RESTORING *AGAVE PALMERI* POPULATIONS: CRITICAL FACTORS FOR SEEDING AND TRANSPLANTING IN DISTURBED LANDSCAPES

by

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Laura Pavliscak
July 2010

DEDICATION

Great truths are sometimes so enveloping and exist in such plain view as to be invisible. ... Millions of years of coevolution have finely tuned the relations between particular plants and their special pollinators. The shapes and colors of the flowers, their scent, their location on the stalks, the season and daily schedule of the pollen and nectar offerings, as well as their qualities we admire but seldom understand, are adjusted precisely to attract particular species of [pollinators]; and those specialists in turn, …are genetically adapted to respond to certain kinds of flowers. Nature, we learn, is kept productive and flexible by uncounted thousands of such relationships. No phenomenon in nature illustrates more vividly the principle that conservation measures must be directed at ecosystems, not just individual species. If the last pollinator species adapted to a plant is erased by pesticides, or habitat disturbance, the plant will soon follow. And as these and other populations decline or disappear, the consequences spread through the remainder of the food net, weakening other interspecific relationships. The evidence is overwhelming that wild pollinators are declining around the world. Their ranks are being thinned not just by habitat reduction and other familiar agents of impoverishment, but also by the disruption of the delicate "biofabric" of interactions that bind ecosystems together. Humanity, for its own sake, must attend to the forgotten pollinators and their countless dependent plant species.

--E.O. Wilson (The Forgotten Pollinators, Island Press, 1996)

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ABSTRACT

Agave palmeri (Palmer's agave) is a semelparous, perennial succulent thought to provide critical forage for the endangered species, *Leptonycteris curasoae* (lesser long-nosed bat). Preserving intact agave populations and mitigating loss of habitat may be critical to *L. curasoae* recovery. Two methods for restoring *A. palmeri* in disturbed habitats were evaluated: seeding and translocation. In a greenhouse, the emergence and establishment of 2700 seeds was tested across four environmental variables: irrigation level, shade, surface mulch, and soil type. The overall emergence of seedlings was low, particularly in low irrigation, unshaded, unmulched treatments—conditions that might be commonly expected in disturbed habitats. In the field, growth responses of 277 wild transplants were assessed in relation to size class, initial water availability, and storage method. Transplants of all sizes responded positively when replanting coincided with seasonal rainfall, suggesting that salvaging and replanting *A. palmeri* plants may be a promising restoration strategy.

CHAPTER 1: RESTORING *AGAVE PALMERI* POPULATIONS: CRITICAL FACTORS FOR EMERGENCE AND ESTABLISHMENT

Abstract

Agave palmeri Engelmann (Palmer's agave) is a semelparous, perennial succulent, thought to provide a critical food source for the USFWS-listed endangered species, *Leptonycteris curasoae* Miller (lesser long-nosed bat). Awareness is growing for the need to conserve intact wild populations of *A. palmeri*, and to mitigate for such impacts to its habitat as mining and urban development. Little is known about the early life history of this agave species and its potential for restoration by seed in disturbed landscapes. I tested four variables associated with seedling emergence and establishment in a multifactor greenhouse experiment: irrigation level (low, average, high), shade (present, absent), surface mulch (straw, gravel, bare soil), and soil type (Arkose, Gila, Glance). Variables were based on environmental conditions from a study site in the northeastern Santa Rita Mountain Range, Arizona, where potential mining disturbance is possible. Twenty-seven hundred seeds were monitored for 5.5 months through a simulated wet summer monsoon and dry autumn period, and a final inventory quantified seedling emergence (total emergence, mortality), and establishment characteristics (height, number of leaves, dry weight). High irrigation levels, straw mulches, shaded treatments, and use of Gila or Glance soils resulted in the highest emergence and the largest seedlings. Straw mulch, as a main effect, was associated with the most

positive seedling response, possibly due to the dual effect in reducing temperature and decreasing evaporative water loss at the soil surface. Treatments involving low irrigation levels, bare soil, absence of shade, and use of Arkose soil resulted in the lowest emergence and the smallest seedlings. The bare soil treatment, as a main effect, was associated with the most negative seedling response, possibly due to a lack of moisture and temperature mitigation at the soil surface.

Introduction

The family Agavaceae has evolved in the warm, humid foothills and lowlands of present-day Central and South America, southern North America, and the Caribbean Islands (Good-Avila et al. 2006). Currently, some 293 species are known, each uniquely adapted to regional environments using diverse physiognomic and reproductive strategies. *Agave palmeri* Engelmann (Palmer's agave) is one such species, inhabiting mountain foothills in southeastern Arizona, southwestern New Mexico, and northern Sonora, Mexico. In recent years, interest has grown in the life history of this agave, due to its potentially critical role as a food source for an endangered species, *Leptonycteris curasoae* Miller (lesser long-nosed bat) (Schaffer and Schaffer 1977, Howell and Roth 1981, Ober and Steidl 2004, Ober et al. 2005). *L. curasoae* is a migratory nectar-feeding bat, known to follow the annual phenological tide of blooming agaves and columnar cacti from central Mexico to the southwestern United States (Gentry 1982, Fleming et al. 1993). Research over the last several decades has suggested that flowering *A. palmeri* may serve as a primary

nectar source preceding the bats southward migration, and that in turn, *L. curasoae* may serve as one of the principal means for pollinating this agave species (Schaffer and Schaffer 1977, Howell and Roth 1981, Ober and Steidl 2004, Ober et al. 2005). This mutualistic relationship has caused a growing awareness for the need to conserve intact wild populations of *A. palmeri*, and to mitigate for impacts to its habitat through restorative methods.

Restoration efforts for re-establishing vegetation in disturbed areas typically involve seeding desired species, however, applying this strategy to restore *A. palmeri* may be problematic. To date, research on *A. palmeri* has largely focused on the pollination ecology of adult plants (Slauson 1994, 1996, 2000; Scott 2004). Little is known about the environmental requirements for seedling, germination, establishment and growth. It is widely acknowledged that agave seedlings in the wild are extremely rare; Nobel's (1977) research on *A. deserti* concluded that only one in 1.2 million seeds survive to maturity. Gentry (1972) made similar predictions after a lifetime of observing agave natural history across the Americas, claiming he had never seen a seedling less than one year old. Agaves are renowned for producing copious quantities of seed, in the range of tens of thousands to hundreds of thousands per plant (Gentry 1972, Nobel 1977), thus, the apparent lack of germination and/or high mortality of seedlings in the wild prompts some question about the efficacy of a seeding restoration effort for this genus.

The present study was conducted because of the possibility of a large-scale disturbance in an area of dense stands of *A. palmeri*, in order to assess the potential

of restoring a functional, independent wild population. Possible mining activity in the northeastern Santa Rita Mountain Range in southern Arizona is currently in review and would impact thousands of *A. palmeri* individuals (WestLand Resources 2009). Seeding of this species has been suggested as a means to re-establish plants within disturbed areas. In order to evaluate the efficacy of a landscape-scale *A. palmeri* seeding effort, knowledge of its early life history and how environmental variables affect seedling emergence and establishment is essential.

Agave palmeri is a monocarpic perennial in the group Ditepalae (Gentry 1972). It is commonly associated with oak woodland and grama grassland plant communities at elevations from 900-1,800 m (3,000-6,000 ft) (Gentry 1982). Mature plants are generally 100-120 cm tall (40-48 in) and 50-120 cm wide (20-47 in) with succulent, yellow-green to silver-gray leaves. Tooth-fringed leaves unfold from a central spike above a fleshy meristimatic shoot (Nobel 1988). Individuals grow for up to 35 years before flowering in late spring through early autumn. The inflorescence is a broad panicle, 3-5 m tall (10-16 ft), with light-colored, tubular flowers in umbellate clusters. Flowers are protandrous, and fertile seeds are uncommonly formed without external pollination (Schaffer and Schaffer 1977, Howell and Roth 1981, Hinman 2003). Capsules are oblong and contain three locules that may be packed with flat, round seeds. After ripening, capsules dehisce and seeds are shaken loose by high winds, perching birds, and other fauna, showering down over the dying parent plant. There is no dormancy period, and seeds are thought to germinate in the warm, wet monsoon season (Freeman 1975,

Jordan and Nobel 1979). *A. palmeri* is thought to rely primarily on sexual reproduction, rather than vegetative cloning (Gentry 1982).

Mature agave plants are adapted to tolerate the extreme variability in temperature and water availability that is common to the arid landscapes they inhabit (Nobel 1988). Such mechanisms as CAM photosynthesis, large volumes of water-storing biomass, and receptive cellular structure in root systems that allow rapid uptake of soil moisture and minimize water loss to drying soils, allow adult plants to avoid desiccation and mortality during extreme temperatures and prolonged drought (Nobel 1976, 1994; Nobel and Cui 1991, North and Nobel 1998). However, during the first few years of life while a seedling is small and developing, a young plant is highly vulnerable to seasonal environmental extremes and mortality rates are high (Nobel 1977, Jordan and Nobel 1979, Gentry 1982). Like many desert succulents, in order to mediate unfavorable conditions, young agave plants are thought to benefit from, and possibly require, facilitative "nurse" relationships. Nurse plants or rocks can provide critical services to seeds and seedlings, including increasing the moisture and nutrient availability of soils, and protection from extreme temperatures, herbivory, and trampling (Turner et al. 1966, Jordan and Nobel 1979, Peters et al. 2008).

In order to assess the response of *A. palmeri* seeds and seedlings to a possible restoration effort in an area of disturbance, I conducted an experiment, testing 2700 seeds to four environmental variables: irrigation level (high, average, low), shade (presence or absence), surface mulch (straw, gravel, bare soil), and soil type

(Arkose, Gila, Glance). Variables were selected based on seasonal conditions specific to a study site in the prospective impact area in the northeast Santa Rita Mountain Range, including temperatures, amount of rainfall, and soil types, as well as what might be expected in a restoration scenario, including surface mulches and the presence or absence of shade. Between October 2008 and February 2010, seed was collected from the study site, preliminary growth chamber trials were conducted to assess viability, and a greenhouse experiment testing seedling emergence and establishment was initiated and monitored for 5.5 months. Results from the experiment offer insights into the preferred growing conditions for *A. palmeri* seedlings at the study site, and suggest the effectiveness of this strategy for restoration of the species in disturbed areas.

The ultimate variable of interest in this study is the long-term reproductive success of plants. However, due to the long life span of the species (≤ 35 years) and the short time period available for this research, mortality was the only certain response to assess this variable in seedlings. I focused on documenting two primary seedling responses, emergence and establishment characteristics. Quantifying emergence involved comparing how many seeds were initially sown with how many emerged into seedlings, and how many then survived until the end of the experiment. The establishment assessment was intended to qualify the condition of surviving seedlings through quantitative measurements. Researchers apply several measures to assess agave productivity, including CO_2 uptake, leaf initiation, and dry weight (Nobel 1984a, 1988, Nobel and Meyer 1985, Nobel and Hartsock 1986b,

Nobel and Quero 1986). Seedling establishment in this experiment involved measuring three characteristics: height, number of leaves, and dry weight (biomass).

