CO_2 plants did not show significant relationships in any case.


y = - 0.10251 + 0.21968x R^2 = 0.486
A.
Mean total seed mass per capitulum
0.0
0.1
0.2
0.3
0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6 1.7 1.8
Mean capitulum diameter (cm)


y = - 34.894 + 229.13x R^2 = 0.591
B.
Mean seed number per inflorescence
100
150
200
250
300
350
400
0.8 1.0 1.2 1.4 1.6 1.8
Mean capitulum diameter (cm)


C.
Mean hundred seed mass (g)
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.10
100 200 300 400
Mean seed number per inflorescence

p=0.056). Also, under high CO_2, seed number varied less between inflorescences (High CO_2: 0.120 ± 0.043, Ambient CO_2: 0.157 ± 0.047; Mann-Whitney U=103, p=0.074). In *Plantago,* there was no effect of CO_2 on the variability pattern for both the traits of seeds per capsule and hundred-seed mass (all $p > 0.10$).

Allometry of leaves and reproductive structures

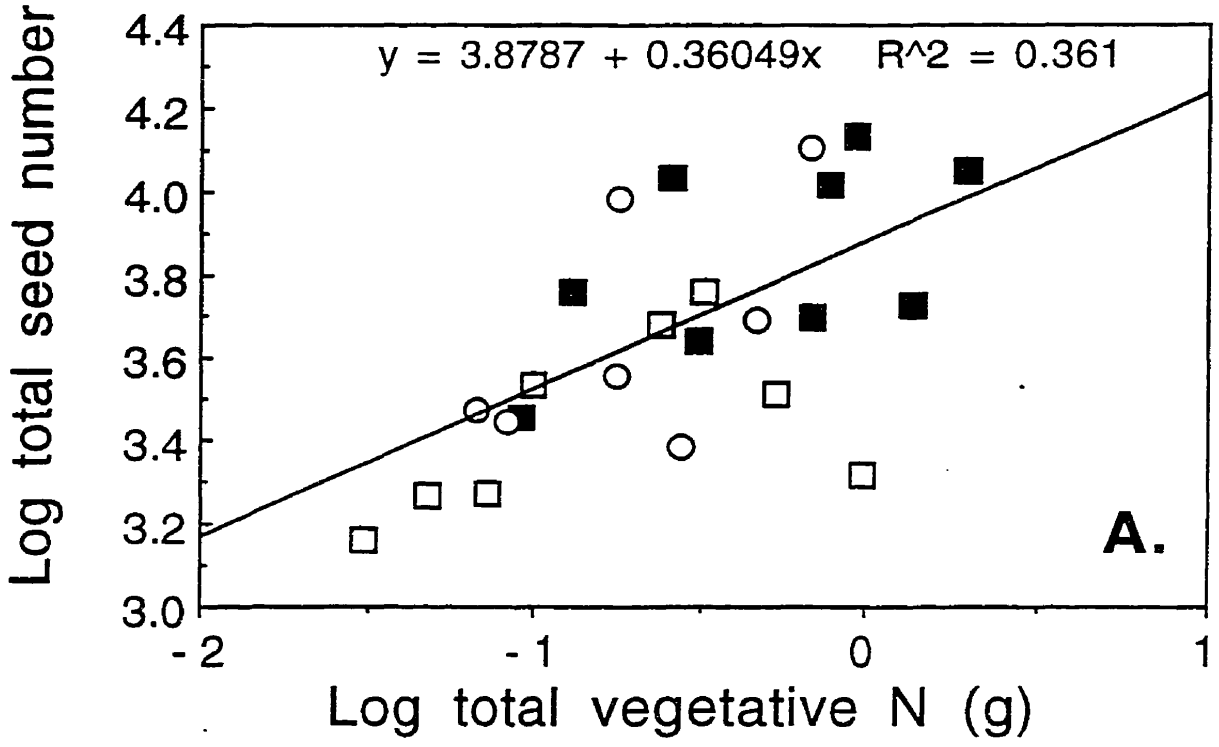
The relationship between leaf shape and reproductive structure size was explored in *Plantago*. There was a very constant allometry that did not differ with treatment between the blade length and traits of petiole length, leaf area, or spike length (data not shown).

