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I. UNINTENDED BIAS IN MULTIPLE COMMON GARDEN EXPERIMENTS: THE EXAMPLE OF A MOJAVE DESERT SHRUB, *AMBROSIA DUMOSA*

Abstract

The need for ecological restoration of disturbed ecosystems around the world is increasing as environmental disturbances occur more frequently, with greater intensity, and spanning greater areas**.** Using genetically appropriate plant materials when local ecotypes are not available is of the utmost importance for long-term restoration success. I used 19 Mojave Desert ecotypes of the restoration species *Ambrosia dumosa* (white bursage) to illustrate how size bias introduced through the growth conditions in a greenhouse carries over into performance of transplants in common gardens and influences fitness parameters. Ecotypes that grew to larger sizes inside the greenhouse had a significant positive correlation with initial growth in the garden. This initial growth had a significant influence on survivorship out to two years with larger plants having increased survivorship over smaller plants. Measuring functional traits during this period to correlate to survivorship would be biased by greenhouse size and waiting for the greenhouse size bias to dissipate could result in mortality of many individuals that could have had functional trait data collected upon.

Introduction

The need for ecological restoration of disturbed ecosystems around the world is increasing as environmental disturbances occur more frequently, with greater intensity, and span greater areas (Pardue and Olvera 2009, Briggs et al. 2017). As a result, there is a high demand for plant materials adapted to persisting not only in the present climate, but under the expected harsher conditions of a future climate (Jones 2013). To address this need, there has been a push to examine genotype × environment interactions in common garden experiments (Kawecki and Ebert 2004). These experiments test performance of plant materials collected from multiple natural populations across a species' range within common environment (garden), but the environments differ in climate norms. The aim is to determine which populations will perform best under specific climatic conditions (Kawecki and Ebert 2004). So far, these experiments have produced some successes (McLean et al. 2014) but also highlighted some challenges. For example, it is rarely possible to select natural garden locations differing only in climate conditions as soil characteristics often also vary among gardens (Gibson, 1995). Here, we highlight a different challenge that arises out of establishing experimental populations from greenhouse transplants.

In common garden experiments with perennial plant species, it is a standard practice to first grow seedlings from seeds in a greenhouse and then to transplant seedlings into garden sites (Richardson 2014, Alba, 2017). The objective is to establish robust plants with substantially reduced mortality risk at the time of transplanting. Seedlings are typically raised for six to nine months inside a greenhouse before being transplanted into the field. Accordingly, greenhouse conditions are maintained to

facilitate fast growth and minimize seedling loss due to stress. However, this practice also means that the environment that seedlings of all ecotypes first encounter is identical and strongly biased towards benign growing conditions. Ecotypes react to these conditions differently from the time of germination, and these differences will interact with their subsequent performance across garden locations (Donohue 2010).

Plant growth strategies vary along a universal ('fast-slow') spectrum ranging from the capacity for fast growth coupled with low stress tolerance to high stress tolerance coupled with slow growth (Bazzaz 1979, Budowski 1965, Pianka 1970, Reich 2014, Smith and Huston 1989). This tradeoff can be found across taxonomic levels, including among ecotypes of the same species (McLean 2014). Ecotypes from more favorable environments are expected to possess traits that are positively correlated with growth rate when resources are plentiful, while ecotypes from harsher environments are expected to have traits indicative of resource use efficiency and stress resilience (Kramer 1980). Therefore, ecotypes adapted to more favorable environments should grow faster in a greenhouse, compared to ecotypes from harsher environments, which could in turn improve field survivorship irrespective of climate conditions.

Across climate zones, plant size is positively correlated with survivorship in juvenile life stages. For example, five-year transplant survivorship for shortleaf pine (*Pinus echinata*) growing in southeast Missouri was positively correlated with initial seedling stem diameters (Kabrick et al. 2015). Similar results were found for natural seedlings of a tropical canopy tree (*Ocotea whitei*) in Panama (Gilbert et al. 2001) and the desert perennial *Ericameria nauseosa* (Benard and Toft 2008). There are many reasons why larger plants are more successful; for example, they may be less suppressed by

competition in high-resource environments (Weiner 1985). Larger plants may also be more resilient to injury or herbivory than smaller plants due to greater carbohydrate storage (Lusk and Piper 2007). In water-limited environments success could be more strongly linked to rooting depth. For example, Donovan et al. (1993), reported that taller transplants of the desert species *Chrysothamnus nauseosus* had higher probabilities of survival during summer drought due to deeper roots that were able to access groundwater (Padilla 2007).

The consistently positive effect of size on survivorship may introduce a deep bias in the analysis of common garden experiments. If under a benign greenhouse environment, ecotypes from mesic, less stressful environments have a growth advantage, then the performance of seedlings of such ecotypes for restoration could be consistently overestimated, especially for more stressful environments. We tested the validity of this conjecture and the magnitude of the potential bias in a multiple common garden experiment conducted in the Mojave Desert, involving the shrub *Ambrosia dumosa* (A. Gray) Payne (white bursage). This shrub is ubiquitous throughout the ecoregion and forms dense co-dominant communities with *Larrea tridentata* (creosotebush) in undisturbed environments. *Ambrosia dumosa* is of high restoration priority because it acts as a shade plant for many sensitive wildlife species. I grew seedlings in the greenhouse from seeds collected from 19 populations spanning most of the Mojave Desert climate variability, and after twelve months, transplanted them into three climatically distinct gardens. I tested the following hypotheses:

1) Average plant size is significantly different among ecotypes in the greenhouse and immediately after transplanting, and the two size measures are positively correlated.
2) Growth of seedlings in the greenhouse will be positively correlated with more mesic conditions in the climate of seed origin (i.e., higher precipitation and/or cooler temperatures);
3) Larger transplants have higher survivorship across all gardens;
4) The influence of initial transplant size on survivorship weakens after several years, and similarities between a specific garden climate and the climate in the regions of population origin increase their influence on survivorship.

Methods

Study Species

Ambrosia dumosa (white bursage) is a drought-deciduous desert shrub that occurs throughout the Mojave and Sonoran deserts and is co-dominant with *Larrea tridentata* (creosotebush) (Shreve 1942), for which it may serve as a nurse plant in early developmental stages (McAuliffe 1988). *Ambrosia dumosa* typically grows to 20-60 cm in height with a hemispherical crown and a root system mainly comprised of lateral roots with the ability to reach soil depths of 70 cm (Fonteyn 1981). This species provides cover for many wildlife species, including the federally listed *Gopherus agassizii* (Mojave desert tortoise), and provides forage for *Lepus californicus* (black-tailed jackrabbit) and many rodent species (Hunter 1987). As an early colonizer in disturbed habitat, *A. dumosa* is a priority species for Mojave Desert restoration.

Seed Sources and Transplant Preparation

Seed collections of *A. dumosa* were provided by the Seeds of Success (SOS) program (Bureau of Land Management) in partnership with many federal and non-federal organizations. The seeds were collected from 19 populations that spanned the precipitation and temperature gradients that are observed within the Mojave Desert (Table 1.1). All seeds were stored at approximately 4.5°C until used.

All seeds were germinated between January and March 2013 in 72 cell flats containing a sandy growing medium in a greenhouse at the College of Southern Nevada, Henderson, NV, USA. The number of available seeds and germination success differed among ecotypes, which required germinating more seeds until a target population of 1100 plants was established, with a range of 16-109 plants per ecotype. Once a majority of seedlings had produced their primary leaves, seedlings were transplanted into 7.62 cm × 7.62 cm × 30.48 cm plant bands (open ended cardboard tubes) (Monarch Manufacturing, Inc., Salida, CO USA) in a 3:1:1 soil mix of sand, organics (mixture of potting soil and wood mulch), and perlite, respectively. In May 2013, plants were assigned a permanent unique ID number stenciled on a metal tag and affixed with metal wire around the basal stem. In February 2014, one-year old seedlings were moved outside for one month for hardening. From early to mid-March all live seedlings were randomly stratified across the gardens based on initial size.

Garden Design

The three gardens were located near St. George in Utah (abbreviated 'UT'), on the grounds of Fort Irwin National Training Center (abbreviated 'FI') and near Twentynine Palms and Joshua Tree National Park in southern California (abbreviated 'JT'). These sites were selected based on their position along an environmental gradient

of mainly climatic factors (e.g. average precipitation and temperatures, minimum and maximum temperatures; Fig. 1.1). All gardens were approximately 2500 m^2 and fenced with 0.64 cm galvanized steel hardware cloth. The hardware cloth was buried approximately 20 cm to deter rodents from digging under the fence and had a height of one meter above the ground to deter large mammals. In addition, a 20 cm wide strip of aluminum flashing was affixed near the top of the fence to deter small rodents. The fence at the UT garden had a 2.15 m tall deer fence to exclude the deer population at this site.

While all other ecotypes were split evenly among gardens, ecotype NV052-275R had few plants available at the time of transplanting so plants from this ecotype were split among the more climatically extreme gardens (JT and UT) and not planted at FI. Seedlings were transplanted into 30 cm deep, previously irrigated holes laid out at a distance of approximately one meter from each other in a square lattice design. Transplants were watered immediately after transplanting from a portable sprinkler system with approximately 2000 gallons of water except in the JT garden where conditions were very dry prior to planting and approximately 8000 gallons were sprayed over the entire planting area to enhance plant establishment. Subsequently, gardens were watered monthly with approximately 1000 gallons from April to June 2014 for the UT garden, April to July 2014 for the FI garden, and April to August 2014 for the JT garden.

Data Collection

Monthly assessments of survivorship were conducted during the first three months after transplanting (April – June 2014) and from February 2015 – December 2016. The status of each plant was recorded as “dead” (no leaves present and non-

flexible stems), “dormant” (brown leaves attached and no green leaves), or “alive” (live green leaves present).

The stem diameters and canopy volumes for a majority of *A. dumosa* plants were measured once in the greenhouse during May 2013. In the gardens, complete sets of plant height from soil (H), greatest crown width (D_1) and the perpendicular crown width (D_2) were measured monthly from April to June 2014 and in March 2015, 2016, and 2017. Canopy volumes were estimated using the following equation (Ludwig et al. 1975):

$$V = \left(\frac{4}{3}\right)\pi\left(\frac{H}{2}\right)\left[\left(\frac{D1+D2}{2}\right)/2\right]^2 \qquad \text{(eq 1)}$$

Climate Variables

The climate variables used in the analyses were obtained from the PRISM Climate Group, Northwest Alliance for Computational Science & Engineering based at Oregon State University (www.prism.oregonstate.edu; accessed 05/15/2016). The database was queried to provide 30-year normals (1981-2010) for monthly precipitation totals, average minimum (T_{min}) and maximum (T_{max}) temperature for each latitude and longitude where *A. dumosa* seeds were collected.

Analyses

Before analysis, data were reviewed for quality and consistency. For example, status data were reviewed for each seedling such that a false assignment of “dead” was revised to “dormant” if the plant greened up at a later time. Conversely, the first report of a “dormant” status was revised to “dead” if the plant never greened up again. Prior to running any survivorship analyses, all “dormant” designations were changed to “alive.” Measured canopy volumes of dead plants were removed.

We used ANOVA to determine if ecotypes differed in average size in the greenhouse and immediately after transplanting, using greenhouse stem diameters and log transformed initial canopy volume, respectively, as the dependent variables. Linear regression was used to determine if there was a positive correlation between the two early size indicators.

A model selection approach, based on minimizing the AICc, was used to determine the best multivariate model to represent the relationship between stem diameter of greenhouse plants and climate data in the region of origin. Starting with all monthly precipitation and temperature averages (T_{min}, T_{max}), models were simplified stepwise by removing the least significant climate variable, until further simplification increased the model's AICc value. The same analysis was conducted for the initial canopy volume data, except that the analysis was separated by garden because initial canopy sizes were significantly different between gardens following one-month of garden exposure.

A Kaplan-Meier survivorship analysis was used to estimate the overall survivorship of *A. dumosa* from April 2014 – June 2016 and determine differences in survivorship between gardens. Monthly viability status checks for each individual were evaluated and coded with a 1 for alive and a 0 for dead. A censor variable (event) was created to indicate at the end of the study period (March 2014-June 2016) which plants were dead (event occurred) and alive (event did not occur).

Linear logistic regression was used to determine effects of initial canopy volume on subsequent survivorship. To examine if the effect of initial size weakened over time, the analysis was conducted for four time periods: one month, three months, one year and

two years after transplanting. Additionally, model selection analysis was used to determine how ecotype's climate of origin influenced survivorship, in addition to initial canopy volume, using the same approach to model selection as described before.

Results

Ecotype Size Differences

The average stem diameters prior to transplanting were significantly different among ecotypes (ANOVA: $F_{18, 681} = 2.892$, $p < 0.001$). Model selection analysis identified two significant predictors of average stem diameter: June precipitation and average T_{min} in December at the population's climate of origin (Table 1.2). Thus, populations from regions with wet summers and warm winters grew faster in the greenhouse.

The average April 2014 canopy volumes (one-month after transplanting) were also significantly different between ecotypes (ANOVA: $F_{18,994} = 5.385$, $p < 0.001$), as well as among gardens (ANOVA: $F_{2,994} = 56.988$, $p < 0.001$), but there was no significant ecotype × garden interaction (ANOVA: $F_{35,994} = 1.168$, $p = 0.233$). Across all ecotypes, canopy volumes decreased from JT > FI > UT. Furthermore, the April 2014 canopy volumes measured in the field had a significantly positive correlation with the stem diameters of greenhouse seedlings (Linear Regression: Est. = 0.290, $p < 0.001$, $R^2 = 0.029$).

