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REPRODUCTIVE AND GROWTH RESPONSES OF MOJAVE
DESERT PLANTS TO A CHANGING CLIMATE

by

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Bachelor of Science
California State University, San Bernardino
1993

Master of Science
California State University, San Bernardino
1996

A dissertation submitted in partial fulfillment
of the requirements for the

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Department of Biological Sciences
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ABSTRACT

Reproductive and Growth Responses of Mojave Desert Plants to a Changing Climate

By

Travis E. Huxman

Dr. Stanley D. Smith, Examination Committee Chair
Professor of Biology
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Global change may impact terrestrial ecosystems through effects on the regenerative capacities of plants. Changes in energy allocation to reproduction, as a result of increasing CO₂, changing temperature or precipitation, could shift the ecological and evolutionary factors that control plant distributions and species interactions. These changes in energy allocation to reproduction are manifested as seed number, viability and mass change. We conducted a meta-analysis of results from a number of different elevated CO₂ experiments with the goal of determining patterns of reproductive response across a large number of species and functional types. In addition, we performed a number of experimental studies that evaluated the patterns of allocation to reproduction in the context of adult performance and offspring consequences. The relative biomass allocation to reproduction is not consistently affected by exposure to elevated CO₂. Accordingly, total seed production generally increases, while seed mass decreases upon exposure to elevated CO₂, but the response is dependent upon plant functional type. Nitrogen-fixing plants do not exhibit reduced
seed mass at elevated CO$_2$. but may actually show enhancement. Changes in seed characteristics based on functional type suggest that the nitrogen economy of plants at elevated CO$_2$ is very important in combination with alterations of allometric relationships, such that both result in potential life history consequences. CO$_2$-affected changes in reproductive characters may have important implications for species distributions and trophic interactions in natural and agricultural ecosystems.
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CHAPTER ONE
OVERVIEW OF CLIMATE CHANGE AND PLANT RESPONSES

There is little scientific doubt that the carbon dioxide (CO\textsubscript{2}) concentration of
the atmosphere is rapidly increasing (Schlesinger 1997), prompting substantial efforts
to determine plant growth responses to elevated CO\textsubscript{2} and a changing climate (Bowes
1993). For the past several decades, patterns of plant vegetative response to an
elevated CO\textsubscript{2} environment have been extensively studied in growth chambers,
glasshouses and open-top chambers. Mechanistic and empirical models used to
predict plant and ecosystem response to future levels of atmospheric CO\textsubscript{2} are based on
these vegetative assessments. Despite the ecological and evolutionary importance of
plant reproduction, the flowering, fruiting and seed characteristics of terrestrial plants
exposed to elevated CO\textsubscript{2} have not been adequately addressed. This is a concern, as the
reproductive biology of terrestrial plants obviously affects the ability of populations to
change in size. Furthermore, patterns of plant reproduction (e.g., seed production) can
subsequently control a number of important ecosystem processes. A better
understanding of plant reproductive responses to a changing climate would be
necessary to construct more accurate models.

The greatest amount of useful information relative to plant reproduction and
climate change may be derived from attempts to understand the relationship between
the physiological effects of plant growth at high CO\textsubscript{2} and plant life history schedules.
This type of analysis would provide information to modelers about the timing and
investment in reproduction, and would be directly comparable to the better-documented vegetative and physiological effects of elevated CO$_2$. For adult plants, increasing CO$_2$ impacts the ability of a plant to capture resources, which may alter patterns of allocation between reproduction, vegetative growth, and tissue maintenance. Changes in these allocation patterns may be illustrated as shifts in the quantity and quality of seeds (including performance of future generations) produced by a plant. This information is invaluable for assessing fecundity. This dissertation is an attempt to better understand the reproductive responses of plants grown at elevated CO$_2$ relative to known vegetative patterns. The projects in this dissertation evaluated these patterns of growth and reproduction within the context of the life-span of an organism, the performance characteristics of offspring, and within the control of real feedbacks in an intact ecosystem. This first chapter provides background information relevant to the research goals in this project. Included is a brief review of predicted patterns of climate change for the southwestern regions of North America, and a review plant responses to elevated CO$_2$. The roles of known changes in plant function at elevated CO$_2$ are evaluated in this chapter to develop a general hypothesis for the effects of elevated CO$_2$ on the regenerative properties of plants.

*Changes in atmospheric CO$_2$, temperature and precipitation*

Records of atmospheric CO$_2$ content show a relatively stable concentration over the time period of 500 years before present (ybp) to 225 ybp, at approximately 285-290 µmol mol$^{-1}$. This stable period was followed by a rapid rise in concentration to current day measurements of greater than 340 µmol mol$^{-1}$ CO$_2$ (Ehleringer and
Cerling 1995). The rise of 60-70 μmol mol\(^{-1}\) CO\(_2\) within 1.5 centuries was more rapid than any measured changes in the past 200 k ybp. This distinct and rapid rise in CO\(_2\) concentration corresponds well to the beginning of industrialization of the European and American continents (Vitousek 1994) and can be directly attributed to fossil fuel sources and deforestation (Stuiver 1978). More recently, atmospheric samples taken from the Mauna Loa volcano in Hawaii have provided direct measurements of changes over the last 40 years (Keeling et al. 1995). These data show a significant rise in CO\(_2\) concentration of the atmosphere from approximately 315 to 360 μmol mol\(^{-1}\) between 1958 and 1995. The data also highlight the intra-annual changes in atmospheric CO\(_2\) concentration that occur as a function of the seasonal changes in photosynthesis and short-term carbon sequestration associated with temperate ecosystems of the Northern Hemisphere (Fung et al. 1987). All of this data lead to a prediction of a doubling (to 700 μmol mol\(^{-1}\)) of CO\(_2\) in the atmosphere likely by the end of the 21\(^{st}\) century.

Most general circulation models (GCM) predict that warming will result from a doubling of atmospheric CO\(_2\) concentration globally (Gates et al. 1996). These same models also predict global increases in precipitation patterns associated with land and ocean surface warming. Two models in particular highlight the range of predictions for global values of temperature and precipitation change. The Oregon State University GCM (Schlesinger and Zhao 1989) predicts a mean global temperature increase of about 2.8 °C, while the United Kingdom Meteorological Office Model (Wilson and Mitchell 1987) predicts an increase of 5.2 °C. The two models predict increases in precipitation globally of 8 % and 15 %, respectively. Regional estimates
of climate change are much more difficult to formulate and potentially deviate substantially from global mean predictions.

The predicted increase in downward infrared radiation (15 W m$^{-2}$) as a result of increased atmospheric greenhouse gas concentration should correspond to increases in mean surface temperatures of about 2-3 °C within the next century (Ramanathan 1981; Watson et al. 1990; Mitchell et al. 1995). Current predictions suggest that increases in temperature for the arid zones of southwestern North America will be greatest in winter months, with only slight enhancements during summer (Kattenberg et al. 1996). Non-GCM modeling efforts and short term environmental monitoring support these predictions (Knox 1991). This change in temperature may be important for regional flora, as temperature is an important factor in determining the distribution of plant species (Grace 1987).

Two models outlined by Giorgi et al. (1994) suggest significant increases in annual precipitation for the Intermountain West region of North America, up to an increase of 1 mm per day (cited from Kattenberg et al. 1996). GCM's of anticipated climate change in response to elevated CO$_2$ in the 21$^{st}$ century predict that the amount of winter-early spring precipitation in the desert regions of North American will remain relatively constant, but the amounts of late spring-summer rainfall might increase (Taylor and Penner 1994). These predictions are in contrast to other modeling efforts, which suggest only slight increases in precipitation for the arid Southwest in winter months, with no enhancement during summer (Knox 1991). Both attempts show that the stimulation in precipitation is greater for northern arid zones (i.e., Great Basin), but falls off with decreasing latitude. In the arid zone of North
America, seasonality in precipitation is partially responsible for large changes in regional flora (Smith et al. 1997).

Predicted impacts of climate change on the arid-southwestern United States

If the predictions of the global concentration of CO$_2$ doubling by the end of the 21$^{st}$ century hold true, along with a concomitant increase in the mean temperature of the Earth's atmosphere of 1.5-4$^\circ$C (Watson et al. 1990), there is considerable potential for a strong response from terrestrial vegetation. This is especially true if past climate effects on vegetation are good predictors of future response to climate change (Gajewski 1993). In addition to a mean increase in annual temperature, there is the potential for an increase in the number and frequency of extreme temperature events (both high and low) under specific climate change scenarios (Wagner 1996). Plant responses to temperature extremes are important in the determination of species distributions and abundances (Grace 1987). It is unclear how such temperature stress may interact with changing CO$_2$ concentrations (Roden and Ball 1996; Sage 1996). In addition, changes in precipitation patterns will significantly affect the responses of vegetation to CO$_2$ and temperature.

Currently, few studies exist in natural systems to evaluate the interactive effects of changing CO$_2$, temperature, and water. A number of projects are currently being built which will aid in the development of patterns of response of terrestrial vegetation (e.g., Jasper Ridge High CO$_2$ Experiment: a factorial CO$_2$, water addition, subtraction, and nitrogen deposition experiment). However, to make predictions of plant response in a future climate scenario at this time requires the use of single factor
experiments, such as precipitation manipulations in the Mojave Desert and the Colorado Plateau. A number of multi-factor glasshouse and growth chamber experiments have been conducted, evaluating the tolerance of seedlings to varying climatic parameters (e.g., Huxman et al. 1998b). However, there is difficulty extrapolating these responses to natural systems, which highlights the need for multi-factorial climate manipulations in intact ecosystems that would represent the full degree of climate change.

Feedbacks on plant growth associated with changes in the salient properties of the environment may become very important in ultimately directing the overall response of many ecosystems to climate change. This is especially true for the aridlands of North America, where feedbacks from water and nitrogen use by terrestrial plants may dictate responses to changing climate variables (Smith et al. 1997). Neilson and Drapek (1998) employed a series of coupled and non-coupled climate models which contain feedbacks of terrestrial vegetation to rising CO$_2$ and temperature (as outlined by Bazzaz 1990) to evaluate an effect of doubling CO$_2$ on the continental United States. The non-coupled models were based on the GCM simulations of OSU and UKMO (described above), with static ocean properties. The coupled models contained three dimensional ocean attributes. Vegetation feedbacks were described by the land surface model MAPSS (an equilibrium model with vegetation dynamics that evaluate land surface performance as a function of water and energy input constraints). This type of coupled model allows for good descriptions of energy flow between GCM compartments and fine-tuned vegetation responses based on evapotranspiration, runoff, temperature, and leaf area index in the MAPSS model.
Neilson and Marks 1994). These models highlight the relative importance of the ocean, and the degree to which plants increase water use efficiency after acclimatory responses of photosynthesis to CO₂ take place reduce growth potential (Eamus 1991).

For the northern Mojave Desert and the Great Basin, two scenarios develop from the models. First (non-coupled model), there was a predicted increase in arid zone expansion in the Mojave and Great Basin (demonstrated as decreased LAI). This resulted from an interaction of precipitation and increased water-use efficiency such that the growth response of plant species brought cover to a level that could not be supported by the water inputs. In addition, biological/ecological feedbacks, such as increased fire-cycling, contributed to the increased degree of aridity throughout the Intermountain West (Neilson 1998). This may occur through increases in surface albedo driving the difference between precipitation input and actual evapotranspiration to a higher level than can be made up by the relative increases in water use efficiency acquired by plant growth at elevated CO₂.

The second (coupled model), increases in precipitation were greater than expected for the North American continent as compared to global estimates (presumably resulting from the dynamic interaction of the ocean aspect of this model), resulting in increased leaf area index for the arid Southwest, primarily through the northern movement of grasslands into the Mojave Desert and Great Basin. The results and predictions of these two models are strongly influenced by land/surface feedbacks that are subjective model inputs of direct CO₂ effects on terrestrial vegetation. All of these additional factors in the models may be very important for the prediction of vegetation patterns in the arid Southwestern U.S. Incorporating seasonally transient
CO₂ effects on terrestrial plants, as influenced by water status, may help to resolve some of these differences highlighted in the current modeling attempts (Huxman et al. 1998a). These generalized predictions do not preclude, but suggest that to accurately predict vegetation response to climate change, a greater understanding of the mechanistic effects of elevated CO₂ on plant function is required. This is especially true considering that these models do not consider plant reproduction in any fashion.

*Plant responses to elevated CO₂*

The initial evolution of terrestrial plants occurred during periods of relatively high CO₂ concentrations (Stewart 1983). Terrestrial plants last experienced CO₂ concentrations comparable to current values approximately 50 million ybp. but since that time, CO₂ concentrations have been comparatively low. The low levels of atmospheric CO₂ is often cited as one of several factors resulting in the proliferation of plant adaptations (and subsequent species radiations) to deal with the ecophysiological problems associated with delivering high CO₂ concentrations to plant chloroplasts (i.e., C₄ and CAM photosynthesis; Ehleringer and Monson 1993). The degree of fluctuation in atmospheric CO₂ concentration currently occurring is comparable with recent variation (occurring in approximately 100 k ybp intervals) yet the magnitude is now becoming much larger (Webb and Bartlein 1992). All of these factors are important for terrestrial plants and suggest that changes in CO₂ concentration may affect plant function on a global scale.

Understanding past climate change with respect to CO₂ concentration allows us the use of data to compare with empirical and modeled results from current studies.
This will help evaluate our prediction of patterns of future climate change (Webb and Bartlein 1992; Beerling 1998). Many models and studies focus on a doubling of current levels of CO₂ to aid in predictions of plant responses to climate change (Bazzaz 1990). By using a doubling approach, the magnitude of change between current levels and future levels can be compared to known events of major change within the last 200 K ybp (Graham 1992; Peters 1992). Specifically, the increase in CO₂ (and concurrent changes in other climatic variables) between 140 K ybp and 120 K ybp (Jouzel et al 1993), and the increase between 18 K ybp and 9 K ybp (Webb 1992) are situations we can directly compare to our current research results when the doubling approach is utilized.

Of particular interest in comparing results from current research to historical events is highlighting the differences between mechanisms that terrestrial plant systems utilize in response to changing climates through: (1) migrations relating to tolerances of existing genotypes (Grace 1987; Bazzaz 1990), and (2) changes in distribution which may be associated with evolutionary forces (changes in genotype frequencies) (Curtis et al. 1996). Both of these mechanisms can be assessed using paleobotanical evidence from a given time period (Beerling 1998). For example, the changes in the distribution of the semiarid pinyon-juniper woodland which occurred between approximately 12.5 and 2.5 K ybp during recent deglaciation (co-occurring with a rising CO₂ concentration), can be directly compared to the current distribution of this vegetation type (Miller and Wigand 1994). The biogeographical data highlight differences between actual terrestrial vegetation responses to changing climatic variables, and predictions based on laboratory and field studies today (Miller and
Wigand 1994). Most research today has focused on physiological responses in plants, not the evolutionary and ecologically important life history characteristics that may be critical for shifts in species distributions and ultimately ecosystem function. Thus, adding information relative to life history characteristics of plants at elevated CO₂ would complement the general understanding of vegetative responses and provide a better means to predict responses in natural systems.

For many ecosystems in the arid southwestern United States, plant distribution is a function of several different processes, depending upon general life history strategy (i.e., perennial versus annual). Perennial plant distributions are a function of seedling survival in the abiotic environment near the soil surface (Smith and Nowak 1990). The high rates of seedling mortality seen in this region are mostly the result of adverse environmental conditions (Fenner 1985; Franklin et al. 1992). For Agave deserti seedlings in the northwestern Sonoran Desert, temperatures at the soil surface and the thermal tolerance of leaves may strongly limit the species distribution (Jordan and Nobel 1979). High temperature tolerance may be a critical factor in seedling establishment and may be driving nurse plant associations commonly seen in aridland systems (Franco and Nobel 1988; Nobel 1989). Seasonality, water, carbohydrate, and nutrient status can also impact tolerance limits of many species in the arid Southwest (Smith et al. 1997). How elevated CO₂ may affect the ability of seedlings to survive the abiotic environment near the soil surface is not well understood (Huxman et al. 1998).

For annual plants and some fast growing perennials, the ability to produce a substantial seed crop is important for long term persistence in the arid-southwestern
U.S. (Mulroy and Rundel 1977). Annual plants have often been categorized as
"stress-avoiders" due to their life history strategy of being present only in the form of
seeds during periods of extended stress. A number of factors influence the ability of a
plant's seed bank to persist in any particular location in arid zones. The total number
of seeds produced by adults in any given year and the quality of seeds produced are
both important factors in seed bank persistence although some aspects of fecundity are
not readily translatable into fitness values for modular growth forms (Venable 1997).
Simple evaluations of the reproductive characteristics of annual plants in arid
ecosystems suggest that seed production may affected by growth at elevated CO₂.
Desert plants produce seed crops as a function of plant size (Bell et al. 1979;
Ehleringer 1985). In addition, plant nutrient status affects the quality and quantity of
seeds produced in many annuals (Venable 1992). Plant size and nutrient status are
commonly influenced by plant growth at elevated CO₂ (Bazzaz 1990 & 1997). and it
would not be unexpected to hypothesize a change in reproductive characteristics as a
result.

Controlled environment investigations of terrestrial plants suggest that growth
rates are promoted in plants exposed to elevated CO₂ as a result of increases in
photosynthetic rate, often increasing final plant size (Bowes 1991). Increased
photosynthesis occurs through greater CO₂ concentrations at the site of carboxylation.
due to the increase in the diffusion gradient of CO₂ from the atmosphere into the leaf
(Sharkey 1985). An increase in CO₂ concentration at the site of carboxylation
decreases the photorespiratory characteristics of the primary CO₂ fixation enzyme in
C₃ plants. Rubisco, increasing the efficiency of carbon fixation. This increased
carboxylation efficiency and decreased photorespiratory drain results in increased instantaneous rates of carbon gain, thus promoting plant growth.

Whether plant growth will be promoted in a natural setting is not well known. The persistence of increased net photosynthesis at elevated CO$_2$ has been suggested to be a short-lived response to elevated CO$_2$, as many C$_3$ plants exposed to elevated CO$_2$ for extended periods of time often exhibit photosynthetic down-regulation (Sage 1994). How patterns of photosynthetic down-regulation occur in the field is currently not well characterized. Water, nutrient and temperature regimes appear to be important in this loss of photosynthetic enhancement at elevated CO$_2$ (Huxman et al. 1998). Down-regulation may help explain findings of no plant growth enhancement at elevated CO$_2$. Increased productivity has been shown in some field conditions (natural high CO$_2$ springs), but is dependent upon species-specific responses with respect to carbon allocation and utilization patterns that may mask the effects of changes in photosynthesis relative to CO$_2$ enhancement (Poorter 1993; Körner and Miglietta 1994). It is unclear how shifts in photosynthetic investment could alter fecundity in plants at elevated CO$_2$. The timing of photosynthetic investment relative to other life history events is important in determining fecundity (Arntz et al. 1998).

Simple increases in plant size should increase seed production as a result of allometric relationships between structures in terrestrial plants. However, allometric shifts in relative sizes of reproductive and vegetative structures occur across a range of
other environmental factors (such as nitrogen availability: Bazzaz and Ackerly 1992).
suggesting CO₂ may also affect relative allocation variables (Bazzaz 1997).
Predictions of plant reproductive responses to elevated CO₂ have been based primarily
on life history strategy paradigms (sensu Grime 1977). Since typical ruderal (weed)
species possess relatively high growth rates and high reproductive outputs, it has been
predicted that they will preferentially allocate the additional photosynthate derived
from increased CO₂ concentrations directly to reproduction (Hunt et al. 1991). By
comparison, plants with stress tolerant and competitive strategies will more
preferentially allocate the additional carbon to growth and resource capture rather than
to reproduction.

If increasing the availability of photosynthetic endproducts can force changes
in allocational patterns, the result may be to affect the trade-off relationships between
growth and reproduction (Bazzaz 1997). If patterns of reproduction change with
respect to the availability of additional carbon resources through increased
atmospheric CO₂, fecundity may change. This, in turn, would result in (1) greater
seed production per individual, and/or (2) changes in the quality of seeds produced.
Establishment of seedlings in intact ecosystems could be affected by changes in these
number-versus-quality relationships if either of these predictions are realized, as seed
quality is often indicative of seed performance (Harper 1978).

Most studies of plant response to elevated CO₂ have not considered the effects
of growth over multiple seasons or the performance of offspring produced under
elevated CO₂ conditions. Recently, Arabidopsis thaliana has been found to have
reduced seed crop viability when parents are grown under elevated CO₂ (Andalo et al.
However, the influence of this change in seed quality on offspring performance in an annual species has not been measured. Since qualitative attributes of seeds such as germination and establishment are processes that play a critical role in determining the composition of plant communities as are the initial stages of seedling growth, they are important to understand in the development of future models of plant response to elevated CO$_2$ (Harper 1978; Thompson 1992; Gloser 1996).

Since the potential for CO$_2$ induced changes in patterns of reproduction is both dependent upon life history strategy and complex patterns of allocation, this dissertation used both glasshouse and field experiments to independently evaluate plant responses to changes in CO$_2$ concentration. The study used a fast growing invasive annual (Bromus madritensis ssp. rubens, which is both ecologically and economically important to the Mojave Desert) as a model system to observe the physiology of adult reproduction and the effects of altered parental growth environment on offspring quality, quantity and subsequent growth patterns. The techniques developed in using this model system was then applied to a wide range of plant functional types, including plants within the same life history strategy that vary in functional morphology. This dissertation tested the simplistic hypothesis that increased plant size at elevated CO$_2$ and potentially improved nutrient status would increase seed production.

Three experimental projects were designed to evaluate reproduction at elevated CO$_2$, using Bromus madritensis ssp. rubens in glasshouse conditions. First, the functional reproductive response of adult plants to a change in CO$_2$ environment was measured by evaluating plant gas exchange, growth and reproduction at three different
carbon dioxide concentrations. The project focused on plant gas exchange and biomass partitioning before, during and following reproduction, in addition to seed production per individual. This study was paired with a second experiment that evaluated the ultimate changes in plant fecundity as related to the quality of seed production. This second study involved a test of the effects of parental CO₂ environment on the ability of offspring to grow and produce leaf area. In addition, several tests of the viability and resource reserve status of the seeds produced were conducted. The third experiment addressed the response of seedlings to a change in seed quality with respect to nutrient supply, in order to understand mechanisms of change in seedling performance and also patterns of expression across the landscape.

The final experiment in this dissertation addressed the reproductive responses of *Bromus madritensis* ssp. *rubens* at the Nevada Desert FACE (Free Air CO₂ Enrichment) Facility. This project evaluated similar factors as were described in the glasshouse experiments but in the context of an intact ecosystem. This project served to link concepts developed from the glasshouse experiments as a general framework for plant reproduction at elevated CO₂. In this study, it was possible to scale individual plant performance to a landscape trait, seed rain, that may influence ecosystem dynamics.

In the final chapter of this dissertation, the responses of many different species to changes in atmospheric CO₂ concentration were evaluated by the use of a meta-analytic approach in order to make broad-based predictions on the reproductive responses of plants to elevated CO₂. This study employed a statistical technique that quantitatively compared the results from multiple independent studies, a strategy that
has been useful in directing research on CO₂ effects on plants (Curtis 1996; Rosenberg et al. 1997; Osenberg et al. 1997). The working hypothesis in this study was that elevated CO₂ would increase the quantitative production of reproductive structures in plants and that elevated CO₂ would increase the quality of reproductive units produced. By comparing the general response in the literature to the specific patterns documented in the experimental studies of this dissertation, a critical evaluation of needed research was formed.
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CHAPTER TWO

REPRODUCTIVE ALLOCATION AND SEED PRODUCTION IN BROMUS MADRITENSIS SSP. RUBENS AT ELEVATED ATMOSPHERIC CO₂.