Much research in arid land ecology has explored the role of moisture as one of the most critical environmental variables affecting germination and establishment (Jordan and Nobel 1979, 1982; Nobel 1984a, 1984b, 1988; Pierson and Turner 1998). Young seedlings of perennial plant species are thought to require above average duration and frequency of precipitation for multiple consecutive years in order to establish through early life stages. Given that precipitation in arid land systems is characteristically sporadic within and between seasons, it is thought that many arid-adapted succulents are episodically recruited, where generational cohorts both germinate and establish primarily in repeated years of above-average rainfall—possibly only a few times a century (Turner 1966, Nobel 1977, Jordan and Nobel 1979, Pierson and Turner 1998). Researchers have suggested that the primary cause of agave seedling mortality is drought during the first year of growth (Gentry 1972; Jordan and Nobel 1979, 1982). The irrigation levels used in this experiment were calculated for a summer monsoon season using study site rainfall records, and were intended to apply amounts that would be expected in low, average, and high precipitation years. Given the sensitivity of agave seeds and seedlings to moisture, I expected irrigation level to have a strong effect, and predicted that high levels will have both significantly higher emergence and larger seedlings than low levels.

In addition to moisture, the affect of biotic or abiotic “nurse” roles has been implicated as critical in facilitating germination and establishment of many arid-adapted plant species (Turner et al. 1966, Steenburgh and Lowe 1969, Jordan and Nobel 1979, Franco and Nobel 1989, Callaway 1995, Peters et al. 2008). Seeds germinating below an adult agave, another plant species, or near a large rock can be facilitated in survivorship and growth through thermal buffering from extreme heat and cold, decreased solar irradiation resulting in reduced water loss from soil and vegetative tissues, more consistent relative humidity, increased organic matter and soil nutrient content, and protection from herbivory (Jordan and Nobel 1979, Franco and Nobel 1988, Callaway 1995, Peters et al. 2008). In order to simulate a component of this critical nurse role, I used artificial shade as a means both efficient to standardize in an experiment as well as previously tested with some success (Turner et al. 1966, Nolasco et al. 1997). I expected shaded treatments to be associated with greater emergence and more robust establishment than unshaded treatments.

Soil surface heterogeneity can affect rainwater infiltration, decrease water loss through evaporation, and provide a thermal buffer to reduce temperature extremes at the soil surface where seeds germinate (Steenbergh and Lowe 1969, Jalota and Prihar 1998, Post et al. 2004, Li et al. 2005). The selection of surface treatments in this experiment was largely motivated by an applied context, specific to the study site and its associated management concerns. Straw is a commonly used mulch in landscape restoration that provides the aforementioned ecological

services, in addition to slowing soil erosion (Jalota and Prihar 1998). Gravel is abundant at the study site, and as a surface mulch has been shown to increase rainwater infiltration and conserve soil moisture (Post et al. 2004, Li et al. 2005). Bare soil was used as a control. I expected straw and gravel mulches to create protected microsites for seeds and seedlings and thus be associated with higher emergence and larger seedlings than unmulched, bare soil treatments.

Like surface mulches, the intent in testing specific soil types in this experiment was applied in focus: to determine how *A. palmeri* seeds would respond to the dominant soil types at the study site. Arkose, Gila, and Glance soil samples used in the experiment were excavated from up to 3 m (10 ft) in order to provide a mixed substrate, indicative of an earth-moving disturbance scenario. Soil texture and color varies between types, from dark-colored, extremely gravelly Arkose soil, to lighter-colored, more fine-textured Gila and Glance soils. I expected Arkose to have the lowest emergence and smallest seedlings due to its dark color and potentially warmer surface temperature, and its extremely gravelly substrate which may inhibit seedling growth (Martre et al. 2002).

Methods

Seed collection

In October 2008, I collected seed capsules from 26 *A. palmeri* plants on the northeast side of the Santa Rita Mountains (31°50’N, 110°45’W) at elevations ranging from 1470-1685 m (4820-5530 ft). Capsules were collected from plants

which had flowered that summer and which were at least 40 m (130 ft) apart in order to minimize double-sampling from potential clones. Descriptions of plant morphology and local environmental variables were documented at each plant, including height and width of rosette, number of leaf whorls, height of inflorescence, proximity to neighboring agaves, dominant plants in adjacent vegetation community, incline and aspect of slope, composition of particle size class on soil surface (gravel, cobble, ect.), and percent canopy cover by other vegetation. Ripe capsules were clipped with a telescoping tree pruner, placed in paper bags, and transported to Tucson where they were stored at room temperature. Capsules were cracked and white sterile seeds were removed and discarded. The remaining seeds were secured in manila envelopes, filed in a loosely covered plastic tub, and stored indoors at approximately 21° C (70° F).

Growth chamber trials

In order to assess the viability of harvested seeds and the variability among parent plants, I conducted germination trials in a growth chamber. I initially performed a pilot test to confirm that seed germination is not affected by light (Freeman 1973, Nobel 1988). In this test, I randomly selected four parents from the group. I used five seeds per petri dish, and three repetitions per treatment. Petri dishes were lined with filter paper, seeds were placed apart from each other in the center of the dish, and dishes were moistened with 10 mL distilled water. The dishes designated for limited-light treatments were placed in individual #2 paper

bags sealed with masking tape. The growth chamber was programmed to produce full light at 25°C (77°F), a temperature thought to be ideal for agave germination (Freeman 1975, Nobel 1988). Petri dishes were monitored and a final count of seed emergence took place 21 days after the experiment began. A seed was considered to have germinated if the radical was clearly visible. No significant differences between light (38% germination) and limited-light (45% germination) treatments were discovered, and subsequent growth chamber trials did not incorporate limited-light treatments.

In the spring of 2009, I tested 2,070 seeds in the growth chamber in order to assess viability of individual parents and the group. All 26 parents from which seed was collected were included in the study (80 seeds/parent), except one parent where only 70 seeds were tested. Ten seeds from each individual were placed on a folded, wet paper towel in a sealed pint-sized ziplock bag. Paper towels were moistened with a spray bottle containing a 1% bleach solution (10mL bleach in 828 mL distilled water). Bags were randomized in a growth chamber and programmed to simulate summer monsoon conditions (including temperature, light, and humidity), when germination is thought to occur in the wild (Freeman 1975, Jordan and Nobel 1979). Day and night environmental conditions were tailored specifically to the field site where seed was collected, with temperatures ranging from 15-37° C (59-99° F) and relative humidity ranging from 7-96%. Four replications of each parent were performed in each of two separate trials, for a total of 8 replications per parent. Each trial lasted 21 days, and concluded with an inventory of seed

germination. Seeds were considered to have germinated if the radical was clearly visible.

Greenhouse experiment

In the fall of 2007 through spring 2008, previous experiments were conducted testing native seed viability and species richness on reclaimed soils from the northeastern Santa Rita Mountains, Pima County, Arizona where my research is focused. Taryn Kong and Jeffrey Fehmi developed a related study design, commissioned soil collection, transport, and preparation, designed an irrigation methodology, and initiated the sowing, maintenance, and harvest of several native species seed mixes. Many aspects of my *A. palmeri* emergence and establishment experiment incorporated this earlier investigation's methods and resources, including previously used pots of soil, calculated amount of straw mulch, and both frequency and method of water application, with the intention to offer comparable results for site-specific restoration.

From Aug 2009-Feb 2010, I conducted an experiment assessing emergence and establishment of *A. palmeri* at the Campus Agricultural Center (CAC) in Tucson, Arizona. In an evaporatively-cooled greenhouse, 54 distinct treatments and 5 replications were employed across 5 blocks in a randomized complete block design. Monsoonal climate conditions were simulated from 15 Aug-17 Nov 2009, with higher temperatures [maximum 35°C (95°F)] and the application of irrigation. From mid-November to the study's conclusion, a dry, cooler autumn period was

simulated with temperatures lowered [maximum 29°C (85°F)] and no irrigation applied. The experiment concluded on 2 Feb 2010 when all seedlings were harvested and measured.

Fifteen L (4 gal), 30.5 cm (12 in) diameter plastic nursery pots were arranged on 0.6 m (2 ft) high tables in the greenhouse. Each table held 14 pots, and 10 tables lined both east and west sides of the greenhouse. Blocks were divided between east and west sides, with the center aisle marking the boundary between them, except block 3, which spanned the aisle on the south end of the greenhouse. The north side of the greenhouse (blocks 1 and 5) was in closest proximity to the evaporative cooling mats, and the south side was farthest from this (block 3).

Irrigation treatments

Three precipitation scenarios (designated low, average, high) were implemented based on 31 years of rainfall records (1976-2006) from Santa Rita Experimental Range weather stations (J. Fehmi unpublished report, Agricultural Research Service 2008). Records were averaged from two gauges (numbers 5, 6), selected due to their relatively close proximity to the study site [within 16 km (10 mi)]. Irrigation levels were determined by summing daily rainfall into an annual total, and ranking the totals from each year by lowest to highest amount. The median of the 31 ranks became the “average” irrigation level, and the fourth and twenty-eighth ranks became the “low” and “high” levels, respectively. The selected ranks were intended to encompass the top and bottom 20% from the median of the

31-year range. The daily interval between monsoonal storm events was calculated and averaged to guide the frequency of irrigation events. This was intended to approximate natural precipitation cycles and allow drying conditions between irrigation events.

The monsoon season in southern Arizona accounts for approximately two-thirds of annual rainfall (Sellers and Hill 1974). Thus, to derive appropriate precipitation levels for a monsoon season in the greenhouse, two-thirds of the annual totals that were calculated to simulate low, average, and high precipitation scenarios were applied. This amounted to 170 mm (6.7 in) in low treatments, 285 mm (11.2 in) in average treatments, and 390 mm (15.4 in) in high treatments. Pots were watered every three days for 94 days (summer season), followed by an 82-day dry period (autumn season).

Lack of sufficient moisture has been suggested as a limitation to germination in succulents (Turner et al. 1966, Jordan and Nobel 1979). Thus, as part of the prescribed irrigation level, a preliminary drenching was enacted across all treatments to initiate germination. In this event, high irrigation treatments received 60 mm (2.4 in) over 4 consecutive days, average treatments received 45 mm (1.8 in) over 3 consecutive days, and low treatments received 20 mm (0.8 in) over 2 consecutive days.

A full circle (360°) downspray sprinkler (model MADASBLK, DripWorks, Willits, CA) with an adjustable water volume output on an 18 cm (7 in) stake was centered in each pot and interred so the downspout was 17-18 cm (6-7 in) above

the surface of the soil, ensuring an even application of water. Each sprinkler was connected to the irrigation system through a wide-diameter, 10-70 PSI pressure compensating emitter (model DNPC6-Woodpecker Pressure Compensator-6 GPH, DripWorks, Willits, CA) and was calibrated in advance to apply 140-150 mL per 20 seconds. To achieve different levels of irrigation (low, average, high), the rate of application between levels was constant, but the amount of time allotted per level varied. For each application, a calibrated test sprayer from the water line of the respective irrigation level was used to fill a graduated cylinder to the calculated amount. Irrigation systems were set up to be watered by block in order to minimize effects from variable water pressure across the greenhouse.

Shade treatments

Simple shade structures were constructed from 9-gauge steel wire and knitted polyethelyne sun screen fabric, and applied to half of all treatments. Wire frames were built in the shape of a circular halo, welded atop three stilts which rested against the inside edges of the pot. Sun screen fabric was light-colored and restricted 75% available light. It was cut to fit over the wire frames so that it draped over the east and west sides, eliminating any direct morning and evening sunlight from reaching the soil surface, and leaving the north and south sides open to airflow. The shade structure "roof" was elevated approximately 25 cm (10 in) above the soil surface, and 8 cm (3 in) above the top of the irrigation spray nozzle.