Average spring or early summer precipitation was a significant predictor of canopy volume across all gardens, again indicating that populations originating in areas with wetter warm seasons had greater canopy volumes in all gardens (Table 1.3). Additionally, average T_{max} in July at the site of origin had a significant negative effect on canopy volume in the UT garden.

Survivorship

Kaplan-Meier survivorship curves were significantly different between the three gardens (Mantel-Cox $\chi^2 = 309.705$, df = 2, $p < 0.001$; Fig. 1.2). During the first three months post-transplanting (April to June 2014), the survivorship of ecotypes in the FI and UT gardens were similarly high and lower in the JT, particularly from May to June 2014 (Fig. 1.2). Over the following year, there were high rates of mortality in the UT and JT gardens, but not in the FI garden (Fig. 1.2). Over the second year, there was little more loss in the FI and UT gardens, but continuing decline in the JT garden. The Kaplan Meier analysis also revealed significant differences between ecotypes (Mantel-Cox $\chi^2 = 92.236$, df = 18, $p < 0.001$).

Canopy volume measured in April 2014 would not have had an effect on survivorship one month after transplanting since both survivorship and size were measured in the same interval, but did have a significant effect on all subsequent survivorship estimates (Tables 1.4, 1.5). Garden also had a highly significant effect on survivorship throughout, but ecotype effects were less consistent. There were significant differences in survivorship between ecotypes in April 2014, but without significant ecotype × garden interactions (Table 1.4). In June 2014, both ecotype and ecotype × garden interactions were highly significant (Table 1.4). One year later (June 2015), the effect of ecotype on survivorship was significant, but not the ecotype × garden interactions, and two years later, ecotype effects were also not significant (Table 1.5).

Seedlings in the UT and FI gardens that were still alive in June 2014 had larger prior canopy volumes than seedlings that died, but in the JT garden seedlings overall had larger canopies regardless of survival status (Fig. 1.3). The same pattern persisted during June 2015 and in June 2016, except that the effect of the initial plant size in JT garden

became either marginally significant (2015) or significant (2016) as live plants had larger prior canopy volumes (Table 1.7 and 1.8, respectively).

Besides prior canopy volume, climate variables from the region of origin had additional effects on survivorship. In the UT garden, ecotypes that originated in areas with lower average April or May precipitation had higher survivorship than ecotypes originating in areas with more precipitation (Tables 1.6-1.8). In the FI garden, ecotypes that came from areas with higher average T_{max} in October had higher survivorship until June 2014 (Table 1.6), but for survivorship in June 2015, average T_{max} in January was the better predictor (Table 1.7). However, the best predictor for two-year survivorship were average local precipitation in August and November in the FI garden (Table 1.8). In the JT garden, higher precipitation at ecotype's origin in August, October or November decreased survivorship, while temperature also at ecotype's origin had mixed effects. At JT, survivorship until June 2014 increased with T_{min} in February at ecotype's origin, survivorship until June 2015 increased with T_{max} in November at ecotype's origin, and survivorship to June 2016 increased with T_{min} in September at ecotype's origin; (Table 1.6-1.8).

In the second and final year, June 2016, initial canopy volume had a positive significant effect on survivorship in all gardens with larger plants having higher survivorship (Table 1.7). Additionally, climate variables at the ecotype's origins also partly influences survivorship. In the UT garden, populations that originated in areas with less average May precipitation had increased survivorship, populations inhabiting areas with less average August and November precipitation had increased survivorship in the FI garden, and populations from areas with less average October precipitation and

higher average September minimum temperatures had increased survivorship in the JT garden (Table 1.7).

Discussion

My study shows that variation in the growth among ecotypes of *A. dumosa* plants under greenhouse conditions carried over to the gardens and influenced transplant survivorship. Greenhouse plants experienced higher winter temperature, higher humidity and wetter soil than typical garden conditions in the Mojave Desert. Ecotypes that originated in regions with higher summer rainfall and warmer winter temperatures benefited from these greenhouse conditions and grew faster on average than ecotypes with drier summers and colder winters. Although not quantified, it may have also been the case that these ecotypes from milder climates had higher germination and establishment success, and would have been several weeks older on average than plants from ecotypes to which new seedlings were added later to replace seedlings that died. In any case, by the time plants were ready for transplanting, ecotypes from more benign climate regions would have a consistent fitness advantage, whether or not their climate of origin matched the garden climate into which they were transplanted.

The stem diameter of greenhouse plants and the canopy volume of recent transplants both exhibited ecotype effects and were significantly correlated, thus confirming my first hypothesis. Although the R^2 value of this model was quite low, this may be expected given that the two measurements were taken nearly one year apart and included a month of growing in different gardens, during which size differences between garden ecotypes developed. The model selection analysis of the climate variable effects on greenhouse stem diameters supports my second hypothesis that ecotype size variation

was a result of the wet and warm climate conditions inside the common greenhouse increasing the growth rates of ecotypes that originated in areas with higher June precipitation and higher minimum December temperatures in relation to the growth rates of ecotypes from locations with less favorable conditions.

The larger size that plants developed in the greenhouse increased survivorship of ecotypes once transplanted to the gardens, which supports the third hypothesis. Greater initial transplant size during the field establishment phase (April – June 2014) resulted in higher survivorship in the FI and UT gardens that had less stressful conditions (Fig. 1.3, Table 1.6). However, transplants for these two gardens were overall smaller than those at the JT garden one-month after transplanting, which may have been due to springtime temperatures in the JT garden increasing at a faster rate than the other two gardens (Mean temperature from 3/15/2014 – 4/15/2014: UT 13.3º C, FI 15.3º C, JT 19.2 º C). Transplants in all gardens also received supplemental water initially at the time of outplanting to ensure the highest possible establishment, which coupled with warmer temperatures may have initially accelerated growth in plants that survived initial transplant shock. Nevertheless, the benefit of initial transplant growth in the JT garden made a small but significant increase in two-year survivorship, and plants of all sizes were subject to mortality. Plants that possessed traits that allowed survival despite the stresses of the much warmer and drier climate at this garden had an advantage, which began to influence survivorship. The absence of small plants during the establishment phase due to plants from all ecotypes having accelerated growth in the JT garden likely caused mainly by warmer garden springtime temperatures coupled with more stressful conditions at JT garden (less rainfall) are likely responsible for the lack of an initial size

effect on survivorship and why ecotypes from areas with less summer precipitation and warmer winters had a survivorship advantage.

The survivorship advantage of size is commonly observed among different organisms, especially at the juvenile stage (Bolopo 2015), although environmental conditions, such as food availability for animals or soil fertility for plants, results in larger differences in the size of individuals of the same species living in different environments (Huston and Wolverton 2011). Larger plants with more extensive root systems or more fine roots access water from deeper soils compared to plants with less fine root material. In *A. dumosa*, above- and below-ground biomass are highly correlated with average belowground biomass slightly greater than aboveground biomass (Wallace et al. 1974), which is typical of plants growing where belowground resources are limited (Bazzaz 1979). It is expected then that plants that exhibited larger canopy volumes in this experiment also had larger root systems that allowed access to deeper soil moisture.

During June 2015, after one year in the gardens, initial size was still strongly influential in the FI and UT gardens and marginally influenced survivorship in the JT garden (Fig. 1.4). Two years after transplanting, initial size significantly influenced survivorship in all three gardens (Fig. 1.5). Average climate at ecotypes origin also had a significant influence on survivorship, but the climate variables that had the most influence often changed from year to year. For instance, in the FI garden in June 2014 the climate variable that influenced survivorship the most was maximum temperature in October at the ecotype's origin, and then in the next year it was maximum January temperature that had the strongest influence on survivorship. In the final year, August and November precipitation at the ecotype's origin had the strongest effect on survivorship of

all climate variables. The changing climate signal through time may be due to the variable climate in the garden from year to year, but the size advantage remained strong out to two years after transplanting. The climate signal varied in the JT garden as well (Tables 1.6-1.8), but less so for the UT garden where the size effect remained very strong throughout the experiment. In the UT garden, ecotypes from regions with increased April and May rain had a negative effect on survivorship. I originally hypothesized that the effect of initial transplant size on survivorship would weaken, and that growth and survivorship would be increasingly governed by the local garden climate and favor populations from similar climates. However, this hypothesis was not confirmed: Initial canopy volume remained a strong predictor, and in two gardens the strongest predictor of 2-year survivorship.

Restoration Implications

For researchers conducting similar common garden experiments, it is important to keep in mind that the greenhouse acts as a selecting environment and has the potential to bias survivorship results up to two years, and possibly longer. The best way to avoid this bias is to sow seeds directly into the gardens and bypass the greenhouse stage. If this is not a possibility, as it may be the case with desert species, running field germination and seedling development trials in situ will provide the data to correct for the greenhouse bias. When using ecotypes that were raised in a greenhouse environment before being moved to the field, the best option for removing the greenhouse bias may be to use the initial size as a covariate in all analyses.

For restoration practice, we should also think about restoration goals. Is a species or community being restored for the short-term success, perhaps to discourage invasion

of exotic species? In this case, it may not pay off to be too picky about seed sources, but just transplant the largest plants possible in the available period of time, simply because larger plants have higher survivorship. However, for restoration at the harshest end of a species' distribution, like our JT garden, using plants from populations that are from similarly extreme climates may be more important because plants continue to die off years after transplanting, more likely due to a lack of drought tolerance than size deficiency.

On the other hand, if a community is restored for the indefinite future, it is more important to consider species' recruitment success under natural conditions. This approach may require different kinds of multiple common garden experiments where seeds are germinated in situ.

II. THE INFLUENCE OF CLIMATE OF ORIGIN ON THE FITNESS OF MULTIPLE *AMBROSIA DUMOSA* AND *LARREA TRIDENTATA* ECOTYPES IN A MULTIPLE COMMON GARDEN EXPERIMENT

Abstract

Restoration of arid landscapes can often be challenging in part due to the extreme climate, but also due to the scarcity of plant materials that are locally adapted to the restoration site. Selecting plant materials from areas that are geographically or environmentally distant from the restoration site can introduce maladapted ecotypes, potentially resulting in restoration failure and outbreeding depression. I used a multiple common garden experiment with two species of desert shrubs (*Ambrosia dumosa* (white bursage) and *Larrea tridentate* (creosotebush)) that are commonly included in restoration seed mixes for the Mojave Desert to determine if ecotypes differed in performance traits, functional traits, and if climate of origin was responsible for the observed variation. Although population × garden interactions were not significant for survival or growth for either species, climate of origin was found to have a significant effect on growth and survivorship of *A. dumosa* and on growth of *L. tridentata*. Functional trait values that were associated with increased survivorship in dry conditions, such as low specific leaf area and high predawn water potentials, were observed in ecotypes originating in dry areas across all gardens.

Introduction

Among the preeminent ecological challenges of our time is the increasing frequency of large-scale disturbances, either natural or anthropogenic, which often render ecosystems more vulnerable to degradation by erosion or the invasion of exotic species. Recovery times can be especially long in low-productivity systems, such as deserts, where plant recruitment is sporadic and growth rates are slow (Horn 2015, McAuliffe

1988). The long time required for natural recovery can be significantly shortened through the facilitated establishment of native species (Bainbridge 2007, Lovich and Bainbridge 1999). However, obtaining sufficient quantities of local plant materials to re-vegetate large areas can be quite challenging because arid landscapes usually produce a limited amount of seed with high variability between years due to climatic variability and other resource limitations (Beatley 1974). It has been standard practice to collect plant materials for restoration wherever they are available in, or using cultivars readily available outside of, the species' range, but there is a danger that transplants originating in some other climate or edaphic zone may be maladapted to local conditions and not capable of producing viable populations over the long term (Broadhurst et al. 2008, Rice and Knapp 2008). Therefore, a major aim of restoration research has been to better understand the relationships between species trait variation and fitness in a given environment so that collection ranges can be broadened without jeopardizing the suitability of the materials collected.

The main tool for linking climate to trait suitability has been the establishment of multiple common gardens, in which plants collected across the entire species range are grown side by side for several years in gardens distributed across the same range. With this experimental design, trait values and their variation can be immediately linked to growth and survivorship under the climatic constraints of a specific garden as well as to climate norms in the area where plant materials were collected. If successful, the multiple common garden approach can pinpoint which 'at home' climate variables have influenced local trait evolution, and how locally evolved traits in turn influence the month-by-month growth and survivorship of transplants 'away from home'. In this way,

seed transfer zones can be mapped based on climate drivers proven to be linked to growth and survivorship at and away from home. Furthermore, the approach may yield improved understanding of trait functionality across climate gradients that may someday serve to develop predictive models linking traits, climate, and ecotype fitness.

Predicting the value of traits that promote growth and survival under specific conditions is the ultimate goal for restoration ecology, as climate change may produce novel combinations of climate and edaphic conditions that are not necessarily reproduced in common gardens. For example, the climate for the southwest United States is projected to become drier and hotter with an increase in the frequency of heavy precipitation events over the next decades (Bachelet et al. 2016, Stocker et al. 2013). Individuals that have a high level of fitness under the current climate at a restoration site must also have the ability to survive the increasingly harsher climatic conditions of the future (Jones 2013). Failure to use source materials that can tolerate the future climate may lead to wasted effort because of maladapted ecotypes with decreased survival, decreased size, and decreased reproduction resulting in a loss of genetic diversity. On the other hand, a deeper understanding of key traits capable of increasing survivorship under harsh conditions and/or better suited to exploit unusually wet conditions, may give adaptive evolution a head start by increasing the frequency of potentially desirable traits and genes, and genetic diversity in general.

