Changes in the stress tolerance of two Mojave Desert perennial shrubs after eight years of growth in elevated carbon dioxide

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CHANGES IN THE STRESS TOLERANCE OF TWO MOJAVE DESERT PERENNIAL SHRUBS AFTER EIGHT YEARS OF GROWTH IN ELEVATED CO$_2$

by

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ABSTRACT

Changes in the stress tolerance of two Mojave Desert perennial shrubs after eight years of growth in elevated CO₂

by

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Global climate change is a significant issue facing modern society. Potential changes include increased atmospheric CO₂ concentrations, increased temperature, altered precipitation patterns, and increased nitrogen deposition. The Nevada Desert FACE (Free Air Carbon dioxide Enrichment) Facility (NDFF) examines the effects of increased atmospheric CO₂ concentration in an arid ecosystem. The effects of elevated CO₂ on plants include increased growth rates and stress tolerance. This study examined the mechanistic changes in drought tolerance of two dominant Mojave Desert shrubs (Larrea tridentata and Ambrosia dumosa) grown in elevated (550 μmol mol⁻¹) or ambient (380 μmol mol⁻¹) CO₂ concentrations. Previous studies at this site included photosynthetic, water potential, and fluorescence investigations. This study added a mechanistic approach, investigating the possible photosynthetic down-regulation and increased drought-tolerance after growth in elevated CO₂, including pigment, sugar, and protein analyses. In contrast to past studies, Larrea tridentata plants growing in elevated
CO₂ exhibited photosynthetic upregulation, but as expected, both species exhibited increased drought tolerance through reduced stomatal conductance, as elevated CO₂ had few effects on sugars, proteins, or protective pigments.
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CHAPTER 1

INTRODUCTION

Background

Global change is an increasingly important area of study in biology as well as the social sciences and economics. Climate change scenarios predict warming temperatures accompanied by increasing variability in regional weather, particularly precipitation patterns and amounts. Global change includes increased atmospheric CO$_2$ concentrations ([CO$_2$])—global [CO$_2$] is expected to reach 550 $\mu$mol mol$^{-1}$ by the year 2050 and 700 $\mu$mol mol$^{-1}$ (an approximate doubling) by the year 2100 (Houghton et al. 2001).

Natural terrestrial environments provide one of the most abundant “sinks” for carbon sequestration (fixation and storage of atmospheric CO$_2$). Plants and soil microbes can take up and process significant amounts of atmospheric CO$_2$ and fix it into biological material; in fact, half of the global sequestration of atmospheric CO$_2$ is through photosynthesis (Bowes 1991).

The Mojave Desert is a transitional desert between the cold deserts to the north and hot deserts to the south, and is the driest desert in North America (Smith et al. 1997). Mean annual rainfall at the NDFF for the past 20 years is 138mm. For the Mojave in general, annual precipitation is less than 150mm and is characterized as a winter rainfall desert, with most predictable rainfall occurring between November and March (Smith and Nowak 1990). Summer rain tends to be highly episodic and may be significant in
wet years, but is not a reliably predictable cycle. Plants living in the Mojave Desert have thus developed various adaptations to survive extreme heat and drought during the summer and below-freezing temperatures during the winter. Regional global change models project increased summer rainfall events in the Mojave (Gordon et al. 2000; Pope et al. 2000; Weltzin et al. 2003). These rains typically occur at the end of the “growing season” but during the hottest months of the year (often, in fact, these rains “break” the heat). Additional summer rain may, therefore, allow plants to benefit from soil moisture derived from winter recharge, but they would still be subjected to the extremely hot conditions experienced currently.

Perennial shrubs use one of two major strategies in order to survive the severe water-stress conditions experienced in the desert environments. Evergreen plants maintain their leaves throughout the year, but may become essentially dormant toward the hottest periods of the summer and throughout the winter when cold winter nights limit metabolism. Drought-deciduous plants allow their leaves to senesce and drop when water becomes too limiting for positive carbon status. These plants remain leafless until the spring, when warmer temperatures and wetter soil make growth favorable again.

The co-dominant species of the Mojave Desert – *Larrea tridentata* (creosote bush) and *Ambrosia dumosa* (white bur-sage) – are evergreen and drought-deciduous shrubs, respectively. *Larrea*’s distribution is limited by excess water availability (greater than ca. 180 mm per year) and presumably increased competition in more mesic communities, though its northern boundary is dictated by freezing temperatures (Beatley 1974; Pockman and Sperry 1997). *Larrea* can be metabolically active during all times of the year, and has relatively high photosynthetic rates for a xerophytic plant (Smith et al. 1997).
1997). It has exceptional photosynthetic acclimation to seasonal temperature variation, and acclimates without a significant reduction in photosynthetic capacity (Mooney et al. 1978). *Larrea* has a moderately high water-use efficiency for a desert plant, which is tied to nitrogen status in the soil (Lajtha and Whitford 1989).

As a drought-deciduous shrub, *Ambrosia* produces leaves in response to favorable growing conditions in early spring and these leaves senesce when water becomes limiting in early summer. Despite being drought-deciduous, *Ambrosia* can resist xylem cavitation to very low water potentials, though it is more vulnerable than *Larrea* (Pockman and Sperry 2000).

*Larrea* can maintain photosynthetic capacity at very low water potentials, and continue to maintain turgor pressure when water is scarce (Meinzer et al. 1986). It stays metabolically active year-round, primarily by regulating stomatal conductance and avoiding xylem cavitation to very low xylem water potentials (Pockman and Sperry 2000). Stomata acclimate to their growing temperature, though this can be augmented by water potential and vapor pressure deficit (Ogle and Reynolds 2002). Huxman et al. (1998) found that *Larrea* exposed to both elevated CO$_2$ and drought conditions exhibited less photosynthetic down-regulation than when well watered, implying that growth in elevated CO$_2$ may help offset plant stress when water is limiting.

Because it is drought-deciduous and not as dominant as *Larrea* across the Southwest, few studies on drought responses have included *A. dumosa*. Our previous work at the NDFF did not find photosynthetic down-regulation (or a reduction in the capacity to do photosynthesis) in *Ambrosia* under elevated CO$_2$ conditions and water stress (Naumburg et al. 2003, 2004), but this has not been carefully evaluated across
contrasting moist and dry conditions. Hamerlynck et al. (2002a) found that the co-occurring drought-deciduous shrub *Lycium andersonii* had increased drought tolerance when grown in elevated CO$_2$ due to decreased stomatal conductance and reduced photosynthetic rates.

**Drought Tolerance**

Drought tolerance is the ability of a plant to withstand low water potentials in the soil and plant without dying. Drying causes membrane and protein damage, as cells become porous under water stress, allowing their contents to leak out of the cell and potentially denature proteins and disrupt metabolism. Water stress can severely limit photosynthesis, in part by decreasing stomatal conductance in order to avoid water loss, but also by substrate limitation for the photosynthesis reactions. Drought stress can also alter plant growth and biomass allocation, usually increasing root mass while limiting shoot mass (Wullschleger et al. 2002; Hopkins and Huner 2004). Plants that tolerate drought use mechanisms to prevent cell membrane rupture. As water becomes limiting, plants can allocate sugars and other solutes (including metabolites and ions) into the cytosol, decreasing the osmotic potential of the cell and thus drawing in more water from the apoplast (Lambers et al. 1998). Sucrose and other soluble sugars can act to protect membranes and proteins from dehydration damage by binding with hydrophilic regions as cell-water content declines, thus maintaining membrane integrity and stabilizing proteins (Lambers et al. 1998).