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Huxman TE. Hamerlynck EP. Smith SD (1999) Reproductive allocation and seed production in Bromus madritensis ssp. rubens at elevated atmospheric CO₂. Functional Ecology. IN PRESS
Summary

1. Two trends are consistent across the response of plant species to growth at elevated CO₂: decreased leaf nitrogen content and increased photosynthetic gas exchange. While both of these are very important to the understanding of plant and ecosystem responses to climate change, little research has evaluated the consequences of these patterns on reproductive allocation and seed production.

2. For this reason, *Bromus madritensis* ssp. *rubens* was grown in ambient (360), 1.5x ambient (550) and elevated (700 μmol mol⁻¹) CO₂ environments to compare the relationship between allocation to growth and reproduction as a function of CO₂ growth environment.

3. There were no differences in final total biomass or reproductive mass between CO₂ growth environments. There were significant decreases in reproductive mass per unit total mass and per unit vegetative mass, but not per unit leaf surface area (LSA), as growth CO₂ environment increased from 360 to 700 μmol mol⁻¹ CO₂. Despite these decreases, the number of seeds produced per unit LSA in elevated CO₂ significantly increased as compared to ambient CO₂. These results may be due to a shift in allocation to greater investment in vegetative growth as compared to reproduction under elevated levels of atmospheric CO₂.

4. Prior to reproduction, there were no significant differences between CO₂ treatments in carbon uptake by leaves. In contrast, plants grown in elevated CO₂ did not show a decline in photosynthetic rate during seed filling, suggesting that nitrogen may not have been re-translocated from leaves to seeds as apparently occurred in ambient plants.
5. These patterns measured here may partially explain the parental effect of CO₂ environment exhibited in *Bromus*, as described in Huxman et al. 1998a.

6. Since the success of *Bromus* is partially due to its ability to produce large numbers of viable seeds, these changes in reproductive allocation and subsequent seedling performance with respect to growth in an elevated CO₂ environment may have impacts on community composition in the Mojave Desert.
Introduction

Two effects of plant growth at elevated concentrations of CO₂ appear to persist across a number of experimental studies: decreases in tissue nitrogen content (Cotrufo, Ineson & Scott 1998), and increases in non-structural carbohydrate concentrations (Curtis, Drake, & Whigham 1989). Increases in non-structural carbohydrate concentrations most likely occur due to increased photosynthesis with growth at elevated CO₂ (Tissue et al. 1995), while decreased nitrogen content may be related to the re-adjustment of nitrogen investment away from Rubisco content (Sage 1994: Cotrufo et al. 1998). These basic changes in carbon and nitrogen balance in a plant can have strong influences on plant-herbivore interactions (Smith & Jones 1998), plant community composition (Tissue et al. 1995), and ecosystem processes (Kirschbaum et al. 1998). However, this change in nutrient status of leaves may also have many whole plant consequences affecting basic function related to resource allocation between growth and reproduction (Bazzaz 1997). A logical consequence of increased carbohydrate availability and redistribution of nitrogen away from Rubisco may be pronounced fecundity. Changes in fecundity in plants exposed to elevated CO₂ would have major implications for community composition and ecosystem processes (Bazzaz et al. 1992: Jackson et al. 1994).

Growth at elevated concentrations of CO₂ increases reproductive biomass in a wide range of plant species (Ackerly & Bazzaz 1995). The changes in reproductive biomass cannot be explained as simple functions of increased vegetative structures associated with enhanced photosynthesis at elevated CO₂. The high level of species specific effects of elevated CO₂ on the relationship between reproductive mass and
total plant biomass indicates that the reproductive response is a complex function of changes in allocation patterns (Ward & Strain 1997). A number of reproductive characteristics are influenced by growth at elevated CO\(_2\) as compared to ambient conditions. Seed production increases with growth CO\(_2\) concentration in *Arabidopsis thaliana*, but some genotypes reduce seed crop viability when grown in elevated CO\(_2\) conditions (Andalo *et al.* 1996; Ward & Strain 1997). Seed mass increases (*Abutilon theophrasti*) or decreases (*Plantago lanceolata*) when parents are grown in elevated CO\(_2\) conditions (Garbutt & Bazzaz 1984; Wulff & Alexander 1985). For *Bromus madritensis ssp. rubens*, an exotic grass of the Mojave Desert, initial seedling growth rates decrease when parents are grown in elevated CO\(_2\) conditions as compared to seedlings from parents grown in ambient conditions, regardless of seedling CO\(_2\) growth environment (Huxman *et al.* 1998). A similar, population specific pattern is present in *Arabidopsis*, which especially impacts belowground structures of the seedling (Andalo *et al.* 1998). All of these responses may be linked to changes in vegetative C:N ratio associated with growth at elevated CO\(_2\). Reduced nitrogen in vegetative tissues could impact the nitrogen content of reproductive structures, potentially decreasing the protein content of the seed (Andalo *et al.* 1996; Huxman *et al.* 1998). Changes in nitrogen content of the seed could have important life history consequences, such as decreased seed mass or viability, and affected patterns of resource allocation between reproduction and growth.

The objective of this study was to determine the reproductive output of *Bromus madritensis ssp. rubens* under elevated and ambient CO\(_2\), and evaluate the reproductive response as a function of vegetative growth. Of interest was any shift in

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the relationship between vegetative growth and reproduction that occurs as a result of different CO₂ growth conditions. We evaluated life history characteristics to test the hypothesis that fecundity would increase in elevated CO₂ as a result of increased photosynthate available for reproductive allocation. We evaluated gas exchange and growth characteristics to attempt to understand patterns of allocation between growth and reproduction, as expressed in a high CO₂ environment.
Materials and Methods

Study Species

*Bromus madritensis* ssp. *rubens* L. (Poaceae), an exotic annual grass, is prominent across the northern Mojave Desert. *Bromus* density has increased in this region over the last century (Mack 1981; Hunter 1991), with the grass potentially replacing a number of perennial shrubs and causing a buildup of litter and standing dead material that could increase fire frequency in the arid Southwest (Mayeux, Johnson, & Polley 1994). It has been observed that productivity in *Bromus tectorum*, another important exotic annual, may be particularly responsive to elevated CO$_2$, which could facilitate a transition from current shrub/steppe landscapes to annual grassland dominated systems in the Intermountain region (Smith, Strain, & Sharkey 1987). Since *Bromus* may have an important place in this transition, population level reactions of *Bromus* species to elevated CO$_2$ may be important for future structure and function of this aridland system.

Seed Source and Growth Conditions

Seed of *Bromus madritensis* ssp. *rubens* (originally from a population within the Nevada Test Site, NV USA; 36°49' N, 115°55' W, 970 m) were planted growing exposed to one of three different levels of atmospheric CO$_2$: ambient (360), 1.5x ambient (550), or 2x ambient (700 μmol CO$_2$ mol$^{-1}$) in three chambers (one chamber per CO$_2$ treatment) of a controlled-environment glasshouse at the University of Nevada, Las Vegas. The glasshouse was constantly monitored for CO$_2$ concentration, temperature and humidity, and all environmental variables within treatment chambers.
were kept within ± 5% of the ambient chamber. The ambient chamber was allowed to
track external atmospheric conditions as closely as possible, between temperature
thresholds of 0°C and 45°C, respectively. Plants were grown individually in large
pots (1.0 m tall x 0.15 m in diameter) with a soil blend of commercially available
sand and silt mix (80:20 mix), which is similar to the soil found on the Nevada Test
Site. Plants were germinated on November 23rd 1996 and allowed to grow until seed
maturation in late March 1997. Plants grew, flowered, set seed, and followed a
normal cycle throughout the season. Plants were watered with a 250 ml 1:40 strength
Hoagland's solution once per week from November 23rd, 1996 until flowers began to
develop in each of the chambers (simultaneous on March 5th). At this time water was
withheld to simulate a normal seasonal drying period. Availability of nutrients and
water were typical of a wet Mojave Desert season and produced plants within the
range of sizes found in natural conditions in the field.

Gas exchange

The instantaneous leaf gas exchange characteristics of Bromus under different
CO₂ growth concentrations were evaluated at two distinct phenological stages: pre-
flowering and during seed filling. Gas exchange was performed under light saturating
conditions with a Li Cor 6200 (Li Cor Inc., Lincoln, Nebraska), on the first fully
developed flag leaf of the largest stalk of each plant. This was consistent in both
stages of the life cycle. Leaf area was determined by measuring the linear dimensions
of the leaf within the gas exchange cuvette with a fine-measurement caliper.
Calculations of stomatal conductance and intercellular CO$_2$ partial pressure were accomplished according to von Caemmerer & Farquhar (1981).

*Allocation to growth and reproduction*

Plants were harvested at two distinct phenological stages: prior to flowering (February 25) and at seed maturation (March 25th). Prior to the initiation of flowering, aboveground structures were harvested and total vegetative leaf surface area (LSA) was determined with a portable leaf area meter (Delta-T Devices, Cambridge, UK). Following drying in an oven at 80°C for 48 hours, dry mass and specific leaf area (SLA: cm$^2$ leaf area g$^{-1}$ dry mass) were determined. At seed maturation (determined by the expansion of the youngest flower of each individual), whole plants were harvested and separated into aboveground and belowground parts. All harvested tissue was dried in an oven at 80°C for greater than 48 h. Dry mass of reproductive structures, vegetative structures and belowground structures were determined. Surface area of leaves was determined for a sub-sample of individual leaves using the portable leaf area meter. Regressions relating surface area to dry mass, specific to each CO$_2$ environment, were used to scale individual leaf area to whole plant leaf surface area (LSA).

The mass of reproductive structures was determined by separating the accessory support structure and seeds and determining their total mass separately. Mean seed mass for each stalk on each plant was determined from individual measurements of 10-30 randomly selected seeds. Total seed mass produced by an individual was divided by mean seed mass to determine the total number of seeds.
produced. Seed viability was determined for each individual by germination of 10-20 seeds. The total number of seeds produced by each plant, multiplied by the specific seed viability allowed for a conservative estimate of fecundity (Huxman & Loik 1997).

Allometric measurements of reproductive allocation were determined from the mass and leaf area measurements taken above. The total mass of reproductive structures was expressed as a function of total biomass, vegetative mass, and leaf surface area (LSA) to compare CO₂ treatments. In addition, total seed number was expressed as a function of LSA, as this gives an estimate of fecundity as a function of the potential for carbon gain of the vegetative structures (Huxman & Loik 1997). These parameters represent investment in reproduction and can be compared allometrically to competing allocation pathways, such as growth. The resulting comparisons between the reproductive variables and such characters as total biomass, vegetative mass and SLA allows for a comparison between the relative trade-off between growth and reproduction under elevated versus ambient CO₂ conditions.

Oven dried leaf tissue and seeds from greater than five randomly selected individuals in each CO₂ treatment were taken and ground to a fine powder (250 μm) and sealed into small 5 x 9 mm tin capsules. These samples were analyzed for total nitrogen and carbon by Micro-Dumas combustion in an elemental auto analyzer (NA1500 C/H/N Analyzer, Carlo Erba Strumentazione, Milan) at the University of Georgia. Nitrogen and carbon values are expressed either as a ratio (C:N), or content (moles g⁻¹), determined from % content and sample mass.
Dried leaf tissue from two distinct phenological stages was ground in alcohol to a fine material for leaf starch extraction and analysis. Leaf residue remaining after alcoholic extraction was gelatinized. Amyloglucosidase (Sigma, St Louis Mo.) was used to digest the starch to glucose in a 45°C incubator for 48h. Glucose was detected enzymatically (Sigma, St Louis, Mo.). Glucose was detected colorimetrically using a broad-beam spectrophotometer (Bio-Rad) at 340 nm. Samples were compared to glucose standards purchased from Sigma Chemical Co. (St. Louis, Mo.).

**Statistical analysis**

One factor ANOVA with three levels of treatment was used to compare the effects of CO₂ growth environment on vegetative and reproductive characteristics of Bromus. For data collected at two distinct phenological stages on different individuals (LSA, SLA, aboveground biomass), a two-factor ANOVA was used to evaluate the effects of CO₂ treatment, phenological stage, and their interaction. For data collected on the same individuals at two distinct phenological stages (gas exchange), a split-plot, repeated measures ANOVA was used to evaluate the effects of phenological stage, CO₂ treatment, and their interaction. Proportional and percent data were arcsine transformed. Post-hoc testing for interaction effects was also performed (Newman-Keuls method), testing significance at α = 0.05. For C:H:N and starch analyses, a sample size of between five and ten individuals from each treatment combination was used. For all other analyses, sample size was between 15 and 25. Analysis of covariance (ANCOVA) was used to evaluate allocation shifts between
growth and reproductive pathways, occurring in concert with changes in treatment CO₂ growth environment (Coleman et al. 1993). Linear regression between reproductive and growth characters highlighted allocation shifts.
Results

Prior to the onset of reproduction, aboveground biomass in *Bromus* was significantly greater in elevated CO₂-grown plants than in ambient CO₂-grown plants (Fig. 2.1). Plants grown at 550 and 700 μmol mol⁻¹ CO₂ accumulated 2.5 and 2.6 fold more mass aboveground, respectively, than did ambient-grown plants. However, by maturity, there were no significant effects of CO₂ environment on aboveground.

Fig. 2.1. Biomass of *Bromus madritensis* ssp. *rubens* in three CO₂ growth environments. Data are means ± one standard error. For the lower panel, error is that of total biomass. Aboveground biomass prior to reproduction is included for comparison with that at seed maturity. Significant differences are indicated with lower case letters (p < 0.05).
belowground, reproductive, or total plant biomass (Fig. 2.1). In pre-reproductive plants, total leaf surface area per plant (LSA) did not increase at elevated CO$_2$, and this lack of CO$_2$ response remained through maturity (Fig. 2.2). In this case, maturity in *Bromus* would also correspond to peak leaf area. In pre-reproductive plants, specific leaf area (SLA) declined at both elevated CO$_2$ treatments as compared to ambient-grown plants, but no such response was detected at maturity.

![Graph](image)

**Fig. 2.2.** Whole plant leaf surface area (LSA) and specific leaf area (SLA) for *Bromus madritensis* ssp. *rubens* at three CO$_2$ growth environments during two distinct life cycle stages: pre-reproduction and seed maturity. Significant differences are indicated with different lower case letters (p < 0.05).

The allometric relationship between aboveground and belowground biomass accumulation prior to reproduction did not change with the application of atmospheric CO$_2$ (data not shown). However, the relationship between reproductive and
vegetative biomass changed significantly with growth at different CO$_2$ concentrations, with the slope of the regression line decreasing with increasing CO$_2$ (Fig. 2.3; Table 2.1). In addition, the relationship between seed number per plant and specific leaf

![Graph](image)

Fig. 2.3. The relationship between reproductive mass and vegetative mass for three CO$_2$ treatments (360, 550, and 700 µmol mol$^{-1}$) in *Bromus madritensis* ssp. *rubens*. Specifics of the linear regression are given in Table 1
Table 2.1. Reproductive and vegetative relationships with respect to growth in different CO$_2$ environments for *Bromus madritensis* ssp. *rubens*. F (F statistic from ANCOVA), df (degrees of freedom), m (slope), b (intercept). $r^2$ (coefficient of determination). All regression equations are significant at $\alpha < 0.05$.

<table>
<thead>
<tr>
<th>Relationship</th>
<th>F</th>
<th>df</th>
<th>CO$_2$</th>
<th>m</th>
<th>b</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproductive and vegetative mass</td>
<td>17.0</td>
<td>2.57</td>
<td>360</td>
<td>1.45</td>
<td>0.18</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>550</td>
<td></td>
<td></td>
<td>0.64</td>
<td>0.33</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>700</td>
<td></td>
<td></td>
<td>1.0</td>
<td>0.22</td>
<td>0.84</td>
</tr>
<tr>
<td>Seed # and SLA</td>
<td>4.50</td>
<td>2.57</td>
<td>360</td>
<td>-7.15</td>
<td>1270</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>550</td>
<td></td>
<td></td>
<td>-8.10</td>
<td>1275</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>700</td>
<td></td>
<td></td>
<td>-26.5</td>
<td>3000</td>
<td>0.48</td>
</tr>
<tr>
<td>Seed # plant$^{-1}$ and reproductive mass</td>
<td>3.54</td>
<td>2.57</td>
<td>360</td>
<td>440</td>
<td>6.9</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>550</td>
<td></td>
<td></td>
<td>470</td>
<td>39</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>700</td>
<td></td>
<td></td>
<td>540</td>
<td>6.6</td>
<td>0.99</td>
</tr>
<tr>
<td>Seed # plant$^{-1}$ and vegetative mass</td>
<td>10.6</td>
<td>2.57</td>
<td>360</td>
<td>638</td>
<td>88</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>550</td>
<td></td>
<td></td>
<td>305</td>
<td>191</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>700</td>
<td></td>
<td></td>
<td>402</td>
<td>235</td>
<td>0.68</td>
</tr>
<tr>
<td>Seed # LSA$^{-1}$ and total mass</td>
<td>3.6</td>
<td>2.57</td>
<td>360</td>
<td>0.0009</td>
<td>-0.00030.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td>550</td>
<td></td>
<td></td>
<td>0.0018</td>
<td>-0.00090.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>700</td>
<td></td>
<td></td>
<td>0.0014</td>
<td>-0.00100.77</td>
<td></td>
</tr>
</tbody>
</table>
area (SLA) changed with CO$_2$ concentration (Table 2.1). The slope of the relationship decreased as CO$_2$ level increased, corresponding to a greater investment in growth (decrease in SLA), as compared to reproduction (seed number), similar to the relationship between reproductive and vegetative biomass (Fig. 2.4). A similar change occurred in the relationship between seed number and vegetative mass (Table 2.1). Greater biomass accumulation in plants grown at 550 and 700 µmol mol$^{-1}$ CO$_2$ resulted in proportionally fewer seeds per plant. Concurrent with these increases in

![Diagram](image)

**Fig. 2.4.** The relationship between seed number produced per plant and specific leaf area (SLA) for *Bromus madritensis* ssp. *rubens* grown at three atmospheric CO$_2$ treatments (360, 550, and 700 µmol mol$^{-1}$). Specifics of the linear regression are given in Table 2.1.
relative growth allocation at elevated CO₂. The relationship between seed number and reproductive mass increased at elevated CO₂ resulting in slightly more seeds of lesser mass (Tables 2.1 & 2.2). An estimate of the efficiency of reproduction (seed number per LSA) and investment in growth (total mass) changed in relation to CO₂ growth environment. The number of seeds per LSA increased more rapidly with increasing biomass accumulation in ambient grown plants as compared to elevated (Table 2.1).

Concurrent with the allometric relationships between growth parameters and reproduction, several estimates of reproductive allocation decreased with the application of higher than ambient levels of CO₂. Reproductive allocation (reproductive mass per unit vegetative mass) decreased by approximately 22% when grown at higher than ambient levels of CO₂ (F = 7.04; p < 0.05; Table 2). Reproductive mass per unit aboveground mass decreased from 3.97 ± 0.17 to 3.34 ± 0.36 at 550 and 3.11 ± 0.08 at 700 μmol mol⁻¹ CO₂ versus 360 μmol mol⁻¹ CO₂ (F = 4.34; p < 0.05). Additionally, reproductive mass per unit total mass also decreased as CO₂ concentration increased, though slightly less (by approximately 10%; F = 10.0; p < 0.05). Despite decreases in reproduction on a per mass basis, reproductive mass per unit LSA did not change, as also with total seed number per plant and total seed number per unit vegetative mass (Table 2.2). An additional measure of reproductive allocation, seed number per unit LSA, increased by approximately 1.4 fold (F = 3.39; p < 0.05). This probably occurred through slight increases in seed number per plant, with decreases in seed mass (Table 2.2 and Huxman et al. 1998), and no significant changes in LSA.
Table 2.2. Reproductive characteristics of *Bromus madritensis* ssp. *rubens* grown at ambient (360 μmol mol⁻¹), 1.5x ambient (550 μmol mol⁻¹) and elevated (700 μmol mol⁻¹) CO₂ concentrations. Data are expressed as means ± S.E. Different letters within a character represent significant differences (p < 0.05).

<table>
<thead>
<tr>
<th>CO₂ environment (μmol mol⁻¹)</th>
<th>Character</th>
<th>360</th>
<th>550</th>
<th>700</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproductive Allocation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reproductive mass per unit vegetative mass (g g⁻¹)</td>
<td>1.805 ± 0.05a</td>
<td>1.409 ± 0.16b</td>
<td>1.339 ± 0.04b</td>
<td></td>
</tr>
<tr>
<td>Reproductive mass per unit leaf surface area (g cm⁻²)</td>
<td>0.036 ± 0.001a</td>
<td>0.032 ± 0.002a</td>
<td>0.035 ± 0.001a</td>
<td></td>
</tr>
<tr>
<td>Seed number per vegetative mass (# g⁻¹)</td>
<td>808 ± 25a</td>
<td>766 ± 98a</td>
<td>716 ± 28a</td>
<td></td>
</tr>
<tr>
<td>Seed number per leaf surface area (g cm⁻²)</td>
<td>4.64 ± 0.57a</td>
<td>4.96 ± 0.75a</td>
<td>6.53 ± 0.36b</td>
<td></td>
</tr>
<tr>
<td>Fecundity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed number (# plant⁻¹)</td>
<td>478 ± 43a</td>
<td>446 ± 51a</td>
<td>575 ± 35a</td>
<td></td>
</tr>
<tr>
<td>Seed mass (mg)</td>
<td>2.26 ± 0.09a</td>
<td>1.96 ± 0.11ab</td>
<td>1.74 ± 0.11b</td>
<td></td>
</tr>
</tbody>
</table>
Growth at elevated CO$_2$ produced an interesting response in the leaf gas exchange characteristics of *Bromus* between pre-reproductive and seed filling stages (Fig. 2.5). Across all three CO$_2$ treatments, light saturated CO$_2$ assimilation rate was 1.9 fold greater pre-reproductive than during seed filling ($F = 25.5; p < 0.05$), and stomatal conductance was 3.3 fold greater pre-reproductive than at seed filling ($F = 30.3; p < 0.05$). Interestingly, this reduction in gas exchange during seed filling became less pronounced at higher CO$_2$ concentrations. For example, net CO$_2$ assimilation pre-reproductive was 2.8 fold, 1.8 fold, and only 1.15 fold greater than at seed filling when *Bromus* was grown at ambient, 550, and 700 $\mu$mol mol$^{-1}$ CO$_2$ concentration, respectively. This reduction over time and phenology was due to maintenance of high net CO$_2$ assimilation during seed filling at elevated CO$_2$ as compared to ambient, whereas in pre-reproductive plants values at all CO$_2$ growth concentrations were similar. Because of this increase in CO$_2$ assimilation during seed filling (for example, *Bromus* plants grown at 700 $\mu$mol mol$^{-1}$ had a 2.8 fold higher CO$_2$ assimilation rate than did ambient-grown plants), there was an overall CO$_2$ effect on net photosynthesis ($F = 9.0; p < 0.05$). Similarly, there was an overall CO$_2$ effect on stomatal conductance ($F = 19.4; p < 0.05$), but in the case of conductance the greatest reductions at elevated CO$_2$ occurred during the pre-reproductive phase (Fig. 2.5). There was no phenological (time) response of internal CO$_2$ concentration ($C_i$) ($F = 5.2; p > 0.05$) in *Bromus*, although $C_i$ did decrease by 17% from pre-reproductive to seed filling stage at 700 $\mu$mol mol$^{-1}$ CO$_2$ concentration (Fig. 2.5).
Fig. 2.5. Gas exchange responses of *Bromus madritensis* ssp. *rubens* at three different CO$_2$ environments during two distinct life cycle phases: pre-reproduction and seed filling. Significant differences (p < 0.05) between CO$_2$ growth concentrations are indicated with different lower case letters, while differences within a CO$_2$ treatment, but between life cycle stages, are indicated with an asterisk.