Relevant traits

Traits are "measurable morphological, physiological, or phenological features of an individual organism" (Violle et al. 2007). "Performance traits", or those that are used to assess the three components of plant fitness – growth, reproduction, and survival – are

described using measures of biomass, reproductive output, and survivorship, but are ultimately influenced by a wider set of "functional traits" closely related to resource uptake, use, and storage (leaf, stem, and root characteristics) (Violle et al. 2007). Functional traits are ultimately controlled by an individual's genotype, or genetic makeup, but a certain degree of plasticity exists in the expression of these traits that is influenced by climate and soils (Bradshaw 1965). In desert environments, low water availability and high summer and low winter temperatures are arguably the most significant abiotic factors limiting plant fitness (Comstock and Ehleringer 1992), suggesting that functional trait variation should express a range of strategies for coping with drought and temperature stresses.

In terms of strategies for dealing with water limitations, desert species fall along a spectrum from drought tolerance to drought avoidance (Smith et al. 1997). Tolerators display traits that allow them to assimilate carbon under water-limited conditions and survive long periods of dry conditions, either by being able to transport water and maintain photosynthesis under low soil and plant water potentials (e.g., anisohydric plants; evergreen shrubs), by having greater stomatal control and closing stomata at a threshold water potential level restricting water loss (e.g., isohydric plants) (Franks et al. 2007), or by internal water storage combined with low transpiration (e.g., stem succulents). Avoiders limit their carbon assimilation, growth, and reproduction to the wettest times of year and then die (e.g. ephemeral annuals) or, if they are perennials, withdraw into a state of dormancy for the dry part of the year (e.g., drought deciduous shrubs).

It is sometimes difficult to associate specific traits with one strategy or another. For example, deep-rootedness could be a strategy of avoidance or tolerance, depending on the degree of hydrological coupling between shallow and deep soils. A shallow water table allows deep-rooted plants to avoid drought conditions (e.g., phraeatophytes) (Lynch 1995, Smith et al. 1997). When a water table is not within reach, deep-rootedness may no longer allow avoidance of drought but the trait can be associated with drought tolerance that supports the continuation of photosynthesis longer into drought periods, much like in the long-lived evergreen shrub *Larrea tridentata* that grows long roots (typically 1-3 m but recorded up to 5 m) that can reach unsaturated water sources deep in the soil allowing this species to retain leaf cover year-round (Schwinning 2009, Smith et al. 1997). Shorter-lived species like the drought-deciduous *Ambrosia dumosa* have shallow roots that take advantage of rainfall events but are unable to retain leaf cover during prolonged drought (Smith et al. 1997).

Because leaves are the site of carbon assimilation, several leaf traits are closely associated with strategies of water use. Drought tolerators tend to have lower photosynthetic capacities and smaller leaves with higher specific leaf area (SLA, area per unit dry mass), and low stomatal density that exhibit stomatal closure only at low plant water potentials. Drought avoiders have higher photosynthetic capacities, larger leaves with lower SLA, high stomatal density, and close stomata at much higher plant water potentials (Smith et al. 1997). Some, if not most, perennial vegetation in the desert may be classified as tolerators, but will use avoidance in the form of leaf shedding (drought deciduous species) or delayed leaf production during more extreme drought conditions (Chew and Chew 1965, Smith et al. 1997). During wet times of year when plant water

potentials are high, drought deciduous species tend to have higher overall rates of carbon gain compared to evergreen species (Lambers 2008). This strategy allows for fast growth and development leading to flowering and seed production prior to the onset of dry conditions. On the other hand, species that tolerate drought such as evergreen shrubs may compensate for the lower rate of carbon gain by being able to photosynthesize year round and, depending on rainfall, flower and set seed multiple times per year.

The strategies for dealing with high temperature often overlap with the strategies for dealing with drought because high temperature is in many ways an amplifier of water limitation (Kramer 1980). However, there are traits that are best understood as temperature adaptations. For instance, temperature has been shown to influence the size and shape of leaves, as reduced leaf size and/or increased lobing of leaf margins can reduce leaf temperature by promoting turbulent air flow (Reich 2014). This leaf trait may reduce the need for transpirational cooling when water is scarce so that plants can operate nearer to their photosynthetic temperature optima (Ehleringer and Mooney1978, Sandquist and Ehleringer 1997). A smaller leaf area inevitably results in less photosynthetic surface area and lower photosynthetic rates (μmol $CO_2 \cdot m^2 \cdot s$) per leaf compared to leaves with fewer lobes and larger surface area. However, by using less water for transpirational cooling, leaves with more lobes may have higher water use efficiency allowing for photosynthesis to continue into hotter parts of the day when leaves with fewer lobes would have stopped transpiring and photosynthesizing.

The amount of resources allotted for leaf area production may also be related to temperature; for instance, leaves of plants from cool, wet, high resource environments are generally large and thin, while leaves of those from hot, dry, and low resource

environments are small and thick. Similarly, leaf thickness, expressed as SLA, may differ among ecotypes of the same species growing in slightly wetter versus drier areas and correlate positively with relative growth rate of ecotypes (Westoby 1998).

Ultimately, whole-plant strategies of water use and temperature tolerance are conferred by combinations of traits, and especially synergistic trait combinations tend to be repeated across taxa. Reich (2014) proposed the existence of a universal "fast-slow" strategic continuum, in which being fast or slow at acquiring carbon at the leaf level means that the plant will be fast or slow acquiring all other resources (e.g. water, nutrients) across all organ levels (Reich 2014). According to Reich (2014), above- and below-ground traits, phenological, and reproductive traits should be highly correlated to each other, to produce one overall growth strategy optimized for the whole-plant level. Furthermore, a species adapted to slow growth has advantages only in a low resource environment, and if introduced to a high resource environment, will likely be outcompeted by fast growing species. Conversely, a species adapted to fast growth may be intolerant to a low resource environment and succumb to environmental extremes.

Many studies have been published on interspecific trait trade-offs but relatively few have focused on intraspecific trait differences, especially for desert plants. A study that focused on the desert shrub *Encelia farinosa,* related intraspecific variation of leaf pubescence to a temperature gradient in the Mojave Desert (Ehleringer 1982) that fits Reich's (2014) general hypothesis of differentiation along fast-slow spectrum. Ehleringer (1982) found that the degree of leaf pubescence in ecotypes varied with water stress and temperature in the ecotypes' home climate. In a common garden study, light absorption was reduced in proportion to the degree of leaf pubescence among ecotypes

(Sandquist and Ehleringer 1997). The ecotype from the wetter climate maintained significantly higher light absorption and higher rates of gas exchange than the dry climate ecotype, maintaining optimal leaf temperature through evaporative cooling. The ecotype from the drier environment maintained the same optimal leaf temperature, but through reduced light absorption and at the cost of reduced photosynthesis. Thus, the ecotype from the drier environment used a more “conservative” (slow) strategy of resource uptake, in this case, of light and water, presumably to maintain carbon assimilation over a longer period of time under water scarcity. The ecotype from the wetter climate employed a more “opportunistic” (fast) strategy, by achieving higher resource uptake rates under favorable conditions, but having less ability to maintain optimal leaf temperature under water scarcity.

Within a species one may also expect a degree of phenotypic plasticity that would skew trait expression towards 'fast' trait values under more benign conditions and 'slow' values under more stressful conditions. It may be the case that ecotypes vary in the degree of phenotypic plasticity, which may itself be regarded a trait. However, the environmental circumstances which render phenotypic plasticity of a trait more or less adaptive are not well-understood in general. The few studies that do address the question suggest that the adaptiveness of plasticity is related to the nature of environmental variability in the ecotype’s home environment (Spitze and Sadler 1996). In general, when current conditions are predictive of future conditions, plasticity may be advantageous, but if they are not, fixed, intermediate trait values may produce the best results.

Goal of the study

I conducted a multiple common garden experiment in the Mojave Desert, USA, with multiple ecotypes of two Mojave Desert shrub species, *Ambrosia dumosa* (white bursgae) and *Larrea tridentate* (creosotebush) The overall goal was to determine the relationships between performance traits, functional traits, and climate variables in the ecotypes' regions of origin.

The following specific hypotheses were tested:

1) *Ambrosia dumosa*, a long-lived drought-deciduous shrub with strong selection for local adaptation to the current climate over short time periods, will have significant differences in growth and survivorship between ecotypes and between gardens, and there will be garden × ecotype interactions.
2) *Larrea tridentata*, a long-lived evergreen shrub with selection for plasticity and averaging adaptations to a range of climate conditions over time, will have significant differences in growth and survivorship between gardens, but not between ecotypes suggesting that seed collected for this experiment are of a single ecotype.
3) Ecotypes of *A. dumosa* from hotter and drier climates will have higher survivorship in the driest garden. Ecotypes of *A. dumosa* from wetter climates will have faster growth in the most productive garden.
4) Ecotypes of *A. dumosa* will have trait values commensurate with the climate of origin, i.e., trait values indicating 'faster' resource uptake will

be associated with wetter and cooler climates and trait values indicating 'slower' resource uptake will be associated with drier, hotter climates.

Methods

Study species and seed sources

The Mojave Desert region, at low elevation, is co-dominated by *A. dumosa* (white bursage) and *L. tridentata* (creosotebush) (Shreve 1942). Both species are long-lived, woody perennial shrubs, but *L. tridentata* is an evergreen shrub that has a markedly longer lifespan than *A. dumosa*, which is a drought deciduous. By some estimates, individuals of *L. tridentata* may live for hundreds of years, even up to 1000 years, while *A. dumosa* shrubs typically live for a few decades (Prose and Metzger 1985, Vasek et al. 1975). These two shrubs cover up to 70% of the Mojave Desert (Shreve 1942), act as important shade plants for many of the desert's sensitive annuals and cacti, as well as wildlife species, and are common species included in restoration project seed mixes (Abella and Berry 2016).

Seeds of *A. dumosa* and *L. tridentata* were collected for the Seeds of Success program (Bureau of Land Management) from all over the Mojave Desert in 2010 or 2011, in late summer when seeds were beginning to naturally drop from plants. *Ambrosia dumosa* seeds were collected from 19 sites and *L. tridentata* seeds from 12 sites spanning an environmental gradient mainly dominated by precipitation differences (Table 2.1).

Transplant establishment

Seeds were kept in refrigerated storage (4°C) until germinated in a greenhouse (College of Southern Nevada, Henderson, NV, USA) between January and March 2013. Seeds of *A. dumosa* were submitted to a cold, wet stratification for 30 d prior to planting to break any form of dormancy. Seeds of *L. tridentata* were soaked in water for at least 12 h with the water changed every three hours (Baskin and Baskin 2001). Seeds were first germinated in 72 cell flats and upon the development of two true leaves, seedlings were transplanted into 7.62 × 7.62 × 30.48 cm plant bands (Monarch Manufacturing, Inc., Salida, CO, USA), which contained a growing mix of 3:1:1 parts of sand, organics (potting soil and wood mulch), and perlite, respectively. After one year in the greenhouse, in February 2014, all plants were moved out of the greenhouse into the open to harden off for one month prior to garden transplanting (March 2014).

The number of transplants per population varied due to variable available seed and differences in germination success, especially for *A. dumosa*. For *L. tridentata* transplants, the main limitation was damping off in the cotyledon stage. Ecotype transplants were randomly assigned and evenly divided among the three common gardens.

Common garden establishment

The three common gardens used in this experiment spanned the Mojave Desert environmental gradient of temperature and precipitation. The garden in St. George, Utah (UT), represented a cooler and wetter climate, the garden at Fort Irwin National Training Center Military Base, California (FI) represented an intermediate climate, and the garden outside of Joshua Tree National Park, California (JT) represented the hottest and driest

climate. The exact locations and climate norms of the three gardens are shown in (Table 2.1).

The UT garden was located on a sandy site previously occupied by a structure and cleared of vegetation for multiple decades. The FI garden was located on decomposed granite and had been cleared of vegetation for at least one decade. The JT garden was located on a former farm field and had also been cleared of vegetation for at least a decade. The UT garden was enclosed by a rodent and deer-proof fence; the FI and JT gardens were enclosed with only a rodent-proof fence as deer are uncommon at these sites. All three gardens were approximately 50 m × 50 m (2500 m^2). Data Garrison weather stations (Upward Innovations Inc., East Falmouth, MA, USA) were installed at each garden and logged air temperature, precipitation, and soil moisture at depths of 5 cm and 20 cm every half-hour since February 2014.

Plants of both species were randomly selected from the number of individuals available from each ecotype and split evenly among gardens. Ecotype NV052-275R of *A. dumosa* had few plants available at the time of transplanting, so plants from this ecotype were split between the two more climatically extreme gardens (JT and UT) and not planted at FI. In early to mid-March 2014, plants were transported to each garden and holes were dug approximately 30 cm deep to accommodate the root plug and spaced approximately one meter apart in a square lattice design. Water was added to each open hole prior to planting to enhance plant establishment. Transplants were watered immediately after transplanting from a portable sprinkler system with approximately 2000 gallons of water except in the JT garden where conditions were very dry and approximately 8000 gallons were sprayed over the entire planting area to enhance plant

establishment. Subsequently, gardens were watered monthly with approximately 1000 gallons from April to June 2014 for the UT garden, April to July 2014 for the FI garden, and April to August 2014 for the JT garden.