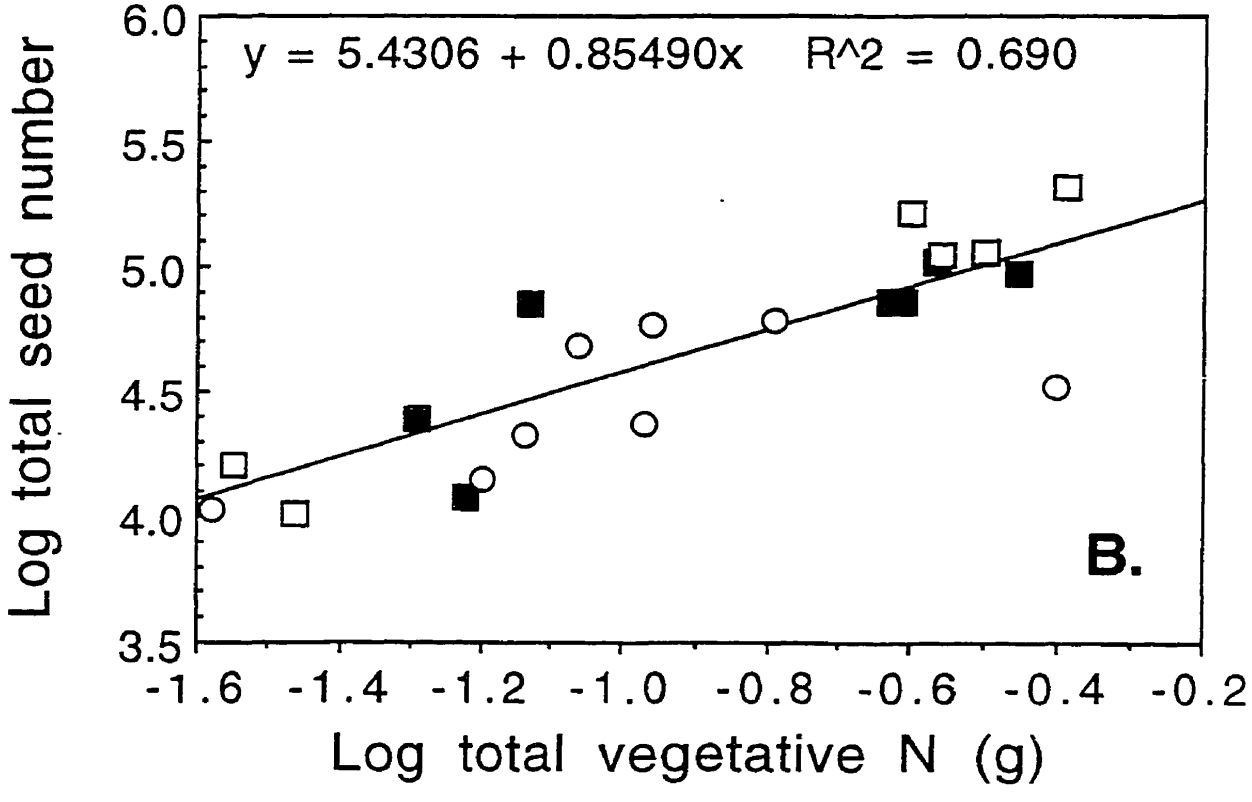
Use of N for seed production

To investigate if there were differences in the vegetative use of N for seed production under elevated CO_2, the relationship between seed number and total vegetative N pools were compared (Figure 6). In *Taraxacum,* there was a significant effect of treatment on total seed number ($F=5.76$, $p < 0.01$) and a marginal effect on total vegetative N ($F=3.11$, $p=0.06$). The high CO_2 plants compared to the ambient CO_2 plants acquired significantly more vegetative N (g) (High CO_2: 0.964 ± 0.709, Ambient CO_2: 0.382 ± 0.332) ($F= 5.84$, $p= 0.025$) and produced significantly more seeds (High CO_2: 7688 ± 3799, Ambient CO_2: 3188 ± 1768) (Figure 6A) ($F = 11.5$, $p = 0.003$). There was no significant effect of the chamber walls. The control plants were intermediate in total N (0.460 ± 0.355) and seed number (5593 ± 4009). There was no significant difference in the relationship between vegetative N use and seed number between treatments, as slopes and interecepts did not differ significantly (all $p > 0.10$). Overall, there was a significant relationship between vegetative N and total seed number (Figure 6A). For each gram N, around 7500 seeds were formed.

Figure 6

Relationship between total vegetative N content and seed production in (A) *Taraxacum officinale* and (B) *Plantago major*. Vegetative mass values were calculated by using % N determinations and total mass of each plant. Symbols as in Figure 1. Sample sizes, n= 9 High CO_2, 8 Ambient CO_2 and 6 Control for *Taraxacum*, and 7 High CO_2, 6 Ambient CO_2 and 8 Control in *Plantago*.


y = 3.8787 + 0.36049x R^2 = 0.361
Log total seed number
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
-2
-1
0
1
Log total vegetative N (g)
A.


y = 5.4306 + 0.85490x R^2 = 0.690
Log total seed number
6.0
5.5
5.0
4.5
4.0
3.5
-1.6
-1.4
-1.2
-1.0
-0.8
-0.6
-0.4
-0.2
Log total vegetative N (g)
B.

In *Plantago,* there was a non-significant trend of less seeds being produced per plant under High CO_2: 64,528 ± 34,213; Ambient CO_2: 103,273 ± 78,241; Control: 32,723 ± 18,852). Vegetative total N did not differ significantly with CO_2: (High CO_2: 0.193 ± 0.111; Ambient CO_2: 0.219 ± 0.155; Control: 0.114 ± 0.115). Overall, there was a very strong relationship between vegetative total N and seed number, with 70% of the variation in plant response accounted for by the vegetative N amount (Figure 6B). For each gram of vegetative N, about 270,000 seeds were formed. Overall, there was no significant effect of elevated CO_2 in altering the proportion (%) of the annual plant N budget that went to seed production, in *Taraxacum* (High CO_2: 32.6 ± 16.4, Ambient CO_2: 37.7 ± 19.6, Control 41.1 ± 14.6), or in *Plantago* (High CO_2: 74.5 ± 8.6, Ambient CO_2 76.9 ± 5.7, Control 73.3 ± 17.1).

DISCUSSION

Changes in reproductive physiology under elevated CO_2

We hypothesized that under elevated CO_2, the altered C and N physiology would increase seed number and quality. Supporting our hypothesis, both species showed significant changes that could contribute to increased fitness. The constancy of seed C:N ratio, while there were increases in seed mass in *Plantago*, and seed number in *Taraxacum*, suggests that more resources were provisioned to seeds under elevated CO_2. This response was in addition to the increased number of reproductive structures produced in both species under high CO_2 (Jablonski et al. unpublished). The consistency of the response suggests this second level of response to CO_2 could be a maternal environmental effect, where resources are provisioned to enhance fitness at the level of the inflorescence structure and physiology in order to increase the seed number and quality (Roach and Wulff 1987). Although the effects could also be due to genetic variability, our results suggest the role of maternal effects might become more predominant under a high CO_2 regime. That across sizes of spikes, seed mass was relatively heavier in *Plantago* under high CO_2, suggests the provisioning of extra resources was consistent. Since a higher seed mass usually correlates with increased seed performance such as greater seedling size and maternal resource provisioning in the second generation (Charest and Potvin 1993, Potvin and Charest 1991), plants that possess this responsiveness to CO_2 could have a competitive advantage (Parrish and Bazzaz 1985, Houssard and Escarre 1991). Because resource effects can be detected for several generations (Miao et al. 1991), a CO_2 effect could accumulate, and be heightened over time. This type of physiological plasticity could effect selection processes under elevated CO_2 by causing a time lag or altering the rate and direction of response (Kirkpatrick and Lande 1989). The lower seed mass in the ambient chambers compared with the control plots may be caused by the higher temperatures