Surface treatments

Based on ecological restoration methods and surface conditions commonly encountered at the study site, three surface treatments were used in the experiment: straw, gravel, and bare soil. In the straw treatment, 34 g (equal to a rate of 2 tons per acre) of certified weed-free straw was applied per pot, based on general restoration industry standards (Jalota and Prihar 1998). Seeds were placed at the soil surface under the straw mulch.

Gravel was included as a surface treatment due to the abundance with which it was encountered at the field site. Over 41% of wild agaves observed in a related study (Chapter 2) were associated with 60% or greater gravel [1x1 cm-5x10 cm (0.4x0.4 in-2x4 in)] and cobble [6x11 cm-20x40 cm (2x4 in-8x16 in)] substrates at the surface. I determined gravel size and density based on the photographs and detailed substrate data recorded at the field site from that study (Chapter 2). A dot grid was placed over digital substrate photographs, taken at equal distances from the ground adjacent to wild agaves, and a percentage of gravel cover was calculated based on these and field observation averages. Sixty percent gravel was concluded as an appropriate surface treatment, comprised of 40% large sizes [>2x4 cm (0.8x1.6 in)] and 20% small sizes [<2x4 cm (0.8x1.6 in)]. Gravel was purchased from a local supplier and was a similar red color to what is found typically at the site. It was washed and dried and distributed evenly across the soil surface. Seeds were placed so that each was in contact with soil.

Bare soil is another common substrate observed in association with *A. palmeri* in the wild. In the same related study (Chapter 2), over 39% of plants were associated with 60% or greater bare soil. The bare soil experimental treatment was unaltered, and seeds were deposited directly on the soil surface.

Soil treatments

Three soil types, dominant on the project site, were collected by Taryn Kong and Jeffrey Fehmi (Fehmi et al. 2008). Samples were collected from a depth of up to 3 m (10 ft) in an effort to simulate potential disturbance conditions, and were trucked to the CAC. Soil was sifted to exclude cobbles greater than 5x5 cm (2x2 in). Subsamples from these collections were set aside for laboratory analysis (Table 1.1).

Arkose is a red, gravelly soil derived from a sedimentary conglomerate mix of siltstones and sandstones from the Willow Canyon Formation. Likely due to the depths from which it was collected, the particle size was very large, with minimal fine particulates less than 2 mm (0.1 in). Glance soil is lighter in color and finer-textured than Arkose, and derived from conglomerate limestone. Gila soil, derived from a late Tertiary alluvium, is also lighter in color than Arkose soil, with a finer texture.

Table 1.1. Characteristics from laboratory analyses of soil samples collected from the northeastern Santa Rita Mountain Range, Pima County, Arizona.

Soil Type	Texture (%)				Classification	pH	NO_3 (ppm)	PO_4 (ppm)	K (ppm)
	Sand	*Silt*	*Clay*	*Gravel*					
Arkose	88	8	4	81	sand	7.1	1	4	64
Gila	68	18	4	43	sandy loam	8.2	2	6	65
Glance	76	18	6	57	loamy sand	8.4	2	3	135

*** Gravel size in analysis was determined to be 2-50 mm (0.1-2.0 in).**

In initiating preparations for the agave germination study, I cleaned the pots of soil used in the previous experiment from roots, stubble, and mulch. I combined like soil types of high, average, and low irrigation treatments from the previous experiment into a cement mixer in order to thoroughly mix any leached or compacted substrates into a uniform soil base. I refilled the pots to a soil level 7 cm (3 in) below the pot rim.

Data collection and abiotic measurements

During the summer monsoon session, pots were inventoried every 2-3 weeks, and emergence and unfurling leaves were documented. A seedling was considered emerged if its cotyledon was clearly visible. Autumn inventories occurred monthly. As leaves unfurled and maturing seedlings developed color and spines, the chronology of this process was recorded. At the end of the autumn period, all seedlings were harvested, and above-ground vegetation was measured, dried at 60° C (140 °F) for 10 days, and weighed.

Abiotic conditions in the greenhouse were recorded. Soil volumetric water content (VWC) was measured using a Field Scout TDR 100 soil moisture meter

(Spectrum Technologies, Inc., Plainfield, IL). Measurements were conducted in every pot on 14 Nov 2009, approximately 24 hours after watering. Temperature and relative humidity were measured in each of the 18-treatment combinations for Gila soil types starting in early September until the end of the experiment using Onset hobo dataloggers (model U23-002, Onset Corporation, Pocasset, MA). Sensor locations were selected randomly from the center of the greenhouse, in order to average any potential temperature and humidity differences between north and south ends. Data-collecting sensors were attached to the base of the irrigation emitter, approximately 5 cm (2 in) above the soil surface.

To create a baseline for what temperature and humidity conditions might be expected in the field during the same time period and how greenhouse conditions may differ, 6 Onset hobo dataloggers (model U23-001, Onset Corporation, Pocasset, MA) were set up within 10 cm (4 in) of young agaves at the study site in the field. Plants were selected arbitrarily based on size [<10 cm (< 4 in)] and number of leaves (≤ 4), as it was impossible to determine age conclusively. Half of the young plants selected were growing in deep shade (>75% canopy cover), and half of the plants were in the open on exposed, grassy knolls. Sensors were 5 cm (2 in) above the soil surface. Temperature and relative humidity measures from both greenhouse and field seedlings were recorded from 19 Sep 2009-2 Feb 2010.

Parent effect

Based on seed viability results from the growth chamber trials, I decided to sow 10 seeds, one from each of 10 parents, in each pot in the greenhouse experiment in order to maximize the potential for at least one seed to germinate. Ten parents with enough seed remaining were selected randomly from the 26-parent cohort.

A circular planting frame was created with 10 equally-spaced divisions. Planting locations were randomized for each parent in each pot, and every seed was carefully placed in its pre-determined planting slot so that parentage could be tracked through emergence and establishment. I sowed each of 270 pots with 10 seeds, amounting to 2700 seeds available to respond to experimental treatments.

Analysis

JMP software (version 8.0) was used for all statistical analyses. Probability values for significance were set at 0.05.

Growth chamber trials

I used ANOVA tests to assess whether *A. palmeri* seeds are photosensitive, with the number of seeds that germinated as the response variable, and parent, repetition, growth chamber shelf (top or bottom), and treatment (light or dark) as the explanatory variables. Simple summary statistics were performed on viability

trial data to determine expected mean rates of emergence of both individuals and the group, and individuals were ranked by their rates of emergence.

Greenhouse experiment

I measured five seedling response variables, averaged across each pot (experimental unit): number that emerged, number that survived to the end of the study, height, number of leaves, and dry weight (biomass). Three of five response variables were transformed to generate a tighter normal quantile plot, including total seedling emergence and total seedlings surviving (arc sine), and seedling dry weight (natural log). To further confirm the appropriateness of each approach to data transformation, I assessed the heterogeneity of variance using the Brown-Forsythe test (Brown and Forsythe 1974).

I used full factorial ANOVA tests for each of the five response variables, with the explanatory variables including: soil type, precipitation level, surface mulch, and shade. Block was added as a random effect. Insignificant explanatory variables were eliminated from the full model, and re-run to further refine the results. I compared multiple means between treatments using Tukey's HSD tests, except for main shade effects where Student's t-test comparisons were used. A Spearman's P rank correlation was used to inspect the relationships between response variables.

I ran an additional multifactor ANOVA test to assess the differences in volumetric water content between treatments. Weekly means for 6 months of

temperature and relative humidity data were compiled and compared between field and greenhouse data loggers.

Parent effects were assessed by comparing the ranking of seedling emergence per parent in the growth chamber trials with the seedling emergence per parent in the greenhouse experiment. A Spearman's P rank correlation was performed on the seed response between parents in growth chamber and greenhouse experiments.

Results

Growth chamber trials

A total of 910 seeds germinated over the course of two, 21-day trials (44% total germination). Per parent, total germination ranged from 8-78%, with 65% of parents ranging between 30-60% germination. There appeared to be significant variation within the growth chamber based on higher germination rates from the bottom shelf (mean over experiments: 66%) than from the top shelf (mean over experiments: 22%).

Greenhouse experiment

Five ANOVA models were used to test significance for both seedling emergence (total emerged, survivorship), and establishment characteristics (number of leaves, height, dry weight). In order to streamline reporting, the

following results are grouped by main effects and interactions for emergence and establishment characteristics. Only significant tests are reported.

Seedling emergence and survivorship

In total, 754 seedlings (28%) emerged over the 171-day experiment (15 Aug 2009 -1 Feb 2010). Thirty-six emerged seedlings died over the same period, resulting in a 5% overall mortality rate. Seedling deaths occurred in 33 pots (out of 270) and 22 unique treatments (out of 54). Based on summary statistics, no trends were identified between seedling mortality and experimental treatments.

In assessing the affect of treatments on surviving seedlings, significance tests were virtually identical to those of total emergence with means varying no more than 4%, and thus results for survivorship are not reported here. However, one additional interacting treatment became significant—shade X surface treatment.

Each of the four main effect treatments was significantly associated with seedling emergence (R^2=0.77). In irrigation level treatments (Table 1.2), high, average, and low levels differed significantly in mean seedling emergence; high levels had 3 times more mean emergence than low levels. Shaded treatments (Table 1.2) had significantly more emergence than unshaded treatments, with more than 3 times more seedlings. Each of the three surface treatments (Table 1.2) exhibited significantly different mean seedling emergence; straw was associated with double the emergence of gravel and five times that of bare soil. In soil treatments (Table

1.2), Glance and Gila soils had similar seedling emergence, and both had three times more emergence than Arkose soil treatments.

Table 1.2. Least square means (± SE) for total *A. palmeri* seedling emergence associated with main effect treatments in experiment from 15 Aug 2009-2 Feb 2010.

Treatment grouping	Treatment	Total mean emergence (%) ◆
Irrigation level	high	32.9 ± 1.16 **A**
	average	26.9 ± 1.16 **B**
	low	10.8 ± 1.16 **C**
Shade	shade	38.0 ± 0.98 **A**
	no shade	10.4 ± 0.98 **B**
Surface	straw	42.1 ± 1.16 **A**
	gravel	21.5 ± 1.16 **B**
	bare soil	8.8 ± 1.16 **C**
Soil type	Glance	30.9 ± 1.16 **A**
	Gila	29.6 ± 1.16 **A**
	Arkose	10.3 ± 1.16 **B**

◆ Means followed by different letters indicate a significant difference (*P* ≤ 0.05) within like treatment groupings, based on Tukey's HSD tests and Student's t-test (shade effect only).

In the irrigation level X soil type interaction (Table 1.3), high and average irrigation levels coupled with Glance and Gila soils had significantly higher mean seedling emergence than both low irrigation treatments on like substrates. Arkose soil exhibited similar emergence across all irrigation levels.

Table 1.3. Least square means (±SE) for total *A. palmeri* seedling emergence associated with irrigation level X soil type treatment interaction in experiment from 15 Aug 2009-2 Feb 2010.