Climate variables

The climate variables used in the analyses were obtained from the PRISM Climate Group, Northwest Alliance for Computational Science & Engineering based at Oregon State University (www.prism.oregonstate.edu; accessed 05/15/2016). The database was queried to provide 30-year normals (1981-2010) for monthly and annual precipitation totals, average minimum (T_{min}) and maximum (T_{max}) temperature for each latitude and longitude where *A. dumosa* and *L. tridentata* seeds were collected as well as for each of the common gardens.

Transplant monitoring

Performance traits. Survivorship was monitored monthly during the first three months in the field, from April to June 2014, and then monthly from February 2015 to March 2017. Survivorship status of each plant was recorded as "dead" (no leaves present and non-flexible stems), "dormant" (brown leaves attached and no green leaves), or "alive" (live green leaves present).

Growth was monitored using two methods. More commonly, the canopy volume was estimated from measurements of two diameters (greatest width of live canopy and length of perpendicular axis) and height (from ground to highest green stem) using the formula for an inverted cone (Ludwig et al. 1975):

$$V = \left(\frac{4}{3}\right)\pi\left(\frac{H}{2}\right)\left[\left(\frac{D1+D2}{2}\right)/2\right]^2 \qquad \text{eq 1}$$

Canopy measurements were taken at least once per year from 2014 to 2016 in spring when plants were actively growing and only on plants that are designated as alive. Additionally, basal stem diameters were measured on live plants with calipers once per year in March from 2015 to 2017.

Functional traits. Due to time and personnel constraints, we were unable to take trait measurements on all transplants. Instead, we focused the trait data collection on ecotypes that a) were representative of the entire environmental gradient at the seed collection sites, b) captured at least six ecotypes from different climates, and c) at the time of sampling had at least six living plants in all three gardens. Different populations were sampled from year to year as mortality reduced the number of individuals below six for some populations.

Leaf gas exchange was measured with a LI-6400 Portable Photosynthesis System (LI-COR, Inc., Lincoln, NE USA). In March and June 2016, gas exchange parameters including photosynthetic rate (A), leaf conductance (g_s) and intercellular CO_2 concentration (c_i) were measured at ambient conditions of light intensity, atmospheric CO_2 concentration, air temperature and humidity. In March 2016, maximal photosynthetic rate (A_{max}) was estimated by increasing chamber CO_2 concentration to 1000 µmol CO_2 mol^{-1} and photosynthetically active radiation (PAR) to 1800 µE m^{-2} s^{-1}, while raising humidity to 80% to discourage stomatal closing. All gas exchange measurements were collected between 0830 h and 1400 h, avoiding the hottest time of the day. In March 2017, photosystem II efficiency (φPSII) was measured in addition to

gas exchange at ambient conditions, using the LI-COR leaf chamber fluorometer. Leaves were wrapped in aluminum foil for at least 30 minutes prior to assessment to allow leaves to dark adapt. Leaves were clamped in the LI-6400 chamber as foil was removed to limit exposure to light and allowed to stabilize in the dark prior to measurement.

Plant water potentials were measured with a Model 1505D Pressure Chamber (PMS Instrument Company, Albany, OR USA) at predawn (0300 h to 0500 h) and midday (1400 h to 1600 h) on the same individuals and at the same time that gas exchange measurements were taken.

Leaf samples for the measurement of stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) were collected once in March 2016 from all live plants. Several of the youngest, fully mature leaves were collected from each canopy into coin envelopes, dried in an oven at 60°C for at least 72 hours. In preparation for analysis, leaves were crushed into a fine powder with a mortar and pestle using small amounts of liquid nitrogen to facilitate the break-up of cell walls. Weighed aliquots were transferred into tin foil sample cups and submitted for analysis at the University of Wyoming Stable Isotopes Facility (Laramie, WY USA). Carbon isotope discrimination (Δ), for the estimation of time-integrated water use efficiency (WUE), was calculated by subtracting 8‰ (the $\delta^{13}C$ of atmospheric carbon) from the $\delta^{13}C$ of leaf carbon (Moghaddam et al. 2013).

Stem samples for the measurement of hydrogen and oxygen stable isotope ratios (δD and $\delta^{18}O$) were collected once in June 2016 following established methods for stem water stable isotope analysis (West et al. 2006). This collection was limited to the plants on which June 2016 gas exchange measurements were collected; however, no samples were obtained from JT garden due to very small plant sizes. Stem samples were stored in

40 mL vials, capped and sealed with Parafilm "M"® (Bemis NA). Then they were stored inside a cooler until they could be transferred to a freezer. Water was extracted by cryogenic vacuum distillation as described in West et al. (2006). Hydrogen and oxygen isotope ratios were determined at the University of Wyoming Stable Isotopes Facility (Laramie, WY USA). Isotope ratios were collected to infer qualitative differences of root distribution (Flanagan and Ehleringer 1991). Without the isotope ratios of soil water for comparison, we assumed that stem water samples that were relatively more depleted in heavy isotopes indicated water uptake from deeper soil layers, as Mojave Desert soils typically develop vertical gradients of isotope ratios with evaporatively enriched, heavier water at the top and lighter water, reflecting the isotope ratio of winter precipitation, deeper down (Goebel et al. 2016).

Specific leaf area (SLA) was determined by collecting multiple leaves from the same pre-selected subset of plants that were measured for gas exchange in March 2016. In the field, just after sampling, permanent images of the leaf samples were cast on blueprint paper. Leaves were held in place on top of blueprint paper by a clear plastic sheet and exposed to sunlight. The paper was then exposed in the dark to ammonia vapor created by shaking a closed container of ammonia and then removing the lid and exposing the blueprint paper to the air just above the ammonia liquid. Leaves and leaf images were placed together into labeled coin envelopes for later processing. Leaf images were later scanned using WinFOLIA software (Regent Instruments Inc. Canada) to obtain leaf areas. Leaf samples were then dried for at least 72 h in a 70ºC drying oven to a constant weight. SLA was calculated by dividing leaf area by the final dry mass.

Data Analysis

Kaplan-Meier survivorship analyses were conducted at the species level to compare overall survivorship curves across gardens. Generalized linear models (GLM, binomial regression) were used to analyze survivorship by garden and ecotype in a fully factorial analysis. This analysis also included the April 2014 canopy volume as a covariate, since prior analysis indicated a strong effect of transplant size on subsequent survivorship that was not necessarily related to in-situ growth rate, but to growth rate under greenhouse conditions (Custer at al., in preparation). Thus, by accounting for this initial size effect on survivorship, I isolated effects that were imposed only by garden conditions.

Analysis of covariance (ANCOVA) was used to analyze growth in terms of increases in stem diameter, with garden, ecotype, and their interaction as factors. In this analysis, April 2014 canopy volumes were also used as covariate. Thus, we examined differences in growth rate while plants experienced ambient garden conditions.

A principal component analysis (PCA) was conducted using all monthly and annual average T_{min}, T_{max}, and precipitation variables at the ecotype's origins to determine the climate variables that account for the most variation in ecotypes. These included February precipitation, October precipitation, January minimum temperature (T_{min}), and July maximum temperature (T_{max}). Principal component results are available in the Supplemental Data.

A GLM was analyzed for each species to examine the influence of the climate factors from the PCA on survivorship and growth in each garden. Survivorship for these analyses was monitored during the entire experimental period, and March 2017 stem

diameters were used as the growth variable. The climate of each garden was very different, therefore the analyses regarding climate variables at the ecotype's origins were conducted by garden.

A bivariate correlation matrix was developed using all functional traits measured during the experiment and four climate variables that encapsulate most of the precipitation and temperature variation determined from the PCA. The correlation matrix revealed correlations between climate variables significant to survivorship and growth and functional traits that could then be analyzed using a GLM to detect the direction of the significant correlations from the correlation matrix.

SPSS was used for all analyses except for the PCA analyses, in which R Statistics was used (R Core Team 2015).

Results

Garden and ecotype effects on survivorship and growth

The Kaplan-Meier survivorship curves show large separation in cumulative survivorship between the three gardens from March 2014 to March 2017 for *A. dumosa* (Figs 2.1A) and *L. tridentata* (Fig. 2.1B). By March 2017, *A. dumosa* plants had the highest survivorship (86%) in the FI garden, followed by the UT garden (71%) and then the JT garden (23%) (Fig. 2.1A). Survivorship for *L. tridentata* was overall higher with 87%, 71% and 48% in the FI, UT and JT gardens, respectively (Fig. 2.1B). High rates of mortality occurred during early establishment when *A. dumosa* were still irrigated in the JT garden (March to June 2014), then another high mortality event occurred prior to the March 2015 census, predominantly in the UT and JT gardens for both *A. dumosa* and *L. tridentata*.

Survivorship was significantly different between gardens and ecotypes for *A. dumosa,* but only garden had a significant influence on *L. tridentata* survivorship (Table 2.2). Interactions between garden and ecotype were not significant for either species. The initial field canopy size following transplantation was a significant covariate for *A. dumosa* but not for *L. tridentata* (Table 2.2).

Growth in the three gardens from the first size assessment in April 2014 to the last size assessment in March 2017 is best illustrated using canopy volumes. For *A. dumosa,* in the first month after transplanting, canopy volumes were largest in the JT garden but in the subsequent months the size of plants in the FI garden soon became largest. At the size assessment in March 2015, FI still had the largest plant sizes followed by UT and then JT, but in March 2016 the UT plants became largest and remained largest until the end of the study period followed by the FI and then JT garden plants (Fig. 2.2A). Growth among the three gardens was somewhat slow during the first two years of the experiment and then in the last year of growth individuals became much larger in the UT and FI gardens with the average size of UT plants overtaking the FI plants. The JT garden also started to gain volume at the same time point but at a much lower rate. For *L. tridentata,* plants during the initial assessment were very close in size and then the FI garden plants became largest followed by the UT garden plants and then the JT garden plants. This pattern remained intact throughout the study period (Fig. 2.2B).

There were significant differences in *A. dumosa* 2017 stem diameters between gardens and ecotypes but no garden × ecotype interaction (Table 2.3). The first field canopy measurement, which was used as a covariate in this analysis, significantly

influenced 2017 stem diameters in the *A. dumosa* analysis (Table 2.3). For *L. tridentata*, there were significant differences in 2017 stem diameters between gardens, but not between ecotypes or a garden × ecotype interaction (Table 2.3). The first field canopy measurement for *L. tridentata* ecotypes had a significant positive correlation with 2017 stem diameters (Table 2.3).

Does climate of origin explain ecotype performance differences?

At the environmentally mild FI garden, where overall survivorship was greatest, October precipitation at the ecotype's home site negatively influenced survivorship while July maximum temperature had a positive effect on *A. dumosa* survivorship (Table 2.4). Stem diameters measured in 2017 were negatively influenced by October precipitation and July maximum temperatures at the ecotype's origin (Table 2.5). Additionally, the initial size covariate had a significant positive influence in both analyses (Tables 2.4 and 2.5).

In the hot and dry JT garden, where survivorship was lowest, October precipitation had a significantly negative effect on survivorship (Table 2.4) and February precipitation negatively influenced stem diameters of *A. dumosa* (Table 2.5). Initial size had a significant positive effect on both survivorship and stem diameter in these analyses (Tables 2.4 and 2.5).

In the winter-cold UT garden, where survivorship was intermediate, the climate variables analyzed did not influence survivorship, however, initial size had a positive effect (Table 2.4). February precipitation and July maximum temperature at ecotype's origin had a significantly negative influence on stem diameters while January minimum temperature had a positive influence (Table 2.5).

Therefore, in the FI garden, warmer and/or drier conditions in *A. dumosa* ecotype's home sites significantly improved survivorship, but cooler and drier conditions increased stem diameter growth. Drier conditions at ecotype's origin in the early part of the year improved growth rates in the JT garden and drier conditions in October increased survivorship. Survivorship in *A. dumosa* does not appear to be influenced by the climate where ecotypes came from, but stem growth was increased in ecotypes that came from areas with drier and warmer winter conditions and cooler summers in the UT garden.

Though no significant ecotype differences were detected for *L. tridentata* in the previous analyses, analyses by climate of origin showed that higher January minimum temperature at the ecotypes' origin increased stem growth in the FI garden, but no other climate effects were significant (Table 2.5). Thus, differently from *A. dumosa*, for *L. tridentata*, warmer winter conditions at the home site improved stem growth under the mild conditions of FI garden.

Climate-functional trait relationships

In *A. dumosa*, a mix of temperature and precipitation variables correlated with functional traits. In FI garden, the June 2016 c_i values were positively correlated with February precipitation and negatively correlated with October precipitation and January minimum temperatures at the ecotype's home sites (Table 2.6). Conditions while collecting c_i values were sunny and windy with temperatures in the morning near 30-35° C increasing to over 40° C by early afternoon. The March 2017 φPSII values had a negative correlation with October precipitation at ecotype's origin (Table 2.6). The stem water collected from plants in the FI garden had significantly higher $\delta\ ^{18}O$ values in ecotypes

that originated in areas with increased average February precipitation while leaf N content and SLA negatively correlated with January minimum temperatures and July maximum temperatures respectively (Table 2.6).

In the hotter and drier JT garden, ecotypes that came from areas with higher average February precipitation had significantly higher predawn WP values that were collected in March 2015 (Table 2.6). This was not the case with predawn WP collected one-year later which had a positive correlation with January minimum temperatures and a negative correlation with July maximum temperatures at ecotype's origin (Table 2.6). All individuals were not measured in both years. The $\delta\ ^{15}N$ of leaves collected in the JT garden had positive correlations with February and October precipitation, but a negative correlation with January minimum temperatures at ecotype's origin (Table 2.6).