within the chambers (Stewart and Potvin 1996), which can decrease seed mass (Charest and Potvin 1993, Potvin and Charest 1991). The direct effect of CO_2 on increasing seed mass may partially counteract the impact of predicted temperature increases. These seed trait results show the importance of not merely relying on the coarser measurement of total biomass to estimate fitness response as these failed to detect the seed mass increase in *Plantago*.

Although increases in seed mass, number and N are typically used as predictors of fitness increase (e.g. Parrish and Bazzaz 1985, Charest and Potvin 1993), to ascertain fitness changes, assessments of seed quality by biochemical analyses and performance criteria of viability, germination and seedling growth are necessary (Primack and Kang 1989). Seed % N, and C:N combined with seed mass are typically good indicators of the quality of reserves. That % N and C:N remained constant in both species, suggests N allocation per seed under elevated CO_2 increased in *Plantago* with seed mass and was unaffected in *Taraxacum*. This N likely indicates a higher protein level which could provide for germination and seedling growth (Parrish and Bazzaz 1985, Charest and Potvin 1992). Seed quality as measured by performance has sometimes shown increases without changes detected in seed mass or %N (Farnsworth and Bazzaz 1995, Alexander and Wulff 1985, Wulff and Alexander 1985). This could have occurred in *Taraxacum* if carbon was used to synthesize seed reserves of higher energy per mass such as protein vs mineral N or lipid vs carbohydrate C. As shown in our study, examining an entire suite of traits, including quantity and quality of seeds, is necessary to assess reproductive response to CO_2 since any one trait alone may not accurately estimate fitness (Farnsworth and Bazzaz 1995).

Morphological constraints on seed traits may account for the distinctly different species responses. In *Taraxacum*, seed number may be favoured over seed mass as a response to increased resources because of the higher cost of achene packaging and

reduced growth rates incurred with seed size (Fenner 1983) and the lack of a seed bank (Grime 1988). Seed mass may also be constrained by the dependence upon wind-dispersal and the precise aerodynamic geometry necessary for effectiveness (Sheldon and Burrows 1973). Production of a higher seed number was likely facilitated by the space of a larger flowering head (capitulum) found under elevated CO_2. In Composites, an increase in number of structures (rather than seed mass) may be an advantage to avert predators, since insect damage is greater with capitulum size (Fenner 1985). The decreased variability (lower coefficient of variation) in *Taraxacum* seed mass and number under high CO_2 suggests that resources were distributed more evenly, that seed-filling was more complete and abortion reduced (Stephenson 1981). Seed size variability usually increases when C and other resources are limited (Winn 1991, Kane and Cavers 1992), so elevated CO_2 may counteract these trends. Variance may have changed at the level of individual seed mass, which was not examined in this study. The strong response of seed mass in *Plantago* may have been due to constraints of the lack of plasticity in leaf and spike morphology which were not altered by elevated CO_2 in this study and others (O'Leary and Potvin in preparation). The trade-off we observed between seeds per capsule and seed mass is typically found in *Plantago* (Primack 1978). Investment in seed reserves may be more critical for *Plantago,* as seeds are long-lived (over 20 years) and seed dispersal is passive and intermittent (Hawthorne 1974, Grime 1988).

Effect of Root and Leaf Physiology on Seed Traits

Seed traits that indicate higher resource provisioning suggest that under high CO_2 more C and N were available to the seeds. Changes in seed traits were expected since any acclimation in foliar physiological processes that are typical under elevated CO_2 may affect seed production. We hypothesized that plants with storage organs would enhance N

acquisition under a high CO_2 regime by the higher storage capacity and sink activity. We examined traits of leaf and root physiology to provide an explanation for seed responses. Supporting our hypothesis, under elevated CO_2 at a whole plant level, N and C both increased, with no change in C:N ratio. This was contrary to common findings of an increased C:N ratio in pot studies (e.g. Wong 1979), C_3 marsh plants (Drake et al. 1996) and deciduous trees (e.g. Lindroth 1996). Similar lack of C:N change has been found in herbaceous species in natural system exposures to CO_2 where there are no root restrictions (Chu et al. 1996, Field et al. 1996, Drake et al. 1996, Korner et al. 1996). Responses may depend upon the functional group as other ruderals have similarly shown constant or increased %N while grassland species have shown decline (Korner and Miglietta 1994, Owensby et al. 1996). That specific leaf mass increased under elevated CO_2 without a change in % N or % C shows that leaf N per area was enhanced along with C. Increased leaf mass under elevated CO_2 has often been viewed as a build-up due to sink limitation which can feedback to lower photosynthesis (DeLucia et al. 1985). However, these surplus resources may provide for increased seed-filling, since seeds are able to draw nutrients in excess of physiological need (Fenner 1986a,b).