In addition to increasing cellular solute contents, plants have stress-tolerance genes that are induced in drying conditions. These genes can up-regulate the production
of enzymes, proteins, and molecules such as sugars that can act to protect important structures and enzymes. For example, in desiccation tolerant plants (plants that can withstand complete cellular water loss) sugars are known to bind to hydrophilic sites on proteins and membranes to prevent denaturing as the cell becomes more hydrophobic (Koster and Leopold 1988; Farrant 2000). Some chaperone enzymes have been shown to protect enzymes and proteins from denaturing by either binding to them or helping them to re-fold to the correct tertiary structure. Some plants are able to increase sugar concentrations until they effectively replace water in the cell, allowing it to remain fully expanded in the relative absence of water. And plants are able to protect against mechanical damage due to water loss, and repair damage when re-hydrated (Oliver et al. 1998).

Evergreen species maintain their chlorophyll and other photosynthetic pigments during times of the year when they are essentially photosynthetically inactive. During this time plants are still absorbing light, and are thus vulnerable to free-radical formation and resulting photo-damage. Plants increase the concentrations of their protective pigments – most notably those in the xanthophyll cycle – in order to dissipate excess light safely (Adams and Demmig-Adams 1992). Xanthophyll cycle pigments act to dissipate excess light energy as heat through non-photochemical quenching and can be very quickly up-regulated from a pool of inactive and partially inactive (violaxanthin and antheraxanthin, respectively) to active species and partially active (zeaxanthin and antheraxanthin, respectively), induced by a change in pH across the thylakoid membrane (Demmig-Adams and Adams 1996). Additionally, the size of the xanthophyll pigment pool can change seasonally in coordination with increasing stress and decreasing
chlorophyll levels (Barker et al. 2002), though this process happens over a longer period than up-regulation from within the pool. Evergreens from alpine environments have very high pools of xanthophyll pigments during the winter months, when they are photosynthetically dormant. Desert species face challenges similar to high-alpine evergreens during their dormant seasons – high light, low water availability, and often-freezing temperatures. Little research has been conducted on the biochemistry of light-induced stress in desert species, but the similarity in environmental challenges to the better-characterized conifer species leads us to believe that desert evergreens may behave similarly.

Plants may also avoid excess water loss through morphological and leaf traits. Both *Larrea* and *Ambrosia* can tolerate low water potential and some localized xylem cavitation. Their shorter growth forms and small branches allow for xylem re-filling and prevent cavitation at lower water potentials than many species (Pockman and Sperry 2000). Both species have small, microphyllous leaves and thick or reflective cuticles which help limit water loss and offer initial protection against light damage. *Larrea* especially exhibits a very compact leaf shape with little or no spongy mesophyll cells and few air spaces. The cuticle is considerable and very sticky, and stomata are small and sunken under the cuticle. *Ambrosia* has very thin leaves less cuticle than *Larrea*, though they are more reflective.

**Elevated CO₂**

Plants growing in elevated CO₂ exhibit changes in physiological processes. Effects of growth in elevated CO₂ are not always consistent and can differ with
environment, temperature, water status, multi-factor changes, and CO$_2$ concentration.

Growth in elevated CO$_2$ has been shown to reduce stomatal conductance and possibly stomatal density over longer time scales (Woodward and Kelly 1995; but see Reid et al. 2003). This can lead to an increase in water-use efficiency (WUE) and improved plant water status and productivity because elevated CO$_2$ tends to simultaneously increase carbon gain through photosynthesis while decreasing water loss through transpiration (Wullschleger et al. 2002).

Rubisco (ribulose-1,5-bisophosphate carboxylase/oxygenase) is the enzyme catalyzing the first step in CO$_2$ fixation (Calvin Cycle) and is considered the most abundant protein on earth (Bowes 1991). Additionally, Rubisco is a slow enzyme with high affinity for O$_2$ at reduced [CO$_2$] and high temperatures. In elevated CO$_2$, more CO$_2$ is available to bind the site of carboxylation at Rubisco, decreasing photorespiration and increasing carboxylation efficiency. At very high levels of CO$_2$ (above those experienced in natural conditions), Rubisco may become saturated, similarly to C$_4$ plants. As a result, plant biomass, root mass, total leaf area, and soluble sugar content all tend to increase (Obrist et al. 2001; Wullschleger et al. 2002). Plants growing in the Mojave Desert are subjected to water stress throughout much of the year, and nitrogen and phosphorous are often severely limiting nutrients (Smith et al. 1997). Even if a plant has excess CO$_2$, it may not have the other resources (water, N, and P) necessary to use additional CO$_2$ in photosynthesis, and thus may not experience higher photosynthetic rates.

Because of the potential for increased WUE, elevated CO$_2$ was initially predicted to have the most profound stimulatory effect for plant production in arid environments.
(Melillo et al. 1993). However, studies at the Nevada Desert FACE (Free-Air Carbon dioxide Enrichment) Facility (NDFD) in the Mojave Desert have found that water is so limiting in most years that differences in photosynthetic rate between plants growing in elevated versus ambient CO$_2$ are minimal much of the time, with significant differences only observed in years (or seasons) with above-average rainfall (Naumburg et al. 2003; Housman et al. 2006). These studies have not included investigations of the physiological and biochemical underpinnings for potential photosynthetic responses to elevated CO$_2$, and thus lacked the mechanistic approach necessary to understand the functional changes causing these observed differences (Huxman et al. 1998; Hamerlynck et al. 2000, Naumburg et al. 2003, 2004). Observing photosynthetic differences is an excellent indicator and predictor of changes in physiological or biochemical processes, but in order to determine the mechanistic basis for those differences – or indeed if functional differences may exist that do not translate to differences in photosynthetic rate – a deeper investigation of physiology and biochemistry is required.

Drought and Elevated CO$_2$

Numerous studies have examined the effects of drought and elevated CO$_2$ in combination. The general expectation is that the increase in CO$_2$ concentration in mesophyll cells will increase the carboxylation efficiency of Rubisco, thereby allowing stomata to partially close while maintaining a favorable CO$_2$ concentration in the leaf, increasing WUE. In practice plants have varying responses, with some exhibiting increased drought tolerance while other species do not (Heath 1998; Obrist et al. 2001; Wullschleger et al. 2002; Naumburg et al. 2003). The mechanisms for drought tolerance
and the effects of CO2 on overall plant physiology are not well enough understood to make definitive conclusions, given these varying results. However, plants that exhibit high drought tolerance tend to increase their tolerance in elevated CO2 (Loik et al. 2000; Hamerlynck et al. 2002).