In the UT garden, March 2015 predawn WP values had a positive correlation with January T_{min} and March 2016 predawn WP values had a positive correlation with October precipitation (Table 2.6). The δD of stem water each had a negative correlation with July T_{max} and SLA had a negative correlation with February precipitation and a positive correlation with October precipitation at the ecotypes' home sites (Table 2.6).

In *L. tridentata* precipitation and temperature variables also correlated with numerous functional traits (Table 2.7). In the FI garden, June 2016 predawn WP had a negative marginally significant correlation with February precipitation and $\Delta\ ^{13}C$ had a negative correlation with January T_{min} at ecotype's origin (Table 2.7).

In the JT garden, predawn WP collected in 2015 and 2016 correlated with climate variables. In March 2015 and June 2016 the correlation was negatively with October precipitation (Table 2.7). March 2017 A_{net} values, g_s values, and φPSII were negatively

correlated with February precipitation (Table 2.7). The $\delta^{15}N$ content of leaves and SLA were positively correlated with February precipitation and SLA was also positively correlated with July T_{max} and negatively correlated with October precipitation and January T_{min} (Table 2.7).

In the UT garden, March 2015, March 2017 predawn WP, and φPSII were negatively correlated with October precipitation and φPSII was also positively correlated with July T_{max} at ecotype's home sites (Table 2.7). The SLA values at the UT garden were negatively correlated with July T_{max} (Table 2.7).

Discussion

The premise of local ecotype adaptation is that there are trait tradeoffs that prevent ecotypes to be equally well-adapted across a climatic gradient, and that ecotypes therefore perform best under environmental conditions most similar to the home environment and worse under less similar conditions. In multiple garden experiments, this pattern should be reflected in garden × ecotype interactions.

In this experiment, survivorship and growth of *A. dumosa* were different between the three common gardens and also between ecotypes, but contrary to expectation, an interaction between garden and ecotype was not significant for either of these performance traits. Survivorship and growth of *L. tridentata* were also different between the three gardens, but there were no differences between the ecotypes or in the garden × ecotype interaction. I anticipated this result for *L. tridentata* due to its difference in life history, which is more likely associated with a generalist strategy.

Both species benefited from larger initial size, *A. dumosa* in terms of both survivorship and growth rate, and *L. tridentata* in terms of growth rate, supporting our

earlier cautionary note (Custer et al, in preparation) that the fitness of transplants in common garden experiments should be evaluated by discounting size effects that may well reflect ecotype responses to a common, benign greenhouse environment.

Nevertheless, when we asked what distinguished the home climate of more or less successful *A. dumosa* ecotypes, we did see that different factors were selected in different gardens. Ecotypes from drier regions (less October precipitation) had greater survivorship in the hottest and driest garden (JT), as well as in the moderate FI garden. Much of the Mojave Desert is a winter-wet desert and strong October precipitation is often the harbinger of a wet winter. The analysis thus suggests that a major cause of death in the FI and JT gardens was drought, favoring ecotypes that were adapted to drier environments.

In the wetter and cooler UT garden, survival was not significantly influenced by the ecotype's home climate variables, but ecotypes from regions with less precipitation in February, warmer winters or cooler summers had higher growth rates. This suggests that the most successful ecotypes in the UT garden were adapted to grow with less water in warmer winters or at lower summer temperatures.

My initial prediction that ecotypes from wetter regions should grow faster in the more productive garden was however not supported. In both the UT and FI gardens, ecotypes from drier regions grew faster.

Climate effects on the survivorship of *L. tridentata* performance traits were generally not significant, further supporting the lack of local adaptation in this species. In fact, the only significant climate effect was a positive effect of January temperature on stem diameter in the FI garden.

I further asked if and how functional traits correlate with climate at the ecotype's home sites. Specifically, we expected traits associated with 'faster' resource uptake to be associated with wetter and cooler climates and traits indicating 'slower' resource uptake to be associated with drier, hotter climate. SLA is a trait commonly positively correlated with growth potential (Lambers and Poorter 1992). Here we found that SLA was positively associated with October precipitation in the JT and UT gardens, suggesting that strong winter rainfall favors ecotypes with leaf traits for fast growth, but that this is simultaneously disadvantage under drier conditions because ecotypes from regions with more October precipitation had lower survivorship in JT garden (Fig. 2.3). However, SLA was negatively associated with February precipitation in the UT garden, suggesting that smaller/thicker leaves may be advantageous when the growing season is cooler, even though this also slows growth (Table 2.4). Additionally, in the FI garden, SLA was negatively correlated with July T_{max}, indicating that high peak temperatures in summer also increase leaf thickness. These ecotypes also had higher survivorship in the FI garden. Overall, the patterns associated with SLA in *A. dumosa* support the hypothesis that ecotypes from more stressful environments, whether due to lack of water or extreme temperatures, have thicker leaves, survivorship advantages and/or reduced growth rates under favorable conditions.

In the intermediate climate FI garden, October precipitation at the ecotype's origin negatively influenced both survivorship and growth. We observed a significant negative correlation between June 2016 c_i levels and October precipitation as well. The conditions in the garden during collection of gas exchange measurements in June 2016 were hot and dry. Ecotypes that were adapted to hotter and drier conditions may have

more control over stomata than ecotypes from wetter regions: under the hot dry conditions, these ecotypes reduce stomatal conductance occurred by closing stomata more quickly when conditions become dry and results in decreased c_i values. The same pattern is also present with the c_i values and January T_{min}, with ecotypes that originated in regions with warmer January temperatures having reduced c_i values. Adaptation to hot and dry climates result in quicker closing of stomata in hot conditions and lower c_i values. Specific leaf area in the FI garden was negatively correlated with July T_{max} such that ecotypes from areas with higher July temperatures had smaller, thicker leaves. Ecotypes from areas with higher July T_{max} also had higher survivorship in the FI garden suggesting that the smaller leaf trait was advantageous in this garden.

In terms of leaf gas exchange parameters, faster growth potential is generally associated with higher capacity of photosynthesis, through higher g_s, A_{max} and leaf N content when conditions are favorable, but a faster decline in gas exchange, often associated with an increase in c_i and loss of PS II efficiency as conditions become more stressful. The conditions for gas exchange were relatively poor during the June 2016 measurement at FI garden (air temperature = 29-36º C, predawn WP = -2.5 MPa, on average). At that time, we observed a significant negative correlation between c_i levels and October precipitation or January T_{min} in *A. dumosa*, suggesting that ecotypes from wetter, warmer regions were less photosynthetically stressed. PSII efficiency measured in March 2017 was also higher for ecotypes from wetter regions in FI garden.

March predawn WP values tended to be positively correlated with February precipitation or January T_{min}, which may be indicative of a difference in root development among ecotypes. In UT garden, ecotypes from regions with lower summer

temperatures had more enriched stem water δD values and in FI garden, ecotypes from regions with higher February precipitation had more enriched $\delta^{18}O$ values. Both patterns may indicate that higher water availability in the surface soil favors a shallower root system.

Overall, correlations between traits and home climate in *A. dumosa*, though not always readily interpretable, suggest a strong tendency for multiple trait adaptations, sensitive to seasonal precipitation patterns and extreme temperatures.

In *L. tridentata*, the strongest apparent interactions between traits and home climate were expressed in the driest garden. Ecotypes from regions with higher October precipitation and higher winter temperature had lower SLA and lower predawn WP in March. Ecotypes from regions with hotter summers had higher SLA values. Gas exchange parameters were more sensitive to February precipitation, indicating greater photosynthetic stress in ecotypes from wetter regions. Overall, these patterns indicate a greater growth potential for plants from wetter regions with more moderate summer and winter temperatures.

In FI garden, $\Delta\ ^{13}C$ values were negatively correlated with January T_{min} values, suggesting an increased water use efficiency in ecotypes from regions with warmer winters. These ecotypes may be adapted to growing in winter, provided the winters are not too cold. Overall, the data suggest that *L. tridentata* does have a degree of local trait variation, but that the effects of these on growth and survivorship are largely negligible or at least too subtle to have been picked up by the sample size available for this experiment.

Restoration implications

A major result of the study is that some species are more variable in their responses to garden climate across the range than other species. For a long-lived, evergreen shrub such as *L. tridentata*, ecotype variation, while not entirely absent, may not have profound effects on the growth and survivorship of transplants across climate gradients. But this may be different for shorter-lived, drought deciduous species such as *A. dumosa*. Even though we could not find direct support that the best-adapted ecotypes are different for different climate regions, numerous interactions between home-climate and functional traits suggest that they exist.

In part, the apparent dissonance between performance traits across climate gradients and climate of origin lies in the fact that only a small fraction of the species' life cycle was examined. It is not surprising therefore, that ecotypes from drier environments had higher survivorship almost everywhere. This is a fundamental weakness of common garden experiments that neither assess establishment success from seed, nor last long enough to evaluate seed productivity. On the other hand, where restoration goals are focused on a relatively short time frame, transplant survivorship may be the primary goal and it would not be a mistake to select the most stress tolerant ecotypes for this purpose.

When using *A. dumosa* as a restoration species and the restoration site is in the range of wet/cold to intermediate, then using climate variables such as October and February precipitation norms and choosing ecotypes from drier regions will increase survivorship and growth. When the restoration site is an extreme hot/dry site, like the JT garden, then it may be more appropriate to select from a similar ecotype in terms of precipitation and temperature. When using *L. tridentata* as a restoration species, it seems

that ecotype is not as important, and seeds may be collected from any local locations to the restoration site within in ecoregion. The slow growth of *L. tridentata* also means that resources are taken up slowly probably giving this species an advantage in many different environments. Faster growth, as with *A. dumosa*, requires rapid uptake of resources, and if resources are not available then survivorship and growth are affected.

Soil types were not taken into account during this experiment but could have strong influences on survivorship and growth within the gardens. The soils across gardens were quite different and soil characteristics between ecotype origins may have been different. Soil characteristics such as texture and sand or clay content can influence how water moves through soil (Hillel 1982) and its availability to plants (Kramer 1983). Matching soil types from collection sites to restoration sites would remove the effect of soil on plant fitness.

Tables

Table 1.1. Origin locations and the climate of origin of *Ambrosia dumosa* populations. Precipitation (PPT) is annual sum of 30-year average (1981-2010), summer maximum temperature (T_{max}) is mean of 30-year average (1981-2010) (June-August), and winter minimum temperature (T_{min}) is the mean of 30-year average (1981-2010) (December-February).

	Origin Location		Climate of Origin		
Population ID	Latitude	Longitude	Annual ppt (mm)	Summer Tmax (˚C)	Winter Tmin (˚C)
AZ010-03	36.2986	-113.994	139.5	40.8	2.2
AZ010-10	36.8275	-113.954	191.7	39.3	1.1
AZ010-22	36.9692	-113.937	199.3	38.9	0.8
CA650-26	35.5706	-117.432	96.0	38.3	1.2
CA930A-44	34.4975	-116.664	162.3	35.6	0.8
CA930A-45	34.7878	-116.377	121.8	38.5	2.0
CA930A-47	35.4296	-117.587	160.4	35.1	4.0
CA930A-58	34.2719	-116.465	172.5	33.8	1.2
MSB15-1245	34.0267	-116.512	227.8	37.4	4.9
NV040-17	35.0971	-114.683	161.7	40.5	5.8
NV040-25	35.4858	-114.808	156.7	38.1	4.5
NV040-33	35.4334	-114.985	176.7	35.4	1.7
NV040-42	35.9749	-115.842	135.0	37.1	0.2
NV040-47	36.7916	-116.623	128.5	36.8	-0.4
NV040-48	36.6937	-114.440	145.5	38.9	1.6
NV040-65	36.9856	-114.924	150.9	37.8	-0.4
NV052-275R	36.5223	-114.163	151.0	37.7	3.5
NV052-343	35.5711	-114.849	190.9	34.9	2.3
NV052-345	36.5229	-114.141	160.3	37.0	3.4
Fort Irwin	35.219	-116.807	142.9	35.9	3.2
Joshua Tree	34.226	-116.108	90.5	39.9	2.8
Utah	37.199	-113.565	304.2	36.3	0.2

Table 1.2. Climate variables that influence stem diameter during early seedling development inside the greenhouse and prior to transplanting into the gardens.

	May 2013 Stem Diameter			
Climate Variables	Estimate	Df	t-value	*p*-value
Precipitation June	0.063	1	3.168	0.002
Minimum Temperature Dec.	0.029	1	2.031	0.043

Table 1.3. Climate variables that influence canopy volume during seedling development shortly after transplanting into the common gardens.

		April 2014 Canopy Volume			
Garden	Climate Variables	Estimate	df	t-value	*p*-value
Utah	Precipitation June	0.242	1	6.047	<0.001
	Max Temperature July	-0.091	1	-3.196	0.002
Fort Irwin	Precipitation May	0.174	1	3.829	<0.001
Joshua Tree	Precipitation June	0.153	1	3.775	<0.001
	Precipitation July	0.037	1	3.107	0.002

Table 1.4. First year survivorship differences in gardens, populations, and garden × population interactions for plants in the gardens during April 2014 and June 2014. Canopy volume was calculated from the dimensions collected in April 2014.