Treatments		**Total mean emergence (%)** ◆
Irrigation level	***Soil type***	
high	Glance	47.0 ± 1.92 **A**
high	Gila	43.4 ± 1.92 **A**
average	Glance	37.0 ± 1.92 **A**
average	Gila	35.3 ± 1.92 **A**
low	Gila	13.1 ± 1.92 **B**
low	Glance	12.1 ± 1.92 **B**
high	Arkose	12.0 ± 1.92 **B**
average	Arkose	11.4 ± 1.92 **B**
low	Arkose	7.7 ± 1.92 **B**

◆ Means followed by different letters indicate a significant difference (*P* ≤ 0.05) based on Tukey's HSD test.

In the surface X soil type interaction (Table 1.4), Glance and Gila soils were associated with the most seedling emergence, particularly when coupled with straw mulch. Although overall emergence on Arkose soil was low, straw mulch had a similar affect on this soil type, increasing total emergence over that of bare soil. Bare soil treatments of all soil types had the least seedling emergence.

Table 1.4. Least square means (±SE) for total *A. palmeri* seedling emergence associated with surface X soil type treatment interaction in experiment from 15 Aug 2009-2 Feb 2010.

Treatments		**Total mean emergence (%)** ◆
Surface	***Soil type***	
straw	Glance	59.5 ± 1.92 **A**
straw	Gila	55.9 ± 1.92 **A**
gravel	Glance	25.5 ± 1.92 **B**
gravel	Gila	24.5 ± 1.92 **B**
gravel	Arkose	15.0 ± 1.92 **B C**
straw	Arkose	14.4 ± 1.92 **B C**
bare soil	Gila	12.5 ± 1.92 **C**
bare soil	Glance	12.0 ± 1.92 **C**
bare soil	Arkose	3.5 ± 1.92 **D**

◆ Means followed by different letters indicate a significant difference (*P* ≤ 0.05) based on Tukey's HSD test.

After excluding seedlings that died during the experiment, shade X surface treatment became a significant factor in affecting emergence (Table 1.5). Shaded straw treatments had the highest mean emergence for survivors, and unshaded bare soil treatments had the lowest.

Table 1.5. Least square means (±SE) for *A. palmeri* survivor and total seedling emergence associated with shade X surface treatment interaction in experiment from 15 Aug 2009-2 Feb 2010.

Treatments		Survivor mean emergence (%) ◆	Total mean emergence (%) ◆
Shade	***Surface***		
shade	straw	52.7 ± 1.59 **A**	55.9 ± 1.59 **A**
shade	gravel	38.1 ± 1.59 **B**	39.4 ± 1.59 **B**
no shade	straw	27.9 ± 1.59 **B**	28.8 ± 1.59 **C**
shade	bare soil	17.8 ± 1.59 **C**	20.2 ± 1.59 **C**
no shade	gravel	7.4 ± 1.59 **D**	7.6 ± 1.59 **D**
no shade	bare soil	1.5 ± 1.59 **E**	1.5 ± 1.59 **E**

◆ Means followed by different letters indicate a significant difference ($P \leq 0.05$) based on Tukey's HSD test.

In the third order interaction irrigation X shade X surface (Table 1.6), shaded, straw treatments had the highest emergence, even across low irrigation levels. Mean emergence was lowest in average and low irrigation, unshaded, bare soil treatments.

Table 1.6. Least square means (±SE) for total *A. palmeri* seedling emergence associated with irrigation level X shade X surface treatment interaction in experiment from 15 Aug 2009-2 Feb 2010.

Treatments			Total mean emergence (%) ◆
Irrigation level	***Shade***	***Surface***	
high	shade	straw	62.8 ± 2.68 **A**
average	shade	straw	59.4 ± 2.68 **A B**
high	shade	gravel	49.2 ± 2.68 **A B C**
low	shade	straw	45.2 ± 2.68 **A B C**
average	no shade	straw	43.0 ± 2.68 **A B C**
average	shade	gravel	39.2 ± 2.68 **B C**
high	shade	bare soil	38.1 ± 2.68 **B C**
high	no shade	straw	38.0 ± 2.68 **B C**
low	shade	gravel	32.1 ± 2.68 **C D**
average	shade	bare soil	30.7 ± 2.68 **C D**
high	no shade	gravel	17.0 ± 2.68 **D E**
low	no shade	straw	9.7 ± 2.68 **E F**
average	no shade	gravel	8.2 ± 2.68 **E F G**
high	no shade	bare soil	4.9 ± 2.68 **E F G**
low	shade	bare soil	1.7 ± 2.68 **F G**
low	no shade	gravel	1.2 ± 2.68 **F G**
average	no shade	bare soil	0.7 ± 2.68 **F G**
low	no shade	bare soil	0* **G**

◆ Means followed by different letters indicate significant differences (*P* ≤ 0.05) based on Tukey's HSD test.
*** No seedlings emerged in this treatment combination.**

Seedling establishment characteristics

The four main effect treatments were significantly associated with each of the seedling growth response variables: number of leaves (R^2=0.55), height (R^2=0.57), and dry weight (R^2=0.53).

High and average level irrigation treatments (Table 1.7) exhibited similar mean seedling heights and were significantly taller than low level treatments. In seedling number of leaves and dry weight responses, each irrigation level was associated with significantly different results. The most leaves and heaviest

seedling weights were consistently supported by high irrigation treatments and the fewest leaves and lightest seedling weights attributed to low irrigation treatments.

Across all measurement variables, seedlings in shaded treatments were significantly larger than those in unshaded treatments, with roughly double the heights and dry weights (Table 1.7).

Table 1.7. Least square means (±SE) for *A. palmeri* seedling establishment characteristic measurements associated with main effect treatments in experiment from 15 Aug 2009-2 Feb 2010.

Treatment grouping	Treatment	Mean number of leaves ⬩	Mean seedling height (mm) ⬩	Mean dry weight (g) ⬩
Irrigation level	high	2.4 ± 0.11 **A**	21.1 ± 1.56 **A**	0.088 ± 0.12 **A**
	average	2.0 ± 0.11 **B**	19.7 ± 1.56 **A**	0.059 ± 0.12 **B**
	low	1.1 ± 0.11 **C**	12.7 ± 1.56 **B**	0.022 ± 0.12 **C**
Shade	shade	2.2 ± 0.09 **A**	23.1 ± 1.46 **A**	0.076 ± 0.11 **A**
	no shade	1.5 ± 0.09 **B**	12.6 ± 1.46 **B**	0.032 ± 0.11 **B**
Surface	straw	2.3 ± 0.11 **A**	24.9 ± 1.56 **A**	0.094 ± 0.12 **A**
	gravel	1.9 ± 0.11 **B**	18.8 ± 1.56 **B**	0.055 ± 0.12 **B**
	bare soil	1.3 ± 0.11 **C**	9.8 ± 1.56 **C**	0.022 ± 0.12 **C**
Soil type	Gila	2.1 ± 0.11 **A**	19.4 ± 1.56 **A**	0.073 ± 0.12 **A**
	Glance	2.0 ± 0.11 **A**	19.1 ± 1.56 **A**	0.060 ± 0.12 **A**
	Arkose	1.4 ± 0.11 **B**	15.1 ± 1.56 **B**	0.027 ± 0.12 **B**

⬩ Means followed by different letters indicate a significant difference ($P \leq 0.05$) within like treatment groupings based on Tukey's HSD tests and Student's t-test (shade effects only).

Each of the three surface treatments exhibited significantly different seedling measurements, with straw mulch consistently promoting the largest seedlings, and bare soil the smallest seedlings (Table 1.7).

Across all measurements, seedlings grown on Gila and Glance soil types were statistically similar to each other, and significantly larger in height, number of leaves, and dry weight than seedlings grown on Arkose soil (Table 1.7).

In the irrigation X surface interaction, high and average irrigation levels coupled with straw and gravel mulches were associated with seedlings with the most leaves (Table 1.8) and the heaviest dry weights (Table 1.9). In contrast, low irrigation levels on gravel and bare soil treatments exhibited the fewest leaves (Table 1.8) and lightest seedling weights (Table 1.9).

Table 1.8. Least square means (±SE) for *A. palmeri* seedling number of leaves associated with irrigation level X surface treatment interaction in experiment from 15 Aug 2009-2 Feb 2010.

Treatments		**Mean number of leaves ◆**
Irrigation level	***Surface***	
high	straw	2.6 ± 0.17 **A**
average	straw	2.5 ± 0.17 **A**
high	gravel	2.4 ± 0.17 **A**
high	bare soil	2.1 ± 0.17 **A B**
average	gravel	2.0 ± 0.17 **A B**
low	straw	1.9 ± 0.17 **A B C**
average	bare soil	1.5 ± 0.17 **B C**
low	gravel	1.3 ± 0.17 **C**
low	bare soil	0.3 ± 0.17 **D**

◆ Means followed by different letters indicate significant differences (*P* ≤ 0.05) based on Tukey's HSD test.

Table 1.9. Least square means (±SE) for *A. palmeri* seedling dry weights associated with irrigation level X surface treatment interaction in experiment from 15 Aug 2009-2 Feb 2010.

Treatments		**Mean dry weight (g)** ◆
Irrigation level	***Surface***	
high	straw	0.121 ± 0.19 **A**
average	straw	0.110 ± 0.19 **A**
high	gravel	0.092 ± 0.19 **A**
average	gravel	0.065 ± 0.19 **A B**
low	straw	0.061 ± 0.19 **A B**
high	bare soil	0.060 ± 0.19 **A B**
average	bare soil	0.026 ± 0.19 **B**
low	gravel	0.025 ± 0.19 **B**
low	bare soil	0.003 ± 0.19 **C**

◆ Means followed by different letters indicate significant differences (*P* ≤ 0.05) based on Tukey's HSD test.

The shade X surface interaction (Table 1.10) produced similar trends in seedling dry weight and mean number of leaves. In general, shaded straw and gravel treatments produced larger seedlings with more leaves and heavier dry weights than unshaded, bare soil and gravel treatments.

Table 1.10. Least square means (±SE) for *A. palmeri* seedling number of leaves and dry weights associated with shade X surface treatment interaction in experiment from 15 Aug 2009-2 Feb 2010.

Treatments		**Mean dry weight (g)** ◆	**Mean number of leaves** ◆
Shade	***Surface***		
shade	straw	0.111 ± 0.16 **A**	2.4 ± 0.14 **A**
shade	gravel	0.087 ± 0.16 **A**	2.3 ± 0.14 **A**
no shade	straw	0.080 ± 0.16 **A B**	2.2 ± 0.14 **A**
shade	bare soil	0.044 ± 0.16 **B C**	1.9 ± 0.14 **A B**
no shade	gravel	0.033 ± 0.16 **C**	1.5 ± 0.14 **B**
no shade	bare soil	0.009 ± 0.16 **D**	0.7 ± 0.14 **C**

◆ Means followed by different letters indicate significant differences (*P* ≤ 0.05) based on Tukey's HSD test.

In the irrigation X soil type interaction (Table 1.11), high irrigation levels coupled with Gila and Glance soils produced the heaviest seedlings, and low irrigation levels of all soil types produced the lightest seedlings.

Table 1.11. Least square means (±SE) for *A. palmeri* seedling dry weights associated with irrigation level X soil type interaction in experiment from 15 Aug 2009-2 Feb 2010.