Research Objectives

Experiments have been conducted at the NDFF that are examining the interaction of elevated CO2 and stress (temperature and water) on the most common species in the Mojave Desert. These past studies concluded that changes in photosynthetic rate and water potential occur (1) only during years where water is not the most limiting resource and (2) that the response is dominated by stomatal control (Naumburg et al. 2003, 2004). However, these studies did not employ techniques with which to investigate the mechanistic response to increased CO2 levels. In order to more thoroughly investigate the biochemical and physiological mechanisms driving the increased stress tolerance in elevated CO2, we conducted a study in which the familiar ecophysiological techniques of photosynthetic gas-exchange, water potential, and fluorescence were coupled with measurements of leaf pigment concentration, soluble sugar concentration, and protein content. Additionally, the 2005 growing season followed a wet winter, the first wet year since the 1998 El Niño event, which was the first full year of operation for the NDFF. Therefore, 2005 represented a year with relatively abundant soil resources during the growing season, but also used plants that had been continuously exposed to elevated [CO2] for a nine-year period rather than the potential “step change” response observed in
1998. The potential to investigate long-term effects of CO$_2$ while simultaneously investigating the mechanistic response to elevated CO$_2$ motivated this study.

Hypotheses

Based on previous studies, we hypothesized that increased [CO$_2$] would lead to increased drought tolerance for both *Larrea tridentata* and *Ambrosia dumosa* not only due to well-established reductions in stomatal conductance and thus potentially improved water relations, but also through (1) changes in soluble sugars acting as osmolytes, (2) changes in pigment concentrations, particularly those associated with the xanthophyll cycle, and (3) protein concentrations and activity. Specifically, we hypothesized that growth in elevated CO$_2$ would lead to increased drought tolerance through reduced stomatal conductance, as previously observed; through increases in soluble sugar pools; through increases in xanthophyll pigments associated with photoprotection; and through photosynthetic down-regulation. Finally, we hypothesized that drought tolerance in *Larrea* would be more positively affected than that of *Ambrosia* due to its year-round metabolic activity and initially higher intrinsic stress-tolerance.
Table 1: List of Terms and Definitions

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<th>Term</th>
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<tr>
<td>Rubisco</td>
<td>Ribulose-1,5-bisphosphate Carboxylase/Oxygenase. Enzyme catalyzing first step in the Calvin Cycle, the CO₂ fixation reaction.</td>
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<tr>
<td>A</td>
<td>Photosynthetic assimilation rate (μmol CO₂ m⁻² s⁻¹)</td>
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<tr>
<td>Cᵢ</td>
<td>[CO₂] in the leaf cavity – internal CO₂ concentration (μmol mol⁻¹)</td>
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<td>Aₙₑₜ</td>
<td>Net assimilation rate measured mid-morning at growth [CO₂]</td>
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<td>A-Cᵢ curve</td>
<td>Comparison of internal [CO₂] with assimilation measured at multiple atmospheric [CO₂] from very low (100 μmol mol⁻¹) to very high (1500 μmol mol⁻¹) [CO₂]</td>
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<td>Jₘₐₓ</td>
<td>Maximum electron transport through photosystem II, modeled from A-Cᵢ curve</td>
</tr>
<tr>
<td>Vₖₘₐₓ</td>
<td>Maximum carboxylation rate of Rubisco, modeled from A-Cᵢ curve</td>
</tr>
<tr>
<td>Fᵥ/Fₘₐₓ</td>
<td>Chlorophyll fluorescence measurement indicating stress to photosynthetic apparatus (the photosystem including PSI, PSII, chlorophyll a + b, etc.)</td>
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<td>Water Potential</td>
<td>Negative pressure under which the water column operates in the plant, measured by creating balance pressure on a cut stem</td>
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<tr>
<td>WUE</td>
<td>Water Use Efficiency – molecules of CO₂ taken up per molecule of H₂O evolved</td>
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<tr>
<td>gₛ</td>
<td>Stomatal conductance, amount of water lost through open pore in the leaf</td>
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<td>Xanthophyll Cycle</td>
<td>Non-photochemical quenching process using the xanthophyll pigments, a class of pigments capable of resonance transfer with chlorophyll, followed by rapid dissipation of the photon energy as heat.</td>
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CHAPTER 2

METHODS

Field Site

The Nevada Desert Research Center (NDRC) is located within the Nevada Test Site, 90K north of Las Vegas, Nevada. The Nevada Desert Face Facility (NDFF) is a Free-Air Carbon dioxide Enrichment (FACE) facility located within the NDRC (Jordan et al. 1999). The NDFF is composed of nine plots, each 23 meters in diameter. Three plots have the full FACE apparatus (stand-pipes and blowers) and continuously expose each plot to elevated CO$_2$ (550 $\mu$mol mol$^{-1}$ [CO$_2$]), three plots have the FACE apparatus, but blow-air onto the plots at ambient CO$_2$ (380 $\mu$mol mol$^{-1}$ [CO$_2$]) and are thus called the “blower controls”, and three plots operate at ambient [CO$_2$] with no FACE apparatus. Thus, six plots are at ambient [CO$_2$], with the blower controls part of the experimental design to insure that there is no “blower effect” on plant performance. The NDFF operates continuously (24 h per day, 365 d per year), with conditional shut-downs occurring only when air temperature drops below 4°C or when wind speed exceeds 7 m s$^{-1}$. The facility, vegetation, and soils are fully described in Jordan et al. (1999).

Sampling Methods

The FACE site is set up for replicates of three rings per treatment. In general, each ring constitutes one data point, so that sampling within one ring is averaged to that
ring, giving a final \( N = 3 \) for all tested parameters. Because of the time-consuming nature of more intensive photosynthetic gas exchange measurements (e.g. A-C\(_3\) curves), potentially short sampling windows for near-maximum photosynthetic rates (only 1-2 h after sunrise to midday, depending on water availability), and the highly variable nature of desert plants, we sampled 5 plants from each of two rings – one elevated and one ambient [CO\(_2\)] – giving \( N = 5 \) for each treatment. This method allowed us to maximize replication, given the sampling constraints.

Due to the long-term nature of the study at NDFF, sampling is limited. This study relied on larger sample sizes than usual for the site and, as a result, use of collected samples was maximized to limit the amount of material removed from the plots. Samples for fluorescence, water potential, pigment analysis, sugar and amino acid contents, and protein contents were all taken pre-dawn. These analyses benefit from a pre-dawn collection because the plant is not subjected to the variable stresses experienced during the day and the biochemical state is a reflection of the plant's longer-term stress. Stomata are closed, so water potential reaches a maximum and photosynthesis is not running, so light and resource stress are minimized and pigments are in their non-activated state. In short, pre-dawn determinations of physiological and biochemical processes represent the capacity for physiological function in the absence of variations in daily conditions.