The significant relationships between total vegetative N and seed number in both species under all treatments suggests that the physiological use of N for seed production was not altered by elevated CO_2. Resource use in the two life history phases is typically tightly coupled (Fenner 1986a,b). Plants showed the pattern of size-dependent reproductive allocation commonly found in these weeds (Thompson et al. 1991, Aarssen and Taylor 1992). The N limitation of seed production provides further evidence of the general N limitation of CO_2 response commonly observed for growth, photosynthesis, productivity and yield (Koch and Mooney 1996). The relationship was tight in *Plantago,* suggesting a stronger limitation by N. We suggest several reasons for the weaker relationship in *Taraxacum.* The estimate was not as precise because of the spring-time

reproduction; thus the autumn 1994 N is expected to be coupled more with seed production the following spring (1995) than of the previous spring. As well, the *Taraxacum* hypocotyl spreads broadly, and may contain reserve stores that are not as directly coupled to reproduction. Also, local variability in light or soil nutrients could be factors since, unlike most weeds, *Taraxacum* shows significant site-dependent variability in its fecundity to above-ground biomass relationship (Aarssen and Taylor 1992).

The ability to acquire more N under elevated CO_2 may be because of the capacity for "luxury" storage of N in *Taraxacum* (Hommels et al. 1989) and *Plantago* (Stulen et al. 1992). This capacity is expected for these pasture species which are adapted to store a resource supply below-ground to survive grazing pressures (Chapin et al. 1990). In perennials, N acquisition is not coupled as much with current physiological need, but is a reserve used for longevity, development of storage organs, regrowth in response to grazing and herbivory, and for future allocation to reproduction. The increased N may in part account for the reproductive fitness enhancement in this study being higher than previously found in wild annuals grown in pots under high CO_2 (Farnsworth and Bazzaz 1995, Curtis et al. 1994b, Tousignant and Potvin 1996). Because perennials are adapted to use larger pools of nutrients than annuals (Chapin 1980), they may have a greater capacity for N acquisition and thus biomass and reproductive response to CO_2.

The relatively larger size of the hypocotyl may have given *Taraxacum* the ability to acquire both a higher seed number and total N under elevated CO_2 while *Plantago* did not. Since nutrient acquisition depends on the soil area foraged (Berntson and Bazzaz 1997, Curtis et al. 1994a), enhancement of the broadly extending *Taraxacum* hypocotyl under elevated CO_2 (Jablonski et al. unpublished) may have facilitated N acquisition. Additionally, the below-ground organs provided a strong sink to draw C that may have provided for the metabolism of N acquisition. *Taraxacum* response may have been facilitated by the high physiological activity of the springtime reproduction, the time when

the soil N availability is usually highest. Known constraints on *Plantago* caudex size and fibrous root growth (Kuiper and Bos 1992) may have also limited N acquisition under high CO_2. In *Plantago,* the increased C in the fibrous root and increased N in the caudex suggest that processes of N acquisition were enhanced under elevated CO_2. This N increase may have supported the increased ramet production found under high CO_2 (Jablonski et al. unpublished)

The constancy in leaf %N and chlorophyll under elevated CO_2 while photosynthesis rate doubled suggests there was no change in the investment of N in the structures and enzymes of photosynthesis. Under ambient CO_2, photosynthesis is typically proportional to leaf N (Field and Mooney 1983). Under elevated CO_2, a decrease in leaf N has been attributed to a decreased carboxylation need, as shown by lower levels of the dominant protein, Rubisco (Sage et al. 1989, Jacob et al. 1995), or reduction in the critical N for crop yield (Conroy 1992). In our study, it is possible that Rubisco was lower, but that N was stored in other pools such as nitrate and these were able to provide for seed-filling without negative consequences on leaf assimilation. Although secondary compounds have also been suggested as a high CO_2 store, they showed no increase in *Plantago* sp. (Fajer et al. 1992). That in *Taraxacum* there were more leaves of larger size (Jablonski et al. unpublished) and no increases in leaf mass or C:N suggests that N was used to produce leaf area. Use of N for increased leaf area is also suggested by increased total N results in other studies (e.g. Owensby et al. 1996)

The two-fold enhancement in reproductive phase photosynthesis we observed under elevated CO_2 suggests that source-sink relations were maintained in both species during the reproductive phase. Sink limitation has often been cited as the reason for photosynthetic acclimation under high CO_2 as it is accompanied by starch accumulation and an increase in the C:N ratio or leaf mass (Arp 1991). The relatively greater degree of growth and reproductive enhancement in *Taraxacum* relative to *Plantago* was likely due to

the greater sink capacity provided by both the larger infloresence and storage hypocotyl. This is supported by the lack of leaf mass build-up in *Taraxacum* compared to *Plantago*. Yield was similarly greater with the higher sink strength of cotton compared with the determinate wheat (Pinter et al. 1996). The high levels of the photosynthetic rates are expected at this life history stage (Curtis et al. 1994b), because of the higher sink strength of reproductive structures (Thorne 1985). Rates for *Plantago* leaves under elevated CO_2 in this study compared with those of *P. major* having sinks of new developing shoots (Fonseca et al. 1996) but were much higher than found when constraints were present such as in morphology of self-shading (Poorter 1988) or sink limitations prior to flowering (Den Hertog et al. 1993). The freedom of the natural soil system conditions may have facilitated response in both species. Photosynthetic response to CO_2 has been greater where sinks have not been constrained, such as in natural environments and in plants with a large sink organ or activity (e.g. Arp and Drake 1991, Idso and Kimball 1991).

Despite similar strong photosynthesis enhancements by CO_2 in this study, *Plantago* showed much less biomass response than *Taraxacum*. (Jablonski et al. unpublished). A major part of the C budget was likely in respiration, which has shown more increase to CO_2 than photosynthesis in *Plantago* shoots (Den Hertog et al. 1993). Reproductive structures are present for over 80% of the growing season, and CO_2 may not enhance the photosynthesis of the green reproductive tissue (Bazzaz et al. 1979) enough to compensate enough for the costs of high respiratory maintenance and production of the increased seed mass. Canopy photosynthesis may also limit overall response, as self-shading occurs earlier in *Plantago* plants under high CO_2 (Poorter and Lambers 1988). *Plantago* leaves were lower in the canopy than those of the longer-leafed *Taraxacum* which likely could outcompete the other vegetation for light.