	April 2014 Survivorship			June 2014 Survivorship		
	Wald χ^2	Df	*p*-value	Wald χ^2	df	*p*-value
Garden	9.688	2	0.008	66.994	2	< 0.001
Ecotype	45.117	18	< 0.001	59.387	18	< 0.001
Garden × Ecotype	27.742	55	0.999	103.181	55	< 0.001
Canopy Volume	NA	NA	NA	3.915	1	0.048

Table 1.5. Second and third year survivorship differences in gardens, ecotypes, and garden × ecotype interactions for plants in the gardens during June 2015 and June 2016. Canopy volume was calculated from the dimensions collected in April 2014.

	June 2015 Survivorship			June 2016 Survivorship		
	Wald χ^2	Df	*p*-value	Wald χ^2	df	*p*-value
Garden	66.053	2	< 0.001	70.326	2	< 0.001
Ecotype	36.692	18	0.006	16.398	18	0.565
Garden × Ecotype	58.851	55	0.336	30.816	55	0.997
Canopy Volume	4.416	1	0.036	3.915	1	0.045

Table 1.6. Effects of April 2014 canopy volume and ecotype specific climate variables on 3-mo survivorship of plants in each garden measured in June 2014.

		June 2014 Survivorship			
Garden		Estimate	St. Error	z-value	*p*-value
Utah	Canopy Volume	3.525	0.827	4.263	< 0.001
	Precipitation Apr.	-0.206	0.106	-1.941	0.052
Fort Irwin	Canopy Volume	9.471	2.687	3.525	< 0.001
	Max Temperature Oct.	0.556	0.236	2.359	0.018
Joshua Tree	Precipitation Aug.	-0.090	0.038	-2.342	0.019
	Min Temperature Feb.	0.276	0.086	3.198	0.001

Table 1.7. Effects of April 2014 canopy volume and ecotype specific climate variables on 1-yr survivorship of plants in each garden in June 2015.

		June 2015 Survivorship			
Garden		Estimate	St. Error	z-value	p-value
Utah	Canopy Volume	0.791	0.194	4.075	< 0.001
	Precipitation May	-0.272	0.108	-2.524	0.012
Fort Irwin	Canopy Volume	2.397	0.677	3.540	< 0.001
	Max Temperature Jan.	0.527	0.206	2.558	0.011
Joshua Tree	Canopy Volume	0.207	0.117	1.766	0.077
	Precipitation Nov.	-0.178	0.053	-3.337	< 0.001
	Max Temperature Nov.	0.165	0.087	1.903	0.057

Table 1.8. Effects of April 2014 canopy volume and ecotype specific climate variables on 2-yr survivorship of plants in each garden measured in June 2016.

		June 2016 Survivorship			
Garden		Estimate	St. Error	z-value	p-value
Utah	Canopy Volume	0.779	0.189	4.117	< 0.001
	Precipitation May	-0.234	0.106	-2.206	0.027
Fort Irwin	Canopy Volume	1.786	0.530	3.369	< 0.001
	Precipitation Aug.	-0.156	0.073	-2.142	0.032
	Precipitation Nov.	-0.212	0.109	-1.945	0.052
Joshua Tree	Canopy Volume	0.264	0.114	2.308	0.020
	Precipitation Oct.	-0.140	0.034	-4.115	< 0.001
	Min Temperature Sep.	0.149	0.064	2.314	0.021

Table 2.1. Origin locations and the climate of origin of *Ambrosia dumosa* and *Larrea tridentata* ecotypes. Precipitation (ppt) is annual sum of 30-year average, summer maximum temperature (t_{max}) is mean of 30-year average (June-August), and winter minimum temperature (t_{min}) is the mean of 30-year average (December-February).

		Origin Location		Climate of Origin		
Species	Ecotype ID	Latitude	Longitude	Annual PPT (mm)	Summer T_{max} (˚C)	Winter T_{min} (˚C)
AMDU	AZ010-03	36.298	-113.994	139.5	40.8	2.2
	AZ010-10	36.827	-113.954	191.7	39.3	1.1
	AZ010-22	36.969	-113.937	199.3	38.9	0.8
	CA650-26	35.570	-117.432	96.0	38.3	1.2
	CA930A-44	34.497	-116.664	162.3	35.6	0.8
	CA930A-45	34.787	-116.377	121.8	38.5	2.0
	CA930A-47	35.429	-117.587	160.4	35.1	4.0
	CA930A-58	34.271	-116.465	172.5	33.8	1.2
	MSB15-1245	34.026	-116.512	227.8	37.4	4.9
	NV040-17	35.097	-114.683	161.7	40.5	5.8
	NV040-25	35.485	-114.808	156.7	38.1	4.5
	NV040-33	35.433	-114.985	176.7	35.4	1.7
	NV040-42	35.974	-115.842	135.0	37.1	0.2
	NV040-47	36.791	-116.623	128.5	36.8	-0.4
	NV040-48	36.693	-114.440	145.5	38.9	1.6
	NV040-65	36.985	-114.924	150.9	37.8	-0.4
	NV052-275R	36.522	-114.163	151.0	37.7	3.5
	NV052-343	35.571	-114.849	190.9	34.9	2.3
	NV052-345	36.522	-114.141	160.3	37.0	3.4
LATR	AZ010-39	36.298	-113.994	139.5	40.8	2.2
	AZ010-43	36.969	-113.937	199.3	38.9	0.8
	CA650-09	36.069	-117.221	86.1	41.0	3.2
	CA930A-105	35.429	-117.587	160.4	35.1	4.0
	CA930A-95	34.271	-116.465	172.5	33.8	1.2
	NV052-299	36.406	-114.093	155.4	38.4	1.9
	NV052-300	35.522	-114.059	236.4	33.5	0.9
	NV052-368	36.264	-114.208	207.6	35.2	0.7
	NV052-369	36.532	-114.935	142.1	37.6	2.2
	NV052-405	35.168	-114.681	180.5	38.6	5.4
	NV052-415	36.281	-115.449	227.9	32.8	-0.1
	NV052-416	36.734	-114.057	174.9	38.6	1.4
Garden	Fort Irwin	35.219	-116.807	142.9	35.9	3.2
	Joshua Tree	34.226	-116.108	90.5	39.9	2.8
	Utah	37.199	-113.565	304.2	36.3	0.2

Table 2.2. Effects on survivorship during entire experimental time period (March 2014 to March 2017).

	Ambrosia dumosa			*Larrea tridentata*		
	Wald χ^2	df	*p*-value	Wald χ^2	df	*p*-value
Garden	270.76	2	< 0.001	29.37	2	< 0.001
Ecotype	59.75	18	< 0.001	3.57	12	0.990
Garden × Ecotype	28.059	35	0.791	10.282	19	0.946
Initial Size	17.17	1	< 0.001	1.50	1	0.221

Table 2.3. Effects on growth during entire experimental time period (March 2014 to March 2017).

	Ambrosia dumosa			*Larrea tridentata*		
	F	df	*p*-value	F	df	*p*-value
Garden	116.595	2	< 0.001	53.566	2	< 0.001
Ecotype	2.409	18	0.007	1.845	12	0.092
Garden × Ecotype	0.971	31	0.514	0.756	20	0.762
Initial Size	15.995	1	< 0.001	22.212	1	<0.001

Table 2.4. Thirty-year average climate variables and the influence on 2017 survivorship for *Ambrosia dumosa* (AMDU) and *Larrea tridentata* (LATR). Bold values indicate significant regressions ($p < 0.05$).

Species	Climate Variable	Fort Irwin		Joshua Tree		Utah	
		β	*p*	β	*p*	β	*p*
AMDU	February PPT	0.069	0.129	0.024	0.372	0.014	0.599
	October PPT	**-0.286**	**<0.001**	**-0.188**	**<0.001**	-0.050	0.124
	January T_{min}	-0.187	0.215	0.078	0.423	0.014	0.874
	July T_{max}	**0.550**	**0.001**	0.156	0.132	-0.028	0.765
	Initial Size	**0.003**	**0.001**	**0.001**	**0.003**	**0.002**	**<0.001**
LATR	February ppt	30.074	0.993	-0.074	0.343	-0.011	0.901
	October ppt	10.474	0.993	-0.009	0.891	0.022	0.771
	January T_{min}	-54.014	0.993	0.090	0.623	0.036	0.870
	July T_{max}	92.569	0.993	-0.145	0.422	-0.064	0.748
	Initial Size	<0.001	0.754	<0.001	0.460	<0.001	0.325

Table 2.5. Thirty-year average climate variables and the influence on 2017 stem diameter of *Ambrosia dumosa* (AMDU) and *Larrea tridentata* (LATR). Bold values indicate significant regressions ($p < 0.05$).

Species	Climate Variable	Fort Irwin		Joshua Tree		Utah	
		β	p	β	p	β	p
AMDU	February PPT	-0.112	0.166	**-0.125**	**0.016**	**-0.201**	**0.017**
	October PPT	**-0.247**	**0.021**	-0.042	0.606	-0.071	0.500
	January T_{min}	-0.155	0.586	0.039	0.835	**0.905**	**0.003**
	July T_{max}	**-0.843**	**0.007**	-0.401	0.069	**-0.834**	**0.005**
	Initial Size	**0.002**	**0.006**	**0.001**	**0.007**	**0.004**	**0.001**
LATR	February ppt	0.138	0.634	-0.208	0.097	-0.130	0.492
	October ppt	0.343	0.211	0.087	0.378	-0.018	0.920
	January T_{min}	**1.296**	**0.049**	0.157	0.571	0.611	0.252
	July T_{max}	0.471	0.493	-0.444	0.128	-0.338	0.433
	Initial Size	<0.001	0.148	<0.001	0.071	**<0.001**	**<0.001**

Table 2.6. Traits measured in each garden that have a significant correlation with climate variables that explain the most variation at ecotype's origin for *Ambrosia dumosa*.

		February PPT		October PPt		January T_{min}		July T_{max}		Initial Size	
Garden	Functional Trait	β	p	β	p	β	p	β	p	β	p
FI	June 2016 c_i	1.962	0.048	-10.038	<0.001	-13.218	0.006	7.310	0.256	-0.010	0.334
	Mar. 2017 φPSII	-0.001	0.616	**-0.008**	**0.049**	0.012	0.165	-0.007	0.600	<0.001	0.310
	δ ^{18}O Stem Water	0.075	0.019	0.066	0.361	-0.207	0.155	-0.007	0.975	<0.001	0.180
	Leaf N content	0.007	0.380	0.001	0.897	-0.063	0.027	-0.006	0.848	<0.001	0.122
	SLA	-0.168	0.253	0.159	0.615	-0.391	0.560	-2.341	0.013	0.002	0.170
JT	Mar. 2015 Predawn WP	0.030	0.014	0.018	0.461	-0.094	0.150	0.015	0.747	<0.001	0.920
	Mar. 2016 Predawn WP	-0.071	0.095	-0.014	0.837	0.395	0.006	-0.676	0.024	<0.001	0.782
	δ ^{15}N Leaf	0.115	0.019	**0.161**	**0.022**	-0.534	0.001	0.329	0.125	<0.001	0.124
	SLA	-0.061	0.748	**1.039**	**0.002**	0.740	0.278	0.456	0.750	-0.005	<0.001
UT	Mar. 2015 Midday WP	-0.008	0.374	-0.010	0.245	0.065	0.003	-0.019	0.391	<0.001	0.040
	Mar. 2016 Predawn WP	-0.009	0.093	0.025	0.023	0.006	0.809	-0.044	0.156	<0.001	0.994
	δD Stem Water	-0.128	0.104	0.195	0.258	0.118	0.754	-1.099	0.021	-0.004	0.030
	SLA	-0.863	0.053	1.972	0.036	3.417	0.113	-1.072	0.685	0.013	0.190

Table 2.7. Traits measured in each garden that have a significant correlation with climate variables that explain the most variation at ecotype's origin for *Larrea tridentata*.