C:N ratio of leaf tissues markedly increased from pre-reproductive to seed maturity in all CO$_2$ treatments (F = 258; p < 0.05), which was primarily a response to
a decline in leaf N concentration during seed filling (F = 387; p < 0.05) (Fig. 2.6). CO₂ concentration had a slight effect on these parameters, as both elevated CO₂ treatments resulted in higher leaf C:N ratios than in ambient CO₂-grown plants (F = 4.76; p < 0.05). For leaf-N content, pre-reproductive plants had highest leaf-N content at ambient CO₂ and lowest leaf-N content at 700 µmol mol⁻¹ CO₂ (F = 8.9; p < 0.05), but there were no CO₂ treatment effects by seed maturity. Carbon content of leaf tissues showed very slight declines from pre-reproduction to maturity (F = 19.0; p < 0.05), and no CO₂ treatment effects were observed for this parameter (F = 0.6; p > 0.05). Growth at 550 and 700 µmol mol⁻¹ CO₂ prior to flowering increased the content of starch in leaves as compared to ambient by 1.4 and 3.0 fold, respectively (F = 41; p < 0.05) (Fig. 2.6). The increase in starch concentration at seed maturity from 360 to 700 µmol mol⁻¹ CO₂ was much more distinct (4.2 fold increase). Overall, a general decrease occurred for each CO₂ treatments from pre-flowering levels to levels at maturity (F = 35; p < 0.05) (Fig. 2.6).
Fig. 2.6. Leaf carbon and nitrogen status of *Bromus madritensis* ssp. *rubens* leaves at three different CO$_2$ growth environments during two distinct life cycle stages: pre-reproduction and seed maturity. Significant differences among treatments and times are indicated with different lower case letters (p < 0.05).
Discussion

*Bromus madritensis* ssp. *rubens* exposed to elevated CO$_2$ for a full life cycle did not increase reproductive biomass or total seed number as compared to counterparts grown in ambient CO$_2$, despite showing increased carbon assimilation and reduced leaf nitrogen investment during seed filling. These findings are not consistent with the hypothesis that enhanced productivity associated with increased carbon fixation and decreased photosynthetic nitrogen investment in plants exposed to elevated CO$_2$ would result in the production of greater numbers of viable seeds per individual. Instead, reproductive allocation, in terms of reproductive mass per unit vegetative mass, decreased in plants grown at elevated CO$_2$, despite seed number per unit leaf surface area increasing with greater concentrations of CO$_2$. This occurred through shifts in allometric relationships between growth parameters and reproductive parameters such that less investment proportionally occurred in reproductive structures at elevated as compared to ambient CO$_2$.

The results of *Bromus* from this experiment are contrary to our original hypothesis, but are consistent with the temporal patterns of gas exchange and nitrogen allocation associated with growth at elevated CO$_2$. Ambient-grown plants showed a typical decrease in photosynthetic rate during seed filling as compared to the values during vegetative growth, corresponding to re-allocation away from growth toward reproduction. The elevated CO$_2$-grown plants did not show differences between gas exchange values at these different phenological stages. The decrease in concentration of nitrogen per unit dry mass of leaf tissue, with no significant changes in total plant biomass, suggests that the pool of nitrogen available for re-translocation to
reproduction is smaller in plants grown at elevated CO$_2$ as compared to ambient CO$_2$ counterparts. These two factors (gas exchange and nitrogen allocation) could potentially explain the differences between hypothesized patterns and those actually measured.

Measurements of reproductive allocation on a biomass basis for annuals such as _Bromus_ are indicative of both standing and total life-time allocation to reproduction (Bazzaz & Ackerly 1992). Biomass allocation to reproduction has been shown to both increase and decrease for plants grown at elevated CO$_2$. Reekie & Hicklenton (1994) showed that increased growth at elevated CO$_2$ co-occurs with reduced reproductive allocation, depending upon species-specific, photoperiod-driven development patterns. Changes are especially pronounced when elevated CO$_2$ increases plants to critical sizes for flowering prior to external phenological triggers. It appears that elevated CO$_2$ can alter the trade-off in allocation between growth and reproduction such that phenological cues from development (plant size, critical leaf number) become asynchronous with environmental cues (photoperiod, water stress, and temperature), resulting in life history consequences, such as reduced seed set. McConnaughay et al. (1993) showed that an increase in reproductive biomass in _Abutilon theophrasti_ occurred to a greater extent when roots were restricted from growth, seemingly re-adjusting allocation away from a typically strong sink in plants grown at elevated CO$_2$ (Rogers _et al._ 1992). Therefore, it seems likely that the reductions we observed in _Bromus_ are related to the effect elevated CO$_2$ may have on allocation trade-offs between growth and reproduction. Specifically, the pattern of carbon fixation late in development suggests that resources were retained in leaves for
a longer period in elevated CO₂-grown plants than in ambient-grown plants. This allows for preferential allocation to vegetative growth over reproduction in *Bromus* at elevated CO₂. Further evidence for this exists in the proportionally larger increase in leaf starch at seed maturation in elevated-grown plants as compared to their enhancement over ambient plants prior to flowering.

Trade-offs in life history traits are usually identified by negative correlations (Stearns 1992). However, depending upon the variable measured, either a negative or positive relationship can be exhibited, and both can be used to identify a trade-off (Schlichting & Pigliucci 1998). Probably the most valid evaluation of growth and reproduction is highlighted in this study by simple biomass accumulation in either pathway, which when compared shows a positive correlation. The trade-off thus is not exhibited in the overall relationship, but in the decrease in slope (Schlichting & Pigliucci 1998). Increased variation in one of the components as compared to the other can lead to a positive relationship that overshadows the variance in the trade-off (van Noordwijk & de Jong 1986). In this study, when two traits that did not show dramatic variation were examined, a negative relationship indicating a trade-off is present. Thus, in the relationship between seed production and SLA at seed maturity, the increased investment in growth, and decreased investment in reproduction at elevated CO₂ is demonstrated.

Changes in allocation to reproduction are often a function of a size-versus-number optimization of flower, fruit, and seed production to new environments (Lee 1988). For a number of species (especially grasses), growth at elevated CO₂ reduces reproductive allocation as a result of reduced seed number per fruit and fruit number.
per plant (Roy et al. 1996; Navas et al. 1997). In contrast, Curtis, Snow & Miller (1994) showed increases in reproductive allocation in *Raphanus raphanistrum* a result of increased seed number per plant. The differences in these two responses may be a result of different meristematic patterns: *Raphanus* has indeterminate meristems, while many grasses have determinant inflorescences (Curtis et al. 1994; Waller 1988). This suggests the two types have different allometric constraints in providing for sink activity relative to source tissue activity. In grasses, the production of leaf area (resource pool) increases to a lesser extent relative to reproductive tissues at elevated CO$_2$ such that the leaf area cannot support similar levels of seed production. Indeterminate plants may maintain source activity by production of sufficient leaf area relative to reproductive structures at elevated CO$_2$ providing resources for the construction of fruit and seed. In *Arabidopsis thaliana*, which has an indeterminate floral meristem, elevated CO$_2$ enhances seed number per plant, which results in increased reproductive allocation (Ward & Strain 1997). However, the increase in seed number may occur at a genotypic-specific reduction in seed size (Andalo et al. 1998).

Despite these changes in seed number and total biomass production, trade-offs associated with seed mass are present. This is probably most evident in *Bromus*, which showed decreases in reproductive allocation on a mass basis, and increases in viable seed number per unit leaf surface area, suggesting that a greater number of seeds could be produced for each unit of leaf area increase. However, the type of seed that could be supported was smaller in mass. The pattern is most evident with the allometric relationship between seed number and reproductive mass, which has a
reduced slope in elevated CO\textsubscript{2} plants. This may occur as a result of the decreased nitrogen pool in the whole plant, when grown at elevated CO\textsubscript{2}. Since leaf biomass was similar between CO\textsubscript{2} environments, and the moles of N per unit leaf mass decreased with increasing CO\textsubscript{2}, total N in leaves, available for re-translocation to seeds also decreased at elevated CO\textsubscript{2}. The reduction in total nitrogen, and the sequestration in leaf tissue until late in flowering, results in seeds with reduced nitrogen contents (Andalo \textit{et al.} 1998; Huxman \textit{et al.} 1998). There appears to be an exception to this trend of reduction in seed mass when other life history characters show increases or no significant changes. Nitrogen fixers can show changes in seed number produced (increases), with decreasing seed viability (resulting in similar viable seed production across CO\textsubscript{2} environments), while seed mass increases (Farnsworth & Bazzaz 1995). This suggests that the nitrogen limitation highlighted by \textit{Bromus} and potentially \textit{Arabidopsis} is overcome in plants that have a greater control over their nitrogen budgets.

Taken together with the reported changes in seed quality for \textit{Bromus} grown at elevated CO\textsubscript{2}, this suggests that there could be population-level responses for \textit{Bromus} in natural conditions. The seeds of \textit{Bromus} harvested from parents grown in elevated CO\textsubscript{2} conditions have lesser mass, greater C:N ratio, greater surface area and produced seedlings that have lower relative growth rates (RGR), achieving lower final biomass than seeds of \textit{Bromus} taken from parents grown under ambient CO\textsubscript{2} conditions (Huxman \textit{et al.} 1998). These characteristics, in combination with the growth patterns in adults presented here, could scale to changes in the ability of \textit{Bromus madritensis} ssp. \textit{rubens} to expand its distribution and persist in the arid southwestern US.
Acknowledgments

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CHAPTER THREE

THE EFFECTS OF PARENTAL CO2 ENVIRONMENT ON SEED QUALITY AND SUBSEQUENT SEEDLING PERFORMANCE IN _BROMUS MADRITENSIS_ SSP. _RUBENS_

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Abstract

Seeds were collected and compared from parent plants of *Bromus madritensis* ssp. *rubens* L. (Poaceae), an exotic Mojave Desert annual grass, grown in ambient (360 μmol mol⁻¹) and elevated (700 μmol mol⁻¹) CO₂ to determine if parental CO₂ growth conditions affected seed quality. Performance of seeds developed on the above plants was evaluated to determine the influence of parental CO₂ growth conditions on germination, growth rate, and leaf production. Seeds of *Bromus* developed on parents grown in elevated CO₂ had greater pericarp surface area, higher C:N ratio, and less total mass than ambient developed seeds. Parental CO₂ environment did not have an effect on germination percentage or mean germination time, as determined by radicle emergence. Seedlings from elevated CO₂ developed seeds had a reduced relative growth rate and achieved smaller final mass over the same growth period. Elevated CO₂ developed seeds had smaller seed reserves than ambient seeds, as determined by growing seedlings in sterile media and monitoring senescence. It appears that increased seed C:N ratios in plants grown under elevated CO₂ may have a major effect on seed quality (morphology, nutrition) and seedling performance (growth rate, leaf production, etc.). Since the invasive success of *Bromus* is primarily due to it’s ability to rapidly germinate, increase leaf area and maintain a relatively high growth rate compared to native annuals and perennial grasses, reductions in seed quality and seedling performance in elevated CO₂ may have significant impacts on future community composition in the Mojave Desert.
Introduction

Increasing atmospheric levels of CO$_2$ have prompted investigations of terrestrial plant responses to altered climatic scenarios. Some of the most common findings suggest that plants exposed to elevated CO$_2$ show increased growth as a result of increases in photosynthetic rate (Bowes 1993). However, this response has been a point of controversy because of the observation that C$_3$ plants exposed to elevated CO$_2$ for extended periods of time exhibit photosynthetic acclimation (Sage 1994). When assessing plants exposed for centuries to elevated CO$_2$ in natural springs, increased productivity has been shown, but it is dependent upon species specific responses with respect to carbon allocation and utilization patterns (Poorter 1993: Körner and Miglietta 1994). In the event that productivity is enhanced in plants exposed to elevated CO$_2$, a logical consequence of increased productivity may be increased fecundity. Changes in fecundity in plants exposed to elevated CO$_2$ would have major implications for community composition and ecosystem processes (Bazzaz et al. 1992: Jackson et al. 1994).

Most studies of plant response to elevated CO$_2$ have not considered the effects of growth over multiple seasons or the performance of offspring produced under elevated CO$_2$ conditions. Recently, *Arabidopsis thaliana* has been found to have reduced seed crop viability when parents are grown under elevated CO$_2$ (Andalo et al. 1996). However, the influence of this change in seed quality on offspring performance in an annual species has not been measured. Seed germination is a process that plays a great part in determining the composition of plant communities as do the initial stages of seedling growth (Harper 1978; Thompson 1992; Gloser 1996).
*Bromus madritensis* ssp. *rubens* L. (Poaceae; referred to here-after as *Bromus*).

an exotic annual grass, is prominent across the northern Mojave Desert (Hunter 1991). Whereas much is known about the biology of *Bromus tectorum* (Mack 1981). *Bromus* is relatively unfamiliar. It is known that the density of *Bromus* on the Nevada Test Site has increased over the last century (Hunter 1991), with *Bromus* possibly replacing a number of perennial shrubs (Mayeux et al. 1994). All *Bromus* species have been shown to cause a buildup of litter and standing dead material and potentially increase fire frequency in the desert Southwest (Mayeux et al. 1994). It has been observed that productivity in *Bromus* may be particularly responsive to elevated CO\(_2\), which could facilitate transitions from current scrub/steppe landscapes to annual grassland dominance in the Intermountain region (Smith et al. 1987; Sage 1996). Since *Bromus* may have an important place in this transition, population level reactions of *Bromus* to elevated CO\(_2\) may be very important in terms of predicting future structure and function of this aridland system.

Predictions of plant response to elevated CO\(_2\) based on life history data (Hunt et al. 1991) indicate that *Bromus* will increase both in growth and allocation to reproduction when grown in elevated CO\(_2\) environments. *Bromus*, with a relatively high growth rate and high reproductive output, is a typical ruderal species (sensu Grime 1977). Ruderal species are predicted to preferentially allocate the additional photosynthate derived from increased CO\(_2\) concentrations to reproduction (Hunt et al. 1991). If so, fecundity may change in elevated CO\(_2\), resulting in either (1) greater seed production per individual or (2) changes in the quality of seeds produced.
Establishment of seedlings in intact ecosystems could be affected if either of these predictions are realized.

In this study, we tested the hypothesis that increased parental CO₂ environment will improve the quality of seeds produced resulting in increased subsequent germination and initial growth in *Bromus* seedlings. This prediction is based upon the assumption that additional reproductive allocation results from greater photosynthesis by parent plants. We also hypothesized that the interaction of parental CO₂ environment and subsequent seedling germination and growth in an elevated CO₂ environment would enhance seed characteristic changes, thus promoting seedling growth. This was tested by germinating seeds from a natural population in a controlled glasshouse and growing them through a complete life cycle in either ambient (360 µmol mol⁻¹) or elevated (700 µmol mol⁻¹) CO₂, characterizing the seed produced and growing them in the same and in reciprocal atmospheric CO₂ conditions. The effect of elevated CO₂ on seed reserve status for seeds from parents grown in different CO₂ levels was also evaluated by germinating and growing seeds on sterile agar and monitoring senescence over time.
Materials and Methods

Seed Source

Seed of *Bromus madritensis* ssp. *rubens* (originally from a natural population within the Nevada Test Site, NV USA: 36°49’ N, 115°55’ W, 970 m) were taken from parents growing exposed to one of two different levels of atmospheric CO$_2$: ambient (360 μmol mol$^{-1}$ CO$_2$) or elevated (700 μmol mol$^{-1}$ CO$_2$) chambers of a controlled-environment glasshouse at the University of Nevada, Las Vegas. Plants were grown in 1 m tall pots, approximately 15 cm in diameter, in a soil solution consisting of an 80:20 mix of commercially obtained sand and silt. Soil analysis showed that nutrients available for uptake by plants were a function of those which were applied during watering. Plants were watered once per week with 500 ml of 1:40 Hoagland’s solution. The glasshouse was constantly monitored for CO$_2$ concentration, along with temperature and humidity, and all values were kept within ± 5% of the ambient room. The ambient room was allowed to track external atmospheric conditions as closely as possible (lower and upper temperature thresholds of 0 °C and 45 °C, respectively). Greater than thirty individuals were used as a seed source from each CO$_2$ treatment room, and pooled according to CO$_2$ treatment.

Seed characteristics and viability

We recorded the mass of seeds number 2, 4, 9 and 15 (numbering refers to the seed position relative to the newest seed produced on an inflorescence) on the inflorescences of ten plants from each CO$_2$ treatment. This positional arrangement is representative of the ontogenetic relationship of these seeds during development.
representing seed filling. For the remainder of the study (viability, growth rate or resource reserve experiments), only seeds in position 14-18 were used to ensure growth experiments were performed on fully developed seeds. Mass and surface area were measured for these seeds and compared between parental sources (ambient versus 2x ambient). Nitrogen content (% dry mass) was determined with a CHN analyzer.

To measure viability, seeds from both parental sources were germinated in petri dishes on 1.4% sterile agar in a darkened room at 25°C. Five seeds were germinated in a single petri dish and monitored each day, with germination recorded as radicle emergence. All dishes were monitored over a period of 14 days. A total of 25 petri dishes were used for each seed source: 125 seeds for each. The mean germination time for each petri dish was computed as:

\[
\text{mean time} = \frac{\sum X_i D_i}{\sum X_i} \tag{1}
\]

where \(D_i = \text{day } i\) and \(X_i = \text{number of germinated seeds on day } i\) (Scott et al. 1984). The combined mean of petri dishes containing ambient CO\(_2\) developed seed was compared to the combined mean of petri dishes containing elevated CO\(_2\) developed seed.

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Seedling Growth Characteristics

Seedlings of *Bromus* were germinated and grown in the following treatments. One hundred and sixty eight seeds which had developed on 30 ambient CO₂ grown plants (ambient developed seeds) and 30 elevated CO₂ grown plants (elevated developed seeds) were equally divided between the ambient and the elevated CO₂ controlled glasshouse rooms, giving a sample size of 42 for each seed source/CO₂ growth environment combination. Pots were randomized within each CO₂ treatment room, and allowed to grow for 22 days. Any seeds not germinating were excluded from data analysis. Seedling emergence from soil was recorded for each seed source and growth environment in order to ensure that seedlings grew for similar periods of time.

Prior to planting, each seed was first measured for mass and surface area, then sown in a moist sandy loam soil in 500 ml containers. The seed was covered with enough soil such that a small portion of the pericarp protruded through the surface. then received an initial watering treatment of 10 ml distilled H₂O. For six days, seeds were watered every other day with 10 ml of a 1:40 Hoagland’s solution. after which seedlings received 10 ml of 1:40 Hoagland’s solution every third day until the end of the experiment (22 days). At 22 days, seedlings were harvested and aboveground and belowground structures were separated. Leaf area (LA) was determined with a Delta T area meter (Delta-T Devices, Cambridge, UK). Roots were washed with distilled H₂O and dry mass was determined for aboveground and belowground structures by oven drying at 90° C for 72 h.
From measurements of seed dry mass and seedling final dry mass, relative growth rate (RGR) was determined as:

\[
RGR = \frac{(\text{seedling mass} - \text{seed mass})}{\text{mean mass} / 22 \text{ days}}
\]

Leaf area ratio (LAR) was calculated as seedling LA per total seedling dry mass. Net assimilation rate (NAR) was calculated as the change in dry mass from seed to seedling per unit LA per day. Specific leaf area (SLA) was determined as seedling LA per unit aboveground mass, and leaf mass ratio (LMR) was the mass of aboveground structures per total seedling biomass. Root-to-shoot ratio was also determined. These parameters have been useful in quantifying differences in growth responses of seedlings (Swanborough and Westoby 1996).

**Resource Reserves**

One hundred twenty five seeds from each parental source were washed with a 0.5% bleach solution and then sown into 1.4% sterile agar within large 35 ml scintillation vials covered with parafilm which had a single hole punctured in the center. Vials were placed on racks in controlled growth room conditions (25°C, 12 hour photoperiod, 300 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) PPFD). Seedlings were monitored daily for radicle emergence, green leaf production and survival. Mean green leaf tissue production was determined by using equation (1) where \(X_i\) is the number of individuals with green
leaves on \( D_1 \). The number of individuals maintaining greater than 50% of their leaves green and turgid to a particular date was expressed as the percentage of the total seedlings which were sown and which germinated.

*Statistical analysis*

Seed viability, mean germination time, and mean time of green leaf production were compared between parental sources by a student's t-test. The survival of seedlings in sterile agar was compared by a student's t-test at each day interval. A two way ANOVA was used to compare the effects of seed source and \( \text{CO}_2 \) growth environment and their interaction on seedling growth characteristics. Proportional and percent data were arcsin transformed. Post-hoc testing for interaction effects was also performed (Student-Newman-Keuls method, SigmaStat, Jandel Scientific), testing significance at \( \alpha = 0.05 \).
Results

For both ambient and elevated CO₂ developed seeds, mass increased with position along the inflorescence, with lower numbered positions being significantly smaller than later, which were up to five times greater in mass (Fig. 3.1). While there was no significant difference between seed sources with respect to seed mass of lower numbered seeds which were typically not viable, for positions number 9 and 15 ambient CO₂ developed seeds had up to 28% greater mass than elevated CO₂ developed seeds (df = 110, P < 0.002). However, elevated CO₂ seeds had a greater pericarp surface area and a higher C:N ratio than ambient seeds (Table 3.1).

Fig. 3.1. Mean seed mass (mg) versus seed position number relative to top of the inflorescence from Bromus grown in 360 μmol mol⁻¹ CO₂ (Ambient seeds) and 700 μmol mol⁻¹ CO₂ (Elevated seeds). Vertical bars represent ± one S.E.; n > 50 at each position number, for each growth environment.
Ambient CO$_2$ developed seeds did not have significantly different percentages of viability than elevated CO$_2$ developed seeds in all experiments. The mean germination time in petri dishes with sterile agar in the dark or in large vials exposed to a normal photoperiod was not significantly different for the different seed sources (Table 3.1). However, mean time to green leaf production in large scintillation vials

Table 3.1. Seed and seedling characteristics for ambient grown seed (360 µmol mol$^{-1}$ CO$_2$) and elevated seed (700 µmol mol$^{-1}$ CO$_2$) sources. Data are expressed as means ± S.E. Sample sizes are located in brackets beside data. (* = p < 0.05)

<table>
<thead>
<tr>
<th>Seed characteristic</th>
<th>Ambient Seeds</th>
<th>Elevated Seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed mass (mg)</td>
<td>2.25 ± 0.12 (140)</td>
<td>1.76 ± 0.10 (140) *</td>
</tr>
<tr>
<td>Seed surface area (cm$^2$)</td>
<td>0.145 ± 0.002 (45)</td>
<td>0.18 ± 0.02 (45) *</td>
</tr>
<tr>
<td>Seed C:N</td>
<td>16.2 ± 0.2 (6)</td>
<td>20.8 ± 0.3 (8) *</td>
</tr>
<tr>
<td>Viable seeds (%)</td>
<td>98.0 ± 0.1 (125)</td>
<td>97.0 ± 0.3 (125)</td>
</tr>
<tr>
<td>Mean day of germination (d)</td>
<td>2.23 ± 0.004 (125)</td>
<td>2.29 ± 0.009 (125)</td>
</tr>
<tr>
<td>Mean day of green leaf prod. (d)</td>
<td>4.38 ± 0.01 (125)</td>
<td>5.59 ± 0.01 (125) *</td>
</tr>
</tbody>
</table>

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was delayed by 20% in the elevated CO\textsubscript{2} developed seed source as compared to the ambient developed seed source (Table 3.1). By comparison, for seeds sown in pots provided with a nitrogen source and exposed to the various CO\textsubscript{2} treatments, there was no difference in the mean day of seedling emergence, as determined by green leaf protrusion through the soil (F = 1.21. df = 1. 131. P > 0.05; Table 3.1).