Treatments		Mean dry weight (g) ◆
Irrigation level	***Soil type***	
high	Gila	0.135 ± 0.19 **A**
high	Glance	0.112 ± 0.19 **A**
average	Gila	0.102 ± 0.19 **A B**
average	Glance	0.077 ± 0.19 **A B**
high	Arkose	0.043 ± 0.19 **B C**
low	Gila	0.026 ± 0.19 **C**
average	Arkose	0.023 ± 0.19 **C**
low	Glance	0.022 ± 0.19 **C**
low	Arkose	0.019 ± 0.19 **C**

◆ **Means followed by different letters indicate significant differences (*P* ≤ 0.05) based on Tukey's HSD test.**

A third order interaction irrigation X shade X surface was significant for all seedling measurement responses. In this treatment combination, no emergence occurred in low irrigation, unshaded, bare soil treatments.

High and average irrigation levels in shaded treatments of all surface types exhibited significantly more leaves, while low irrigation, unshaded gravel and bare soil treatments had significantly fewer leaves (Table 1.12).

Table 1.12. Least square means (±SE) for *A. palmeri* seedling number of leaves associated with irrigation level X shade X surface treatment interaction in experiment from 15 Aug 2009-2 Feb 2010.

Treatments			Mean number of leaves ⬩
Irrigation level	***Shade***	***Surface***	
high	shade	straw	2.8 ± 0.24 **A**
high	shade	bare soil	2.7 ± 0.24 **A B**
high	shade	gravel	2.5 ± 0.24 **A B**
average	no shade	straw	2.5 ± 0.24 **A B**
average	shade	bare soil	2.5 ± 0.24 **A B**
average	shade	straw	2.4 ± 0.24 **A B**
high	no shade	straw	2.4 ± 0.24 **A B**
average	shade	gravel	2.3 ± 0.24 **A B**
high	no shade	gravel	2.3 ± 0.24 **A B**
low	shade	gravel	2.1 ± 0.24 **A B**
low	shade	straw	2.0 ± 0.24 **A B**
average	no shade	gravel	1.8 ± 0.24 **A B C**
low	no shade	straw	1.7 ± 0.24 **A B C**
high	no shade	bare soil	1.6 ± 0.24 **B C D**
low	shade	bare soil	0.6 ± 0.24 **C D E**
average	no shade	bare soil	0.5 ± 0.24 **D E**
low	no shade	gravel	0.4 ± 0.24 **E**
low	no shade	bare soil	0* **E**

⬩ Means followed by different letters indicate significant differences (*P* ≤ 0.05) based on Tukey's HSD test.
*** Zero emergence occurred in this treatment combination.**

Shade and surface mulch seemed to have the strongest influence on mean seedling height (Table 1.13). Shaded straw mulched treatments of all irrigation levels were significantly taller than unshaded bare soil and gravel treatments.

Table 1.13. Least square means (±SE) for *A. palmeri* seedling heights associated with irrigation level X shade X surface treatment interaction in experiment from 15 Aug 2009-2 Feb 2010.

Treatments			**Mean height (mm)** ◆
Irrigation level	***Shade***	***Surface***	
high	shade	straw	31.4 ± 2.64 **A**
average	shade	straw	31.2 ± 2.64 **A B**
low	shade	straw	27.4 ± 2.64 **A B C**
average	shade	gravel	24.8 ± 2.64 **A B C D**
high	shade	gravel	24.2 ± 2.64 **A B C D**
average	no shade	straw	23.4 ± 2.64 **A B C D**
low	shade	gravel	23.1 ± 2.64 **A B C D**
high	shade	bare soil	21.1 ± 2.64 **A B C D E**
high	no shade	straw	20.3 ± 2.64 **A B C D E**
high	no shade	gravel	19.7 ± 2.64 **B C D E**
average	shade	bare soil	18.9 ± 2.64 **C D E**
average	no shade	gravel	16.3 ± 2.64 **C D E F**
low	no shade	straw	15.5 ± 2.64 **D E F G**
high	no shade	bare soil	10.0 ± 2.64 **E F G H**
low	shade	bare soil	5.8 ± 2.64 **F G H**
low	no shade	gravel	4.5 ± 2.64 **G H**
average	no shade	bare soil	3.3 ± 2.64 **H**
low	no shade	bare soil	0* **H**

◆ Means followed by different letters indicate significant differences ($P \leq 0.05$) based on Tukey's HSD test.
*** Zero emergence occurred in this treatment combination.**

Shaded, high and average irrigation level treatments of all surface types were associated with the heaviest seedlings, while unshaded, low irrigation levels had the lightest weight seedlings, particularly in bare soil treatments (Table 1.14).

Table 1.14. Least square means (±SE) for *A. palmeri* seedling dry weights associated with irrigation level X shade X surface treatment interaction in experiment from 15 Aug 2009-2 Feb 2010.

Treatments			Mean dry weight (g) ◆
Irrigation level	***Shade***	***Surface***	
high	shade	straw	0.147 ± 0.25 **A**
average	shade	straw	0.116 ± 0.25 **A B**
high	shade	gravel	0.114 ± 0.25 **A B**
average	no shade	straw	0.104 ± 0.25 **A B**
high	shade	bare soil	0.100 ± 0.25 **A B**
high	no shade	straw	0.100 ± 0.25 **A B**
average	shade	gravel	0.085 ± 0.25 **A B**
low	shade	straw	0.078 ± 0.25 **A B**
average	shade	bare soil	0.075 ± 0.25 **A B**
high	no shade	gravel	0.075 ± 0.25 **A B**
low	shade	gravel	0.069 ± 0.25 **A B**
average	no shade	gravel	0.049 ± 0.25 **A B**
low	no shade	straw	0.047 ± 0.25 **A B**
high	no shade	bare soil	0.034 ± 0.25 **B C**
low	shade	bare soil	0.007 ± 0.25 **C D**
low	no shade	gravel	0.006 ± 0.25 **C D**
average	no shade	bare soil	0.005 ± 0.25 **C D**
low	no shade	bare soil	0* **D**

◆ Means followed by different letters indicate significant differences (*P* ≤ 0.05) based on Tukey's HSD test.
*** Zero emergence occurred in this treatment combination.**

In the final interaction, irrigation X shade X soil type, high and average irrigation levels coupled with Gila and Glance soil types had significantly more leaves than low irrigation levels of all soil types, particularly in unshaded treatments (Table 1.15). Unshaded Arkose treatments had the fewest leaves, regardless of irrigation level.

Table 1.15. Least square means (±SE) for *A. palmeri* seedling number of leaves associated with irrigation level X shade X soil type treatment interaction in experiment from 15 Aug 2009-2 Feb 2010.

Treatments			**Mean number of leaves ◆**
Irrigation level	***Shade***	***Soil type***	
high	shade	Gila	2.9 ± 0.24 **A**
average	shade	Gila	2.8 ± 0.24 **A B**
high	no shade	Gila	2.7 ± 0.24 **A B**
high	shade	Glance	2.6 ± 0.24 **A B**
high	no shade	Glance	2.6 ± 0.24 **A B**
average	shade	Glance	2.6 ± 0.24 **A B**
high	shade	Arkose	2.5 ± 0.24 **A B**
average	no shade	Gila	2.0 ± 0.24 **A B C**
average	no shade	Glance	1.9 ± 0.24 **A B C D**
average	shade	Arkose	1.8 ± 0.24 **A B C D E**
low	shade	Gila	1.8 ± 0.24 **A B C D E F**
low	shade	Glance	1.6 ± 0.24 **B C D E F**
low	shade	Arkose	1.3 ± 0.24 **C D E F**
high	no shade	Arkose	0.9 ± 0.24 **C D E F**
average	no shade	Arkose	0.8 ± 0.24 **C D E F**
low	no shade	Glance	0.8 ± 0.24 **D E F**
low	no shade	Arkose	0.7 ± 0.24 **E F**
low	no shade	Gila	0.6 ± 0.24 **F**

◆ Means followed by different letters indicate significant differences (*P* ≤ 0.05) based on Tukey's HSD test.

Emergence and establishment correlations

Spearman's P rank correlation results compiled by treatment combinations (N=54) indicate that all seedling emergence and establishment variables are positively and significantly correlated (Table 1.16). Total emergence and total survived data were mean proportions. Establishment data (number of leaves, dry weight, height) were mean measurements.

Table 1.16. Spearman's P rank correlation coefficients from five *A. palmeri* seedling response variables in experiment from 15 Aug 2009-2 Feb 2010. (N=54) [Significance threshold: r = 0.27 (α=0.05) (Snedecor 1956)].

	Dry weight	Height	Total emergence	Total survived
Number of leaves	0.970	0.800	0.866	0.864
Dry weight		0.874	0.906	0.905
Height			0.906	0.910
Total emergence				0.996

Abiotic influences

Volumetric water content, based on one day of measurements (14 Nov 2009) was significantly influenced by 3 main effects: irrigation level, shade, and soil type (R^2=0.87). High and average irrigation levels were almost equal in VWC, and both were significantly higher than the low irrigation level (Table 1.17). Shaded treatments were significantly higher than unshaded treatments (Table 1.17). Each soil type had a significantly different VWC content; Glance soil had the highest content and was almost 4 times greater than Arkose soil with the lowest content (Table 1.17).

Table 1.17. Least square means (±SE) for soil volumetric water content associated with significant main effect treatments in experiment on 14 Nov 2009.

Treatment grouping	Treatment	Mean VWC (%) ◆
Irrigation level	high	42.1 ± 0.72 **A**
	average	42.0 ± 0.72 **A**
	low	31.4 ± 0.72 **B**
Shade	shade	39.6 ± 0.65 **A**
	no shade	37.3 ± 0.65 **B**
Soil type	Glance	60.1 ± 0.72 **A**
	Gila	42.9 ± 0.72 **B**
	Arkose	15.5 ± 0.72 **C**

◆ Means followed by different letters indicate significant differences ($P \leq 0.05$) within like treatment groupings based on Tukey's HSD tests and Student's t-test (shade effect only).

In the irrigation X soil type interaction, Gila soil treated with high and average irrigation levels had the highest VWC, and Arkose soil treatments of all irrigation level combinations had the lowest VWC (Table 1.18).

Table 1.18. Least square means (±SE) for soil volumetric water content associated with irrigation level X soil type treatment interaction in experiment on 14 Nov 2009.

Treatment		Mean VWC (%) ◆
Irrigation level	***Soil type***	
average	Gila	67.0 ± 1.04 **A**
high	Gila	64.8 ± 1.04 **A**
low	Gila	48.1 ± 1.04 **B**
average	Glance	47.1 ± 1.04 **B**
high	Glance	46.7 ± 1.04 **B**
low	Glance	34.9 ± 1.04 **C**
high	Arkose	17.1 ± 1.04 **D**
average	Arkose	15.4 ± 1.04 **D**
low	Arkose	14.1 ± 1.04 **D**

◆ Means followed by different letters indicate significant differences ($P \leq 0.05$) based on Tukey's HSD test.

The irrigation X surface interaction showed average and high irrigation levels on bare soil surface treatments were highest in VWC, and low irrigation levels of gravel and bare soil surface treatments were lowest in VWC (Table 1.19).

Table 1.19. Least square means (±SE) for soil volumetric water content associated with irrigation level X surface treatment interaction in experiment on 14 Nov 2009.