Two sources of evidence suggest leaf senescence was delayed under elevated CO_2. First, was the lack of a decline in photosynthesis rate in *Taraxacum* in high CO_2. Because the flowering period continued later in the season under elevated CO_2 (Jablonski et al. unpublished), the reproductive sink strength was likely maintained longer, feeding back to keep photosynthesis high. Second, the small decline in *Plantago* chlorophyll in high CO_2 suggests a greater leaf longevity. Both species results suggest that individual leaf photosynthetic duration was enhanced which could contribute to an increase in productivity. In *Plantago,* there was no effect of CO_2 on leaf number production, nor on the phenology of leaves as assessed visually (Jablonski et al. unpublished). However, individual leaf senescence and photosynthesis may have been extended physiologically. In studies of senescence in natural systems the rate was also delayed in *Scirpus* (Curtis et al. 1989) and the percentage during the reproductive peak was reduced (Arp and Drake 1991). Senescence results in annuals and crop species have been variable in response and coupled more with developmental changes, such as being hastened in wheat (Pinter et al. 1996)

Community and Ecosystem Implications

Changes in the allocation of C and N between the storage and fibrous roots in *Plantago,* along with the two-fold increase in *Taraxacum* total C and N contribution (Jablonski et al. unpublished) suggests an increased contribution of these species to below-ground processes under elevated CO_2. In time it could be expected that an accompanying increase in fine roots, might contribute to soil changes (Berntson and Bazzaz 1997). The increased nutrient supply could enhance below-ground processes involving nematodes (Wilsey, unpublished) or other soil herbivores.

The N availability to the plants may have changed during the course of the study, (Al-Traboulsi et al. unpublished) which could have contributed to the responses observed. It is expected that this recently abandoned pasture was a nutrient rich system, and that response might decline with time. The increased root growth and N acquisition in these species may partially explain the continued maintenance of their presence in the community under high CO_2, while grasses took over in others (Potvin and Vasseur 1997). Differential responses of the two species may have occurred because of differences in nitrate or ammonium N forms available in the community (Al-Traboulsi et al. unpublished), and differential preferences for N forms between the two species which can be altered under elevated CO_2 (Bassirirad et al. 1996).

Our results suggest that in communities and ecosystems where plants possess storage organs and reproduce by abundant seed there could be a significant amount of resource movement through the seeds under elevated CO_2. A very high amount of the annual plant N budget, 1/3 in *Taraxacum* to 3/4 in *Plantago*, was utilized in seed production in the growing season. That seed production was significantly enhanced along with plant size in *Taraxacum* with elevated CO_2, suggests the role of these plants will be enhanced in the community. At an inter-specific competition level, those that have the capacity for increased provisioning to seeds would be expected to be favoured. The ability to acquire N along with C may have allowed this response.

Ecosystem models have typically focused primarily on above-ground productivity and measurements of nutrients (Koch and Mooney 1996). The high N in seeds suggests this could be a significant pool in pastures and disturbed communities such as roadsides where seed-production is abundant. Since biennials also have the life history of below-ground storage and later seed production, our results suggest this group would also show strong reproductive enhancement to elevated CO_2. Models need to incorporate this movement via seeds, which expresses a dramatic shift from below to above-ground

resources. This shift might particularly impact the balance of resource use between decomposers, frugivores and herbivores. With increases in seed number in *Taraxacum,* and seed mass in *Plantago* under elevated CO_2, a greater seedling establishment is expected in the community. This would provide an increased food source for the seedling herbivores, which can eat over 50% of *Taraxacum* seedlings in nutrient-rich regimes (Fenner 1985).

CONCLUSIONS

Seed trait responses of an increased seed number and decreased variability in mass and number in *Taraxacum* and increased seed mass in *Plantago* without a change in C:N ratio suggest a relative fitness increase and potential role of maternal effects under long-term elevated CO_2. Both storage hypocotyl and seed metabolism appeared to enhance sink strength such that photosynthesis rate and duration was enhanced. Lack of a plant increased C:N ratio suggests the functional trait of a storage organ may allow for increases in N and C acquisition and seed production under elevated CO_2. The enhancement of N storage in *Plantago major* under elevated CO_2 may contribute to future ramet production and suggests differences in N acquisition or use under elevated CO_2. This study emphasizes the importance of studying physiological traits in the reproductive stage of life history within the natural ecosystem to best predict future responses to high CO_2.

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SUMMARY & GENERAL CONCLUSIONS

This thesis has examined the roles of carbon (C) storage, nitrogen (N) availability and the seed traits underlying the reproductive response to elevated CO_2. My goal has been to elucidate why reproductive responses to CO_2 have been both so variable and on average less than vegetative phase responses. I focused upon plants with storage organs, the functional group that has shown the greatest vegetative response to elevated CO_2. Two hypotheses were tested: 1) that reproductive response to CO_2 would be greater in plants with a higher non-foliar storage capacity and 2) that the increased C storage and decreased N need in foliar physiology under elevated CO_2 would cause an increase in seed traits of number and quality. A resource-based, physiological, "seed to seed" approach was adopted to examine the effects of CO_2 on C and N contents of all plant parts. To assess reproductive response in as realistic a situation as possible, I used a diurnally and seasonally simulated controlled environment and plants growing within an intact pasture community in open-top chambers. Also of interest was identifying the traits of vegetative phase physiology that could best predict reproductive response to CO_2, and examining the role of phenology in enhancing or constraining response to CO_2. In the reproductive phase, functionally the plant becomes like a "new organism" because of the changed patterns of source-sink relations and resource flow, and this must be incorporated in models predicting reproductive response. Seed traits most closely related to fitness were not predicted by total biomass responses. This research provides a framework for a functional approach to predicting reproductive responses to CO_2. The main conclusions, implications of the work, and future research directions are outlined.