After 22 days of growth in the glasshouse in both CO\textsubscript{2} treatments, final aboveground biomass for seedlings produced by ambient developed seeds was greater than final aboveground biomass for seedlings produced by elevated CO\textsubscript{2} developed seeds (Fig. 3.2A; F = 6.79. df = 1. 131, P < 0.01). Shoot mass was not affected by changes in CO\textsubscript{2} growth environment and there was not an interaction between CO\textsubscript{2} environment and seed source. While seedlings differed significantly in aboveground biomass, leaf area did not significantly differ between seed source or CO\textsubscript{2} growth environment (Fig. 3.2B). Seed source and CO\textsubscript{2} growth environment had the greatest effect on total biomass (Fig. 3.2C; F = 58.5. df = 1. 131. P < 0.0001 for seed source; F = 13.6. df = 1. 131. P < 0.001 for CO\textsubscript{2} environment; F = 6.59. df = 1. 131. P < 0.01 for their interaction). Ambient developed seeds produced larger seedlings than elevated CO\textsubscript{2} developed seeds and the 700 \textmu mol mol\textsuperscript{-1} CO\textsubscript{2} growth environment only stimulated growth for the ambient developed seed source (Fig. 3.2C). The difference in seedling biomass between seed sources was a result of ambient developed seeds producing seedlings with larger roots (Fig. 3.2D) than elevated CO\textsubscript{2} developed seeds. A similar interaction between CO\textsubscript{2} growth environment and seed source on root mass was present (F = 54.1. df = 1. 131, P < 0.0001 for seed source; F = 15.8, df = 1. 131. P < 0.001 for CO\textsubscript{2} environment; F = 8.13. df = 1. 131, P < 0.01 for their interaction).
Fig. 3.2. Mean biomass production of the two seed sources (ambient seeds and elevated CO₂ grown seeds) of *Bromus* under two CO₂ growth treatments (360 μmol mol⁻¹ CO₂ and 700 μmol mol⁻¹ CO₂). Vertical bars represent one S.E. in each case. Lower case letters denote significant differences among groups (p < 0.05).

The rate of biomass increase of the seedling (RGR), in this case seed mass, was greater for seedlings produced from ambient developed seeds as compared to seedlings produced from elevated CO₂ grown seeds (Fig. 3.3A; F = 49.93, df = 1, 131, P < 0.0001). There was an interaction between seed source and CO₂ growth environment resulting in greater RGR for seedlings produced from ambient seeds grown under elevated CO₂ conditions (F = 3.40, df = 1, 131, P < 0.05). NAR differed significantly between seed sources and CO₂ growth environments (Fig. 3.3B; F = 43.3, df = 1, 131, P < 0.0001 for seed source: F = 15.6, df = 1, 131, P < 0.001 for growth environment).
Fig. 3.3. Growth parameters of seedlings grown from the two seed sources (ambient seeds and elevated CO\textsubscript{2} grown seeds) of \textit{B. rubens} under two CO\textsubscript{2} growth treatments (360 μmol mol\textsuperscript{-1} CO\textsubscript{2} and 700 μmol mol\textsuperscript{-1} CO\textsubscript{2}). Vertical bars represent one S.E. in each case. Lower case letters denote significant differences among groups (p < 0.05).

and seed source and CO\textsubscript{2} growth environment interacted to nearly double NAR from ambient to elevated CO\textsubscript{2} growth environment in seedlings produced by ambient seeds (F = 9.35, df = 1.131, P < 0.01). A majority of the interaction effects between seed
source and CO₂ growth environment can be explained through the increase in root:shoot ratios in the seedlings produced from ambient seeds within the elevated CO₂ growth environment (Fig. 3.3C; $F = 15.07, df = 1, 131, P < 0.001$).

LAR differed significantly between seed sources regardless of CO₂ growth environment (Fig. 3.3D; $F = 32.95, df = 1, 131, P < 0.0001$) and followed a non-significant trend of reduction in the elevated CO₂ growth environment as compared to the ambient CO₂ growth environment for both seed sources. LAR can be partitioned into the product of the fraction of biomass in leaves (LMR) and measures of leaf thickness (SLA), and most of the variation in LAR can be explained by mixed effects from these two parameters. LMR was significantly greater for seedlings produced from elevated CO₂ grown seeds as compared to seedlings produced from ambient seeds (Fig. 3.3E; $F = 12.4, df = 1, 131, P < 0.001$). In addition, SLA differed significantly between seed sources with leaves produced from elevated CO₂ seedlings having more mass per unit area than leaves from seedlings produced by ambient seeds (Fig. 3.3F; $F = 9.5, df = 1, 131, P < 0.01$). The trend of smaller LMR and SLA values for seedlings produced from ambient seeds as compared to those produced by elevated CO₂ developed seeds, led to a greater difference in LAR between the seed sources.

When plants were grown in sterile agar, seedlings produced from elevated CO₂ grown seeds senesced leaves at a greater rate than seedlings produced from ambient seeds (Fig. 3.4). Leaf survival over time was reduced by 10% in seedlings from elevated CO₂ grown seeds as compared to those produced by ambient seeds, and this represented a measure of internal reserve status.
Fig. 3.4. Mean relative leaf senescence for seedlings of the two seed sources (ambient seeds and elevated CO$_2$ grown seeds) of *Bromus* grown in sterile agar over the same growth period. Data are mean ± S.E. Error bars are all smaller
Discussion

Seeds of *Bromus madritensis* ssp. *rubens* harvested from parents grown in elevated CO\(_2\) concentration had lesser mass, greater C:N ratio, greater surface area and produced seedlings which had reduced relative growth rates (RGR) and achieved smaller final biomass than seeds of *Bromus* taken from parents from ambient CO\(_2\) conditions. Seedlings produced by these seeds had smaller root and shoot masses, but produced the same leaf area as their counterparts produced in ambient CO\(_2\).

Additionally, ambient CO\(_2\) seeds produced seedlings with greater NAR and root:shoot ratio, but with smaller LAR, LMR, and SLA than for elevated CO\(_2\) seeds. Seed reserves were greater in ambient seed source seedlings, as indicated by longer retention of green foliage than seedlings from elevated CO\(_2\) grown seeds when both were grown in sterile agar. Taken together, these results are not consistent with our hypothesis that increased photosynthate production by plants grown in elevated CO\(_2\) would increase photosynthate allocation to seeds, thereby improving seed quality.

Whereas the results for plant growth from ambient seed source seedlings are consistent with widely reported improvements in plant performance under elevated CO\(_2\), the growth pattern results in elevated CO\(_2\) seed source seedlings are not. For instance, total biomass has been found to increase in *B. tectorum* exposed to elevated CO\(_2\) (Smith et al. 1987), and RGR increases in a wide number of species growing in elevated compared to ambient CO\(_2\) (Allen et al. 1991; Dippery et al. 1995).

Additionally, most elevated CO\(_2\) growth studies have found that NAR increases while LAR decreases when compared to ambient grown plants (Norby and O’Neill 1991; Bowler and Press 1993; Dippery et al. 1995). In the current study, all these parameters
(total biomass, RGR, NAR and LAR) change accordingly under elevated CO$_2$ (Figs. 3.2 and 3.3), but only in seedlings from seeds produced by parents grown in ambient CO$_2$. Seedlings from elevated CO$_2$ source seeds, in contrast, showed little or no response in these growth parameters when exposed to elevated CO$_2$ conditions. This has substantial implications for plant growth in populations exposed to multiple generations of elevated CO$_2$.

An unexpected result in the current study was that elevated CO$_2$ did not stimulate an increase in leaf area in seedlings (Fig. 3.2B). In a related plant, $B$. tectorum, Smith et al. (1987) found that leaf area increased by as much as 145% when exposed to elevated CO$_2$. These plants were grown over 120 days as compared to 22 days in the present study. Over such a short period of initial growth, the development of belowground biomass may be more important than the development of leaf tissue, particularly in Bromus (Harris and Wilson 1970). In addition, at such an early stage of development, it is uncertain that there is sufficient plasticity in allocation patterns to respond to elevated CO$_2$ with a change in leaf area or display. Seedlings of this size may be constrained in their response to increasing resources (such as increasing CO$_2$), but may instead be more sensitive to decreases in resources (such as seed reserves).

Changes in reproductive biomass have been reported for several species grown under elevated CO$_2$ conditions. Seed mass of Abutilon theophrasti, a C$_3$ dicot annual, increased on exposure to elevated CO$_2$ (McConnaughay et al. 1993), as did reproductive biomass in Achillea millefolium, Campanula isophylla, Xanthium pensylvanicum, and Pharbitis nil (Reekie and Hicklenton 1994). In B. madritensis (not same sub-species), the ratio of reproductive to vegetative biomass decreased when
grown under elevated CO$_2$ (Roy et al. 1996). The majority of species studied by Roy et al. (1996) showed reductions in the number of seeds per fruit and seed number per plant, although the effect of CO$_2$ on reproductive output between species was highly variable. As valuable as these studies have been, they did not investigate specific seed viability and germination characteristics. In a follow up to the results presented here, reproductive biomass and seed number per plant did not change for *Bromus* upon exposure to elevated CO$_2$, rather there was a decrease in reproductive biomass per unit vegetative biomass with exposure to elevated CO$_2$ as compared to ambient CO$_2$ counterparts (T. Huxman, unpublished data).

Garbutt and Bazzaz (1984) found that seed production in populations of *Datura stramonium* and *Abutilon theophrasti* was genotype specific, but individual seed mass increased with increasing CO$_2$. The difference between the results in the current study and the results from Garbutt and Bazzaz (1984) suggest that differences in life history may result in unique allocation patterns differentially mediated by changes in CO$_2$ environment. Future studies on different functional groups with respect to life history may lead to a greater understanding of patterns of allocation within these plants. To our knowledge, only seeds of *Arabidopsis thaliana* have been shown to respond to an elevated parental CO$_2$-growth condition by reducing seed viability. In *Arabidopsis*, genotype specific decreases in seed viability occurred in plants exposed to elevated CO$_2$ (Andalo et al. 1996), but no data has been reported on seedling performance from these seeds.

Seed size and morphology can reflect the amount of stored reserves available for germination, and can therefore influence germination success (Bewley and Black
1994). Despite differences in seed size and morphology (Table 3.1) between seeds from different parental CO₂ environment plants, germination was not limited in elevated CO₂ grown seeds. However, seedlings from these seeds grow more slowly over the initial period of establishment (Fig. 3.3). These findings suggest that the establishment phase of growth in *Bromus* may more closely affect population structure in a high CO₂ environment. This stands in contrast to models utilizing *B. tectorum* (Sage 1996), which suggest step increases in annual seedling germination and establishment.

The results from this study suggest that predictions of plant reaction to elevated CO₂ based upon a simple three category life history model (e.g., Grime 1977; Hunt et al. 1991) may be too simplistic. Differences in species within categories with respect to seed production and seed type may also be important factors. For example, seedlings which utilize lipid or starch seed reserves may differentially respond to increases in available photosynthate under elevated CO₂ conditions compared to plants with seed processes based more on nitrogen allocation. Numerous studies have highlighted the importance of species specific and population specific reaction to elevated CO₂ growth condition across a wide range of plant processes (Andalo et al. 1996; Knapp et al. 1996). While the population in this study reacted to elevated CO₂ by producing seeds with lower initial performance, populations within this species may exist that could react differently to elevated CO₂. Based on these results, climatic factors interacting with the reduced potential for growth and establishment induced by elevated CO₂ may ultimately control the distribution of *Bromus* within the Mojave Desert much more strongly than elevated CO₂. Studies of physiological responses of
plant offspring in an elevated CO₂ environment may add significantly to predictions of plant reaction to future climate scenarios.

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Jackson RB. Sala OE. Field CB. Mooney HA (1994) CO₂ alters water use, carbon gain, and yield for the dominant species in a natural grassland. Oecologia 98:257-262


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CHAPTER FOUR

THE EFFECTS OF PARENTAL CO₂ AND OFFSPRING NUTRIENT ENVIRONMENT ON INITIAL GROWTH AND PHOTOSYNTHESIS IN AN ANNUAL GRASS

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Abstract

Seeds of *Bromus madritensis* ssp. *rubens* (red brome; an invasive Mojave Desert annual grass), from parents grown at three CO$_2$ levels (350, 550, & 700 µmol mol$^{-1}$), were grown in factorial CO$_2$ (360, 550, & 700 µmol mol$^{-1}$) and nutrient (zero addition, 1:40, & 1:10 strength Hoagland's solution) environments to evaluate parental CO$_2$ effects on offspring performance characteristics across a range of developmental environments. We evaluated growth rates, leaf nitrogen content and photosynthetic gas exchange over an initial 3 week growth period. Seedlings from elevated CO$_2$ parental seed sources (2xAMB Seeds or Seedlings) had reduced growth rates as compared to seedlings from ambient CO$_2$ grown parents (AMB Seeds or Seedlings). Elevated CO$_2$ stimulated RGR as compared to ambient CO$_2$ growth conditions for most seedlings, but the degree of stimulation was greatest for the AMB Seedlings and least for the 2xAMB Seedlings. Instantaneous rates of photosynthesis mirrored the pattern of RGR across the parental CO$_2$ and seedling CO$_2$ treatment combinations. At ambient CO$_2$, photosynthetic rates of 2xAMB Seedlings were half that of AMB Seedlings. Analysis of A-Ci response curves suggests that 2xAMB Seedlings had a reduced allocation of nitrogen to Rubisco activity as compared to AMB Seedlings, in addition to less total nitrogen investment in leaves. AMB Seedlings responded to low levels of nutrient input (1:40 Hoaglands) with increased growth rates and leaf N content as compared to zero nutrient addition. 2xAMB Seedlings required the application of 1:10 Hoaglands prior to an increase in these two parameters, as compared to zero nutrient addition. These results suggest elevated CO$_2$ affects
Bromus offspring performance through changes in seedling nitrogen dynamics. such that reductions in photosynthesis and growth rates occur in successive generations. Establishment patterns of this invasive grass across a range of nutrient conditions typical of the heterogeneous Mojave Desert indicates potentially differential success across the landscape. Ultimately the distribution of seed quality in seed rain may be important for population response to elevated CO\textsubscript{2} in natural systems.
Introduction

Plant responses to climate change have been evaluated from the perspective of vegetative growth and physiological parameters over relatively short time periods. While the response of a single generation of individual plants to increasing CO$_2$ is very important for understanding the basis for CO$_2$ control over many levels of biological organization, responses over multiple generations will most likely have the primary effect on species distributions in a future high CO$_2$ world. Until recently, very little information has been generated concerning the reproductive responses of plants to a changing climate as compared to vegetative responses (Bazzaz 1997). This includes potential cross-generation effects that may be important in seedling establishment (Andalo et al. 1996; Huxman et al. 1998). Changes in reproductive output, quality of reproductive units, and the subsequent performance characteristics of offspring have profound effects on population dynamics in natural systems (Lee 1988), an aspect of global change that has not yet been sufficiently addressed. In addition, changes in reproductive characteristics may lead to altered selection patterns as compared to current conditions, increasing the potential for rapid evolutionary responses (Curtis et al. 1996). While changes in gene frequencies may be important in understanding species distributions in a high CO$_2$ world, parental effects (characteristics that affect the quality of offspring produced by a plant) may also be important in determining species distributions. Understanding the impact of parental CO$_2$ growth environment on offspring performance should be very important in gaining a full understanding of climate change effects on the terrestrial biosphere.
Parental CO$_2$ environment can affect future offspring performance (Huxman et al. 1998). In *Arabidopsis thaliana*, seed viability can be reduced when parents are grown at elevated CO$_2$ (Andalo et al. 1996). Decreases in growth potential also occur, most likely as a result of decreased ability to produce root tissue early in development (Andalo et al. 1998). Initial seedling growth rates in *Bromus madritensis* ssp. *rubens*, an exotic annual grass in the Mojave Desert, are also a function of parental CO$_2$ growth conditions (Huxman et al. 1998). Seeds from *Bromus* plants grown at elevated CO$_2$ produce seedlings that have reduced growth rates as compared to offspring from plants grown in ambient CO$_2$. In addition, seedlings from elevated CO$_2$ grown parents did not show as strong of a growth stimulation to repeated growth at elevated CO$_2$. This pattern may be imposed upon seeds as a function of complex allocation trade-offs between growth and reproduction in the adult plant that functionally results in a reduction in the pool of nitrogen available for re-translocation to seed production at elevated CO$_2$ (Huxman et al. 1999).

Reductions in growth rates of *Bromus* seedlings from elevated CO$_2$ parents can be explained by a number of mechanisms. Initial seedling photosynthetic performance may be responsible, as reduction in net assimilation rate (biomass derived), is partially responsible for the overall reduction in relative growth rate (Huxman et al. 1998). Mechanistically, this hypothesis suggests that reduced in seed nitrogen content in seeds from elevated CO$_2$ grown plants has a smaller pool available for initial investment in photosynthetic tissues. In this paper, we test the hypothesis that the reduction in nitrogen content of seeds from plants grown at elevated CO$_2$ leads to seedlings that have a decreased ability to fix carbon in general. In addition, it is
hypothesized that seedlings with reduced seed nitrogen reserves do not have the ability to take advantage of the increased potential for CO$_2$ fixation available as a result of enhanced CO$_2$ growth environment that typifies seedlings with adequate nitrogen reserves. We also test the hypothesis that this response (reduction in growth potential) is not plastic, and addition of supraoptimal nutrient supplements will not offset the reduced growth potential, as it is a function of seed quality, not seedling growth environment.
Materials and Methods

Seed Source and Growth Conditions

Seeds of *Bromus madritensis* ssp. *rubens* used in this experiment (originally from a natural population within the Nevada Test Site, NV, USA) were taken from parents growing exposed to one of three different levels of atmospheric CO$_2$ - ambient (350 µmol mol$^{-1}$ CO$_2$), 1.5x ambient (550 µmol mol$^{-1}$) or 2x ambient (700 µmol mol$^{-1}$ CO$_2$) - in a controlled-environment glasshouse at the University of Nevada, Las Vegas. These seeds (and subsequent seedlings) will be referred to hereafter as: AMB Seeds, 1.5xAMB Seeds, and 2xAMB Seeds (or Seedlings), respectively. The glasshouse was constantly monitored for CO$_2$ concentration with a Li-Cor 6252 infrared gas analyzer (LiCor, Lincoln, NE, USA) under computer control and maintained at above set points with a CO$_2$ injection system. Air temperature and humidity were maintained within ± 5% of the ambient room, which was allowed to track external atmospheric conditions as closely as possible.

Seedling Growth Design

Seeds harvested from the above plants were planted in a factorial design to evaluate the effects of parent CO$_2$ growth environment, seedling CO$_2$ growth environment, and seedling nutrient amendment treatment. The three CO$_2$ environments exposed to parents were also used for seedlings (we will refer to seedling growth environment as: 360, 550, or 700 µmol mol$^{-1}$ CO$_2$). Three levels of nutrient treatments were administered: distilled water, 1:40 strength Hoagland's
solution, and 1:10 strength Hoagland's solution (Hoagland and Arnon 1950). Greater than thirty individuals were used as seed sources from each CO₂ treatment, pooled according to CO₂ treatment. Prior to planting, each seed was first measured for mass and then sown in moist soil in 500 ml containers. The seed was covered with enough soil such that a small portion of the pericarp protruded through the surface, then received an initial watering treatment of 10 ml distilled H₂O. For six days, seeds were watered every other day with 10 ml of the appropriate nutrient treatment. After six days, seedlings received the appropriate solution every third day until the end of the experiment (3 weeks).

After the 3 weeks of growth, seedlings were harvested and aboveground and belowground structures were separated. Leaf area (LA) was determined with either a Delta T area meter (Delta-T Devices, Cambridge, UK) or hand held calipers. Leaf area estimates were compared between techniques to insure consistency of values. Roots were washed with distilled H₂O and dry mass was determined for aboveground and belowground structures following oven drying at 90° C for 72 h. From measurements of seed dry mass and seedling final dry mass growth parameters were determined as in Huxman et al. (1998). Relative growth rate (RGR) was determined as the incremental growth over the 3 week period, relative to the average mass of the seedling over the growth period. Leaf area ratio (LAR) was calculated as seedling LA per total seedling dry mass. Net assimilation rate on a biomass basis (NAR) was calculated as the change in dry mass from seed to seedling per unit LA per day. Specific leaf area (SLA) was determined as seedling LA per unit aboveground mass, and leaf mass ratio (LMR) was the mass of aboveground structures per total seedling
biomass. Root-to-shoot ratio was also determined. These parameters have been useful in quantifying differences in growth responses of seedlings, especially *Bromus* (Swanborough and Westoby 1996; Huxman et al. 1998).

Following oven drying at 90 °C for 3 days, carbon and nitrogen contents were determined for leaf samples. Samples were ground to a fine powder (250 μm) and sealed into 5 x 9 mm tin capsules. These samples were analyzed for total nitrogen and carbon by Micro-Dumas combustion in an elemental auto analyzer (NA1500 C/H/N Analyzer, Carlo Erba Strumentazione, Milan, Italy) at the University of Georgia. Nitrogen and carbon values are expressed either as a ratio (C:N), or content (moles g⁻¹), determined from % content and sample mass.

**Photosynthetic Characteristics**

Prior to harvesting, seedling photosynthetic characteristics were determined with a Li Cor 6400 portable photosynthetic gas exchange system. The A-C₁ (assimilation rate-internal CO₂ concentration) response of one to three seedlings from each treatment combination was determined with the programmable, open-flow gas exchange system. Block temperature was held at 25°C and RH at 25%, typical of recent glasshouse environmental growth conditions. All measurements were made with a red light source at photosynthetic photon flux density (PPFD) values of 1500 μmol m⁻² s⁻¹. Prior to the initiation of the A-C₁ curve, instantaneous gas exchange parameters (net photosynthesis, Aₙₑᵦ: stomatal conductance, gₛ) were determined for selected individuals at their respective growth CO₂ concentration. Following these measurements, CO₂ concentration of the cuvette (Cₐ) was reduced to 200 μmol mol⁻¹.
for five minutes to stimulate stomatal opening and then reduced to 75 \( \mu \text{mol mol}^{-1} \). \( C_a \) was incrementally increased to 150, 250, 350, 550, 700, 800, 900, 1200, and 2000 \( \mu \text{mol mol}^{-1} \) and gas exchange properties were logged at each \( C_a \) once the system had reached a pre-determined stability point (coefficient of variation = 1%). The data from the A-C\(_{\text{i}}\) response was fit to a nonlinear least-squares regression using an exponential model from which maximum CO\(_2\) saturated photosynthetic rate (\( A_{\text{max}} \)) and carboxylation efficiency (CE) were determined (Jacob et al. 1995).