Treatment		Mean VWC (%) ◆
Irrigation level	***Surface***	
average	bare soil	45.3 ± 1.04 **A**
high	bare soil	43.6 ± 1.04 **A B**
high	gravel	42.5 ± 1.04 **A B**
average	straw	41.0 ± 1.04 **A B**
average	gravel	40.1 ± 1.04 **A B**
high	straw	39.8 ± 1.04 **A B**
low	straw	38.0 ± 1.04 **B C**
low	gravel	31.8 ± 1.04 **C**
low	bare soil	24.8 ± 1.04 **D**

◆ Means followed by different letters indicate significant differences (*P* ≤ 0.05) based on Tukey's HSD test.

In general, irrigation X surface X soil treatment combinations on Gila soil had the highest VWC, and all like treatment combinations on Arkose soil had the lowest (Table 1.20). On each soil type, bare soil seemed to have the highest VWC of all surface types.

Table 1.20. Least square means (±SE) for soil volumetric water content associated with irrigation level X surface X soil type treatment interaction in experiment on 14 Nov 2009.

Treatment			**Mean VWC (%) ◆**
Irrigation level	***Surface***	***Soil type***	
average	bare soil	Gila	70.6 ± 1.67 **A**
high	bare soil	Gila	67.2 ± 1.67 **A**
average	straw	Gila	67.2 ± 1.67 **A**
high	gravel	Gila	65.2 ± 1.67 **A**
average	gravel	Gila	63.3 ± 1.67 **A B**
high	straw	Gila	61.9 ± 1.67 **A B**
low	straw	Gila	60.0 ± 1.67 **A B C**
low	gravel	Gila	49.7 ± 1.67 **B C D**
high	bare soil	Glance	49.2 ± 1.67 **B C D E**
average	bare soil	Glance	49.0 ± 1.67 **C D E**
average	gravel	Glance	46.9 ± 1.67 **C D E**
high	straw	Glance	46.1 ± 1.67 **C D E**
average	straw	Glance	45.5 ± 1.67 **D E**
high	gravel	Glance	44.8 ± 1.67 **D E**
low	straw	Glance	42.5 ± 1.67 **D E F**
low	gravel	Glance	37.5 ± 1.67 **E F**
low	bare soil	Gila	34.9 ± 1.67 **F G**
low	bare soil	Glance	25.3 ± 1.67 **G H**
high	gravel	Arkose	19.6 ± 1.67 **G H**
average	bare soil	Arkose	18.3 ± 1.67 **G H**
high	bare soil	Arkose	17.0 ± 1.67 **G H**
low	bare soil	Arkose	15.4 ± 1.67 **G H**
high	straw	Arkose	14.9 ± 1.67 **G H**
low	straw	Arkose	14.9 ± 1.67 **G H**
average	straw	Arkose	14.1 ± 1.67 **G H**
average	gravel	Arkose	14.0 ± 1.67 **G H**
low	gravel	Arkose	12.1 ± 1.67 **H**

◆ Means followed by different letters indicate significant differences (*P* ≤ 0.05) based on Tukey's HSD test.

Differences between greenhouse and field temperatures were observed, but shaded and unshaded treatments within those respective locations were not captured using present study methods (Figure 1.1). Weekly mean greenhouse temperatures averaged 9.3°C higher than field temperatures, and ranged from 14-

29°C (57-84°F) over the 20-week monitoring period, while field temperatures ranged from 5-23°C (41-73°F).

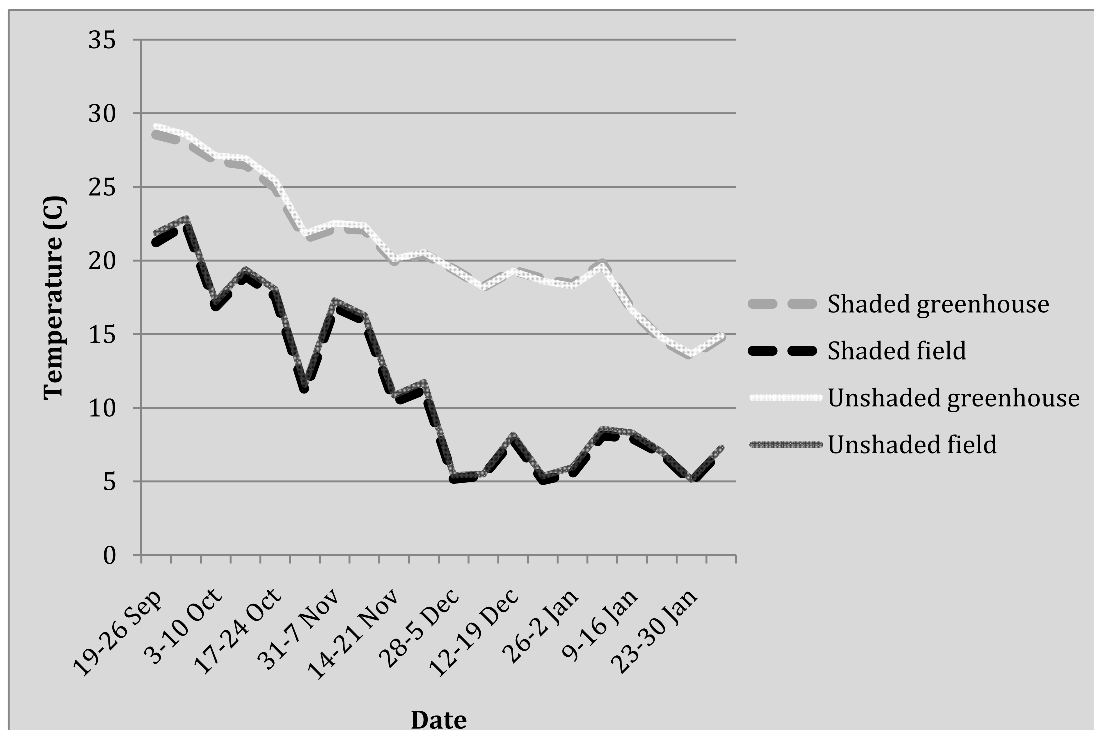

Figure 1.1. Weekly mean greenhouse and field temperatures in shaded and unshaded experimental treatments at 5 cm (2 in) from soil surface from 19 Sep 2009-2 Feb 2010.

Weekly mean differences in relative humidity between shaded and unshaded treatments in greenhouse and field locations were also negligible (Figure 1.2). The average difference in relative humidity between greenhouse and field weekly means was 3%. Weekly means in the greenhouse appear to be less variable than in the field, ranging from 33-68% over the 20-week monitoring period, compared to 23-80% in the field.

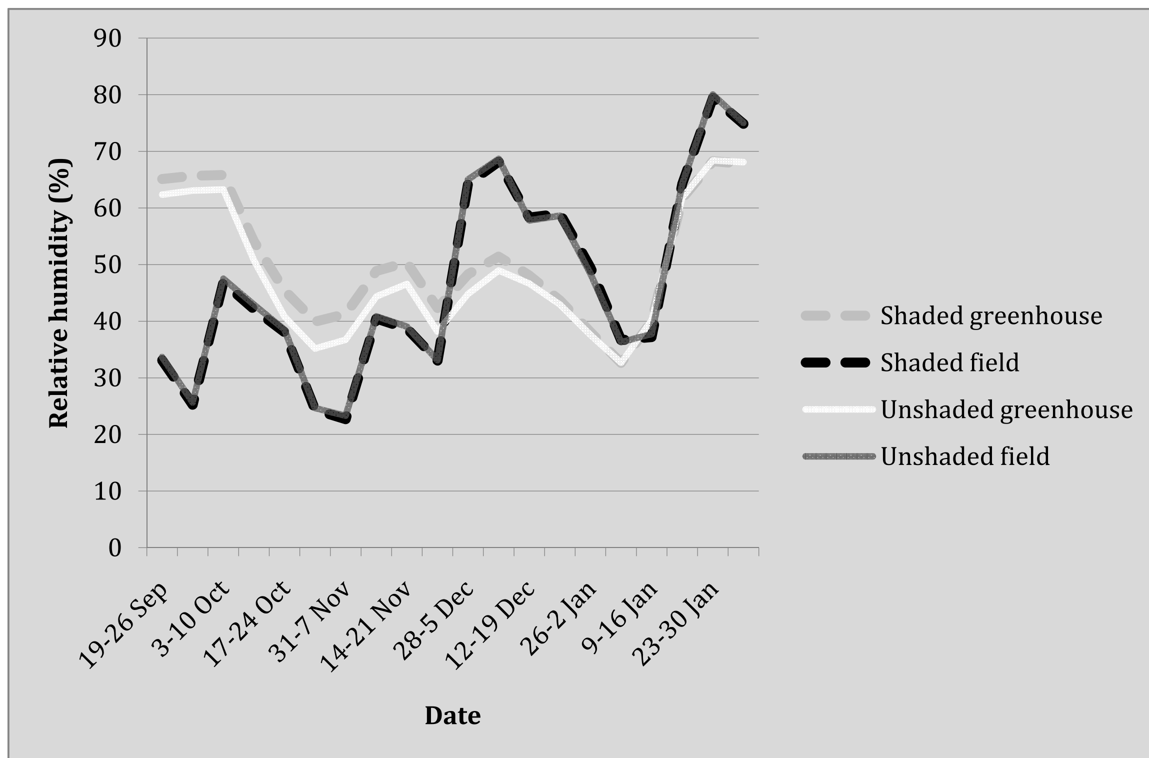

Figure 1.2. Weekly mean greenhouse and field relative humidity measures in shaded and unshaded experimental treatments at 5 cm (2 in) from soil surface from 19 Sep 2009-2 Feb 2010.

Parent effect

Differences in germination/emergence between parents were larger in the growth chamber (8-78%) than in the greenhouse (6-42%) (Table 1.21). Few patterns in individual parent viability were revealed between experiments, with the exception of parents 7 and 10, exhibiting consistently lower emergence than their cohort. Spearman's P rank correlation found no relationship between parent emergence in growth chamber and greenhouse experiments (r = 0.41) [significance threshold: r = 0.63 (α=0.05)] (Snedecor 1956).

Table 1.21. Mean *A. palmeri* germination/emergence per parent and associated rank from highest to lowest in both growth chamber and greenhouse experiments.

Parent #	Growth chamber		Greenhouse	
	% Germination	Rank	% Emergence	Rank
1	61.3	**3**	30.1	**6**
2	47.5	**6**	41.7	**1**
3	77.5	**1**	31.0	**5**
4	52.5	**4**	28.2	**7**
5	66.3	**2**	31.3	**3**
6	33.8	**8**	32.8	**2**
7	7.5	**10**	5.9	**10**
8	48.8	**5**	31.1	**4**
9	38.8	**7**	28.1	**8**
10	12.5	**9**	10.1	**9**
AVERAGE	44.7 %		27.0 %	

Discussion

Irrigation treatment

As has been reported for many other arid adapted species, water availability appeared to have a great affect on *A. palmeri* seedling response. High irrigation level treatments were associated with 1.2 times more emergence than average levels, and more than 3 times the emergence of low level treatments (Table 1.2). Further, high irrigation treatments produced significantly larger plants (number of leaves and dry weight) than average and low irrigation treatments (Table 1.7). Studies researching growth of agave seedlings from multiple species have shown that C_3 photosynthesis may be used by young plants, which then switch to CAM months or years later (Jordan and Nobel 1979, Nobel 1988, Nobel 2010). Employing this metabolic strategy allows young plants which are vulnerable to temperature extremes,

variable water availability, and herbivory, to grow more rapidly while they are developing defensive mechanisms to protect themselves. The utilization of this strategy requires ample water availability, which is also requisite for germination and seedling establishment (Jordan and Nobel 1979, Nobel 1988).