1. Vegetative source-sink relations were used to test the hypothesis of greater response to elevated CO_2 being found in the functional group of plants with a higher

hypocotyl: leaf storage ratio. It is clear from the radish study (Chapter 1) that the hypocotyl storage sink alone did not contribute to the reproductive response, but other vegetative sinks such as new leaf growth and leaf area increase must also be considered. The source-sink relations in the reproductive phase also appear to be critical, as sink constraints limited reproductive response to CO_2 in *Raphanus* (Chapter 1). Enhancements in reproductive structures in response to elevated CO_2 were evident (*Taraxacum* in Chapter 2 & 3). There is a need for development of models to predict response to elevated CO_2 based on reproductive phase traits and criteria including the switch in phenological timing. I advocate that to better predict CO_2 response, functional groups should be re-defined based on phenological and reproductive phase morphological criteria and broader source-sink models should be developed that include the reproductive phase. Applying hypothesis testing to con-generics of different source-sink relationships as well as to other storage species such as biennials and other perennials would confirm the importance of this approach.

2. Enhancement of leaf area in response to elevated CO_2 was the greatest vegetative predictor of reproductive biomass response. The enhancement occurred in all species and by different means: by the leaf area increase in *Raphanus*, by the number of modules in *Plantago* (increased ramet production) and by the size of individual leaves in *Taraxacum*. This trait is typically an indicator of N amount in the plant, and appears to be important in predicting the degree of reproductive response. In this thesis, I explored relationships between vegetative and reproductive responses by looking primarily at the mean response within the sample. Examination of the relationships within the same individuals of the whole suite of vegetative and reproductive traits are necessary to verify the complete path of influence from vegetative leaf CO_2 fixation to seed traits. Experimental use of genetically identical individuals via known genotypes or clonal forms, and applying path analysis

would be useful approaches to this end.

3. Taken at a whole plant level and over the entire life history, there was no evidence of a change in C:N ratio and no evidence of a different physiological use of N in these hypocotyl-storing species in seed production. Few studies have examined N use in both vegetative and reproductive traits. This thesis suggests that vegetative physiological use of N was similar under elevated CO_2, but that seed quality was enhanced. An unanswered question is the interaction of reproductive response with soil fertility levels, since assessments at the field site have not yet been completed (Al-Traboulsi et al. unpublished), and a lower near-limiting level was not provided in the *Raphanus* study. It is also important to examine the impact of CO_2 on the ability of roots to acquire different soil N forms, and on shifts in allocation among the different N chemical forms within the plant.

4. Seed traits and clonal growth suggest fitness was consistently enhanced under elevated CO_2. Fitness increase was suggested by the increases in size of inflorescences and seed number, along with decreases in variability in *Taraxacum* , the seed mass increases without loss of C:N ratio in *Plantago*, and the decreased abortion rate in *Raphanus*. Seed quality should be verified by seed performance testing for seed viability, germination and initial seedling growth. An unexplored avenue is the examination of quality contents of lipids, carbohydrates and proteins for seeds produced throughout the growing season under elevated CO_2 to examine if typical responses to changing seasonal resources are found, and the ultimate impact on fitness. The seed trait results, particularly the very interesting decreased variability in seed traits in *Taraxacum*, suggest that maternal provisioning may be important under a high CO_2 environment. *Taraxacum*, as an apomictic species, provides a test system by which environmental effects can be isolated

from paternal. The importance of maternal environmental effects and possible carry over effects in both species should be verified by experiments that control for genotypes, and that examine second and third generation seedlings in a high CO_2 environment. Seeds hold much promise as a bioassay or indicator. Because the interactions of vegetative and reproductive phase physiology and morphology are complex, examination of both the number and quality of seeds may serve as an index of the physiological integration of the plant's response to the global change environment and may be able to be used as a predictive tool of response for community and ecosystem level studies. Several results suggest that clonal propagation may be enhanced under an elevated CO_2 regime. The enhanced production of new ramets in *Plantago* and rosettes in *Taraxacum* suggests both species have a capacity for clonal increase and subsequent reproductive (fitness) benefit. That there was no significant difference in mortality, but the trend of a decrease under elevated CO_2, suggests the trend of a later fitness gain. Even longer-term studies should be done to verify this.

5. Phenology can play an important role in enhancing or restricting response to elevated CO_2. A shift in phenological timing was not present in response to elevated CO_2 in *Raphanus* and likely constrained the response, but was observed in delaying the onset of phases in *Taraxacum* and in extending the flowering season in *Plantago*. Shifts in response due to temperature changes may be critical, as the open-top chamber results suggested temperature enhanced the onset of phases in *Plantago*. Future models should include a time switching component in predictions and follow the seasonal phenological pattern at the community and ecosystem level, given the responses of individuals. Phenological plasticity may be critical in enabling response to global change as demonstrated by seasonal shifts such as maturation rates in *Taraxacum*. Limits of growing season length or lower temperature regime may limit the expression of potential response to

elevated CO_2, as observed in the responses of the *Raphanus* cultivars and the *Plantago* flowering season. Plasticity of response under these conditions should be investigated. The open-top chambers used in the pasture study did partially simulate the increased temperature regime of global change. But the factors were confounded by the decrease in light and precipitation contributed by the wall. Isolating the influences of only temperature and CO_2 are important because of the interactive roles evident.