Statistical analysis

A three way ANOVA was used to compare the effects of parental CO\(_2\) environment seed source, seedling CO\(_2\) growth environment, and seedling nutrient treatment, and their interactions on seedling growth characteristics, leaf photosynthetic parameters, and leaf carbon and nitrogen contents. Proportional and percent data were arcsin transformed to meet ANOVA assumptions. Post-hoc testing for interaction effects was also performed (Student-Newman-Keuls method), testing significance at \( \alpha = 0.05 \). An ANCOVA was used to evaluate relative growth rate, which can be size dependent, with total mass included as a covariate in the model.
Results

Both parental seed source and seedling nutrient addition affected total biomass production in *Bromus*. In 1.5x and 2xAMB Seedlings, total biomass was reduced by 20% as compared to AMB Seedlings ($F = 9.5; \text{df} = 2.238; p < 0.05$) as a function of proportional decreases in both root biomass and shoot biomass. CO$_2$ growth environment and parental CO$_2$ seed source had no effect on root:shoot ratio. Increasing the amount of Hoagland's solution applied to seedlings increased total seedling biomass ($F = 19.0; \text{df} = 2.238; p < 0.05$). Plants grown with only distilled water were approximately $6.1 \pm 0.2$ g, as compared to $7.0 \pm 0.2$ and $8.0 \pm 0.2$ g for 1:40 strength and 1:10 strength Hoagland’s, respectively. In addition, root:shoot ratio decreased from $2.62 \pm 0.05$ to $2.36 \pm 0.06$ and $2.10 \pm 0.06$ with increasing nutrient supply (distilled water, 1:40 Hoaglands and 1:10 Hoagland’s, respectively), suggesting greater allocation to shoots as plants were provided with more nutrients ($F = 18.8; \text{df} = 2.238; p < 0.05$).

1.5x and 2xAMB Seedlings showed overall reductions in RGR (by about 10%) as compared to AMB Seedlings ($F = 3.83; \text{df} = 4.238; p < 0.05$; Fig. 4.1a). When the seedlings were grown at 550 and 700 µmol mol$^{-1}$ as compared to 360 µmol mol$^{-1}$ CO$_2$, there were small increases in growth rate for each parental seed source. However, at each CO$_2$ growth condition, RGR of 1.5x and 2xAMB Seedlings was less than that of AMB Seedlings (Fig. 4.1a). The degree of stimulation for seedling growth at 700 µmol mol$^{-1}$ CO$_2$ was greatest for AMB Seedlings (18 % greater as compared to 2xAMB Seedlings). Total mass did not influence the change in RGR with different
Fig. 4.1. Relative growth rate of *Bromus madritensis* ssp. *rubens* seedlings as a function of parental CO$_2$ growth environment (AMB, 1.5xAMB, or 2xAMB Seedlings): (A) Interactions with seedling (offspring) CO$_2$ growth environment (360, 550, or 700 µmol mol$^{-1}$ CO$_2$): (B) Interactions with seedling (offspring) nutrient addition amount. In panel A, letters above general parental CO$_2$ growth environment categories represent significant differences among groups. In panel B, small letters above bars represent differences among treatment combinations. Values are means ± S.E.

parental CO$_2$ growth environments across the seedling growth CO$_2$ environments ($F = 1.9; df = 2. 238; p > 0.05$).

Nutrient supply affected the growth rates of each parental seed source differently ($F = 12.96; df = 2. 238; p < 0.05$: Fig 4.1b). In general, AMB Seedlings increased relative growth rate as the strength of the Hoagland’s solution increased.
(from 0.010 ± 0.003 g g\textsuperscript{-1} d\textsuperscript{-1} at 0% Hoagland's to 0.120 ± 0.002 g g\textsuperscript{-1} d\textsuperscript{-1} at 1:10 strength Hoagland's). Seedlings grown from 2xAMB Seeds did not show such a significant increase in RGR with increasing nutrients. For 1.5x ambient CO\textsubscript{2} parental seed source seedlings, the increase was only from 0.092 ± 0.002 to 0.116 ± 0.002 g g\textsuperscript{-1} d\textsuperscript{-1}, and for 2x ambient CO\textsubscript{2} parental seed source the increase was only 0.094 ± 0.002 to 0.109 ± 0.003 g g\textsuperscript{-1} d\textsuperscript{-1} when nutrients were increased from 0% Hoagland's to 1:10 strength Hoagland's. As compared to growth in distilled water, growth rates at 1:40 strength Hoagland's was stimulated for AMB Seedlings, but for the 1.5x and 2xAMB Seedlings, a similar increase did not occur. The amount of nutrients in the 1:40 strength Hoagland's was not sufficient to increase growth rates for these elevated seedlings.

Net assimilation rate (on a biomass basis) changed with nutrient supply (F = 4.1; df = 2. 72; p < 0.05). Values decreased from 1.43 ± 0.1 to 1.20 ± 0.1 and 1.02 ± 0.1 g m\textsuperscript{-2} d\textsuperscript{-1} with the increasing nutrient supply (0. 1:40 and 1:10 Hoagland's, respectively). This decrease in NAR was due to increased total allocation to leaves versus roots, which constitute the greater proportion of biomass allocation in these small seedlings. Likewise, LMR changed only with nutrient supply (F = 18.9; df = 2. 238; p < 0.05), in concert with root:shoot ratio. That is, LMR increased with increasing nutrient supply (from 0.284 ± 0.005 to 0.306 ± 0.006 and 0.330 ± 0.005 g g\textsuperscript{-1} for 0. 1:40 and 1:10 Hoagland's, respectively). Leaf area ratio also increased with increasing nutrient supply (F = 7.3; df 2. 72; p < 0.05). along with slight changes in
Fig 4.2. Carboxylation efficiency (A) and net photosynthesis (B) as a function of parental CO₂ growth environment (AMB, 1.5xAMB, or 2xAMB Seedlings) and seedling CO₂ growth environment (360, 550, and 700 μmol mol⁻¹ CO₂). Different letter combinations above bars represent differences among treatment combinations. Values are means ± S.E.

specific leaf area. These interactions with nutrient supply and CO₂ growth environment made it difficult to determine patterns across the entire nutrient supply. CO₂ growth environment and parental CO₂ seed source treatment combinations for these growth parameters.

There were significant reductions in carboxylation efficiency (CE) with increasing CO₂ growth environment for AMB Seedlings (Fig. 4.2a). This reduction in
CE was greater than 50% for plants grown at 700 as compared to 360 μmol mol\(^{-1}\)CO\(_2\). A similar reduction in carboxylation efficiency as in the AMB Seedlings was not present for seedlings grown from 1.5x and 2xAMB Seeds when they were grown at 700 as compared to 360 μmol mol\(^{-1}\) CO\(_2\). Effects on carboxylation efficiency due to interactions with seedling nutrient supply were difficult to detect for the sample sizes used.

Net photosynthesis (A\(_{\text{net}}\)) was greater at 550 and 700 μmol mol\(^{-1}\) seedling CO\(_2\) growth concentrations as compared to 360 μmol mol\(^{-1}\) for each parental seed group (Fig. 4.2b). For AMB Seedlings, net photosynthesis increased about 50% as growth CO\(_2\) increased from 360 to 700 μmol mol\(^{-1}\), as compared to a 3-fold increase in A\(_{\text{net}}\) for 2xAMB Seedlings. Despite this dramatic increase in response between growth CO\(_2\) environments, there was a general decrease in A\(_{\text{net}}\) with increasing parental CO\(_2\) growth environment in the 360 μmol mol\(^{-1}\) seedling growth conditions (8.3 ± 0.9 μmol m\(^{-2}\) s\(^{-1}\) in AMB Seedlings to 5.7 ± 2.8 in 1.5xAMB Seedlings and 3.8 ± 2.3 in 2xAMB Seedlings). Rates of net photosynthesis were similar for all parent seed groups at 550 and 700 μmol mol\(^{-1}\) CO\(_2\) growth environments.

Across all parental CO\(_2\) seeds, there was a decrease in leaf % N when grown at greater than 360 μmol mol\(^{-1}\) CO\(_2\) growth environments (F = 7.0; df = 2. 53; p < 0.05). Values for plants grown at 550 and 700 μmol mol\(^{-1}\) CO\(_2\) were 25 % less than those at 360 μmol mol\(^{-1}\) CO\(_2\). Different CO\(_2\) growth environments, different parental seed sources, and different nutrient amendments interacted to affect the percentage of nitrogen in leaf tissue for the seedlings of *Bromus* (F = 2.4; df = 4. 53; p < 0.05).
Fig. 4.3. Leaf nitrogen content (% dry mass) for different parental CO$_2$ growth environments (AMB or 2xAMB CO$_2$) and nutrient addition (0 added, 1:40 Hoagland’s solution added, or 1:10 Hoaglands added) treatment combinations. Different lower case letters above bars represent significant differences between treatments. Values are means ± S.E. 1.5x ambient seed source seedlings were not used in this assay due to a technical problem with sample combustion resulting in a small sample size.

AMB Seedlings were much more responsive to addition of Hoagland’s than 2xAMB Seedlings (Fig. 4.3). AMB Seedlings increased leaf % N from 2.44 ± 0.22 % to 3.01 ± 0.19 % by the addition of 1:40 strength Hoagland’s solution, and did not further increase it’s value with an increase in Hoagland’s to 1:10 strength. In contrast, 2xAMB Seedlings did not show an increase in leaf % N until 1:10 strength Hoagland’s solution was applied.
Discussion

Growth at elevated concentrations of carbon dioxide results in reductions in seed mass for glasshouse grown *Bromus madritensis* ssp. *rubens* (Huxman et al. 1998). These reductions in seed mass occur through decreases in seed nitrogen content, resulting in seedlings with a reduced potential for growth as compared to seedlings from ambient CO$_2$ parental seed sources. In this study, seedlings that developed from parents who were exposed to high CO$_2$ (2xAMB Seedlings) had lower photosynthetic rates as compared to AMB Seedlings when grown at 360 $\mu$mol mol$^{-1}$ CO$_2$, over an initial 3 week period (Fig. 4.2). Repeated growth at elevated CO$_2$ does not overcome this reduction in growth potential, despite increasing instantaneous rates of leaf photosynthesis. The pattern of photosynthesis in these seedlings at 360 $\mu$mol mol$^{-1}$ CO$_2$ is consistent with our hypothesis that reductions in seed nitrogen content would result in reduced photosynthetic rates for 2xAMB Seedlings as compared to AMB Seedlings. In this study we found that 550 and 700 $\mu$mol mol$^{-1}$ CO$_2$ stimulated $A_{\text{net}}$ for each parental seed source (Fig. 4.2). This is not consistent with our hypothesis, but may be explained by the pattern of change in carboxylation efficiency for the different parental seed sources and CO$_2$ growth environments.

Carboxylation efficiency decreased for AMB Seedlings grown at 550 and 700 $\mu$mol mol$^{-1}$ CO$_2$ as compared to 360 $\mu$mol mol$^{-1}$ CO$_2$ growth conditions (Fig. 4.2). Reductions in CE are associated with a down-regulation of the photosynthetic apparatus that effectively reduces the stimulation in net photosynthesis from potential values, had the apparatus not re-adjusted. Thus, plants that down-regulate do not have a large stimulation in net photosynthesis at elevated CO$_2$ as theoretically possible, or
often lose the stimulation altogether (Sage 1994). The nitrogen that is re-allocated away from the photosynthetic apparatus is suggested to be available for other uses in the context of the whole plant (additional leaf production, root construction, etc...). For 2xAMB Seedlings, an increase in photosynthesis at 550 and 700 μmol mol$^{-1}$ CO$_2$ growth conditions could occur primarily as a result of reductions in Rubisco photorespiratory activity as compared to 360 μmol mol$^{-1}$ CO$_2$ (Sage 1994). Thus, the two plants have similar values of net photosynthesis, but 2xAMB Seedlings would have lesser biomass accumulation than AMB Seedlings, most likely because reductions in seed nitrogen limits another factor important for growth. This was consistent with the data collected here.

Reductions in growth potential as a result of parental growth at elevated CO$_2$ does not diminish with the application of greater nutrients to the growing seedling in an effort to supply supraoptimal growth conditions (Fig. 4.1b). This is consistent with our hypothesis that increasing nutrient supply to the seedling would not increase the growth rate of the 2xAMB Seedlings as compared to AMB Seedlings. Ecologically, this suggests that patches of high nutrient availability in a natural ecosystem would not overcome a reduction in seed growth potential.

*Bromus madritensis* ssp. *rubens* in an important invasive grass in the Mojave Desert, as it may competitively displace native shrub species and potentially increases system-wide fire cycles (Smith et al. 1987; Hunter 1991). Population dynamics in a future high CO$_2$ world is therefore important to understand. The Mojave Desert is a spatially heterogeneous environment with respect to soil nutrient availability. Soil nitrogen concentrations beneath shrub islands can be greater than 10 times the
concentration in surrounding interspaces (Nishita and Haug 1973). Desert annual species are restricted in many instances to these shrub canopy fertile islands for establishment, as a result of lack of survival in open spaces (Patten 1978). This is especially true for *Bromus*, which can have greater than 20 times the densities beneath shrubs as in open areas in the Mojave Desert (Rundel and Gibson 1996). The pattern of establishment under shrubs is not only a function of nutrient availability, but also that of soil water content and reductions in solar irradiance (Shreve 1931; Muller 1953). In light of the results of this experiment, seedlings of *Bromus madritensis* ssp. *rubens* would show reduced growth rates as a result of parental growth at elevated CO₂. In addition, shrub fertile islands would not promote establishment even though it is a preferential locations for establishment. Increasing nutrient supply in glasshouse conditions did not lead to as great an increase in growth rate for elevated CO₂ parental seed source seedlings as compared to ambient CO₂ parental seed source seedlings (Fig. 4.1).

The response of whole plants to elevated CO₂ are much more complicated than the leaf level increases in photosynthesis and subsequent homeostatic adjustments may indicate (Wolfe et al. 1998). Increased photosynthetic rates have whole plant allocation consequences that can influence plant fitness in multiple directions. Increases in growth as a result of increased photosynthesis are important in determining reproductive success (Arntz et al. 1998), but greater investment in vegetative structures late in ontogeny can also lead to a forced allocation trade-off with reproductive investment (Huxman et al. 1999). Inherent in this trade-off are potentially conflicting carbon and nitrogen currencies in the allocation schedule. By
enhancing carbon content, but not nitrogen, in the plant. Elevated CO₂ appears to have potentially negative consequences for nitrogen investment between vegetative and reproductive functions, which may have long term consequences for plant performance in N limited species or life forms.

Acknowledgments

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CHAPTER FIVE

WINTER-ANNUAL BIOMASS AND SEED PRODUCTION IN A WHOLE ECOSYSTEM ATMOSPHERIC CO₂ MANIPULATION IN THE MOJAVE DESERT

This chapter has been prepared for submission to Ecology and is presented in the style and format of that journal. The complete citation is:

Abstract

Year to year variation in annual net primary production (ANPP) is strongly influenced by annual plant behavior in deserts. Thus, an evaluation of the CO2 effects on annual plants is essential for understanding climate change impacts on deserts. Annual plant growth may be a particularly responsive to elevated CO2, along with potential shifts in allocation. During the wet El Niño year of 1998, we evaluated growth and biomass allocation of four dominant Mojave Desert winter annuals to a 1.5 x ambient CO2 enrichment at the Nevada Desert FACE (Free-Air CO2 Enrichment) Facility (NDFF). Although all annual plant species increased in size at elevated CO2 as compared to ambient, the increase in total biomass was a function of species-specific changes in allocation to leaf and reproductive structures. Microsite affected the response to elevated CO2, as plants growing under perennial plant canopy cover (evergreen / drought-deciduous shrubs and perennial grasses) showed the greatest biomass enhancement as compared to growth within open-intercanopy spaces. Elevated CO2 accelerated phenology in Bromus madritensis ssp. rubens and Lepidium lasiocarpum, but not in Vulpia octoflora and Eriogonum trichopes. Bromus and Lepidium may have exhibited an ontogenetic shift at elevated CO2. Vulpia changed the allometric relationship between plant size and fecundity and increased allocation to reproduction at elevated CO2. Eriogonum showed little reproductive or vegetative response to elevated CO2. Bromus, Vulpia and Lepidium all increased seed number at elevated CO2. Bromus showed adjustments in reproductive characteristics such that individual seed mass and % N all decreased. Individual seed mass increased in Vulpia, despite slight decreases % N. Lepidium showed no changes in seed mass, but slight decreases in seed % N. These CO2
impacts on seed size and number, reproductive phenology and changes in size hierarchies suggest the potential for alterations in species interactions and ecosystem processes at elevated CO$_2$. 
Introduction

Deserts are expected to increase annual net primary production (ANPP) in response to rising atmospheric CO$_2$ concentration, perhaps more than any other biome type (Melillo et al. 1993). This enhancement is expected primarily due to (1) system-wide reductions in water use due to lower stomatal conductance and canopy transpiration, (2) increasing plant and soil water reserves (3) prolonged growth seasons, and (4) enhancements in photosynthesis (Long 1991; Jackson et al. 1994; Hunt et al. 1996; Senock et al. 1996; Field et al. 1997). Although small-scale, CO$_2$ enrichment has been conducted on desert plants in controlled environments (e.g., Smith et al. 1987; BassiriRad et al. 1997; Huxman et al. 1998 a, b, 1999a), no large-scale studies of the effects of global change on desert ecosystems have been conducted. The Mojave Desert is an ideal area to test the response of aridland plants and ecosystems to changes in atmospheric CO$_2$.

Productivity in the Mojave Desert is a function of precipitation, with relatively wet years enhancing ANPP by up to 50% over average years (Noy-Meir 1973; Le Houerou 1984). Functional plant types in deserts contribute differently to ANPP with respect to available soil water (Smith et al. 1997). Shrubs contribute a greater proportional amount during dry years, when germination and establishment of annuals is low or nonexistent. During wet years the contribution of annuals to ANPP can exceed that of perennials (Turner and Randall 1989). Thus, year-to-year variation in ANPP can be characterized by the relative contribution of annuals. In order to make accurate predictions of how global change will impact productivity in deserts, it is important to understand how changes in atmospheric CO$_2$ concentration affect performance of annual plants.
Winter annuals comprise the largest group of Mojave Desert plant species (Beatley 1974). Recently the flora has been invaded by the prolific annual grass. *Bromus madritensis* ssp. *rubens* (red brome) with most locations (including the site of this study) having densities greater than 20 plants m⁻² (Hunter 1991). Similar to *B. tectorum* and its influence on the structure and function of the Great Basin (e.g., Young and Evans 1978), *B. madritensis* ssp. *rubens* is important to understand with respect to its potential role in ecosystem function in the Mojave Desert (Smith et al. 1997). *Bromus* spp. compete with native species for soil moisture and nutrients and may have the potential to alter the frequency and intensity of wildfire in the Mojave Desert (Hunter 1991). Native annuals and the invasive grasses have similar phenological patterns — they germinate in response to winter precipitation, develop relatively high growth rates when daytime temperatures rise in the spring and rapidly advance to reproductive maturity (Beatley 1966). Reproduction usually occurs in the months of April and May, with the length of the growth period dependent upon the degree of soil moisture availability (Beatley 1967).

In general, plants are predicted to increase biomass as a result of increased photosynthesis associated with growth at elevated CO₂ (Bowes 1991). Increases in biomass are suggested to be a function of proportional increases in both vegetative mass and reproductive mass (Ackerly and Bazzaz 1995; Huxman and Smith, in review). In addition, increases in leaf area are predicted to occur, although the increases may be slightly smaller than hypothesized due to concurrent adjustments in specific leaf area (Poorter 1993). While changes in proportional biomass may occur, potential changes in phenology, seed production as a function of whole plant leaf surface area, and re-allocation of nutrients may have impacts of the performance of annuals and their role in
ecosystem productivity (Huxman et al. 1999b). A large proportion of the biomass of desert annuals can be present as seeds, and these seeds persist in seed banks (Mulroy and Rundel 1977), making patterns of reproductive allocation in this functional type critical to understand in deserts.

It has been suggested that annual species will be more responsive to elevated CO$_2$ than longer-lived, more stress tolerant species due to their greater sink tissue strength (Hunt et al. 1994), and that invasive annuals may be particularly responsive (Smith et al. 1987). Thus, Mojave Desert annuals are an important functional group to understand, with respect to increasing CO$_2$, as: (1) their relative contribution to ANPP is quantitatively and qualitatively important to the whole ecosystem. (2) their growing season is a function of ecosystem water status, which may be altered at elevated CO$_2$. (3) their life history strategy may be very responsive to elevated CO$_2$, and (4) their overall strategy for persisting in deserts is tied to their ability to make viable seeds, which has been shown to change in elevated CO$_2$ in glasshouse experiments (Huxman et al. 1999b), and (5) the interaction between invasive and native species may be particularly important to desert ecosystem function (D’Antonio and Vitousek 1992; Smith et al. 1997).

For deserts, it is difficult to predict individual plant responses and whole-ecosystem consequence of elevated CO$_2$ based on extrapolations from glasshouse experiments. Factors such as microsite growth location in deserts, which can strongly dictate plant growth and phenology (Ehleringer 1985), leads to spatial variation that cannot be adequately replicated in glasshouse experiments. Feedbacks from plant-plant interactions (such as overstory canopy type) can potentially dictate final outcomes, and are important to understand for making accurate predictions of plant response to elevated
CO₂, particularly for annual plants (Bazzaz 1996). As a result, experimental data from intact ecosystems are necessary in order to determine the response of desert annuals to elevated CO₂.