Starting approximately 1.5 hours before sunrise on each sampling date, two terminal shoot (stem + leaves) samples were removed from each study plant. Samples were placed in plastic bags, stored in a cooler and moved to a field lab just off the
research plots where they were prepared and analyzed. Fluorescence was determined by
removing a small number of leaves from one of the collected branches; light flash
exposure occurred away from all other samples. Pigment samples (approximately 4-6
leaves) were removed from the same branch as fluorescence samples, but remained in the
dark and were not attached to the fluoresced leaves. Water potential was determined as
described below, using the other, intact shoot sample. Sugar and amino acid analyses
required significantly larger samples, so all the remaining samples from both
fluorescence and water potential determinations were pooled. Leaves for sugar, amino
acid and pigment analyses were removed from the stems in the dark and placed in liquid
nitrogen within an hour after harvest. All pre-dawn measurements were completed
before sunrise and all work was conducted with headlamps as the only artificial light
source.

Photosynthesis

Gas exchange was measured with a Li-Cor 6400 photosynthesis system (Li-Cor
Inc., Lincoln, NE, USA) at approximately ambient temperature with a PPFD
(Photosynthetic Photon Flux Density) of 1500 μmol m⁻² s⁻¹. Curves showing the
relationship between photosynthetic assimilation (A) and internal [CO₂] (Cᵢ), called A-Cᵢ
curves, were generated by sequentially taking measurements at 120, 200, 250, 380, 550,
700, 900, and 1500 μmol mol⁻¹ with minimum and maximum measurement times 100 and
120 seconds, respectively (determined by observation of minimum stabilization time).
Because both Larrea and Ambrosia have small, microphyllous leaves, more than one leaf
(leaflets in Larrea) was inserted into the gas exchange cuvette. After the A-Cᵢ curve was

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generated, all material inside the cuvette was collected and leaf area was determined using a flatbed scanner and analyzed with software from Scion Imaging (Scion Corporation, Frederick, MD, USA).

Gas exchange data were analyzed using Photosynthesis Assistant software (Dundee Scientific, Dundee, Scotland, UK), choosing the A-C_i mechanistic option. This model is based on the models of Farquhar et al. (1980) and modified by von Caemmerer and Farquhar (1981), Sharkey (1985), Harley and Sharkey (1991), and Harley et al. (1992) and uses raw Assimilation rate and calculated C_i (internal [CO_2]) to calculate mechanistic data such as V_{c_{max}} (maximum carboxylation rate of Rubisco) and J_{max} (maximum electron transport rate). Net photosynthesis (A_{net}) measurements were taken directly from the raw A-C_i data at each growth CO_2 concentration. Previous experiments have shown that, for these species, mid-morning A_{net} is a good estimation for diurnal integrated CO_2 assimilation (A_{day}; Naumburg et al. 2003).

Pre-dawn fluorescence was determined using a portable Hansatech fluorimeter (Hansatech Group, Cambridge, UK) on one to two leaves using the F_o/F_m (a measure of light stress on the photosystem) measurement program on the fluorimeter. Measurements were recorded by hand. Leaves were placed at a constant distance from the fluorimeter head using a leaf clip that positioned the leaf and fluorimeter head in their respective positions.

Water Potential

Water potential was determined pre-dawn using a pressure chamber (Soil Moisture Equipment Corporation, Santa Barbara, CA, USA) very soon after detachment.
The stem was re-cut with a razor (< 1 mm from the previous cut in order to ensure a clean-cut surface) and placed in the pressure chamber. Pressurized gas (N\textsubscript{2}) was released at a steady flow rate into the chamber and water potential was read as the balance pressure at which water was visible on the cut surface of the stem.

Sugar Analysis

Samples for sugar analysis were taken pre-dawn, as described above. After removing samples for pigments and fluorescence, and after determining branch water potential, all remaining leaves from each individual were removed and placed in liquid nitrogen. Fresh mass was subsequently determined while the material remained frozen in liquid nitrogen. After fresh mass determination, samples were lyophilized. Samples were stored in a −80°C freezer between collection and lyophilization.

Samples for soluble sugars were ground in the dark to a fine powder on liquid nitrogen with a mortar and pestle and 80% ethanol. Samples were vortexed on highest speed for 1 minute and incubated at 60°C for one hour, vortexing for one minute every ten minutes. Samples were then incubated at room temperature for five minutes and centrifuged at room temperature for ten minutes at highest speed (>10,000 rpm). 400 μl of supernatant was transferred to a new Eppendorf tube and samples were dried under vacuum and medium heat (Speed-Vac) for approximately three hours. For analysis, samples were resuspended in 200 μl HPLC grade water, vortexed for one minute, and incubated overnight at 4°C in a refrigerator. Samples were then vortexed for one minute and centrifuged on high (>10,000 rpm) for five minutes. Finally, 100 μl of supernatant was transferred to an HPLC vial for sugar analyses and the remaining 100 μl of
supernatant was transferred to an HPLC vial for amino acids. Samples were stored at -20°C until ready for analysis. A sugar standard was prepared using sucrose, fructose, maltose, and glucose in known concentrations.

A second analysis for soluble sugars was conducted in order to determine cellular contents of cyclitols, a class of osmotically active alcohol-sugars. Approximately 20 mg of ground lyophilized leaf tissue was extracted in 1.5 ml of 80% ethanol. Samples were centrifuged (10,000g, 15 min, 4°C), the supernatant decanted, and pellets resuspended in 80% ethanol. Ethanol extraction was repeated a total of five times, fractions pooled, and the ethanol evaporated using a compressed air stream. To remove pigments, dried fractions were resuspended in 1.5 ml of 2:1 water: chloroform. Soluble carbohydrates were analyzed by high-performance anion-exchange chromatography-pulsed amperometric detection (HPAE-PAD) essentially as described by Moore et al. (1997), using a Beckman Model 126 HPLC (Fullerton, CA), a CarboPac PA-1 column (Dionex, Sunnyvale, CA, USA) and a Dionex ED40 electrochemical detector. PAD was carried out with a gold working electrode as described by the manufacturer for carbohydrate detection. Each sample was eluted with 0.2 M NaOH isocratic at a flow rate of 1.0 cm$^3$ min$^{-1}$. Carbohydrate standards were purchased commercially (Sigma).

Pigment Analysis

As stated above, pigment samples were taken pre-dawn and separated from the shoot before any other measurements were made. Samples were immediately frozen in liquid nitrogen and stored at -80°C prior lyophilization.
Before HPLC analysis the dry weight of each sample was determined and approximately 10 mg dry weight of leaf material was used for pigment extraction. Samples were ground to a fine pulp in the dark in ice-cold 80% acetone (v/v) with an addition of MgCO₃ (spatula tip) using a tissue grinder (Kontes Duall K885450-0021, Kontes, Vineland, New Jersey). Pigment extracts were then centrifuged 7 minutes and 10,000 x g (Centrifuge 5402, Eppendorf AG, Hamburg, Germany). The supernatant was transferred to a new microfuge vial and stored on ice. The pellet was then washed with 100% acetone to extract the remaining pigments and centrifuged for 7 min and 10,000 x g (Centrifuge 5402, Eppendorf AG, Hamburg, Germany). The supernatant was added to the first extract. This step was repeated until the pellet appeared to be free of pigments. The final pigment extract was then centrifuged again for 7 min and 10,000 x g before its volume was determined and filtered using a nylon syringe filter (Cameo Disposable Syringe Filters, 0.45 µm pore size, No. DDN0400300, MSI, Westboro, MA). The pigment extracts were then transferred to 1.5 mL Eppendorf tubes with a piercable top and bubbled with gaseous nitrogen prior to capping and stored on ice until being processed for HPLC analysis.