In assessing significant interactions, it is apparent that irrigation level is a crucial component to emergence, but its affect is difficult to extricate from related environmental variables. Of the 12 significant interactions (of 6 unique treatment combinations), 9 interactions (of 4 unique treatment combinations) included irrigation level, indicating that in addition to water availability, shade, surface mulches, and soil type were integral in affecting seedling response.

In the interaction irrigation level X soil type, emergence was highest and seedlings were largest in the fine-textured soils with greater silt and clay content (Glance and Gila) when treated with high and average irrigation levels (Table 1.3, 1.11). This is possibly due to the ability of these soils to retain moisture longer and lose water to evaporation more slowly than the almost exclusively coarse gravel content of Arkose soil. Indeed, Glance and Gila soils had significantly higher VWC in all analyses than Arkose soil (Tables 1.17, 1.18, 1.20). Additionally, the lower gravel content of Glance and Gila soils may have facilitated root permeability through the soil column, allowing root growth to develop more extensively and take advantage of soil nutrients, as well as access moisture deeper in the soil column where it would be slower to evaporate during the dry fall period (Martre et al. 2002). Gila and Glance soils coupled with high irrigation levels, were associated with the heaviest

seedling dry weights (Table 1.11). In contrast, Arkose soil treatments of all irrigation levels, and low irrigation levels of all soil types had the lowest emergence rates, as well as the lightest weight seedlings, suggesting that water availability is significantly affected by substrate (Table 1.3, 1.11).

In irrigation treatments X surface mulch interactions, seedlings had more leaves and heavier dry weights when associated with straw mulches and high or average irrigation levels (Tables 1.8, 1.9). The same treatment interactions tended to have fewer leaves and lighter dry weights in low irrigation treatments of all surface types, as well as average irrigation treatments coupled with bare soil. This may be attributable to mulches creating favorable microclimates at the soil surface through buffering high temperatures, conserving soil moisture, and maintaining a more consistent relative humidity (Jordan and Nobel 1979, Jalota and Prihar 1998, Post 2004).

Shade treatment

Shade appeared to provide an unequivocally favorable environment for seedlings, with more than three times the emergence, almost twice the height and dry weight, and 1.2 times more leaves than unshaded seedlings (Tables 1.2, 1.7). Decreased PAR can be limiting to plant growth, and it has been suggested that this is one of several tradeoffs for young agaves associated with nurses; slower growth in exchange for increased security against desiccation, tissue damage from extreme temperatures, herbivory, and access to soil nutrients (Nobel and Hartsock 1986,

Franco and Nobel 1988, 1989, Nolasco et al. 1997). However, in this experiment, where no herbivory or lack of moisture (in some treatments) was limiting, shaded treatments were associated with significantly larger and heavier seedlings than unshaded treatments, across all treatment combinations (Table 1.7, 1.10, 1.12-1.15). This may suggest that in addition to water limitation, another factor, like soil surface temperatures, had a strong affect in seedling response differences between shaded and unshaded treatments. Research by Turner et al. (1966) found that unshaded saguaro (*Carnegiea gigantea*) seedlings that were irrigated had significantly higher rates of mortality than shaded seedlings, with or without irrigation, and concluded that temperature plays an integral role in the germination and seedling establishment of succulent species. Studies on agave species have offered similar conclusions, indicating that seedlings are much more sensitive to soil and air temperature extremes than older plants, and that temperature tolerance increases as the plant volume and height (distance from soil surface) increases (Nobel 1984b, 1988; Franco and Nobel 1988).

Seven of 12 interactions (3 of 6 unique treatment combinations) involved shade treatments, with similar trends between them. In the shade X surface interaction, shaded treatments of all surface mulches had the most emergence, except straw mulches, which had high emergence even in unshaded treatments (Table 1.5). Straw mulches stood out consistently as associated with increased seedling emergence and size, independent of shade (Tables 1.5, 1.6, 1.10, 1.12-1.14), and it is possible that this mulch may be performing a similar function by lowering

soil temperatures and preserving moisture near the soil surface. The trend in shaded treatment seed response continued in the irrigation X shade X surface interaction; shaded, mulched treatments had higher emergence, particularly in high and average irrigation levels (Table 1.6). The highest overall emergence seen in the greenhouse experiment was in the high irrigation, shaded, straw mulch treatment combination [63% (SE=2.68)] (Table 1.6). In addition to high emergence, the largest values for seedling height, number of leaves, and longest leaf all appear to be driven by the presence of shade in this interaction (again, except unshaded straw treatments) (Tables 1.12-1.14). VWC was significantly higher in shaded verses unshaded treatments, but varied only by 2.3%, another indication that soil moisture may not have been the only driving factor for the positive seed response (Table 1.17). In contrast, unshaded, bare soil treatments, even coupled with high irrigation had ≤ 5% (SE=2.68) emergence and were consistently smallest and lightest, further suggesting that water limitation was not an exclusive driving factor in affecting seed response in this experiment (Tables 1.6, 1.12-1.14). Low irrigation, unshaded, bare soil treatments were the only treatment combination with zero emergence, suggesting that the low water availability and the exposed soil surface with minimal protected microsites and potentially high temperatures were unfavorable for seedling growth (Tables 1.12-1.14).

Although surface temperatures may be an important factor in emergence and establishment across treatments, differences between treatments were not observed with present study methods. Across the 18 greenhouse and 6 field data

loggers, measured temperatures in shaded and unshaded treatments were essentially the same. In the greenhouse, temperatures between shade, surface mulch, and irrigation level treatments were also very similar (Figure 1.1). The lack of any apparent difference in temperatures between treatments, particularly between shade treatments, is likely attributable to the location of the sensor. Temperature and relative humidity measurements were conducted 5 cm (2 in) from the soil surface, and not at the surface itself; thus differences were not captured.

Surface treatment

Although surface mulches (or the lack thereof) might not be considered a primary factor in affecting seedling growth, they clearly played an important contributing role in both emergence and early establishment of *A. palmeri* seedlings in this experiment. Of the 12 total interactions determined to be significant in analyses, 9 terms included surface treatment, including 4 out of 6 unique treatment combinations.

As a main effect, seedlings in the straw mulch treatment had the highest emergence [42% (SE=1.16)] in addition to the longest leaves, the tallest seedlings, the heaviest dry weights, and the second most number of leaves (high irrigation level was slightly more) (Tables 1.2, 1.7). The positive seedling response to straw as a main effect surpassed that of both high irrigation level and shade, the two factors that I had thought would have the strongest affect. Previous work researching the facilitative association between the bunchgrass *Hilaria rigida* and the hosted species

A. deserti and *Ferocactus acanthodes*, found that one of the primary benefits for seedlings associated with the bunchgrass were decreased soil temperatures, even though competition with the nurse plant for water and PAR negatively affected their growth (Franco and Nobel 1988, 1989). Similar features in straw mulch may be acting in an analogous way as this bunchgrass, decreasing soil temperatures and water loss, and increasing relative humidity-- thus providing favorable microsites for germination and establishment, but without competition for resources. Although I was not successful capturing temperature differences using my methods, straw has been shown in other studies to reduce temperatures significantly at the soil surface (Jalota and Prihar 1998).

Gravel mulches are common at the study site in association with a large population of *A. palmeri*. In a study by Li et al. (2005), gravel of the size used in this experiment was found to effectively intercept precipitation and retain moisture in the underlying soil, thus increasing the potential to facilitate emergence and establishment at low irrigation levels. Additionally, it is possible that gravel would create heterogeneous texture on the soil surface, maintaining favorable microsites for seed emergence with temporary shade and potentially cooler temperatures a result of increased soil moisture (Post et al. 2004, Peters et al. 2008). Although emergence was not as high as in straw treatments, gravel mulches were associated with more than double the emergence and almost double the seedling height and weight of bare soil treatments (Table 1.2, 1.7), indicating that the presence of surface texture was more favorable than none.

Bare soil was the least productive treatment, with only 9% (SE=1.16) emergence (Table 1.2). Without exception, interactions associated with the lowest emergence and smallest seedlings involved bare soil treatments, accompanied by low irrigation levels, Arkose soil, and unshaded treatments (Tables 1.4-1.6, 1.8-1.10, 1.12-1.14). This, again, indicates that a complexity of microsite conditions is critical to evaluate when considering the germination and establishment of this species.

Soil treatment

Laboratory analyses indicate that of the three soil types, Arkose had the highest sand and lowest silt and clay content in the fine earth fraction, while Gila had the lowest sand and highest silt and clay content (Table 1.1). In both emergence and growth, the most positive response of seedlings was in Gila and Glance soils. Soil treatments involved the fewest significant interactions, suggesting that effects in seedling response were fairly straightforward and not as interwoven with other environmental variables.

Gila and Glance soils were never significantly different from each other in mean emergence and seedling size main effects, and were consistently associated with significantly more emergence, more leaves, and taller and heavier seedlings than Arkose soil (Tables 1.2, 1.7). High irrigation levels and straw mulch accentuated this trend, with higher emergence and larger seedlings, while the opposite was found with low irrigation levels and bare soil treatments (Tables 1.3, 1.4, 1.11, 1.15).

It is unknown how deep the developed soil profile was at the excavation sites for the samples collected for this experiment, but because collections were made up to a depth of 3 m (10 ft), it is possible that parent material was incorporated unevenly within each type. I have observed relatively shallow, but well-developed soil profiles with good clay formation in Arkose soil at the study site. Thus, it seems possible that the high gravel content of Arkose soil samples in this experiment may be attributable to the depth of collection, resulting in an unfavorable substrate for seedling growth—an important issue to consider in a landscape restoration context on this soil type. The Arkose samples used in this experiment were approximately 81% gravel and appeared to contain a minimal amount of particulates <2 mm (0.8 in) in size. In research by Martre et al. (2002) on root development of agave species, soils with high gravel content incorporated within the profile (>14% of bulk soil) decreased the available water capacity of the soil and reduced the space penetrable by roots. The study documented fewer leaves, decreased root growth, and less root surface area by agave seedlings grown in rocky soils. In the present study, seedlings growing in Arkose soil were smallest and lightest in weight (Table 1.7). Although the coarse particles may have facilitated increased percolation, the minimal fine earth fraction may have stymied root growth, as well as decreased water holding capacity. Arkose soil exhibited the lowest VWC, and was significantly drier in all interactions (Tables 1.17, 1.18, 1.20). In addition to the rocky substrate, this soil was a dark red color and may have been higher in temperature than the lighter colored Gila and Glance soils. Straw and gravel mulches did significantly increase

mean emergence in Arkose soil, and these treatment combinations had higher total emergence than bare soil treatments of all soil types (Table 1.4). It is possible, as previously suggested, that mulches decreased soil temperature, and helped curb water lost through evaporation in this coarse substrate.

Although undisturbed Arkose soil currently supports dense populations of wild *A. palmeri* at the study site, the results of this experiment suggest that disturbed Arkose soil may be highly unproductive. In contrast, the fine particle texture, lighter color, and higher water holding capacity of Gila and Glance soils may be more successful at supporting *A. palmeri* seedlings after disturbance. However, without further research it is unknown if the trends in seedling response by soil type observed in this study would be realized in a field context where a multitude of additional ecological factors would affect seed response.