6. Photosynthesis rates consistently showed an enhancement response in these hypocotyl storing species measured during the vegetative phase in *Raphanus*, and during the reproductive phase of growth in both *Plantago* and *Taraxacum*. There was no evidence of negative build-up of carbon in any of the three species even after three years of exposure. In fact, increased leaf mass corresponded with positive effects on reproductive traits related to fitness in *Raphanus* (decreased seed abortion) and *Plantago* (increased seed mass). This provides evidence that the reproductive structures were a very strong sink for resource translocation. Though these results suggest that a negative photosynthetic acclimation did not occur, downregulaion during the reproductive phase should be tested by instantaneous high CO_2 application, and examination of A/Ci curves. It is also important to examine the different N pools within the leaves (predominantly protein and nitrate) to verify where the storage N was located, and that there was no decline in leaf Rubisco. In radish, the absolute enhancement in photosynthesis rate failed to predict traits most closely connected to fitness, and photosynthesis was enhanced nearly equally in *Plantago* and *Taraxacum* with very different biomass enhancement responses detected. Where did all the carbon go? To complete an understanding of carbon budget, it is important to assess dark respiration and maintenance costs. It is also important to examine the possible exudation of excess C out of the roots and the impact on soil biota.

7. These findings have implications at both community and ecosystem levels for processes involving seed-producing plants and plants with below-ground storage. This study showed that seed-producing plants contain a substantial pool of N, a resource flux that has not been included in traditional ecosystem models. This thesis suggests that the functional group containing storage organs (biennials and perennials) show the highest potential for response to CO_2. Ecosystems where these dominate (such as in north temperate regions) may show more response than others. These results suggest that agricultural, pasture and lawn weed management may be even more troublesome in the future. This type of ruderal weed is also prevalent in disturbed areas such as roadsides and urban areas, which are increasing with urban development. These results may also ultimately apply to longer-lived woody perennials whose storage capacity may already be contributing to them being favoured. Currently growing season and temperature may limit the response of many perennials. As these also shift with global change, the growth enhancement response to CO_2 might increase. Response to elevated CO_2 varied in time, suggesting that short-term studies on these species may not reveal the long-term impact of CO_2. The shifts in phenology and degree of response detected in just these two species suggest that species effects may have an important role in altering processes at the ecosystem level. In particular, the strong acquisition of N along with C, and enhancement in below-ground organs, could be a significant influence on herbivore, frugivore, soil decomposer and other microbial processes.

8. This study has identified the main vegetative and reproductive traits affected by elevated CO_2 in storage plants. Effects may have been obscured because of variability at the level of the individual due to the genetic make-up and the local environment experienced. Relationships between some pairs of traits within the same individual have been explored thus far in my work by correlation and regression, but the pathway of effect

through multiple traits of the vegetative and reproductive phases together has not been identified. The data set I have gathered for *Taraxacum* and *Plantago* will allow me to do this as the traits of the local environment, physiology of C and N use, vegetative growth,morphology, biomass, phenology, inflorescence and seed traits have all been measured on the same individuals. Using path analysis, I will test directly the influence of storage and other physiological factors on seed traits in order to identify those most important. I plan to apply principles of classical source-sink relations to build a model that shows which responses would maximize source and sink strength in both vegetative and reproductive phases (and thus responsiveness to elevated CO_2) and to test it with my data.

9. This experimental approach, which utilized both a natural intact community system and controlled environmental chambers to examine and test particular mechanisms, should be extended. In the controlled environment, a seasonal and diurnal rhythm was chosen that mimicked natural conditions as closely as possible. In the open-top chambers, the natural community was used as the background, which provided a more realistic milieux for assessing response. However, there were several limitations to this latter approach. Over time, the background species changed so there was an accumulation effect which may have inevitably limited the ability to detect a response to elevated CO_2. As an experimenter, I had no knowledge of the age or previous history of the plants, nor control over the positioning of the plants relative to the chamber walls or to their particular neighbours. I used a large sample size and covariates to account for as much of this variability as I could. Examining the impact of the chamber environmental factors including light reduction by the walls, identity of neighbours and their cover would provide some indication of the roles of these secondary factors in contributing to the response. Future experiments could control for neighbour and density differences. Even though these parameters were not controlled, I argue for the use of the natural community

background, for these were the natural soil processes and community members these species will be interacting with in the future. Even with the complexity, it was a more "real" situation. FACE (Free-air CO_2 enrichment) ring studies which allow for a greater surface area to be studied, and remove the artifact of the chamber wall (and probably add other new artifacts!) provide much promise. Controlled environmental chambers, with artificial soil should be used sparingly to examine perennial reproduction (only to test very particular mechanisms), because of the importance of phenology and difficulties in controlling soil fertility.

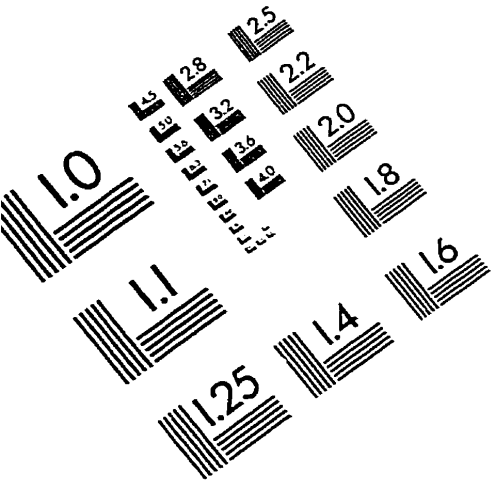
In summary, this thesis has examined the interaction of elevated CO_2 and N in the plant functional group containing hypocotyl (below-ground, non-photosynthetic) storage organs. The experiments used three species representing both wild and arable forms, and typical morphologies found in storage perennials, biennials and annuals. The examination has been comprehensive and has applied principles of both physiological ecology and reproductive ecology that have broad applications including to community and ecosystem contexts. Evidence from the several approaches taken in this thesis including enhancements in physiology, vegetative and reproductive traits support that this functional group will be enhanced under a future elevated CO_2 regime, provided there are no other limiting constraints such as growing season length or increased competition from other species. This thesis has opened the field of global CO_2 change to two important areas: 1) physiological ecology responses of both vegetative and reproductive traits in storage plants; and 2) examination of long-term growth and reproductive response including phenology within the natural community setting. The ability to store both excess C and N resources are important for reproductive response. This enhancement has implications for community competitive interactions and ecosystem belowground processes and nutrient cycling. Future global change studies and models should include the reproductive phase of