Samples were analyzed via HPLC using a Bio-Rad AS1005 autosampler, a low pressure ternary gradient system (Model 220B pump and Model 231 pump controller, Scientific Systems, State College, PA), a Spherisorb ODS1 5U column (Alltech Associates, Inc., Deerfield, IL, USA) maintained at 30°C and a flow rate of 1 mL min⁻¹, and a Dynamax UVD II dual-wavelength detector (measuring at two wavelengths: 445 nm and 654 nm). Data acquisition and analysis was performed using Allchrome Plus
data acquisition software and interface. Total running time was 35 minutes for each sample with additional two minutes for sample loading.

For each run the following protocol was followed: 14 minutes of solvent C (900 mL methanol, 100 mL hexanes, and 38 mL 0.1M Tris-HCl pH 8.0); a six-minute linear gradient from solvent C to solvent B (800 mL methanol and 200 mL hexane); 100% solvent B until minute 25; a linear gradient back from solvent B to solvent C between min 25 and min 27. Solvent A (900 mL acetonitrile, 100 mL methanol, and 38 mL H₂O) was then used to flush the system for 45 minutes before system shutdown.

Individual pigment contents were calculated as mg pigment per g dry weight for neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin, chlorophyll b, chlorophyll a, and β-carotene.

Statistics

Significance relationships were tested using a one-way ANOVA for each sample pair on each date. When data were non-transformable to meet the assumptions of the model (e.g. homogeneity in error), the test was run as non-parametric Kruskal-Wallis Test.
CHAPTER 3

RESULTS

Environmental Conditions

The hydrologic year for this experiment was a wet year with significant amounts of rainfall occurring between October 2004 and March 2005 (Figure 1b). This wet winter allowed high germination rates of annuals and plenty of early-growing-season moisture for perennial shrubs. The temperatures were not out of the ordinary, with the highest average monthly temperature occurring in August (Figure 1a). Of note, however, was that the average minimum temperature did not rise above freezing until late April, so the spring “growing season” was characterized by frequent freezing temperatures at night. The driest part of the year corresponds with the hottest, though the heat was interrupted by a few significant rainfall events (Figure 1).

Water Potential

Predawn water potentials of Larrea tracked seasonal changes in temperature and precipitation, declining as soils dried and temperature/VPD (vapor pressure deficit) increased (Figure 2a). Later in the growing season (July), water potentials were lower, and the largest drop in water potential occurred as soils dried but while photosynthesis (see Fig. 3) and therefore stomatal conductance was still high. The difference in water potential between the CO₂ treatments was greatest later in the growing season, and a
Figure 1: FACE environmental data. Panel A represents average maximum, average minimum, and average monthly temperatures (bars are ±1 standard deviation). Panel B represents individual precipitation events. All data ranges from 1 October 2004 through 1 January 2006 (the hydrologic cycle affecting this experiment).
Figure 2: Pre-dawn water potential for, A, *Larrea tridentata*, and, B, *Ambrosia dumosa* at ambient and elevated [CO$_2$]. Bars are + 1 S.E. Note the difference in scales for both axes. "**" indicates $p \leq 0.05$ between ambient and elevated [CO$_2$] at each specific date.
significant difference was not observed until predawn water potentials dropped below -4 MPa. At this time plants growing in elevated CO₂ had significantly higher water potentials than those growing in ambient CO₂. Water potential in *Ambrosia* was similar to the pattern observed in *Larrea*, although water potential was only recorded during the spring growing season due to the drought-deciduous nature of *Ambrosia*. When water potential dropped below -1.5 MPa, a significant difference became apparent in that *Ambrosia* plants growing in elevated CO₂ exhibited significantly higher water potential than in ambient CO₂ (Figure 2b).

**Photosynthesis**

*Larrea* exhibited maximum net assimilation rates (ANet) between 25 May and 7 July 2005 (Figure 3a). Early season values showed no CO₂ treatment effect, but as the growing season progressed – increasing in temperature and decreasing in moisture – ANet rates increasingly separated, reaching maximum differences between treatments just after maximum ANet values were reached on 25 May and 7 July, respectively. Late in the season, even as ANet decreased to below levels measured in March, ANet in elevated CO₂ remained significantly higher than ANet in ambient CO₂ (Fig. 3a).

Peak net assimilation rates (ANet) in *Ambrosia* occurred between 25 May and 7 July (Figure 3b). ANet was not significantly different between CO₂ treatments in *Ambrosia*, although the measured ANet in elevated CO₂ was always higher than in ambient CO₂. As with *Larrea*, ANet increasingly separated through the growing season, reaching maximum differences between treatments just after maximum ANet values were reached on 25 May. Measurements were not conducted on *Ambrosia* later in the season.
**Figure 3:** Net assimilation rate, measured at growth (ambient or elevated) [CO₂] for *Larrea tridentata* (panel A) and *Ambrosia dumosa* (panel B). Note the different scales on X- and Y-axes for each species. Bars are ± 1 S.E.; "*" indicates p ≤0.05 between ambient and elevated [CO₂] at each specific date.
because it was leafless, thus the rate of photosynthetic down-regulation cannot be determined from this data.

Water use efficiency (WUE; calculated as $A_{\text{net}}$/transpiration; Figure 4a) shows a steady decline seasonally for both treatments. In late July there is a slight increase in ambient WUE and a larger increase in elevated WUE – this spike is most likely in response to a late-July rain event. There are several dates showing a significant treatment effect beginning 6 April and ending on 25 July; the final date of measurement, on 13 October, indicates that the water potential values are re-converging at the end of the growing season. WUE in *Ambrosia* showed a steady decline throughout the measurement period with the highest rates and most significant ($p = 0.0544$) CO$_2$ treatment effect occurring on 17 March (Figure 4b).

Carboxylation capacity ($V_{\text{cmax}}$, maximum rate of carboxylation by Rubisco) and maximum electron transport rate ($J_{\text{max}}$) were modeled from the A-C$_i$ curves using the Photosynthesis Assistant program and, like $A_{\text{net}}$, showed an increase from spring to summer in *Larrea* as temperatures warmed, but then a modest decrease occurred as soils dried through the summer (Figure 5a,c). However, the maximum difference between CO$_2$ treatments for these parameters occurred on the first date of measurement (3 March). At this time, plants growing in elevated CO$_2$ showed significantly lower $V_{\text{cmax}}$ and $J_{\text{max}}$ than ambient-grown plants, indicating photosynthetic down-regulation in the early season. However, the later seasonal trends in $V_{\text{cmax}}$ and $J_{\text{max}}$ matched that of $A_{\text{net}}$, with plants growing in elevated CO$_2$ showing consistently higher rates (Fig. 5a,c). These differences were not significant on most dates, but the trend indicates photosynthetic up-regulation in plants growing in elevated CO$_2$. $V_{\text{cmax}}$ and $J_{\text{max}}$ showed a seasonal increase...
Figure 4: Water Use Efficiency, calculated as $A_{\text{net}}$/transpiration for, A, Larrea tridentata and, B, Ambrosia dumosa at ambient and elevated [CO$_2$]. All figure details are as in Fig. 3.
Figure 5: $V_{\text{cmax}}$ (maximum Rubisco carboxylation efficiency) for *Larrea tridentata* (A) and *Ambrosia dumosa* (B), and $J_{\text{max}}$ (maximum electron transport rate) for *Larrea tridentata* (C) and *Ambrosia dumosa* (D) at ambient and elevated [CO2]. All figure details are as in Fig. 3.
and decrease, respectively, in *Ambrosia*, with the maximum difference between CO$_2$ treatments corresponding with maximum photosynthetic rates in late May (Figure 5b,d).