		February PPT		October PPT		January T_{min}		July T_{max}		Initial Size	
Garden	Functional Trait	β	p	β	p	β	p	β	p	β	p
FI	June 2016 Predawn WP	-0.094	0.059	-0.062	0.253	**0.231**	**0.044**	-0.104	0.337	<0.001	0.336
	Δ ^{13}C Leaf	0.005	0.931	-0.045	0.411	**-0.270**	**0.041**	0.050	0.716	<0.001	**0.037**
JT	Mar. 2015 Predawn WP	0.043	0.402	-0.090	0.027	-0.010	0.922	0.118	0.319	<0.001	0.214
	June 2016 Predawn WP	0.141	0.641	**-0.397**	**0.013**	-1.668	0.150	0.438	0.489	<0.001	0.217
	Mar. 2017 A_{net}	**-1.509**	**0.003**	-1.222	0.100	-1.584	0.239	1.090	0.471	<0.001	0.977
	Mar. 2017 g_s	**-0.022**	**0.002**	-0.004	0.676	-0.010	0.583	-0.012	0.565	<0.001	0.202
	Mar. 2017 φPSII	**-0.008**	**<0.001**	-0.003	0.366	-0.003	0.584	0.001	0.856	<0.001	**0.004**
	δ ^{15}N Leaf	0.242	0.019	-0.074	0.397	-0.161	0.471	0.302	0.202	<0.001	0.592
	SLA	2.316	0.010	-2.446	0.005	-7.838	0.030	**5.178**	**0.011**	<0.001	0.875
UT	Mar. 2015 Predawn WP	-0.002	0.909	-0.023	0.020	-0.033	0.206	-0.006	0.870	<0.001	0.896
	Mar. 2017 Predawn WP	-0.029	0.451	**-0.169**	**<0.001**	-0.058	0.563	0.152	0.134	<0.001	0.764
	Mar. 2017 φPSII	0.001	0.765	**-0.009**	**<0.001**	-0.006	0.256	**0.013**	**0.013**	<0.001	0.196
	SLA	-0.608	0.074	0.414	0.183	0.434	0.614	**-1.730**	**0.025**	<0.001	0.231

Figures

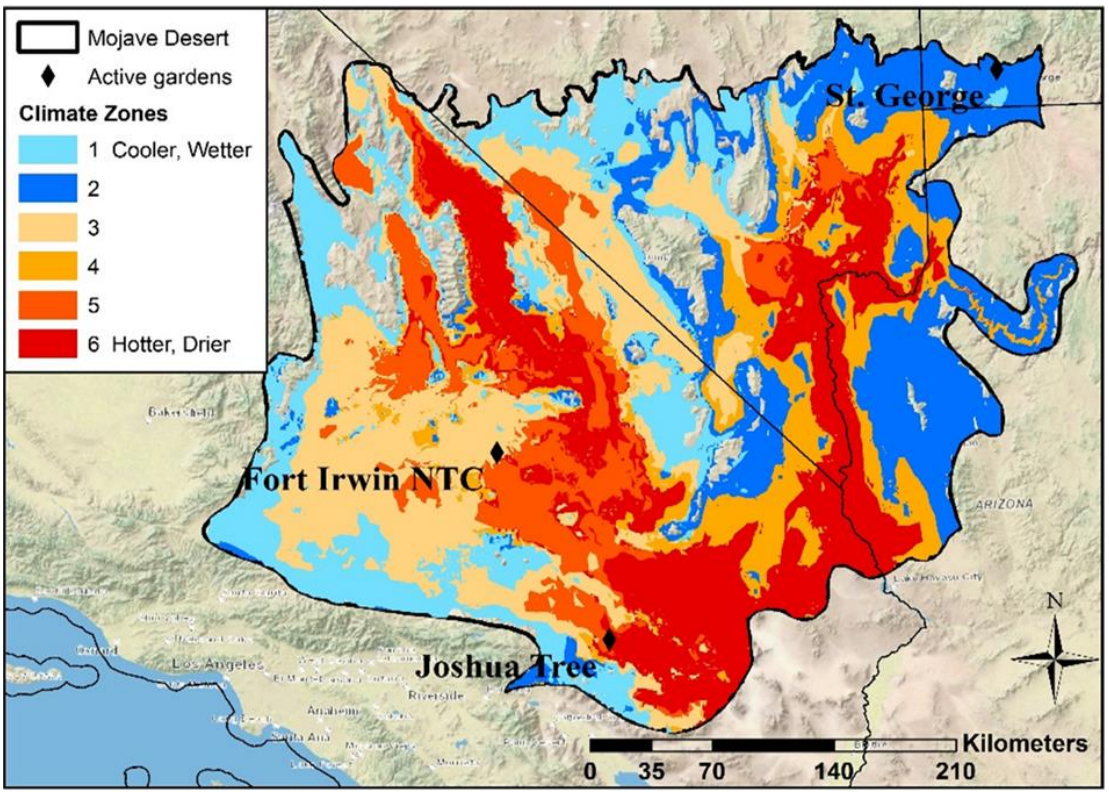

Figure 1.1. Map of the Mojave Desert, USA with colors representing average climate in terms of precipitation and temperature. The three common gardens are designated.

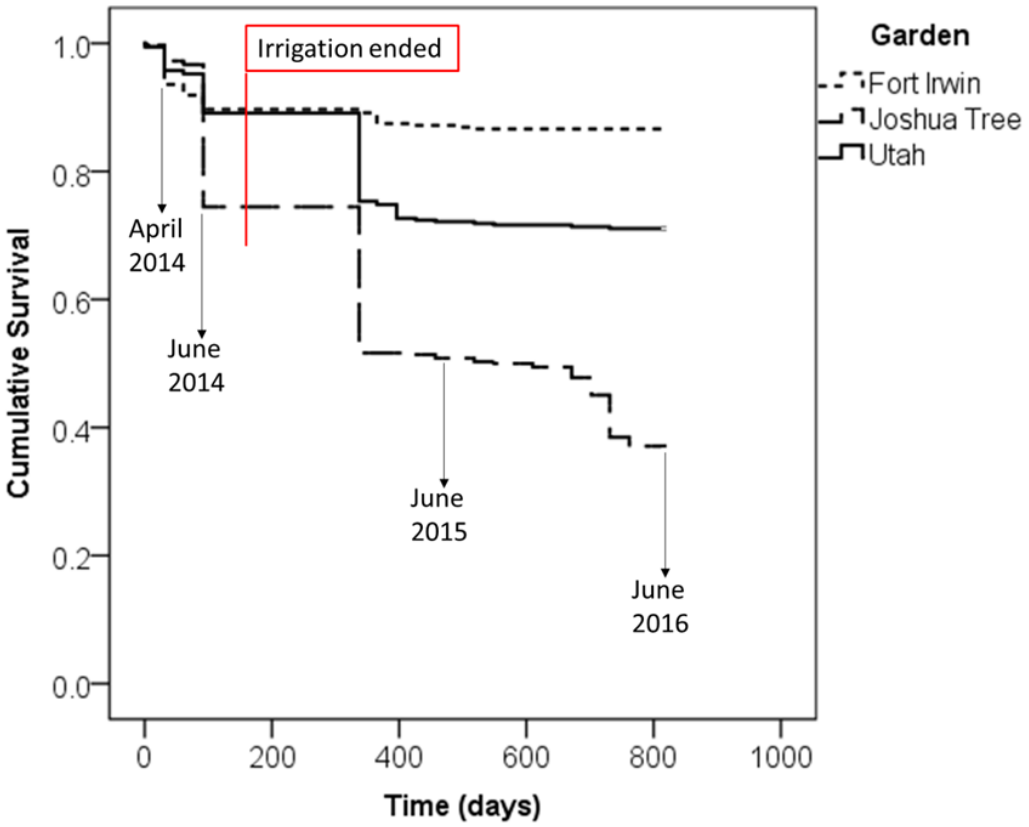

Figure 1.2. Survivorship averaged across ecotypes of *Amrosia dumosa* in three common gardens along an environmental gradient.

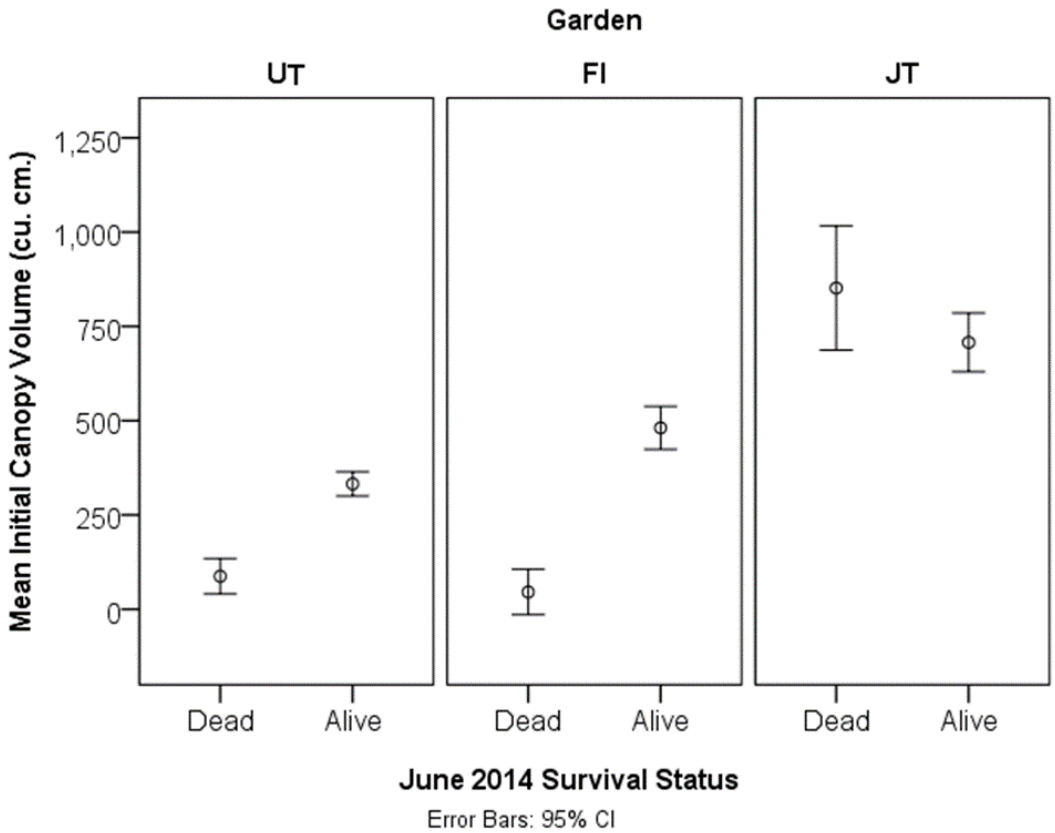

Figure 1.3. Effect of initial canopy volume on survivorship of *Ambrosia dumosa* plants in three common gardens along an environmental gradient in 2014. FI = Fort Irwin, CA; JT = Joshua Tree, CA; UT = St. George, UT.

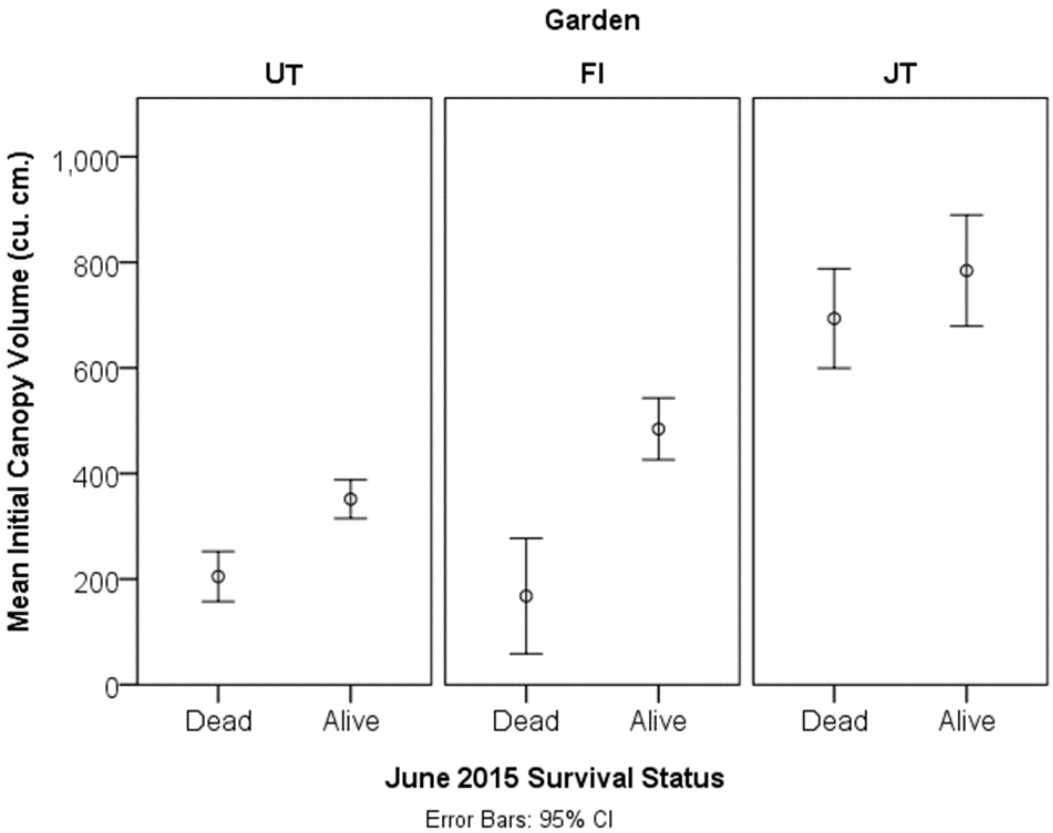

Figure 1.4. Effect of initial canopy volume on survivorship of *Ambrosia dumosa* plants in three common gardens along an environmental gradient in 2015. FI = Fort Irwin, CA; JT = Joshua Tree, CA; UT = St. George, UT.