Here we present data from an experiment conducted in the Mojave Desert that manipulated an intact ecosystem by raising CO₂ levels to 1.5 times the current ambient atmospheric concentration (to 550 μmol mol⁻¹ CO₂). In this experiment, we evaluated biomass production along with potentially important changes in allocation patterns in winter annuals within several microsites. We tested the following hypotheses. First, annual plants exposed to elevated CO₂ in an intact ecosystem will increase in size. Second, the increase in size at elevated CO₂ will be greater for invasive species as compared to native annuals due to the invasive species having a greater potential for growth and seed production (i.e., sink strength). Third, increases in plant biomass at elevated CO₂ will be greater in “fertile island” microsites beneath perennial plants where soil-N contents are substantially higher (Schlesinger et al. 1990). Finally, increases in biomass will result in proportional increases in reproductive allocation and seed production. It is expected that these factors are important to understand for whole-ecosystem response to climate change in deserts, and allow for the spatial features of the Mojave Desert to be included in the experimental design.
Materials and Methods

Site Description

The Nevada Desert FACE (Free Air CO\textsubscript{2} Enrichment) Facility (NDFF) is located on the Nevada Test Site (36° 49' N, 115° 55' W, 970 m elev.), a National Environmental Research Park operated by the U.S. Department of Energy. This is a relatively pristine portion of the Mojave Desert that has been free from livestock grazing for ca. 50 years. Average precipitation is 138 ± 62 mm. falling mostly as rain during winter months, although the region can receive unpredictable precipitation during any portion of the year (Bowers 1987; Hunter 1994). The NDFF is located within a Mojave Desert scrub community (< 20% total perennial cover) dominated by two evergreen shrubs (*Larrea tridentata* and *Ephedra mojavensis*) and four deciduous shrubs (*Ambrosia dumosa, Lycium andersonii, L. pallidum, and Krameria erecta*). A number of perennial grasses occur on site, including *Achnatherum hymenoides* and *Plueraphis rigida* (*C\textsubscript{3} and C\textsubscript{4} photosynthetic pathways, respectively*). In addition to the perennial cover, more than 60 species of native winter annuals and 6 species of summer annuals occur at the Nevada Test Site depending on seasonal rainfall (Bowers 1987). A minimum of ca. 25 mm of rainfall between October and March is necessary for germination, growth, and viable seed production of Mojave Desert winter annuals (Beatley 1974). The lack of recent historical disturbance on the Nevada Test Site has important ecosystem implications. Slow growing crusts of N-fixing cryptogams are in pristine condition at the NDFF. These crust cover 35-65% of the ground surface, in contrast to off-site areas where they are easily destroyed by grazing.
The NDFF consists of nine circular plots, each 25 m in diameter (491 m²). Three were maintained at 550 µmol mol⁻¹ CO₂, three have a full FACE assembly each but were maintained at ambient CO₂ (360 µmol mol⁻¹ in this remote location), and three were non-FACE controls (Jordan et al. 1999). The NDFF maintains continuous CO₂ enrichment except when the 5-minute wind speed average exceeds 6.0 m s⁻¹ (7.0 m s⁻¹ in the primary growing season) and a low temperature cut off of 3°C. This protocol resulted in elevated CO₂ conditions for greater than 95% of the time during daylight hours. Plants were accessed from an overhead moveable walkway system that allowed sampling without disturbing soil in the plots. A more extensive facility description and performance evaluation is available in Jordan et al. (1999).

Plant Sampling

During the winter and spring of 1998, the four dominant winter-annual plant species (with C₃ photosynthetic pathway) were repeatedly sampled from plots at the NDFF. These were: (1) *Bromus madritensis* ssp. *rubens*, an exotic annual grass; (2) *Vulpia octoflora*, a native grass; (3) *Lepidium lasiocarpum* (Brassicaceae); and (4) *Eriogonum trichopes* (Polygonaceae). These four species represented greater than 70% of the total plant density for all annual plants in each plot. In each treatment ring, four microsites were chosen for harvesting locations, representative of greater than 90% of the total area within each plot. The microsites were: (1) open space, greater than 1 m from the edge of any perennial plant canopy; (2) within the canopy of the evergreen shrub *Larrea tridentata*; (3) within the canopy of the drought-deciduous shrub *Lycium andersonii*; and (4) within the canopy of *Plueraphis rigida*, a C₄ perennial bunchgrass.
These four different microsites allowed for the evaluation of annual plant responses to the spectrum of light, water and nutrient availabilities in the plots over a growing season.

Five harvests were conducted at two week intervals from April 7 to June 1, 1998. All species were present (with green tissues) for the first four harvests, but only *Eriogonum* was alive for the fifth harvest. A sixth harvest for *Eriogonum* was conducted on July 16, 1998. To determine the initial harvest date, plants were monitored in transects throughout the plots to determine the point at which rapid leaf expansion began. This point in the late winter/early spring provided a good starting point for annual species growth in the Mojave Desert, because most of the vegetative and reproductive expansion for a season occurs as temperatures warm in the early spring, despite seedlings having germinated earlier in the winter (Beatley 1974; Ehleringer 1985).

At each harvest date, within each plot, for each microsite location, five plants of each species were harvested (n = 15 plants for each species x time x cover x CO₂ treatment combination). Plants for harvesting were selected by randomly choosing a cover-type and removing the individual closest to the center of that cover type. This process was repeated at each treatment ring until all combinations of species and cover types were sampled five times. The plant was cut at the soil surface and all aboveground portions of the plant were removed. Roots could not be harvested in order to protect the long-term integrity of the NDFF. However, since Mojave Desert annuals have a root:shoot ratio of approximately 0.1 (Bell et al. 1979; Forseth et al. 1984) aboveground harvests should account for ca. 90% of total plant biomass. Harvested plants were immediately separated into vegetative and reproductive structures. The number of leaves on each individual was counted and total leaf area was determined with a leaf area meter.
Similarly, the total number of flowering and/or fruiting stalks were also counted, then the entire plant was placed in an oven to dry at 70°C for four days. Dry mass of each individual plant part was determined separately.

To estimate total seed number, a sub-sample of seeds and accessory reproductive structures was selected and mass determined, both combined and individually. A ratio of these structures was used to adjust the total reproductive mass into total seed mass and total accessory reproductive structure mass. A sub-sample of seeds (greater than ten per individual) chosen at random was weighed and used to estimate total seed production (from total seed mass). A sample (greater than five) of mature seeds was selected, and mass determined for each individual seed (Huxman et al. 1998b). Mean mature seed mass was only determined for the harvest at which the plants had the greatest relative allocation to reproduction, greatest seed production and greatest mean seed mass.

Oven dried leaf tissue and seeds from greater than three randomly selected individuals in each CO₂ treatment and microsite combination were taken and ground to a fine powder (250 μm) and sealed into 5 x 9 mm tin capsules. These samples were analyzed for total nitrogen and carbon by Micro-Dumas combustion in an elemental auto analyzer (NA1500 C/H/N Analyzer, Carlo Erba Strumentazione, Milan, Italy) at the University of Georgia. Nitrogen and carbon values are expressed either as a ratio (C:N), or content (moles g⁻¹), determined from % content and sample mass.

Several parameters that estimate reproductive allocation were evaluated. The mass ratio of reproductive and vegetative structures was a good estimate of reproductive allocation and allowed for an evaluation of phenology. In addition, the number of seed per unit leaf surface area of the whole plant was used to estimate the efficiency of
reproduction. This parameter can also be used to evaluate potential shifts in life history characteristics (Huxman and Loik 1997).

**Statistical Analysis**

A split-plot, repeated measures ANOVA was used to determine the effects of elevated CO\textsubscript{2}, microsite, time, and their interactions (SPSS Inc., Chicago, IL, USA). The CO\textsubscript{2}-by-time-by-cover-by-replicate (plot) factor was used as the sub-plot F-test denominator, while the whole-plot F-test denominator was CO\textsubscript{2}-by-replicate, cover-by-replicate, or harvest-by-replicate (plot) for the appropriate whole-plot factor. Plot means (n=3 for each CO\textsubscript{2} x cover x harvest date combination) were used as the statistical unit. All perennial cover microsites consistently acted as a group, and is therefore presented in figures as combined data. This improved illustration of major trends. For mean mature seed mass and C:N ratio, the effect of elevated CO\textsubscript{2} treatment, microsite type and their interaction was determined by a two-factor ANOVA for each species individually. Post-hoc tests were performed as following the detection of significance (α = 0.05).

Allometry of reproductive and vegetative structures was evaluated non-linear regression of the natural log of reproductive and vegetative mass (leaf mass) for all data for each species (Coleman et al. 1994). We eliminated the smallest and largest plants from each harvest, species, cover-type, and replicate combination, which resulted in a sample size of approximately 400 individuals per species. A second order polynomial was fit through the complete data for each species (both CO\textsubscript{2} treatments), then individually (each CO\textsubscript{2} treatment separately) and F-statistics were constructed from the residual sum of squares from each regression line following Potvin et al. (1990).
Results

Precipitation

The 1997-1998 hydrologic year (Oct 97-Sept 98) was a period of greater than average precipitation. Total rainfall was ca. 2.4-fold greater than average for the entire year (Table 5.1). This dramatic increase in annual precipitation was a function of increased rainfall from the January-March and April-June three-month time periods, which corresponded with the time of most active growth in these annual plants.

Table 5.1. Rainfall (mm) as a function of season for the Nevada Desert FACE Facility (NDFF) in a wet year (1997-1998). The 14 year average was taken from Rundel and Gibson (1996) at a location within several kilometer of the NDFF location.

<table>
<thead>
<tr>
<th>Time Period</th>
<th>14 year Average (mm)</th>
<th>Current Study (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>October – December</td>
<td>36.4</td>
<td>24.4</td>
</tr>
<tr>
<td>January – March</td>
<td>51.7</td>
<td>150.8</td>
</tr>
<tr>
<td>April – June</td>
<td>19.5</td>
<td>82.4</td>
</tr>
<tr>
<td>July – September</td>
<td>30.7</td>
<td>67.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>138.1</strong></td>
<td><strong>327.7</strong></td>
</tr>
</tbody>
</table>
Growth in Individual Species

*Bromus madritensis* ssp. *rubens*

Most factors in the repeated measures ANOVA were statistically significant for total individual plant mass in *Bromus madritensis* ssp. *rubens*. The general response was dictated by a three-way interaction of CO\(_2\), cover type, and harvest date (F=1.87; df= 9.48; p = 0.06) that led to different dates of peak biomass production on an individual basis for each canopy type with respect to CO\(_2\) treatment level. Peak biomass in *Bromus* was recorded by the third harvest date (May 5) at elevated CO\(_2\) for all cover types (Fig. 5.1). However, at ambient CO\(_2\), peak biomass production did not occur until the fourth harvest. The three-way interaction is present because *Bromus* at elevated CO\(_2\) produced greater total mass than at ambient CO\(_2\) in all overstory canopy types except in the open intercanopy spaces.

Leaf mass was greatest for elevated CO\(_2\)-grown *Bromus* in the perennial plant canopy microsite while, *Bromus* grown in the open had the smallest leaf mass with no differences between CO\(_2\) environments (Fig. 5.1). There was a three-way interaction with CO\(_2\) environment, harvest date and microsite cover (F=1.96; df= 9.48; p<0.05) such that total plant leaf surface area nearly doubled in *Bromus* at elevated CO\(_2\) in the evergreen canopy overstory microsite location (ca. 28 cm\(^2\) versus 15 cm\(^2\)). Leaf surface area generally followed the same trend as leaf mass with respect to CO\(_2\) enhancement. Across the perennial cover types, leaf surface area increased, but for *Bromus* growing in the open, there were no significant differences between CO\(_2\) environments.

The increase in total biomass production in *Bromus* at elevated CO\(_2\) didn’t occur as a shift in the allometry of vegetative and reproductive biomass production (Table 5.2).
However, the mass ratio of reproductive and vegetative structures (leaves) was significantly influenced by overstory canopy type ($F=32.4; \text{df}=3.6; p<0.05$) and an interaction between CO$_2$ and harvest date ($F=3.83; \text{df}=3.48; p<0.05$). The interaction is present as a result of accelerated phenology at elevated CO$_2$ such that *Bromus* reached total reproductive investment earlier (Fig. 5.1). Early in the season, reproductive allocation was similar between CO$_2$ environments and microsite locations. The ratio of reproductive mass and leaf mass increased from approximately 0.25 g g$^{-1}$ at the first harvest date to about 2.2 g g$^{-1}$ at the third harvest. At the fourth harvest, reproductive allocation at elevated CO$_2$ in each microsite location was similar to the values from the third harvest, but significantly less than ambient CO$_2$ values at the fourth harvest. While the ambient-grown plants eventually allocated proportionally similar biomass to reproduction as plants in elevated CO$_2$, allocation occurred later in the season.

Table 5.2. Allometric analysis of vegetative and reproductive mass for the four dominant species at the Nevada Desert FACE Facility in the relatively wet year, 1998. Methods followed those of Potvin et al. (1994). Significance indicates that elevated CO$_2$ affected the relationship between reproductive and vegetative structures, whereas non-significance indicates no allometric shifts by elevated CO$_2$.

<table>
<thead>
<tr>
<th>Species</th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bromus madritensis</em> ssp. <em>rubens</em></td>
<td>3.470</td>
<td>1.26</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><em>Vulpia octoflora</em></td>
<td>3.474</td>
<td>3.07</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><em>Lepidium lasiocarpum</em></td>
<td>3.412</td>
<td>1.02</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><em>Eriogonum trichopes</em></td>
<td>3.129</td>
<td>0.04</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

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Figure 5.1. Biomass, seed production and seed production per leaf surface area for *Bromus madritensis* ssp. *rubens* at the Mojave Desert FACE Facility in the wet 1998 season.
Peak seed production occurred earlier for *Bromus* at elevated CO$_2$ in all canopy overstory types (Fig. 5.1), as exhibited by a significant CO$_2$-by-harvest interaction ($F= 8.77; df= 3.48; p<0.05$). Plants at elevated CO$_2$ had produced all their seeds by the third harvest, while plants at ambient CO$_2$ did not show a peak in seed production prior to the fourth harvest, consistent with the temporal pattern of relative biomass allocation to reproduction. In addition, there was a CO$_2$ and cover-type interaction that led to the greatest enhancement at elevated CO$_2$ in plants growing in the canopy of perennial plants ($F=5.56; df= 3.48; p<0.05$). *Bromus* growing in the open did not show a significant stimulation in total seed production once phenology had been accounted for in the comparison (Fig. 5.1).

Despite differences in total seed production in *Bromus*, the efficiency of reproduction, seed number per total plant leaf surface area (LSA), showed greater variability with microsite location and across time ($F= 4.79; df= 3.6; p<0.05$; and $F=18.1; d= 3.6; p<0.05$, respectively) than elevated CO$_2$ (non-significant). Within each cover type, CO$_2$ did not have an effect on the total numbers of seeds produced per LSA, just a shifting of the date of the maximal value (Fig. 5.1). *Bromus* at elevated CO$_2$ had a maximal value at the third harvest, as compared to the fourth harvest for ambient CO$_2$-grown *Bromus*. All of these shifts in reproductive variables in time, along with maintenance of allometry, suggest that *Bromus* experiences an ontogenetic shift related to increased growth rates at elevated CO$_2$. 

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*Vulpia octoflora*

The three-way interaction between CO$_2$, harvest date and cover type that was present for the invasive grass, *Bromus*, in total biomass production did not occur for the native grass, *Vulpia octoflora*. Instead, there was an effect of elevated CO$_2$ (F=7.0; df= 1.2; p < 0.10) and microsite cover type (F=5.0; df= 3.6; p<0.05). Plants in perennial canopies were generally larger, but showed a similar CO$_2$ related increase in size. The clear shift in phenology that was evident in the invasive *Bromus*, was not present in this native grass, as peak biomass production did not consistently occur at a particular harvest date in any of the CO$_2$ and overstory cover-type combinations (Fig. 5.2). Increases in total plant biomass did not occur as a result of increased leaf mass at elevated CO$_2$; rather, increases in total biomass were a function of greater biomass in reproductive structures. This is especially evident for *Vulpia* growing under the canopy of perennial plants, where large proportional increases in reproductive biomass occurred relative to leaf biomass in harvests 3 & 4 (Fig. 5.2).

There was a significant interaction of harvest date and CO$_2$ on leaf area in *Vulpia*, as well as a significant effect of cover type (F=3.2; df 3.48; p<0.05; and F=13.9; df= 3.6; p<0.05, respectively). Ambient CO$_2$-grown plants typically had greater total individual leaf surface area early in the spring, and the elevated CO$_2$ plants did not match these high values (about 1.25 x the maximum elevated value). Despite this, ambient CO$_2$ plants reduced leaf area over time, and the elevated plants showed a variety of responses depending on microsite location.

For *Vulpia*, the allometric relationship between leaf biomass and reproductive biomass changed with application of elevated CO$_2$ and growth under different canopy-
Figure 5.2. Biomass, seed production and seed production per leaf surface area for *Vulpia octoflora* at the Mojave Desert FACE Facility in the wet 1998 season.
types (Table 5.2). This change in allometry, as well as the greater proportional increases in reproductive mass as compared to vegetative mass, resulted in greater seed production at elevated CO₂ similar to that seen in *Bromus*. At peak biomass allocation to reproduction, the ratio of reproductive mass and leaf mass was significantly greater at elevated CO₂ as compared to ambient CO₂ in several canopy-types. Within the perennial canopy cover type, plants at elevated CO₂ had a relative allocation to reproduction of 3.3 ± 0.3 g g⁻¹ as compared to 2.6 ± 0.3 g g⁻¹ at ambient CO₂. *Vulpia* at elevated CO₂ within the intercanopy spaces had a value of 3.5 ± 1.2 g g⁻¹ as compared to 3.1 ± 0.8 g g⁻¹ for the peak value at ambient CO₂.

*Vulpia* increased seed production at elevated CO₂ for all cover types (Fig. 5.2), but interactions with canopy type and CO₂ resulted in relatively greater enhancement within the beneath perennial cover microsites as compared to open (F=2.2, df= 3.48; p<0.10). There was a significant effect of harvest date (F=38.2; df= 3.6; p<0.05) on the number of seeds produced by *Vulpia* per unit leaf surface area, but there were no clear effects of elevated CO₂, just a slight increase for all plants through the second and third harvest date (Fig. 5.2).

*Lepidium lasiocarpum*

In the native mustard, *Lepidium lasiocarpum*, there was a CO₂-by-harvest interaction (F=3.4; df= 3.48; p<0.05) that led to the greatest relative biomass enhancement due to CO₂ during the third and fourth harvest dates (Fig. 5.3). In addition, there was a significant interaction of harvest and cover type (F=1.8; df= 3.48; p<0.05), illustrated by the greatest overall biomass production occurring beneath perennial
Figure 5.3. Biomass, seed production and seed production per leaf surface area for *Lepidium lasiocarpum* at the Mojave Desert FACE Facility in the wet 1998 season.
canopies. Similar to total biomass, CO₂ and harvest date affected leaf production (F=2.2; df= 3.48; p<0.05), along with an interaction between harvest and microsite type (F=1.9; df= 3.48; p<0.05), resulting in plants growing under the perennial locations having the greatest leaf biomass and greatest CO₂ enhancement (Fig. 5.3). *Lepidium* in the open had the smallest leaves and relative enhancement of leaf area of elevated CO₂. Similar to that of *Bromus*, there were trends towards an acceleration of phenology, such that peak biomass production occurred by harvest number three at elevated CO₂, as compared to harvest number four in ambient CO₂ (Fig. 5.3). In contrast to these strong effects of elevated CO₂, leaf area was only influenced by overstory canopy type (F=20.0; df= 3.6; p<0.05). *Lepidium* within the evergreen canopy had the greatest leaf area production, while those growing within the open had the least individual plant leaf area (Fig. 5.3).

In *Lepidium*, increases in total biomass production at elevated CO₂ resulted from proportional increases in both leaf and reproductive biomass across all overstory cover types (no shift in allometry: Table 5.2) with no major effects of CO₂, microsite or higher interactions on the ratio of reproductive mass and vegetative mass. Peak seed production occurred earlier in plants exposed to elevated CO₂ (Fig. 5.3). In all canopy types, seed production at harvest three was not significantly different from seed production at harvest four for plants in elevated CO₂. For plants at ambient CO₂, typically, harvest four was the date of peak seed production. There were no effects on the number of seeds produced per unit leaf surface area, that is any increases in leaf area resulted in proportional increases in seed production over time in all canopy types (Fig. 5.3).
Eriogonum trichopes
grew over a much longer season than Bromus, Vulpia or Lepidium, persisting with green tissue well into July. Plants did not begin to put on a large amount of biomass until the fourth, fifth, and sixth harvests, when reproductive maturation had occurred, and large photosynthetic inflorescences developed. Eriogonum only grew in the open spaces, with densities in the other overstory cover types too small to contribute significantly to annual production. There was a two-way interaction between CO₂ application and harvest date on total biomass production (F=3.63; df= 5.10; p<0.05), leading to the greatest relative CO₂ enhancement late in the season, after harvest number three (Fig. 5.4). Concurrently, there were three-way interactions for both leaf mass and reproductive mass (F= 4.63; df= 5.10; p<0.05; and F=3.2; df= 5.10; p<0.05, respectively) that led to production of each biomass component paralleling total mass (Fig 5.4).

Despite enhancements due to elevated CO₂ in leaf mass, there were no significant effects of CO₂ on leaf area production (Fig. 5.4). As expected, harvest date significantly affected leaf area (F=2.96; df= 5.10; p<0.10), leading to the greatest values between harvests number three and five, but with no differences associated with elevated CO₂.

The large enhancement in total biomass in Eriogonum was due to proportional increases in both reproductive biomass and leaf biomass, neither of which were affected by elevated CO₂ (no shift in allometry; Table 5.2). Despite the increases in reproductive mass, there were no significant differences between elevated and ambient CO₂ (and their pattern through time) for flower production or the number of flowers produced per unit.
Figure 5.4. Biomass, seed production and seed production per leaf surface area for *Eriogonum trichopes* at the Mojave Desert FACE Facility in the wet 1998 season.
leaf surface area (Fig. 5.4). Seed production was difficult to determine, as seed maturation and dehiscence occurred rapidly, preventing the collection of a sufficient sample size for any one individual. By observation in the field with intact plants, the fruit-to-flower ratio was estimated for both ambient and elevated CO₂ grown plants, and no significant differences were found. Since this ratio was not different among treatments, flower production gives a good estimate of total seed production on an individual basis. Consequently, mean mature seed mass was not determined for this species.

Seed Quality

For mean mature seed mass in Bromus, elevated CO₂ resulted in a significant reduction in seed mass of about 8% as compared to ambient CO₂-grown plants (F = 13.4: p < 0.05). In addition, there was a significant CO₂-by-overstory cover type interaction that resulted in microsite-specific responses to elevated atmospheric CO₂ enhancement (F = 4.78: p < 0.05). Bromus growing at elevated CO₂ under the evergreen and drought-deciduous cover types and in the open all showed reductions in seed mass of approximately 10%, while seeds from elevated CO₂ plants within a Plueraphis canopy did not change mean seed mass (Fig. 5.5). Concurrent with these changes in seed mass, seed nitrogen content (% N) decreased at elevated CO₂ as compared to ambient CO₂ from 1.56 ± 0.08 % to 1.46 ± 0.10 %.

In contrast to the patterns seen in Bromus, Vulpia mean mature seed mass increased at elevated CO₂ for all cover types by about 5% (F = 31.5: p < 0.05; Fig. 5.5). However, there was a CO₂ and cover-type interaction that led to the greatest degree of
enhancement at elevated CO₂ within the drought-deciduous cover-type (F=8.1; p<0.05).