While these data support the $A_{\text{net}}$ data, the seasonal changes and CO$_2$ treatment differences in $V_{\text{cmax}}$ and $J_{\text{max}}$ were not statistically significant in *Ambrosia*.

Pre-dawn chlorophyll fluorescence gives information about the stress level of the photosystem; higher $F_v/F_m$ levels indicate that the photosystem is not stressed and that all photosystems are open and accepting electrons for photosynthesis; lower $F_v/F_m$ levels indicate that the photosystem is experiencing light stress and is not accepting all possible photons. Pre-dawn chlorophyll fluorescence in *Larrea* exhibited seasonal changes, with reduced levels in early spring (presumably due to freezing temperatures) and in the fall, but no statistically significant CO$_2$ effects (Figure 6a). $F_v/F_m$ tracked $A_{\text{net}}$, increasing to a maximum in May before decreasing in October to near those levels measured in March. The most statistically significant $F_v/F_m$ measurements ($p = 0.16$ and 0.13, respectively) corresponded with significant differences in $A_{\text{net}}$, the first observed significant difference in water potential, and the first observed difference in Rubisco content (see below). Pre-dawn chlorophyll fluorescence in *Ambrosia* increased from early to late spring, and a significant difference between ambient and elevated CO$_2$ was observed only on the last measurement, 30 May (Figure 6b). As with *Larrea*, plants growing in elevated CO$_2$ had higher $F_v/F_m$ at the point where $A_{\text{net}}$ was maximum but stress was starting to set in.

**Photosynthetic Biochemistry**

Rubisco content was analyzed in *Larrea* only with a general protein extraction and run on a standard protein electrophoresis gel. Protein concentrations were not quantified, but
**Figure 6:** Pre-dawn Fv/Fm for, **A**, *Larrea tridentata*, and, **B**, *Ambrosia dumosa* at ambient and elevated [CO2]. All figure details are as in Fig. 3.
showed a clear pattern with respect to Rubisco content in elevated versus ambient CO\textsubscript{2} (Figure 7). Plants growing in elevated CO\textsubscript{2} maintained higher levels of Rubisco later in the growing season (25 July) than those plants growing in ambient CO\textsubscript{2}. These data support the observed separation of photosynthetic rates between CO\textsubscript{2} treatments beginning in July. The date at which the ambient Rubisco band density is appears lighter than the elevated band (25 July) is the second date at which significant treatment differences in A\textsubscript{net}, water potential, and F\textsubscript{v}/F\textsubscript{m} were observed. This point also corresponds with the observed significant decrease in A\textsubscript{net} in both treatments, although Larrea growing in elevated CO\textsubscript{2} maintained significantly higher A\textsubscript{net} on this date. Additionally, this date corresponds with peak temperature, and lies in the middle of the longest dry period for the year (Fig. 1).

Soluble sugar contents showed few significant treatment differences (Figure 8). In both species, sucrose levels were highest in mid-March, but dropped significantly in April and did not recover. During maximum sucrose concentrations, Larrea plants growing in elevated CO\textsubscript{2} had higher sucrose concentrations, though they were not statistically significant. Plant material was screened for sucrose, glucose, fructose, and maltose (no maltose was present in either species). The sugar contents do, however, follow the seasonal trends observed in other data, though the treatment effect is less consistent or apparent, with elevated CO\textsubscript{2} plants showing higher concentrations of sucrose (Fig. 8a) on a few dates and ambient CO\textsubscript{2} plants showing higher concentrations of fructose (Fig. 8c) and glucose (Fig. 8e) at nearly all times of the year. Soluble sugar contents in Ambrosia are generally higher in plants growing in elevated CO\textsubscript{2}, especially later in the growing season. The overall sucrose (Fig. 8b), fructose (Fig. 8d), and glucose

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Figure 7: Protein electrophoresis gel for *Larrea tridentata* leaf samples across the 2005 growing season. The left panel represents elevated [CO$_2$] samples, the right panel ambient [CO$_2$] samples taken on the dates indicated. The left-hand arrow points to the Rubisco band at ca. 50 kD MW.
Figure 8: Non-structural carbohydrate concentrations for the respective growing seasons of *Larrea tridentata* (left panels) and *Ambrosia dumosa* (right panels) at ambient and elevated [CO$_2$]. Representative sugars were sucrose (A,B), fructose (C,D), and glucose (E,F). All other figure details are as in Fig. 2.
(Fig. 8f) pools decreased as the season progressed, even when $A_{\text{net}}$ was increasing, but the treatment effect became more important as overall pools decreased and seasonal water stress increased.

A second screening for soluble sugars was conducted in order to detect the presence and concentration of cyclitol sugars (Figure 9). This second screening confirmed the results of the previous determinations of fructose, glucose, and sucrose among other similar classes of sugars, though, due to limited sample availability we were not able run a full statistical analysis. However, the trends are clear and indicate that the concentration of cyclitols in the leaf tissues of *Larrea* (Fig. 9a,c) was a significant proportion of the sugar pool and, later in the season, made up the majority of soluble sugars. As with fructose, glucose, and sucrose, no differences between ambient (Fig. 9a) and elevated (Fig. 9c) CO$_2$ treatment were observed in cyclitol concentrations. Cyclitols were also present in *Ambrosia*, although the pattern was notably different from that observed in *Larrea*. There was no apparent CO$_2$ effect on cyclitol pools (Fig. 9b,d), although there was a significant effect on the other soluble sugars. The result is that, later in the growing season, plants growing in ambient CO$_2$ have a much higher percentage of their sugars as cyclitols, while the concentration of the non-cyclitols in elevated CO$_2$ plants remained high in *Ambrosia*.