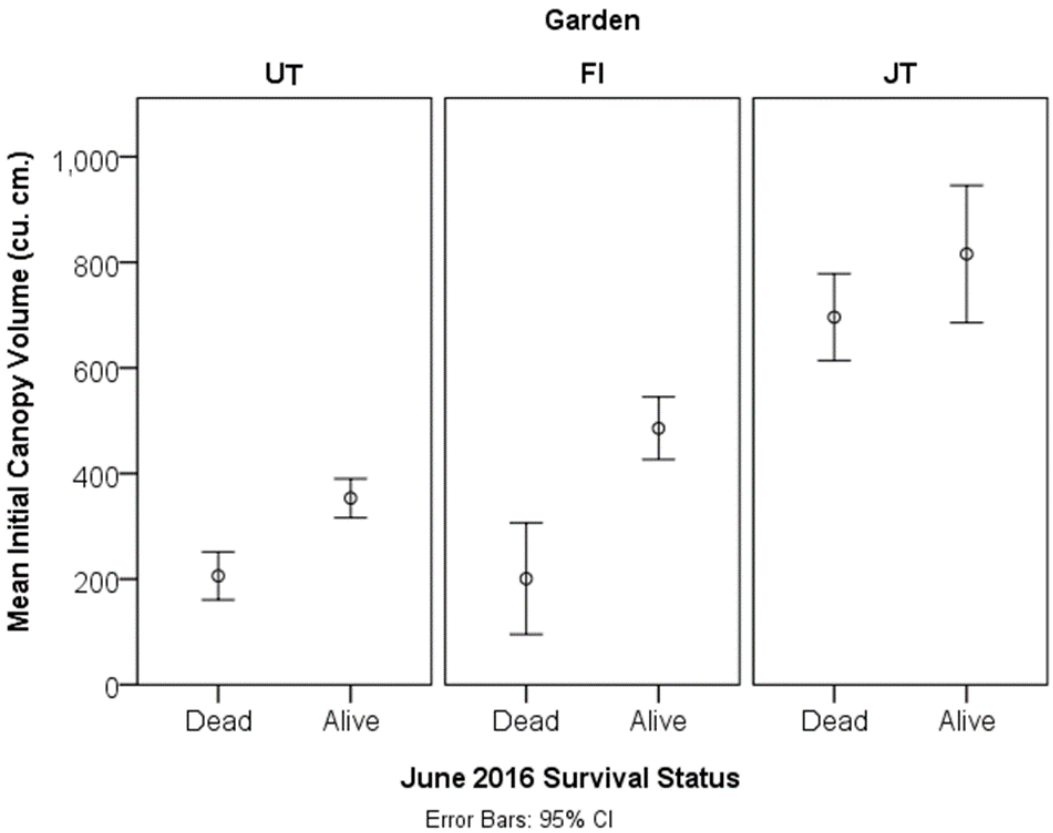

Figure 1.5. Effect of initial canopy volume on survivorship of *Ambrosia dumosa* plants in three common gardens along an environmental gradient in 2016. FI = Fort Irwin, CA; JT = Joshua Tree, CA; UT = St. George, UT.

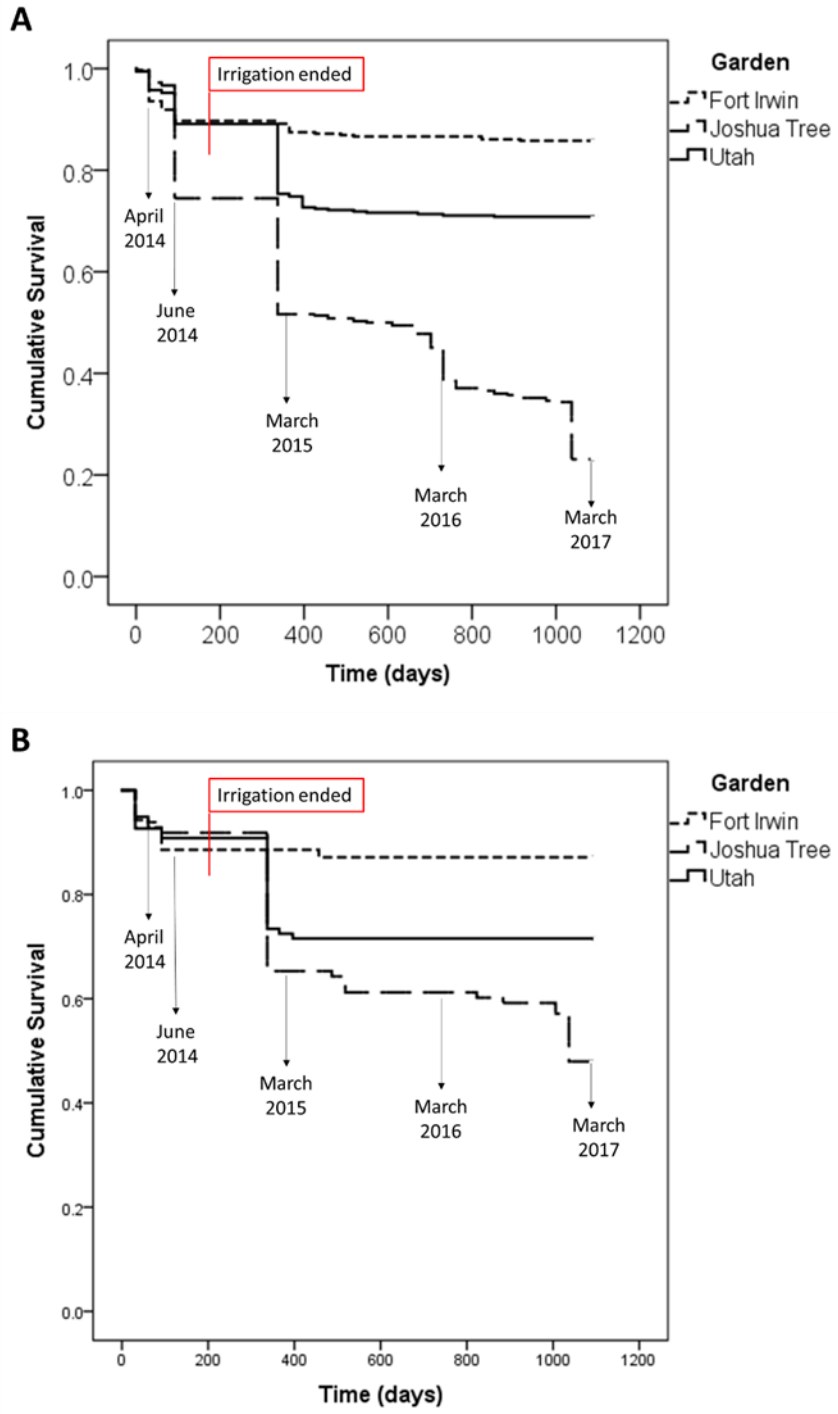

Figure 2.1. A) *Ambrosia dumosa* and B) *Larrea tridentata* Kaplain-Meier survivorship curves in three common gardens.

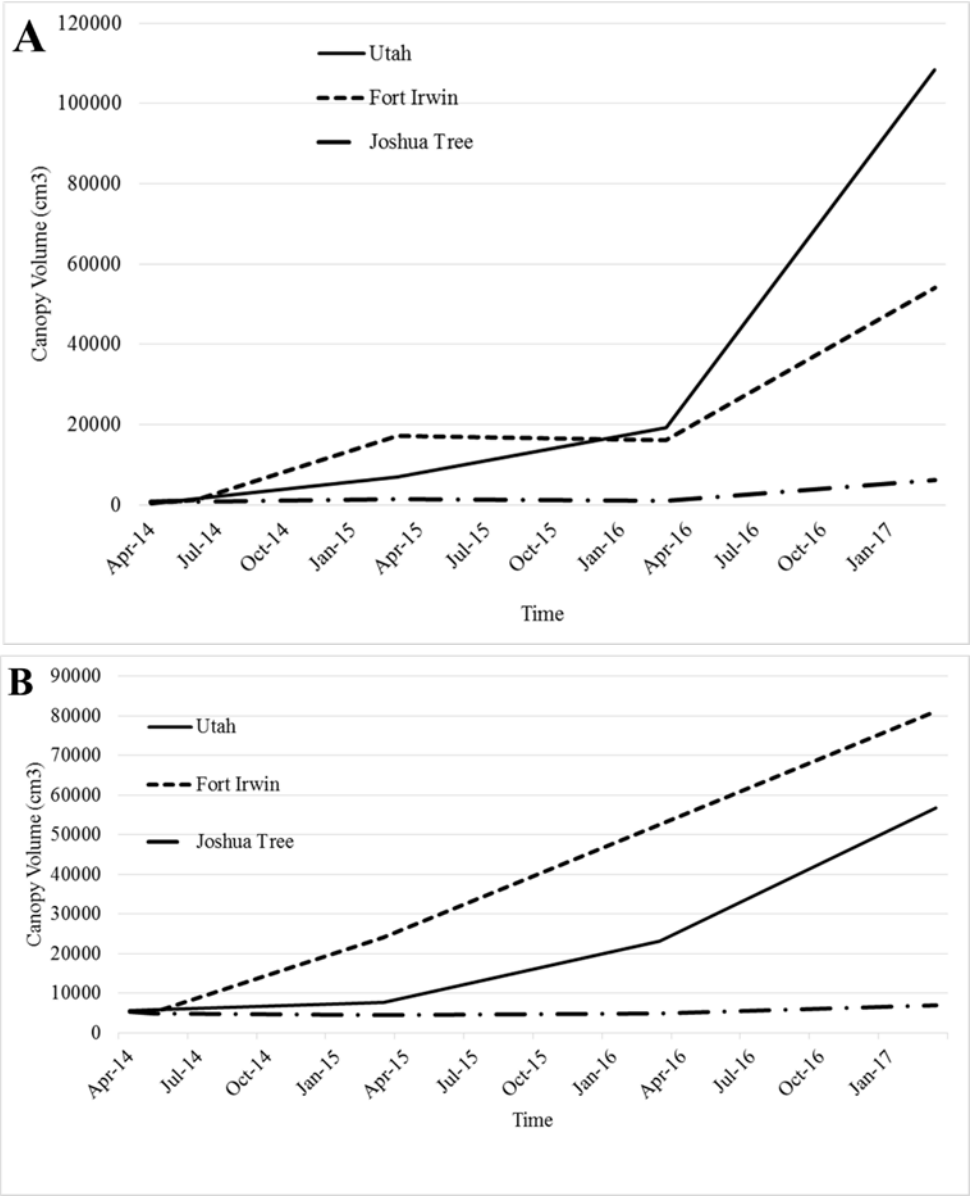

Figure 2.2. Growth of A) *Ambrosia dumosa* and B) *Larrea tridentata* canopies in the three common gardens from April 2014 to March 2017.

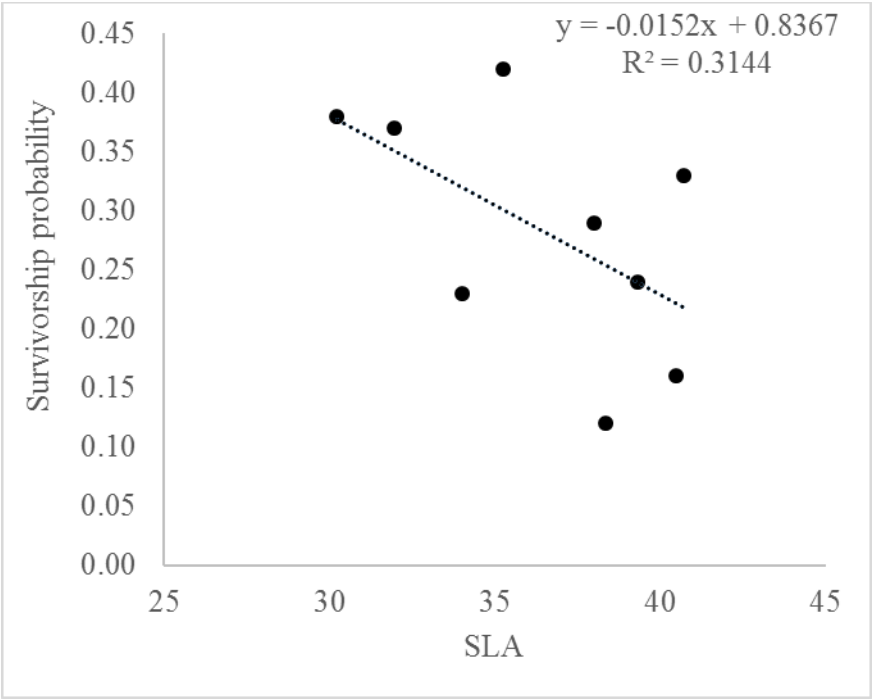

Figure 2.3. Survivorship of ecotypes based on mean specific leaf area (SLA) in the Joshua Tree garden.

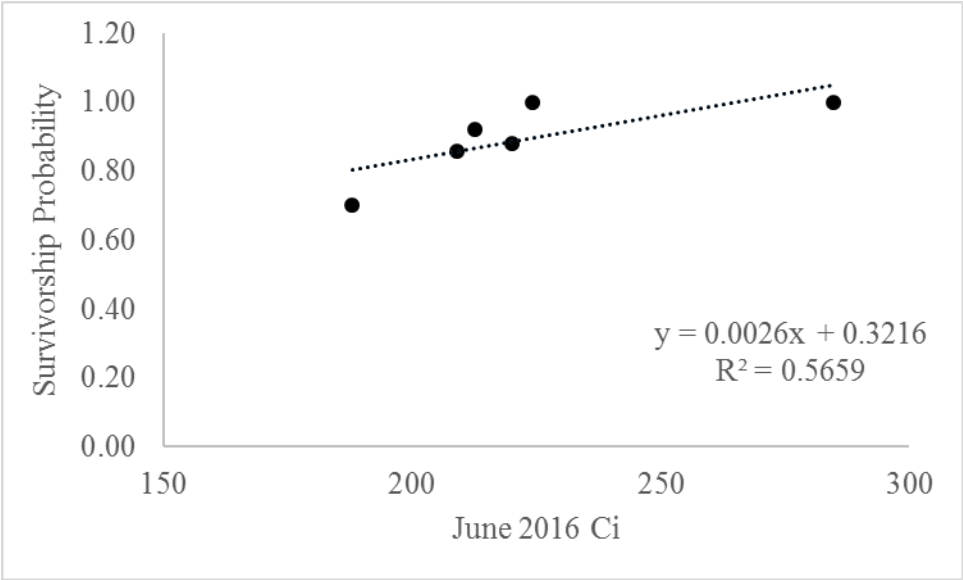

Figure 2.4. Survivorship of ecotypes based on mean intracellular CO_2 (c_i) measured in June 2016 in the Fort Irwin garden.

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