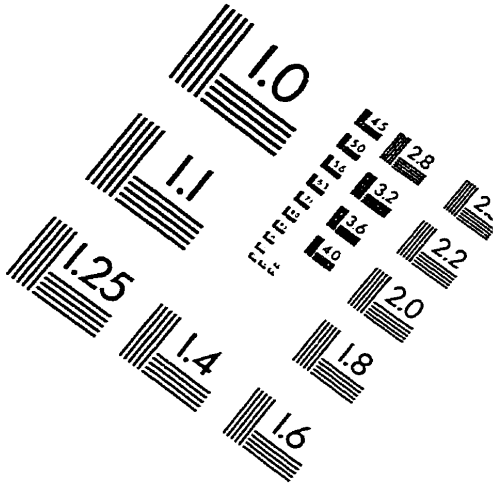
the life history, as this can have a significant influence at all levels of organization, from the interpretation of response to CO_2 of the individual, to the ecosystem level.

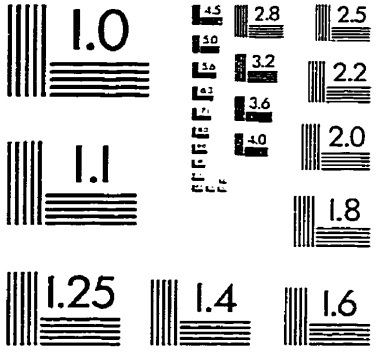
APPENDIX I - List of abbreviations used in the thesis

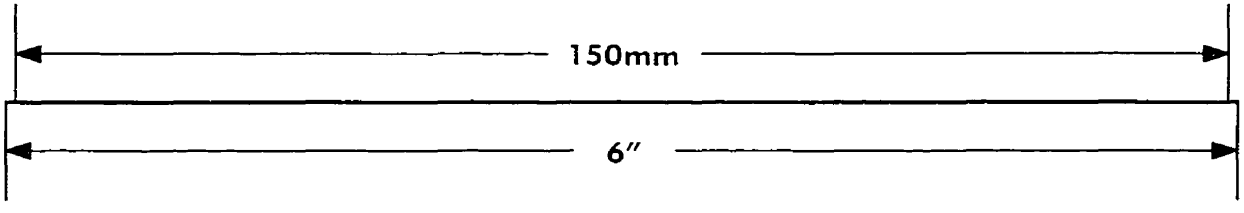
Abbreviation	Definition
A/Ci	net rate of CO_2 uptake per unit leaf area/ CO_2 concentration in the leaf intercellular space
AFP	aborted flowers and pods
ANOVA	analysis of variance
ANOVAR	repeated measures analysis of variance
CER	carbon dioxide enhancement ratio, the value of a trait at elevated (high CO_2): value of the trait under ambient (low) CO_2
CHL	chlorophyll mass per unit leaf area
CO_2	carbon dioxide
CV	coefficient of variation, CV = standard deviation / mean
C_3	plants that use the Calvin cycle photosynthetic pathway
C_4	plants that use the Hatch-Slack photosynthetic pathway
LAR	leaf area ratio, the ratio of leaf area to total plant mass
RA	reproductive allocation, the ratio of reproductive mass to total vegetative (root + shoot) dry mass
RUBISCO	ribulose 1,5-bisphosphate carboxylase enzyme
RSR	root to shoot ratio, the ratio of root dry mass to shoot dry mass
SLM	specific leaf mass, the mass per unit interveinal leaf area
SPAD	machine units measured with a Minolta SPAD-502 meter

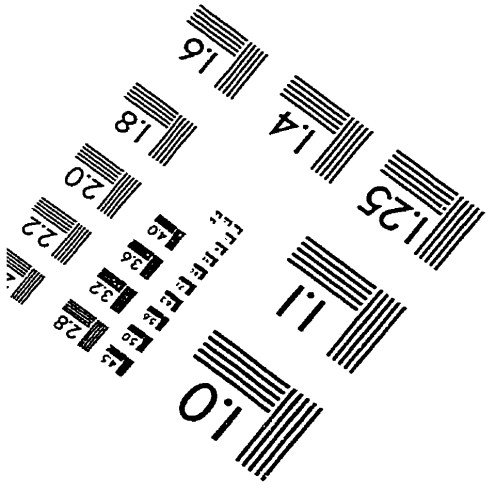
IMAGE EVALUATION TEST TARGET (QA-3)

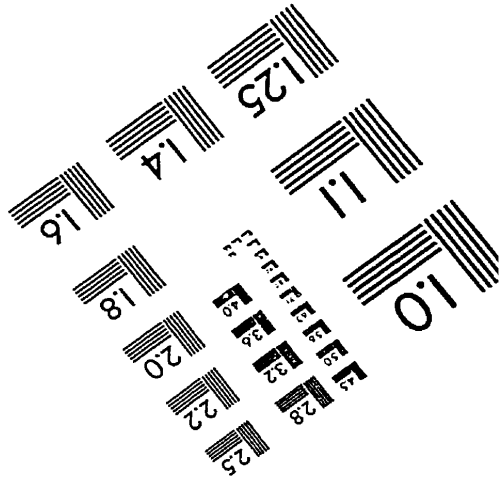












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