No pigments measured (Chlorophylls a and b, zeaxanthin, antheraxanthin, and violaxanthin) showed differences in concentration due to CO$_2$ treatment in *Larrea* (Figure 10a-c), although they did follow the seasonal trends observed in other data. Chlorophylls a and b began at low levels and increased to maximum concentrations during peak $A_{\text{net}}$ in late June for *Larrea*, after which they began to decline through the
Figure 9: Percent cellular sugars as Aldose + Ketose or Cyclitol sugars in Larrea tridentata grown in ambient CO$_2$ (Panel A), and elevated CO$_2$ (Panel C); and in Ambrosia dumosa grown in ambient CO$_2$ (Panel B), and elevated CO$_2$ (Panel D). Bars are ± 1 S.E. standard error.
Figure 10: Pigment contents of *Larrea tridentata* at ambient and elevated [CO2]. Panel A represents total Chlorophyll a + b contents. Panel B represents the ratio of active:inactive xanthophyll pigments. Panel C represents the ratio of active xanthophyll pigments:chlorophyll a + b. All other figure details are as in Fig. 2.
rest of the growing season (Fig. 10a). The activation state of the xanthophyll pool changed throughout the growing season (Figure 10b). Maximum activation, calculated as zeaxanthin or zeaxanthin + antheraxanthin (fully and half-active, respectively) divided by the total xanthophyll pool (zeaxanthin + antheraxanthin + violaxanthin), occurred during periods of least photosynthetic activity. Therefore, the highest ratios of active-to-inactive xanthophyll pigments were observed in March, and the lowest observed active ratio was observed in May. In July, the active ratio began increasing and continued through the end of the growing season. The ratio of active xanthophyll pigments to Chlorophyll a + b pools provides insight into the efficiency of photosynthesis, and showed a steady decrease from spring to summer, followed by an increase in July (Fig. 10c).

In Ambrosia, chlorophylls a and b were present in highest concentrations in Ambrosia during peak A_{net}, in April-May (Fig. 11a). The activation state of the xanthophyll pool changes throughout the growing season. Maximum activation, calculated as zeaxanthin or zeaxanthin + antheraxanthin (fully and half-active, respectively) divided by the total xanthophyll pool (zeaxanthin + antheraxanthin + violaxanthin), occurred during the period of least photosynthetic activity in early spring (Fig. 11b). Therefore, the highest ratios of active to inactive xanthophyll pigments were observed in March for both species. The lowest observed active ratio occurred in April for Ambrosia and May for Larrea. In July, the active ratio began increasing in Ambrosia and a significant increase was apparent beginning in July and continuing through the end of the growing season for Larrea. Additionally, the ratio of active xanthophyll pigments
to chlorophyll a + b pool size showed a steady decrease throughout the growing season (Fig. 11c).
Figure 11: Pigment contents of *Ambrosia dumosa* at ambient and elevated [CO₂]. Panel A represents total Chlorophyll a + b contents; Panel B represents the ratio of active:inactive xanthophyll pigments; and Panel C represents the ratio of active xanthophyll pigments:chlorophyll a + b. All other figure details as in Fig. 2.
CHAPTER 4

DISCUSSION AND CONCLUSION

Results from this study indicate that growth in elevated CO$_2$ enhances the apparent drought stress tolerance of the desert shrubs *Ambrosia dumosa* and *Larrea tridentata*. Photosynthetic rates during periods of higher stress – high temperature, high light intensity, and low water availability – remained higher in plants grown in elevated CO$_2$ than those grown in ambient CO$_2$ at the Nevada Desert FACE Facility. The most convincing data in favor of increased stress tolerance are measured mid-morning $A_{net}$ and pre-dawn water potential, and these data are supported by a qualitative examination of Rubisco content and the apparent up-regulation of photosynthesis during the summer at elevated CO$_2$. All other data, although showing seasonal changes and occasional CO$_2$ effects, did not show a consistent or convincing response of these desert perennials to elevated CO$_2$.

The differences in $A_{net}$ and water potential due to elevated CO$_2$ concentration were not apparent until mid-growing season, and corresponded with peak temperatures in the middle of the longest rainless period of the year. Late June is perhaps the time of the growing season with the least favorable environmental conditions. In other words, as stress levels increased and plants were still photosynthetically active, plants growing in elevated CO$_2$ were able to maintain higher rates of photosynthesis and more positive
water potentials. A qualitative evaluation of Rubisco content in *Larrea* clearly showed at this same date that plants growing in elevated CO$_2$ had higher levels of Rubisco than plants growing in ambient CO$_2$. At this date, all data reflect the observation that ambient CO$_2$ plants were nearly dormant, while elevated CO$_2$ plants were still maintaining moderately active photosynthesis.

Water use efficiency (WUE) calculations show that these plants adjust conductance rates in response to growth in elevated CO$_2$. $A_{\text{net}}$ is shown to correlate with stomatal conductance in the field (Naumburg et al. 2003), and our $A_{\text{net}}$ data imply significant reductions in stomatal conductance ($g_s$) in response to high CO$_2$. When WUE and $A_{\text{net}}$ are considered together, the argument for reduced $g_s$ is strengthened. Increased WUE coupled with increased $A_{\text{net}}$ (and photosynthetic up-regulation) must be a result of decreased $g_s$. These observations offer strong evidence that reduced $g_s$ is a major component of the observed increase in drought tolerance of elevated *versus* ambient CO$_2$-grown plants.

Rubisco content is variable and often reduced when photosynthesis becomes down-regulated. Proteins are expensive (in terms of available resources, such as nitrogen) and Rubisco is generally regarded as the most abundant protein on earth (and in leaves) and is also one of the largest repositories of organic nitrogen (Bowes 1991). If a plant no longer requires the higher Rubisco concentration needed during the peak growing season, it will reduce the concentration of Rubisco, potentially diverting amino acids to stress tolerance functions. It is, therefore, surprising that the reduction in Rubisco observed in this study did not correspond to an up-regulation of protective pigments. Desert plants are exposed to high irradiance throughout the year, and must
employ protective mechanisms if photosynthesis is not active, in order to avoid serious
damage to the photosynthetic apparatus due to free radical formation (Demmig-Adams
and Adams 1996). In addition, a reduction in chlorophyll $a$ and $b$ contents should
accompany the decrease in Rubisco and expected increase in protective pigments.
However, as with the xanthophyll cycle, *Larrea* and *Ambrosia* did not exhibit a change
in pigment concentrations corresponding to significant changes in photosynthesis and
apparently significant changes in Rubisco content. It may be that, given the xerophytic
nature of desert plants, protective pigments are high at all times of the year, since the
short Mojave Desert growing season, during which soil moisture is adequate for
photosynthesis, is also accompanied by freezing or near-freezing nocturnal temperatures.
Thus, these plants are exposed to either cold or drought stress in almost all months of the
year, except in rare high rainfall years in which soil moisture persists well into late spring
and early summer.

The hypothesis that increased CO$_2$ increases drought (or stress) tolerance through
increased levels of soluble sugars in the cell was rejected. Although soluble sugars may
be important osmolytes during periods of high stress, *Larrea* did not develop
significantly different levels of the three photosynthetically important sugars – sucrose,
glucose, and fructose – due to growth in elevated CO$_2$. Unlike *Larrea*, osmolyte
regulation due to increased soluble sugar pools may play a role in the differences
observed in physiology between ambient and elevated CO$_2$-grown *Ambrosia* plants,
however, these alone were not enough to explain changes in drought tolerance. Soluble
sugars are assumed to have osmolytic functions, but may not be the primary
carbohydrates used for osmotic regulation in these species. As highly drought-tolerant
desert plants, *Ambrosia* and *Larrea* likely have specialized compounds that help maintain proper osmotic and water potentials throughout the year.