Unlike *Bromus* and *Vulpia*, there were no shifts in the size of seeds produced by

*Lepidium* at elevated CO₂ (F=1.8; p>0.05). *Lepidium* seeds were smaller than those

produced by *Vulpia* and *Bromus* (0.33 ± 0.06 and 0.34 ± 0.07 mg for ambient and

elevated CO₂, respectively). In both *Lepidium* and *Vulpia*, seed % N showed significant

decreases. In *Vulpia*, % N in seeds decreased from 1.37 ± 0.08 % at ambient CO₂ to 1.24

± 0.07 % at elevated CO₂, while in *Lepidium* the decreased was from 2.03 ± 0.15 % to

1.83 ± 0.07 %, respectively.
Figure 5.5. Mature seed mass at ambient and elevated CO2 for both Bromus and Vulpia in four different microsites, from the NDFF in the wet 1998.
Discussion

In four Mojave Desert winter annual species evaluated at the Nevada Desert FACE Facility during the anomalously wet 1998 winter/spring season, individual plant biomass increased considerably at elevated atmospheric CO$_2$. However, the CO$_2$ response of this system was species specific and influenced by plant microsite location. Plants growing under drought-deciduous, evergreen and perennial grass cover-types were generally larger and more responsive to elevated CO$_2$ than those growing in open inter-shrub spaces (Fig. 5.1, 5.2, 5.3). These data are consistent with our original hypotheses; that plants would increase in size at elevated CO$_2$ and the fertile island effect of concentrated resources under perennial plant canopies would enhance the CO$_2$ effect on plant growth stimulation. Total biomass enhancement at elevated CO$_2$ was predicted to be greatest for the invasive annual grass, *Bromus madritensis* ssp. *rubens*, but the stimulation in production for *Vulpia octoflora* was essentially equivalent (both approximately produced 2 fold more biomass at elevated as compared to ambient CO$_2$). The native mustard, *Lepidium lasiocarpum* was the next most responsive (1.75 fold increase), followed by *Eriogonum trichopes* (1.25 fold increase). These results were in contrast to our original hypothesis that the invasive grass would be much more responsive to elevated CO$_2$. Finally, the increases in plant size resulted in greater seed production at elevated CO$_2$ (Fig. 5.1, 5.2, 5.3, 5.4), but allometric and allocation shifts resulted in species specific responses (Table 5.2), contrary to our original hypothesis.
Biomass Production

The spatial pattern of biomass enhancement at elevated CO$_2$ is related to the location of high soil resources in shrub islands (Ludwig et al. 1988; Schlesinger et al. 1990, 1996). In arid systems, intershrub spaces are locations of decreased resource availability as compared to within the area immediately surrounding shrubs. Soil nitrogen pools beneath *Larrea tridentata* shrub islands are 2-10 times the size of the pools in surrounding interspaces (Nishita and Haug 1973; Cox et al. 1984). Annuals growing in open interspaces may therefore be limited by the amount of soil nutrients available for uptake and use, and thus achieve smaller final biomass. This response may have been amplified by elevated CO$_2$ in the relatively wet year of 1998 when comparing the differences in biomass enhancement between intercanopy and within-canopy microsites. Nutrient availability can be a significant factor that limits the potential for growth enhancement at elevated CO$_2$ in a number of plant species (McConnaughay et al. 1993). The non-enhancement of total biomass by elevated CO$_2$ in the open spaces is thus not unexpected and is apparently a result of low soil nutrients. How increased understory growth in these resource islands at elevated CO$_2$ will impact the dynamics of shrub island development and persistence is not presently known, but may be important for processes such as desertification in a future high CO$_2$ environment.

The strong growth response of annuals under the canopy of shrubs and perennial grasses may have occurred due to greater water-use efficiency of the overstory plants at elevated CO$_2$ in addition to the nutrient concentrations (Huxman et al. 1998a; Hamerlynck et al. 1999). Increased WUE in perennial cover should translate into greater soil moisture during the growing season being available for annual plant use. While
elevated CO₂ impacted water-use efficiency at this site (Hamerlynck et al. 1999). the change was most likely due to photosynthetic enhancements and not whole plant water loss (Pataki et al. 1999). However, there were not detectable increases in soil moisture at elevated CO₂ over the time period of annual plant growth in 1998 (S. Zitzer. unpublished data). If a growth response as a function of increased soil water did occur, it would be expected that these winter annuals would have shown an increase in life cycle duration, as soil water status would be expected to remain favorable longer into the summer dry season. Since we typically saw the opposite response, i.e., an acceleration of phenology at elevated CO₂, we can conclude that improved water status under shrubs did not lead to this biomass enhancement.

One major allocation sink in many plants is root systems, a factor that was not possible to consider for this analysis due to non-destructive sampling protocols at the NDFF. Many plants have shown an increase in root-to-shoot ratio when grown at elevated CO₂, as compared to ambient CO₂ (Saxe et al. 1998), although alternative changes in the relationship between aboveground and belowground structures are possible, such as changes in the root and leaf surface area ratio (Huxman et al. 2000). In most desert annual species, roots represent a small fraction of the total production in a given year, as compared to aboveground structures (Bell et al. 1979; Forseth et al. 1984). In that case, small changes in biomass allocation to roots would not change the interpretation of results given here, or may occur in tandem with leaf allocation patterns. In Bromus grown at three CO₂ levels in a glasshouse, there were no shifts in root-to-shoot ratio, along with similar patterns of allocation to leaf and reproductive tissue, as seen in the present study (Huxman et al. 1999b).
Reproductive Allocation and Seed Production

Seed production is important in desert annual species as it is the life history stage responsible for long term persistence, and therefore represents a primary allocation endpoint (Mulroy and Rundel 1977). The responses of these winter annuals to elevated CO₂ fell into two general categories with respect to seed production. *Bromus* and *Lepidium* showed a distinct acceleration of phenology, such that peak seed production generally occurred earlier in the spring (Fig. 5.1, 5.3). In contrast, *Vulpia* and *Eriogonum* did not show a shift in the date of peak seed production (Fig. 5.2, 5.4). This change in phenology may be related to the ability of these plant species to shift resource allocation to either vegetative or reproductive structures late in the season, restricting the potential for maximum final size of the plant.

Changes in resource levels following initiation of seed production can differentially influence species with indeterminate and determinate meristematic structures (Pyke 1989). At elevated CO₂, both *Bromus* and *Lepidium* have a decreased ability to grow vegetatively following floral initiation, most likely as a result of determinate meristematic activity. Thus, these species reached a size threshold for reproductive initiation that was accelerated compared to the ambient CO₂ plants because they had accelerated growth rates in vegetative stages and did not increase in vegetative size further. *Vulpia* and *Eriogonum* both have mechanisms that allow for further biomass development following floral initiation. In *Vulpia*, lateral meristems remain indeterminate and leaf production can occur later in the plant's life cycle. In *Eriogonum*, the basal rosette gives way to a large determinate inflorescence, but the inflorescence has
the ability to fix carbon and may contribute to a considerable portion of the plant’s total carbon budget, as has been documented in *Eriogonum inflatum* (Smith and Osmond 1987). At that time, resource pools in vegetative structures (particularly nitrogen) may have been sequestered to a greater extent than in ambient CO$_2$ plants, resulting in less total resources available for re-translocation to seeds. This has been the explanation for a decrease in seed mass for *Bromus* grown at elevated CO$_2$ in a glasshouse (Huxman et al. 1999b). Thus, the significant increase in seed (or flower) production at elevated CO$_2$ occurs in concert with increased total plant size, without the loss of quality in seeds.

These two contrasting response patterns highlight potential trade-offs between growth and reproductive pathways that may be altered by increased carbon gain at elevated CO$_2$.

There were changes in the mean size of mature seeds at elevated CO$_2$ that may be related to patterns of growth and allocation to reproduction. In *Bromus*, there were no changes in the number of seeds produced per unit leaf surface area (Fig. 5.1). but there were decreases in nitrogen content per unit leaf surface area (as evidenced from gas exchange, data not shown). This functionally resulted in a smaller pool size of nutrients available for allocation to reproduction and a decrease in the size of seeds produced. This pattern has also been observed with *Bromus* in a glasshouse CO$_2$ experiment (Huxman et al. 1998b, 1999b). However, total numbers of seeds produced by *Bromus* at elevated CO$_2$ was greater, as proportional increases occurred in the plant with respect to leaf mass, leaf area, and reproductive mass (Fig. 5.1). In *Vulpia* at elevated CO$_2$, there were increases in seed mass (Fig. 5.5), suggesting the opposite trend should have occurred with respect to N-content per unit leaf area, as compared to *Bromus*. However, there were slight changes in the allometric relationship between reproductive mass and leaf mass in
Vulpia, along with a greater proportional allocation to reproduction and no changes in seed number per unit leaf surface area, all of which may have contributed to greater seed mass. In addition, there were no obvious changes in phenology for Vulpia, suggesting that elevated CO$_2$ did not affect the potential for re-translocation of resources to seeds from leaves. These patterns suggest that simple schemes of predicting plant response to elevated CO$_2$ may miss important life history characteristics that ultimately dictate plant response (Huxman et al. 1998b). For example, in a glasshouse study, prolonged investment in photosynthesis at elevated CO$_2$ late in the life cycle of Bromus may have contributed to decreases in reproductive allocation and diminished seed quality (Huxman et al. 1999b). These functional differences in plant allocation behavior need to be further investigated.

**Implications and Conclusions**

Seed production by annual plants is an important component of aridland ecosystems. Seed input affects the replenishing of seed banks (critical for annual plant persistence), the transfer of carbon and nitrogen to soil pools, and is a dominant food source in this granivore-dominated desert ecosystem. Scaling seed production from individual plant production to total seed rain across the landscape allows us to understand some of the potential effects of elevated CO$_2$ on these processes. By adjusting individual plant seed production with microsite specific plant density and total microsite cover of each plot at NDFF, we can estimate total seed rain for each species presented here individually, and in combination. Neither Vulpia nor Lepidium significantly increased total seed production per ground area at elevated CO$_2$, due to adjustments in density and
substantial variation (density data not shown). *Vulpia* produced approximately 6.400 and 8.500 seeds m\(^{-2}\) at elevated and ambient CO\(_2\), respectively. *Lepidium* produced approximately 30.000 and 22.000 seeds m\(^{-2}\) at elevated and ambient CO\(_2\), respectively. *Bromus* dramatically increased seed production per ground area at elevated CO\(_2\) (13.000 seeds m\(^{-2}\)) as compared to ambient CO\(_2\) (4.300 seed m\(^{-2}\)). When combined, there was a general trend of increasing seed production of all annuals at elevated CO\(_2\) as compared to ambient CO\(_2\) (49.000 and 34.000 seeds m\(^{-2}\), respectively).

Quantitatively, the changes in total seed production may not be dramatic, but qualitatively, the change in proportion of *Bromus* seeds in the total seed rain may be important. Both the long term persistence of this invasive annual grass in this ecosystem and interactions with higher tropic levels may be affected by this CO\(_2\) effect on seed rain. *Bromus* seeds are much smaller at elevated CO\(_2\) as compared to ambient. We have shown in earlier studies that the reduction in seed size is a function of reduced nitrogen content that results in a diminished ability to grow as a small seedling (Huxman et al. 1998b). How this large increase in total seeds of *Bromus* will interact with a reduced ability for offspring growth potential is unknown. The reduction in growth potential can be up to 50 % (in a glasshouse study), but the increase in seed production is over 3x greater at elevated CO\(_2\). It may be that the dramatic increase in systemwide seed production overwhels any reduction in seedling growth potential that may occur. However, the reduction in offspring performance still may restrict *Bromus* activity in the Mojave Desert, especially if the dramatic increase in seed production does not occur in relatively drier years.
The large increase in the proportion of *Bromus* seeds in the seed rain, along with reduction in their overall nitrogen content, has implications for trophic interactions in the desert. *Bromus* seeds are not often favored in feeding trials with desert granivores (Everett et al. 1978). Thus, their reduction in quality and increase in overall proportion may have impacts on granivores. Desert granivore activity can affect species composition of plant communities (Inouye et al. 1980), but it is unclear how this change in the proportion of *Bromus* seeds in the seed rain will affect processes in the Mojave Desert. In addition, bighorn sheep use the annual bloom as a major diet source during lamb production (K. Longshore, personal communication). How the decrease in tissue quality and increase in total mass (along with a change in phenology) may impact large mammals is unknown.

A large proportion of litter produced in the Mojave Desert during wet years is in the form of reproductive structures of annuals. How increased annual plant growth at elevated CO$_2$ and subsequent increases in seed number, along with reductions in seed quality may affect carbon and nitrogen cycling in desert ecosystems is unknown. This may be an area of important research as invasive annuals such as *Bromus* ssp. have been known to dramatically influence whole desert systems through their impacts on N-cycling (Naeem et al. 1994). The dynamics of this large increase in *Bromus* reproductive litter production on whole system nitrogen dynamics is currently being investigated at the NDFF.

Overall, the patterns of growth and biomass allocation measured here for the dominant annual plant species in the Mojave Desert at elevated CO$_2$ may be important for a number of biological scales. First, elevated CO$_2$ changes the patterns of allocation...
between growth and reproduction in a way that is important for individual plant functioning. This change in function and the spatial pattern of CO$_2$ effects suggest that species interactions may be altered in the desert, with the balance between invasive and native species potentially changing. The composition of species in aridland ecosystems may change as a result of the quantitative and qualitative CO$_2$ effects on seed rain. In addition, there is the potential for changes in ecosystem processes through the changes in plant growth, affecting productivity, litter quality, nitrogen cycling and interactions with other trophic levels.

It has been suggested that elevated CO$_2$, through effects on plant water-use efficiency, may lead to the expansion of invasive and native species into the open intercanopy spaces or promote increases in individual plant size (Strain and Bazzaz 1983; Smith et al. 1987; Mayeux et al. 1994; Sage 1996). This pattern could lead to the development of greater plant cover across a Mojave Desert landscape that currently only supports ca. 20% perennial cover. The addition of fine fuels and concomitant increased probability of episodic fire could lead to larger-scale burns that eliminate shrubs from the landscape (Torn and Fried 1992; Sage 1996). The lack of a strong biomass response in the inter-shrub spaces in response to elevated CO$_2$ implies that increased annual cover and size in inter-shrub spaces may not necessarily lead to the development of sufficient fuel to carry fire across the landscape, as has been previously hypothesized, without dramatic increases in density with increasing CO$_2$. Increasing densities could lead to the same ecological outcome, increased total plant cover. Previous research from glasshouse experiments had suggested that this change in cover may be a general possibility, but the contrasting results reported here highlight the importance of using larger, ecosystem-
scale experiments to evaluate the potential effects of global change. Glasshouse studies
do not encompass all plant level feedbacks that control plant growth, particularly low soil
resource levels, as are present in a natural ecosystem setting. The interaction of growth
with other global change variables, such as temperature, moisture, and land-use patterns
may ultimately dictate the response of annual plants to elevated CO₂ in desert
ecosystems.
Literature Cited


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Pataki DE, Huxman TE, Jordan DN, Zitzer SF, Coleman JS, Smith SD, Nowak RS, Seemann JR (1999) Water use of Mojave Desert shrubs under elevated CO\textsubscript{2}. Global Change Biology, IN REVIEW.


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CHAPTER SIX

REVIEW OF TERRESTRIAL PLANT REPRODUCTIVE RESPONSES TO ELEVATED ATMOSPHERIC CO₂.

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Introduction

Increasing atmospheric levels of CO₂ have prompted investigations of terrestrial plant responses to altered CO₂ scenarios. Some of the most common findings suggest that plants exposed to elevated CO₂ show increased growth as a result of increases in photosynthetic rate (Bowes 1993). However, this general response has been subject to re-evaluation due to the observation that C₃ plants exposed to elevated CO₂ for extended periods of time exhibit photosynthetic acclimation (Sage 1994). Increased individual plant size and ecosystem productivity at elevated CO₂ in natural springs has been shown, but it is dependent upon species-specific responses with respect to carbon allocation (Poorter 1993; Körner and Miglietta 1994). One logical consequence of increased productivity may be greater fecundity, as is predicted by the straightforward relationship between plant size and seed production in many species (Ackerly and Bazzaz 1995). Changes in fecundity in plants exposed to elevated CO₂ could have major implications for community composition, ecosystem processes and plant evolution (Bazzaz et al. 1992; Jackson et al. 1994).

Reproduction is a complex function of allocation, driven evolutionarily by trade-offs between competing resource sinks and ecologically by the availability of limiting resources (Reekie and Bazzaz 1987; Bazzaz 1997). In addition to trade-offs in allocation, there are also many size-versus-number trade-offs that occur with regard to structure (i.e., offspring size and number; Stearns 1992). This may lead to complex patterns of reproductive response for plants exposed to a changing environment relating to both allocation and reproductive structure production.
Reproductive allocation is often evaluated on a mass basis relative to vegetative biomass investment (Bazzaz and Ackerly 1992), although other conceptual models based on determining fecundity relative to the ability to capture resources (Huxman and Loik 1997) or relative costs to current and future growth (Zhang and Jiang 1997) can be used. In addition, reproduction in plants can also be evaluated in terms of seed production, seed viability or seed mass, all of which are ecologically and evolutionarily important. Evaluating plant reproduction by these characters, researcher have found a good relationship between patterns of reproduction and environment. For example, plants from early successional ecosystems are frequently found to have high reproductive allocation and seed production. In plants from later successional ecosystems, the values of reproductive allocation are relatively lower (Harper 1967; Silvertown and Dodd 1997). This line of thought can be extended to stressful environments, such that reproductive allocation in deserts is often found to be high, whereas closely related species in more mesic environments have relatively lower values of reproductive allocation and seed production (Young 1981; Noy-Meir 1986). Thus, how patterns of reproductive allocation and seed production are impacted by a changing climate has implications for the relationship between reproductive strategy and environment.

Changes in plant reproduction potentially affect both ecosystem structure and function. Plant reproduction affects physiological processes in individual plants that are important to how an ecosystem functions. Reproduction can influence (either positively or negatively) such processes as photosynthetic carbon sequestration, respiratory activity, and nutrient partitioning (Ashman 1994; Bazzaz 1997). Changes
in these processes can influence primary production and trophic interactions in
natural ecosystems. Alteration of reproductive characteristics also has demographic
consequences such that plant population dynamics can be strongly influenced by the
rate and degree of offspring production (Harper 1978). These changes in population
dynamics can influence ecosystem structure if they result in a shift in the relative
representation of functional groups. Changes in the functional makeup of species in
an ecosystem can impact the ability of an ecosystem to sequester carbon or persist
following disturbance (Tilman et al. 1997). For these reasons, the manner in which
increasing atmospheric CO₂ may influence plant reproductive characteristics is
extremely important.

Using an economic analysis, and considering that carbon often represents a
good currency for estimating reproductive effort in plants, a logical consequence of
increased carbon gain at elevated CO₂ may be enhanced investment in reproduction.
This hypothesis has been examined in a number of experiments using a wide range of
plant functional types (Wulff and Alexander 1985; Bazzaz et al. 1992; Farnsworth
and Bazzaz 1995; Ward and Strain 1997; Huxman et al. 1998, 1999). Despite these
research efforts, no general patterns have been identified in plant reproductive
response to elevated CO₂. In fact, these research efforts suggest that much more
complex responses can be expected from plants, with respect to reproduction, as
compared to physiological and vegetative responses.

Since the potential for changes in patterns of reproduction may depend both
upon life history strategy and complex patterns of allocation, factors difficult for any
one experiment to examine, a meta-analysis combining results from a range of
existing experiments may elucidate some a response. In this paper we evaluate the
general responses of plant reproduction to elevated CO₂ from a number of
experiments and relate these responses to life history or functional categories. We
had two main goals: (1) to elucidate patterns across species that may not be detectable
from individual studies and (2) to compare directly our results from the Nevada
Desert FACE Facility with the overall response identified in the literature. This may
be helpful in generating hypotheses that help guide future research on plant
reproductive responses to elevated CO₂. Since reproduction has rarely been the main
focus of elevated CO₂ research projects, the work is dispersed through the literature
and currently has not been synthesized (but also see Ackerly and Bazzaz 1995 for
additional review). Thus, probabilities associated with inappropriately rejecting a
null hypothesis (of no effect of elevated CO₂) are small. This characteristic of the
data makes the questions asked in this paper ideal for a meta-analysis approach
(Osenberg et al. 1997). In this review of the literature we present data on the
response to increased CO₂ of biomass allocation to reproduction, total seed
production by individuals, seed viability, and mean seed mass from several
independent studies performed on a wide range of natural and agricultural species.
Methods

In order to only make valid comparisons among many independent studies, a study had to meet the following four requirements for inclusion in the meta-analysis (conceptually similar to Curtis 1996). First, the ambient CO$_2$ treatment was required to be $<450 \mu$mol mol$^{-1}$ CO$_2$ and the elevated CO$_2$ treatment had to fall between 600 and 900 $\mu$mol mol$^{-1}$ CO$_2$, respectively. Second, several reproductive variables had to be recorded (preferably more than one), including seed number per plant, total seed mass, reproductive mass, total mass (in several cases, only aboveground mass was available), individual seed mass, and seed viability. Third, at least one of the treatments within the experimental design was a "non-stressed" or ambient condition for all other environmental variables (see Curtis 1996). For this third requirement, data were only taken from a non-stressed or ambient (fertilization; water regime) condition even though several treatments may have been applied. The fourth requirement of the study was that the plants were exposed to elevated CO$_2$ for the full duration of their growth and reproductive cycle. A list of species, variables, and citation of information is presented in Table 6.1.

For any reproductive variable used in this analysis, the mean (X), standard deviation (s) (or standard error) and sample size (n) of that mean reported in the manuscript were tabulated by study, species, and nitrogen fixing ability of the species. Where data were presented in a figure, we enlarged the graph to facilitate data extraction.
Table 6.1. Species used in meta-analysis, listed by citation. Characteristics used in analysis are indicated by either R (reproductive allocation), S# (seed number), S% (seed viability) or SS (seed mass).

<table>
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<tr>
<th>Species</th>
<th>Characteristic</th>
<th>Citation</th>
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<tr>
<td>Arabidopsis thaliana</td>
<td>R – Sm - S#</td>
<td>Ward and Strain 1997</td>
</tr>
<tr>
<td>Bromus madritensis ssp</td>
<td>R – Sm - S# - S%</td>
<td>Huxman et al. 1998 &amp; 1999</td>
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<td>Rubens</td>
<td></td>
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<td>R – Sm - S#</td>
<td>Larigauderie et al. 1988</td>
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<td>Camissonia claviformis</td>
<td>Sm - S%</td>
<td>Huxman, unpublished data</td>
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<td>Cassia fasciculata</td>
<td>R – Sm - S# - S%</td>
<td>Farnsworth and Bazzaz 1995</td>
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<td>Cassia nictitans</td>
<td>R – Sm - S# - S%</td>
<td>Farnsworth and Bazzaz 1995</td>
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<td>Farnsworth and Bazzaz 1995</td>
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<td>Baker et al. 1989</td>
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<tr>
<td>Ipomoea perpurea</td>
<td>R – Sm - S# - S%</td>
<td>Farnsworth and Bazzaz 1995</td>
</tr>
<tr>
<td>Oryza sativa</td>
<td>R – Sm - S# - S%</td>
<td>Baker et al. 1990</td>
</tr>
<tr>
<td>Plantago lanceolata</td>
<td>Sm</td>
<td>Wulff and Alexander 1985</td>
</tr>
<tr>
<td>Polygonum hydropiper</td>
<td>R – Sm - S# - S%</td>
<td>Farnsworth and Bazzaz 1995</td>
</tr>
<tr>
<td>Polygonum lapathifolium</td>
<td>R – Sm - S# - S%</td>
<td>Farnsworth and Bazzaz 1995</td>
</tr>
<tr>
<td>Polygonum persicaria</td>
<td>R – Sm - S# - S%</td>
<td>Farnsworth and Bazzaz 1995</td>
</tr>
<tr>
<td>Raphanus raphanistrum</td>
<td>Sm - S#</td>
<td>Curtis et al. 1994.</td>
</tr>
<tr>
<td>Triticum aestivum</td>
<td>Sm</td>
<td>Mitchell et al. 1993</td>
</tr>
<tr>
<td>Zea mays</td>
<td>Sm</td>
<td>Rogers et al. 1983</td>
</tr>
</tbody>
</table>

NOTE: Several species were taken from Navas et al. (1997), where species were listed by group (ie. grasses, composites, legumes) and individual data points could not be linked with a particular species. However, the species used in the Navas et al. (1997) experiment did not overlap with the species listed above. We used Navas et al. (1997) in seed production and reproductive allocation data sets (at least one measure for a grass, composite, and legume). *Cassia, Glycine* and the legume presented in Navas et al. (1997) are considered a part of the N-fixing functional group.
We pooled data for Arabidopsis and Raphanus genotypes (greater than five were listed in each study) to develop an overall mean for each species. Otherwise data were taken from text, tables or appendices. Where all statistical parameters were available in the original studies, we used a parametric fixed model for analyzing effect size, following Gurevitch et al. (1992) from commercially available software (MetaWin Software: Rosenberg et al. 1996). The effect size is the difference between the experimental and control means divided by the pooled standard deviation of the means (s), and weighted by a term, J, that approaches 1 as sample size increases (Curtis 1996). This weighting corrects for an overestimation bias of effect size when sample sizes are small (Hedges and Olkin 1985). The 95% Confidence Interval (CI) of the effect size was used as a determination of the significance of any particular effect. Inclusion of zero within the 95% CI was considered a non-significant effect of elevated CO₂. Positive 95% CI bounds were considered a positive effect on the trait, while negative 95% CI bounds were considered to indicate a negative effect of elevated CO₂ upon the trait. Similarly, the response of two groups were considered significantly different if their CI bounds did not overlap.