One such group of compounds, cyclitols (cyclic, alcohol sugars), were measured and found to be an important component of the carbohydrate pool, especially later in the growing season, although again no CO$_2$ effects were apparent. Cyclitols are also found in alpine plants, which face similar environmental stresses as desert species: high light, low water availability, and nighttime freezing temperatures. Cyclitols are thought to confer drought tolerance through osmotic adjustment (Monson *et al.* 2006). Given the large cyclitol pool found in *Larrea* especially, and the high ability to withstand stress in general, this is apparently the case in these species as well. However, the cyclitol pools were not sensitive to atmospheric [CO$_2$] and were, apparently, a constitutive aspect of drought tolerance.

Pre-dawn chlorophyll fluorescence ($F_v/F_m$) is a good indicator of stress to the photosynthetic apparatus. Higher ratios correspond to lower levels of stress and higher photosynthetic efficiency. Therefore, during the dormant season (when nighttime temperatures are freezing) *Larrea* maintained extremely low rates of photosynthesis and $F_v/F_m$, indicating that the photosystems were closed (or inactive) until nighttime temperatures were no longer freezing. During the growing season, peak $A_{\text{net}}$ corresponded with peak $F_v/F_m$; the photosystems were open (or active) and accepting maximum amounts of light with efficient electron transport. These mechanistic assumptions are supported by $V_{\text{cmax}}$ and $J_{\text{max}}$ rates calculated from A-$_{\text{C}_i}$ curves using a modeling program as described above. Plants growing in elevated CO$_2$ tended toward up-regulation in this study. This finding is in opposition to previous experiments
conducted at the NDFF and in a glasshouse experiment, which found photosynthetic down-regulation in Larrea plants growing in elevated CO₂ (Huxman et al. 1998). We conclude that up-regulation is related to water availability in this system. In the cool, moist early spring, photosynthesis is either down-regulated at elevated CO₂ (Huxman et al. 1998) or shows no difference (this study). But as temperatures warm, soils dry, and the environment transitions into the drier, hotter summer months, stomatal conductance has a more controlling effect, potentially resulting in photosynthetic up-regulation, as we observed in this study. In a year with abundant water supply, photosynthesis is up-regulated in elevated CO₂ plants during this seasonal transition (spring to summer). In years with limiting water supply, any photosynthetic response to elevated CO₂ is apparently overridden by strong stomatal control of gas exchange. Therefore, in the spring-summer transition, when water is not strongly limiting, elevated CO₂-grown plants are able to increase their photosynthetic capacity while improving WUE and, therefore, gain a significant advantage compared to ambient CO₂-grown plants.

In comparison with A_{net} and water potential, in particular, the lack of difference in F_{v}/F_{m} measurements between CO₂ treatments is perhaps surprising. If the A_{net} and water potential data show an increased level of stress tolerance, and apparent up-regulation of photosynthesis, as a result of growth in elevated CO₂, we expect F_{v}/F_{m} to reflect some change in photosynthetic stress. However, these species are capable of maintaining photosynthesis under conditions of severe stress and are perhaps able to isolate water, nutrient, light, and heat stress from the photosynthetic machinery, insuring that the resources available after allocation to protective roles can be used at the maximum photosynthetic efficiency even during times of high overall plant stress.
In early spring all ecophysiological measurements started at a basal level, at which time there was no consistent CO$_2$ effect. Sugar and pigment data showed some of the greatest differences as a result of CO$_2$ at this time. However, the fact that photosynthesis (including $A_{net}$, $V_{cmax}$, and $J_{max}$), chlorophyll fluorescence, and water potential began the season with no CO$_2$ effects implies that plants can respond seasonally to CO$_2$ availability, although the long-term effects are less apparent. In previous studies, differences due to CO$_2$ treatment were minimal following dry years and apparent during wet years (Naumburg et al. 2003). The 2005 growing season followed a wet winter; any CO$_2$ effects from the previous growing season were not carried through the winter and plants in both CO$_2$ treatments began the growing season with essentially the same resources. As the season progressed and seasonal water stress increased, growth in elevated CO$_2$ enabled plants to maintain higher rates of photosynthesis and higher water potentials than plants growing in ambient CO$_2$. The previous studies showing little or no response as a result of CO$_2$ took place during very dry years, and indicated that plants were not able to respond to changing CO$_2$ availability when water was severely limiting. This study supports those data, implying that the response to increased CO$_2$ will be most important during wet periods and that plants in these environments may exhibit significant physiological changes, especially during periods of above-average resource availability.

Additionally, this study was conducted during the eighth full growing season of CO$_2$ fumigation at the NDFF. Therefore, the plants had been growing for seven years at their respective CO$_2$ concentrations. We would expect plants growing under different conditions for a number of years to exhibit long-term effects. In other words, any long-
term effects of elevated CO$_2$ on growth would be expected to carry over across growing seasons and accumulate over time. We did not observe any such effects in this study, and conclude that the physiological responses to elevated CO$_2$ are seasonal and not accumulated. Secondary effects such as increased growth are likely to be more indicative of a long-term response, and growth does not exhibit a sustained response to elevated CO$_2$ (Housman et al. 2006). Alternatively, it could very well be that deserts are such episodic environments that it is not possible for a “new equilibrium” to be attained at elevated CO$_2$, in which higher levels of photosynthesis, growth, and metabolite levels are sustained over time. Strongly oscillating cycles between wet years and droughts may largely preclude the potential for sustained functional changes at elevated CO$_2$.

This study did not address multi-factor resource changes. Global change will also affect nitrogen availability, timing and amount of rainfall, and temperature. Other multi-factor studies have shown that the effects of elevated CO$_2$ alone do not always persist when combined with other projected global change factors (Shaw et al. 2002). In order to gain a more complete understanding of the potential changes in physiology and ecology as a result of global change, multi-factor studies need to be conducted in conjunction with FACE studies.

Conclusions

This study confirmed that stomatal control of transpiration is the most significant contributor to the differences seen in drought tolerance between desert shrubs growing in elevated and ambient CO$_2$ at the Nevada Desert FACE Facility. While *Ambrosia dumosa* did exhibit some biochemical changes to growth in elevated CO$_2$, these were not
sufficient to fully explain the apparent differences observed in drought tolerance. *Larrea tridentata* exhibited essentially no biochemical adaptations to growth in elevated CO$_2$, yet had significantly higher stress tolerance when grown in elevated CO$_2$. Previous studies concluded that stomatal conductance drove the response to elevated CO$_2$, however, these studies lacked the biochemical investigation to back up those findings. This study employed a variety of analyses, including biochemical and ecophysiological, in order to more deeply investigate the mechanistic underpinnings of photosynthetic differences at ambient and elevated CO$_2$. We thus conclude that stomatal control is the primary cause of changes in drought tolerance resulting from growth in elevated CO$_2$. 

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REFERENCES


*Climate Dynamics*, 16, 123-146.


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