In many cases, data sets from published papers did not present complete descriptive statistics for each of the parameters of interest. In an effort to maximize studies and species representation, a non-parametric approach was conducted with resampling tests, following Rosenberg et al. (1996). Response ratios were constructed for each species and study combination by the use of natural log ratios. To perform the statistical analysis, variance is set to one and bootstrapping (with 4999 iterations) is performed to construct confidence intervals in the MetaWin protocol.
Bias-corrected bootstrap intervals were used to determine significance, as described for the parametric approach above. Results from the non-parametric tests were compared to the smaller, but complete data set of descriptive statistics and analyzed by parameteric methods. We performed this dual analysis for values that showed a significant (from zero) response. All reported data in this paper are presented with this duel analysis implied, and the more conservative response is presented. The inclusion of nitrogen-fixing plants in the analysis, a functionally important variable, required the use of this non-parametric technique.
Results and Discussion

While a search of the literature for any index (e.g., BIOSIS, AGRICOLA) yields greater than 1000 citations for the keywords “elevated CO₂ and plants”, few papers contain reproductive data. Data fitting selection protocol could be determined for 20 species, from a range of agricultural and natural groups (Table 6.1). Different sample sizes were used for each character in the analysis, as follows: (1) reproductive allocation – 13 species, (2) seed production – 15 species, (3) seed viability – 12 species, and (4) 18 species – seed mass.

The four characters included in the analysis are presented in Figure 6.1. Reproductive allocation (ratio of reproductive mass and vegetative mass) and seed production (seed number per individual), showed contrasting patterns at elevated CO₂. The mean effect size for the meta-analysis was not significantly different from zero for reproductive allocation across all species, whereas the mean effect size was significantly greater than zero for seed production. For both variables, separating the data into nitrogen-fixing plants versus non-N fixing plants did not aid interpretation. Seed viability showed no effect of elevated CO₂, with all species grouped, or when categorized by functional type. A significant decrease in seed mass was identified for plants exposed to elevated CO₂, but there is an interaction with functional type. Non-nitrogen-fixing plants show a significant decrease in seed mass, but nitrogen fixing plants did not have a significant response at elevated CO₂.
Fig. 6.1 Mean effect size of elevated CO$_2$ on reproductive characteristics from a number of different studies. Data are plotted for reproductive allocation, seed production, seed viability, and seed mass. Data plotted are means, with bias-corrected bootstrap confidence intervals (intended to be uneven). A negative effect size indicated a decreased value in the character at elevated CO$_2$, whereas a positive value indicates an increase. An asterisk indicates significance (zero not included within the confidence interval).

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Reproductive Allocation and Seed Production

A blended view of reproductive characteristics is helpful when evaluating changes or differences in plants across environments (Reekie and Bazzaz 1987). This is due to the difficulties understanding the appropriate currency for evaluating reproductive allocation and the non-linear relationships between seed number and fitness for many plants (Bazzaz and Ackerly 1992; Venable 1997). Since there were no shifts in reproductive allocation at elevated CO₂, plants increased in size, with proportional changes in vegetative and reproductive biomass. Increased size at elevated CO₂ most likely contributed to the greater seed production per individual. Reproductive allocation and seed production are important performance characteristics of plants, related to fitness and tightly coupled to the environment. These changes in reproductive allocation and seed production as affected by elevated CO₂ in this meta-analysis are important for species in natural systems.

Plant response from the Nevada Desert FACE Facility (NDFF) contrasts the general pattern in the literature. At NDFF, in a wet year, the invasive annual Bromus madritensis ssp. rubens increased in plant size, with reduced reproductive allocation, but greater seed production (Huxman et al. 2000). Vulpia octoflora increased in size, but the response occurred along with increasing reproductive allocation, and greater seed production (Huxman et al. 2000). Since we can consider reproductive characteristics tightly coupled to the environment, a decrease in reproductive allocation could indicate an alleviation of stressful conditions. Indeed, Bromus actually increased in density at NDFF. An increase in reproductive allocation could indicate increased stress, which also fits with the decreases in density of Vulpia at
NDF. However, these changes in reproductive characteristics occurred with specific changes in seed quality (decrease for *Bromus* and increase for *Vulpia*) suggesting that the functional relationships that led to this pattern of reproduction is critical to understand.

The mean reproductive response to elevated CO$_2$ does not take into account variation within individual species in these traits that may have ecological and evolutionary consequences for any one species, as in the example of *Bromus* and *Vulpia*. Likewise, in a review by Ackerly and Bazzaz (1995), the similar conclusion (larger plants, more seeds) is determined from a comparison across species. An increase in seed production, with no change in allocation is the most frequently reported response of elevated CO$_2$ on plant reproduction (Bazzaz et al. 1992; Ackerly and Bazzaz 1995; Farnsworth and Bazzaz 1995; Huxman et al. 1999; Huxman et al. 2000), but few studies have considered the possibility for a shift in allometric relationships. Two studies that have used an allometric analysis have both identified shifts in the relationship between reproduction and growth (Huxman et al. 1999; Huxman et al. 2000). It is possible that slight differences in allocation schemes occur upon exposure to elevated CO$_2$ to such an extent that the mean responses do not detect the change.

A shift in allometry has been documented for *Bromus madritensis* ssp. *rubens* in a glasshouse, where the slope of the regression line between reproductive mass and vegetative mass decreased (Huxman et al. 1999). This indicates a decreased investment in reproduction. For *Vulpia octoflora* at the Nevada Desert FACE Facility, a change in allometry at elevated CO$_2$ resulted in increased proportional
allocation to reproduction (Huxman et al. 2000). These results suggest that elevated CO₂ lead to species specific variation in reproductive output that will require larger data sets, with greater numbers of representative species for evaluation. Since evolutionary responses will be a function of the breadth of response for these species, and ecological impacts will be a result, it is important to consider these responses in future studies.

Seed Quality (Viability and Mass)

Seed quality is an important component of fecundity, as total seed production, total viable seed production, and seed size and can vary significantly between environments and species (Huxman and Loik 1997). Seed performance in natural ecosystems (patterns of dormancy, survival, and germination) are important for species persistence (Baskin and Baskin 1983), especially in deserts (Mulroy and Rundel 1977). Both seed number and quality affect offspring potential in natural systems. Many studies have looked at seed production (number), but few studies in the global change literature have evaluated seed quality or patterns of subsequent seedling performance (Wulff and Alexander 1985; Andalo et al. 1998; Huxman et al. 1998). Seed viability was not impacted by elevated CO₂ in our analysis. However, genotype specific changes in seed viability have been documented for Arabidopsis thaliana at elevated CO₂ (Andalo et al. 1998). This again highlights the importance of species specific responses in reproductive traits and blended assessments of reproductive response to elevated CO₂.
One of the most interesting findings from our meta-analysis comes from data on seed mass. Seed mass is both an ecologically and evolutionarily important life history trait that is also a measure of seed quality. Many interspecific interactions, such as seed herbivory, dispersal potential, and early competitive ability, impose strong selection on both seed size and mass (Janzen 1969; Rees 1997; Westoby et al. 1997). Seed mass is also important in terms of trophic interactions, as seeds provide a large source of energy transfer to consumers, both naturally and agriculturally. Understanding the relationship between changes in reproductive allocation, seed production and seed quality is critical.

For *Bromus madritensis* ssp. *rubens*, the change in seed mass was a function of reduced nitrogen content (Huxman et al. 1998, 2000). Likewise, changes in the nitrogen content of *Vulpia octoflora* and *Lepidium lasiocarpum* have also been documented at elevated CO$_2$. A potential insight may come a comparison with the meta-analysis and the nitrogen fixing plants, which did not show a reduction in seed mass at elevated CO$_2$. Perhaps the changes in nitrogen dynamics associated with nitrogen fixation potential allows for the maintenance of seed quality under different allocation and seed production scenarios. Increasing the number of studies that describe reproductive responses of nitrogen fixing plants would be extremely helpful in developing patterns of response to CO$_2$. In many studies, the application of superoptimal nitrogen fertilization to adult plants results in no seed mass change at elevated CO$_2$ for non-N-fixing plants (Larigauderie et al. 1988; Mitchell et al. 1993).
Implications and Conclusion

The pattern of CO$_2$ effects on the reproductive characters evaluated in this meta-analysis suggest that the relationship between plant structure (allometry) and patterns of biomass allocation, in light of total seed production is important. It specifically suggests that changes in these relationships may alter the internal nitrogen budget of plants may be important in linking the response of growth and reproductive allocation pathways to elevated CO$_2$. Nitrogen - by - CO$_2$ effects are a common and interesting finding in climate change experiments (Luo et al. 1994), suggesting that internal factors such as nutrient balance may ultimately dictate whole plant responses to elevated CO$_2$ (Geiger et al. 1999). The ability to produce greater carbon compounds at elevated CO$_2$ may not be reproductively advantageous for plants that cannot concurrently increase overall nutrient status. A greater relative increase in carbon accumulation compared to nitrogen accumulation may result in a significant size-versus-number trade-off between seed number and seed size. For example, in *Bromus madritensis* ssp. *rubens*, the overall pool of nitrogen available for re-translocation to reproduction decreases at elevated CO$_2$ (Huxman et al. 1999). The relationship between plant size and nutrient pools may change such that the allometric relationship between plant size and number of seeds initiated remains constant, but insupportable by the nutrient pool available for re-translocation within the plant.

Nitrogen-fixing plants apparently overcome the allometric limitation that prevents production of viable seeds or increases in seed mass with respect to plant C:N balance. Perhaps the increased carbon compounds available at elevated CO$_2$ allow for increased exudation, supporting symbiotic bacteria that can accumulate
nutrients relative to other plant compound pools. The ability to proportionally increase all compounds would maintain a balance between allocation to growth and reproduction in these plants. Under optimal conditions, this could result in increased reproductive capacity simply due to increases in overall plant size, similar to the pattern of C:N change in plants grown at elevated CO\textsubscript{2} (Coleman et al. 1993).

These changes in reproductive characteristics provide another mechanism by which a changing global environment can alter vegetation makeup of ecosystems. With the commonly found benefits of elevated CO\textsubscript{2} on seedling establishment and survival, these variable changes in reproductive characters may provide a feedback on increasing productivity in terrestrial ecosystems due to reduced rates of population growth. The relationship between changes in plant growth and reproductive characteristics in more natural conditions, such as at CO\textsubscript{2} springs or Free Air CO\textsubscript{2} Enrichment (FACE) sites, and changes in population dynamics are critical to understand in order to predict the long-term effects of increasing CO\textsubscript{2} on terrestrial ecosystems.

Key components of plant reproduction change upon exposure to elevated CO\textsubscript{2}. The mass of seeds produced by non-nitrogen fixing plants decreases at elevated CO\textsubscript{2}, as compared to their ambient values when plants are grown at levels of nitrogen typical of most natural ecosystems. Changes in this ecologically and evolutionarily important character could have implications for both the distribution of species in a future high CO\textsubscript{2} world, and the selective interactions that dictate the potential for evolutionary changes. This analysis also indicates that plant reproductive response to elevated CO\textsubscript{2} may vary across ecosystems as a function of nutrient availability.
**Literature Cited**


CHAPTER SEVEN

SUMMARY OF MAJOR RESULTS

How will plant reproduction in natural ecosystems be impacted by levels of atmospheric CO$_2$ predicted to occur within the next century? This is an important question, especially since reproduction is necessary for regenerating plant populations, maintaining the structure of ecosystems, and driving evolution. Elevated CO$_2$ impacts the manner in which resources are acquired by plants, affecting total resource status and thus the potential for reproduction. Elevated CO$_2$ has been shown to affect seed production, seed quality and subsequent seedling performance (Chapters Two, Three). How concurrent changes in these parameters interact to affect higher levels of biological organization (populations, communities, and ecosystems) is unknown. Variation in these parameters create fitness hierarchies and dictate survival in model systems (Stanton 1985) and may therefore be important to how biological systems respond to climate change. Experiments designed to scale these CO$_2$ induced changes in plant function to ecosystem response have yet to be accomplished. However, in light of the research reported in this dissertation, we can make some statements as to the ecological and evolutionary consequences possible as a function of CO$_2$-induced change.

*How reproduction is influenced by elevated CO$_2$*

Two trends are consistent across the response of plants to growth at elevated CO$_2$:
decreases in leaf nitrogen content and increases in photosynthesis (Bowes 1991; Cotrufo et al. 1998). These two parameters reflect plant resource status, which is important in determining patterns of reproduction for terrestrial plants. Decreases in leaf nitrogen and increases in photosynthesis potentially affect plant seed production in different ways. For a plant of constant size, increasing photosynthesis relieves limitations on performance due to carbon resources, promoting reproduction in some species (Reekie and Bazzaz 1987), while decreased leaf nitrogen potentially aggravates a nitrogen limitation in allocation to seeds. Nitrogen can be a strong limiting factor for reproduction in many desert annuals (Williams and Bell 1981). Thus, for plants exposed to elevated CO₂ (where these two factors occur concurrently), two contrasting patterns of seed production could be possible; both increases and decreases in relative biomass allocation to reproduction.

The most complete data set on plant reproduction and elevated CO₂ exists for the invasive Mojave Desert annual grass, Bromus madritensis ssp. rubens. When grown at ambient and elevated CO₂ in a glasshouse, (1) there were no differences in final total biomass. (2) reproductive allocation decreased, and (3) seed production remained constant (Chapter Three). These results reflect a shift in allocation as a result of decreased nitrogen availability (decreasing potential re-allocation to reproductive structures) late in the life cycle. During this time, nitrogen remained in leaves and promoted photosynthesis. This effectively was a shift towards greater vegetative versus reproductive investment at elevated CO₂. Such a pattern of biomass accumulation and seed production leads to decreases in individual seed mass for plants at elevated as compared to ambient CO₂, as a result of reduced total nitrogen content in the seed.
(Chapter Two). The straightforward, take-home message is that *Bromus* plants at elevated CO$_2$ make proportionally fewer seeds (relative to vegetative biomass) that are of lesser quality than at ambient CO$_2$.

Seed quality is an important determinant of the performance characteristics (germination and growth) of subsequent seedlings in plants. For *Bromus*, reduced nitrogen in seeds from plants grown at elevated CO$_2$ reduces offspring growth potential (Chapter Three). Seedlings from elevated CO$_2$ parents (2xAMB Seeds) grew slower and achieved smaller final mass over an initial growth period as compared to seedlings from ambient CO$_2$ parents (AMB Seeds). Photosynthetic performance of 2xAMB Seedlings was half that of AMB Seedlings, as an additional result of this nitrogen deficiency. Adding nutrients to the soil solution in developing seedlings did not overcome this reduced-growth performance of 2xAMB Seedlings. A similar trend is present in *Arabidopsis thaliana*, where reduced germination and root production occur in 2xAMB Seeds (Andalo et al. 1996, 1998). The results from both *Bromus* and *Arabidopsis* suggest elevated CO$_2$ affects offspring performance through changes in parent-seed-seedling nitrogen dynamics, such that reduced leaf nitrogen pools in adults leads to reduced potential nitrogen re-allocation to seeds and thus reduced photosynthesis and growth rates in successive generations.

The changes described above occurred in a controlled environment setting, but if such changes in the relationship between vegetative size, seed number and seed quality in plants imposed by elevated CO$_2$ occur in a natural setting, they may be important to understand relative to predictions of plant responses to future climate. Since fitness of different genotypes is related to fecundity, and fecundity is altered by size / number
change in seed production relative to vegetative growth, there are potential evolutionary consequences of this CO₂ effect. Since fecundity is tightly coupled to environment (e.g., desert annual species tend to have a relatively high reproductive output as compared to closely related mesic species), these changes in fecundity may have ecological implications relating to species abundances and distributions. Ultimately the distribution of seed quality in seed rain scaled up from the individual to the landscape may be important for population and ecosystem response to elevated CO₂ in natural systems.

*How does elevated CO₂ affect plant reproduction in a natural setting?*

How can the results from these glasshouse studies be extrapolated to a real ecosystem? Data from an intact ecosystem is critical to identify similar responses in the field. At the Nevada Desert FACE Facility (NDFF), similar patterns of reproduction occurred at elevated CO₂ as in glasshouse studies, with two major differences (chapter Five). Total individual plant mass increased at elevated CO₂, concurrent with species-specific patterns of reproduction. However, the same logic of parent-seed-seedling nitrogen dynamics helped explain the results. *Bromus madritensis* ssp. *rubens*, *Vulpia octoflora* and *Lepidium lasiocarpum* all increased seed number at elevated CO₂ as a result of increased plant size. *Eriogonum trichopes* showed little reproductive or vegetative response to elevated CO₂. All species decreased their leaf nitrogen pool relative to plant size, providing proportionally less total nitrogen to re-allocate to seeds during reproduction relative to size. *Bromus* had decreased individual seed mass and N-content, similar to glasshouse studies. Individual seed mass increased in *Vulpia*, despite slight decreases N-content. *Vulpia* changed the allometric relationship between plant size
and fecundity and increased allocation to reproduction at elevated CO\textsubscript{2}. \textit{Lepidium} showed no changes in seed mass, but slight decreases in seed N-content. Elevated CO\textsubscript{2} accelerated phenology (i.e., earlier reproduction) in \textit{Bromus} and \textit{Lepidium}, but not in \textit{Vulpia} and \textit{Eriogonum}. These CO\textsubscript{2} impacts on seed size and number, reproductive phenology and changes in size hierarchies suggest the potential for alterations in species interactions and ecosystem processes at elevated CO\textsubscript{2}.

\textit{What are the ecological implications of changes in reproduction for deserts?}

How do changes in reproduction affect how desert ecosystems function? Seed production is an important component of aridland ecosystems. Year to year variation in annual net primary production (ANPP) is strongly influenced by annual plant behavior, and a large proportion of ANPP comes from seeds of desert annuals. At the NDFF, increased growth of individual plants at elevated CO\textsubscript{2}, along with increased seed number and decreased seed nitrogen content, have led to greater numbers of seeds produced in the landscape, but with a decrease in overall quality of reproductive units and a shift in species proportions. The patterns of growth and reproduction in the Mojave Desert at elevated CO\textsubscript{2} may impact a number of important processes for ecosystem functioning, including (1) species interactions, (2) nitrogen and carbon cycling, (3) trophic interactions, and (4) patterns of anthropogenic disturbance.

First, elevated CO\textsubscript{2} changes the patterns of allocation between growth and reproduction in a way that fecundity is altered for individual plants, potentially affecting survival. This change, and the spatial pattern of CO\textsubscript{2} effects on reproductive characters, suggest that species interactions may be altered in the desert, with the balance between
invasive and native species potentially changing. Simply scaling the increase in seed number to a population response at elevated CO$_2$ would suggest that *Bromus* (invasive annual) will increase in density dramatically as compared to either small increases or no response for the natives annuals (*Vulpia, Lepidium*, and *Eriogonum*). As a result, the composition of species in aridland ecosystems may change as a result of the quantitative CO$_2$ effects on seed rain. How the concurrent changes in seed rain quality may limit the impact of the large increase in seed quantity and species composition is currently being studied. One potential area of research that may facilitate understanding would be to evaluate all species at the NDFF for a 'parental effect' as described above for *Bromus* (reduction in offspring growth potential; Chapter Three). This would allow us to compare the potential competitive abilities of future offspring and adjust estimates of population growth based only on seed production in the landscape. For example, a slight increase in the seed mass of *Vulpia* may promote growth of subsequent seedlings at elevated CO$_2$, offsetting slight reductions in their representation in seed rain.

How increased annual plant growth at elevated CO$_2$ and subsequent increases in seed number, along with reductions in seed quality may affect carbon and nitrogen cycling in desert ecosystems is unknown. Litter production in deserts consists in a large part of reproductive structures. This may be an area of important research as invasive annuals such as *Bromus* ssp. have been known to dramatically influence whole desert systems through their impacts on N-cycling (Naeem et al. 1994). The dynamics of this large increase in *Bromus* litter production on whole system nitrogen dynamics is currently being investigated at the NDFF.
The large increase in the proportion of *Bromus* seeds in the seed rain, along with reduction in their overall nitrogen content, has implications for trophic interactions in the desert. *Bromus* seeds are not favored in feeding trials with desert granivores (Everett et al. 1978). Thus, their reduction in quality and increase in overall proportion may have contrasting impacts on granivores. Desert granivore activity can affect species composition of plant communities (Inouye et al. 1980), but it is unclear how this change in the proportion of *Bromus* seeds in the seed rain will affect processes in the Mojave Desert. In addition, bighorn sheep use the annual bloom as a major diet source during lamb production (K. Longshore, personal communication). How the decrease in tissue quality and increase in total mass (along with a change in phenology) may impact large mammals is unknown. A better understanding of the nutritional effects of these changes in seed N is required, along with feeding trials to attempt to understand dietary impacts on consumers.

It has been suggested that elevated CO$_2$, through effects on plant water-use efficiency, may lead to the expansion of invasive and native species into the open intercanopy spaces or promote increases in individual plant size (Strain and Bazzaz 1983; Smith et al. 1987; Mayeux et al. 1994; Sage 1996). This pattern could lead to the development of greater plant cover across a Mojave Desert landscape that currently only supports ca. 20% perennial cover. The addition of fine fuels and concomitant increased probability of episodic fire could lead to larger, more frequent burns that eliminate shrubs from the landscape (Torn and Fried 1992; Sage 1996). The lack of a strong biomass response in the inter-shrub spaces in response to elevated CO$_2$ implies that increased annual cover and size in inter-shrub spaces may not necessarily lead to the development
of sufficient fuel to carry fire across the landscape, as has been previously hypothesized, without dramatic increases in density with increasing CO₂. Increasing densities could lead to the same ecological outcome, increased total plant cover. Previous research from glasshouse experiments had suggested that this change in cover may be a general possibility, but the contrasting results reported here highlight the importance of using larger, ecosystem-scale experiments to evaluate the potential effects of global change. Glasshouse studies do not encompass all plant-level feedbacks that control plant growth, particularly low soil resource levels, as are present in a natural ecosystem setting. The interaction of growth with other global change variables, such as temperature, moisture, and land-use patterns may ultimately dictate the response of annual plants to elevated CO₂ in desert ecosystems.

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