In this experiment, I attempted to identify the importance of specific environmental variables on seedling emergence and establishment. However, there were several notable differences between greenhouse and field conditions that deserve to be mentioned, in order to frame the context of these results. The greenhouse location at the Campus Agricultural Center in Tucson, Arizona, is located at approximately 732 m (2400 ft) in elevation, roughly 770 m (2500 ft) lower than the field site in the foothills of the Santa Rita Mountain Range. Although the facility is reasonably controlled, the evaporative cooling system did not reproduce the same seasonal temperature and relative humidity of the cooler, higher elevation study site, particularly during the monsoon season when ambient relative humidity is very

high and temperature by evaporative cooling is more difficult to control. Temperatures were continuously higher in the greenhouse and had much slighter diurnal differences than in the field (Figure 1.1). These persistent increased temperatures may have had a negative influence on seedling response (Nobel 1984, Nobel and Hartsock 1986, Nobel 1988).

Irrigation levels and application methods were carefully calculated based on 3 decades of local daily precipitation records. However, the amount and/or frequency of water applied did not seem appropriate for the combination of soil volume and environmental conditions present in the experiment for two reasons. Foremost, the high and average irrigation treatments appeared to be wet at the surface most of the time. During irrigation events, water pooled on the soil surface and drained out of the bottom of the pot for several minutes afterwards in the high and average level treatments. Several pots had visible mold or algae growing on the soil surface. Secondly, although differences in seedling response occurred, VWC did not differ significantly between high [42.1% (SE=0.72)] and average [42.0% (SE=0.72)] irrigation treatments (Table 1.17). Due to the disturbance caused by the soil probe, measurements were collected only once, however, repeated measures averaged together may have offered more representative results. The appearance of persistent wetness of the soil, as well as the seemingly minute differences between high and average irrigation levels are both notably deviant from what one would expect in field conditions, potentially skewing the results observed in this experiment. Several studies have noted that bare soil has higher rates of

evaporation than soils with organic or inorganic mulches (Jalota and Prihar 1998, Post et al. 2004, Li et al. 2005). Although bare soil treatments were associated with the lowest emergence and smallest seedlings, the VWC tended to be highest across all high and average irrigation treatments involving bare soil (Tables 1.19, 1.20), further suggesting an anomaly in calculating irrigation levels or in measuring VWC.

Recommendations

Although differences between greenhouse and field conditions are inherent in this study, much can be learned about the early life stages of this species in the results presented here. Previous research on arid adapted species has largely determined that temperature and water availability are probably the most critical factors for successful germination and establishment (Turner et al. 1966, Steenbergh and Lowe 1969, Jordan and Nobel 1979, Nobel 1988). Present study findings suggest support for this assertion for the species *A. palmeri* at the study site. The manner in which these critical factors interact with other environmental variables, as observed through present study treatments, allude to the relative complexity involved in re-establishing this species from seed into a disturbed landscape.

Irrigation appeared to play an integral role in seedling emergence and establishment, however, it is clear that other interacting treatments had a strong influence on seed response, at all irrigation levels. Given that rainfall is characteristically variable and unpredictable in the arid regions that *A. palmeri*

inhabits, the incorporation of other favorable treatments would appear to offer a greater chance for more successful seeding of this species in a disturbed landscape. Shade had a notably positive affect in increasing seedling emergence and early establishment in all treatment combinations and has been implicated in the literature as a critical factor in the survivorship of the early life history of many arid adapted species (Turner et al. 1966, Nolasco et al. 1997). Thus sowing seed under established vegetation, debris, or near large rocks could increase emergence and survivorship rates. The utilization of surface mulches could also have a positive affect on *A. palmeri* seedlings, as gravel and straw had significantly higher emergence and early establishment rates than bare soil. Finally, soil type appears to have an influence on both emergence and early establishment of seedlings, as Arkose soil was associated with a significantly poor seedling response. It is possible that without the mitigating effects of shade or surface mulches, the extremely gravelly substrate and/or dark color of this soil type may be unfavorable for both emergence and establishment.

In conclusion, it would appear that meaningful, landscape-scale restoration of *A. palmeri* populations by seed is less probable without protected microsites facilitating temperature mitigation and water retention, in combination with above average rainfall. Although only 28% total emergence occurred in this study, other factors known to threaten the survivorship of young plants in the wild such as herbivory and long-term variability in temperature extremes and water availability were not tested. Given the apparent challenge involved in growing *A. palmeri* from

seed, long-term, field-based research on seedling response, in addition to testing other restoration methods such as transplanting older plants, should be considered. A particular concern in any restoration scenario involving *A. palmeri* should be providing long-term, uninterrupted annual foraging opportunities for *L. curasoae* within disturbed habitat, which would not be possible if solely employing a seeding strategy in a landscape-scale disturbance. Thus, an integrated approach may be required for developing an effective restoration plan for re-establishing this long-lived species in the wild.

CHAPTER 2: RESTORING *AGAVE PALMERI* POPULATIONS: CRITICAL FACTORS FOR SALVAGE AND TRANSPLANTATION

Abstract

Agave palmeri Engelmann (Palmer's agave) is a semelparous, perennial succulent thought to provide a critical food source for the USFWS-listed endangered species, *Leptonycteris curasoae* Miller (lesser long-nosed bat). Awareness is growing for the need to conserve wild populations of *A. palmeri*, and to mitigate for such impacts to its habitat as mining and urban development. Transplanting of wild plants was tested as a potential method for restoring and maintaining agave populations within areas of disturbance. Specifically of interest was the growth response of various size classes to the prolonged drought stress from storage before replanting, and the affect of water availability directly after replanting. In January 2009, 387 wild plants were collected from the Santa Rita Mountains, Arizona, and transported 80 km (50 mi) to a storage facility in Tucson, Arizona. Roughly half the plants were potted in soil, and the remainder were placed in pots without soil (bare) and covered with burlap cloth. In July 2009, a 2400 m^2 (26,000 ft^2) denuded and scarified field plot was planted with the surviving 277 *A. palmeri* individuals from 5 size classes. One-third of plants received each of three water treatments: a 90-day slow-release gel irrigation supplement, 8 L (2 gal) of water, and a dry treatment. After transplanting, plants were monitored for 9 months and survivorship and growth were documented. Mortality did not differ among size classes, but the

smallest plants (size classes 1 and 2) had significantly more proportional growth (number of leaves, leaf length) than the largest plants (size class 5). Bare root transplants had significantly more proportional leaf production, but exhibited higher mortality (11%) than soil treatment plants (2%). Lack of significant differences in growth or mortality of plants in the three water treatments may reflect unusually high seasonal rainfall during the study period.

Introduction

Each spring, a large population of nectivorous bats, *Leptonycteris curasoae* Miller (lesser long-nosed bat), migrate north from central Mexico following a phenological tide of blossoming columnar cacti and paniculate agaves (Gentry 1982, Fleming et al. 1993). Traveling thousands of kilometers, the bats reside briefly in southern Arizona before making the return flight in autumn (Cockrum 1991). In the United States, *L. curasoae* is listed as an endangered species with potential threats including human disturbance of foraging and roosting sites in their limited habitat north of the border (USFWS 1995). Several studies have suggested that *Agave palmeri* Engelmann (Palmer's agave) provides a critical food resource for *L. curasoae* preceding its southward migration (Schaffer and Schaffer 1977, Howell and Roth 1981, Ober and Steidl 2004, Ober et al. 2005). This species appears to have a reproductive strategy strongly associated with chiropterophilious pollination, including peak nectar production and concentration coinciding with

seasonal and diurnal bat activity periods (Schaffer and Schaffer 1977, Howell and Roth 1981, Ober and Steidl 2004).

Recent studies have emphasized the importance of preserving *A. palmeri* populations in order to facilitate the recovery of *L. curasoae* (Hinman 2003, Ober et al. 2005). Additionally, there is strong evidence that pollination by *L. curasoae* is critical in maintaining the stability and longevity of future *A. palmeri* populations (Schaffer and Schaffer 1977, Howell and Roth 1981). In order to assist in the preservation of this mutualistic relationship, conserving intact populations of *A. palmeri* and restoring disturbed areas of its habitat is crucial.

Proposed mining activity in the northeastern Santa Rita Mountain Range in southern Arizona is currently in review and would impact thousands of *A. palmeri* individuals (WestLand Resources 2009). Salvaging agaves from impact zones and transplanting them outside of disturbed areas, or within disturbed areas undergoing restoration, are proposed strategies for attempting to mitigate the loss of habitat. However, little is currently known about the life history stages of *A. palmeri* and its potential response to the stress of transplanting, thus limiting the effective implementation of a large-scale restoration plan.

Agave palmeri is a monocarpic perennial in the group Ditepalae (Gentry 1972). It is commonly associated with oak woodland and grama grassland plant communities in southern Arizona, southwestern New Mexico, and northern Sonora, Mexico, at elevations from 900-1,800 m (3,000-6,000 ft) (Gentry 1982). Mature plants are generally 100-120 cm tall (40-48 in), and 50-120 cm wide (20-47 in) with

succulent, yellow-green to silver-gray leaves. Tooth-fringed leaves unfold from a central spike above a fleshy meristimatic shoot (Nobel 1988). The inflorescence is a broad panicle, 3-5 m tall (10-16 ft), with light-colored, tubular flowers in umbellate clusters. Capsules are oblong and contain three locules that may be packed with flat, round seeds. Flowers are protandrous, and fertile seeds are uncommonly formed without external pollination (Schaffer and Schaffer 1977, Howell and Roth 1981). Individuals grow for up to 35 years before flowering in late spring through early autumn. *Agave palmeri* is thought to primarily rely on sexual reproduction, rather than vegetative cloning (Gentry 1982).

Agaves persist through extremes in temperature, water availability, competition for limited resources, and herbivory for several decades before attempting a single opportunity to sexually reproduce (Gentry 1982, Nobel 1988). Thus, they have developed multiple biological mechanisms to mediate the stress associated with the relative variability in their environment. Agaves use CAM photosynthesis, transpiring at night to conserve water in hot and dry climates. Leaf surfaces are covered by a protective waxy cuticle with a relatively low stomatal frequency, further measures to preserve water. The unique cellular structure in their root systems restricts water-loss to a drying soil, but allows them to rapidly uptake water when it becomes available during infrequent storms (Nobel 1976, Nobel and Cui 1991, North and Nobel 1998). They have thick, succulent shoots and leaves that enable them to store water, and they can maintain physiological function with up to 95% water loss before cellular damage (Nobel 1994). Like many other

arid-adapted succulents, young agave plants are more vulnerable to environmental extremes than adults (Turner et al. 1966, Jordan and Nobel 1979). Research by Nobel (1977) has estimated that only 1 in 1.2 million seedlings of *A. deserti* in the northwestern Sonoran Desert survive to adulthood, and Gentry (1982) attributed similar survival estimates to representatives of the genus. In order to mediate the stress from environmental extremes, young plants are thought to require facilitative 'nurse' relationships (Gentry 1982, Jordan and Nobel 1979). Nurse plants or rocks provide critical services to seeds and seedlings, including increasing the moisture and nutrient content of soils, and protection from extreme temperatures, herbivory, and trampling (Turner et al. 1966, Jordan and Nobel 1979, Gentry 1982, Peters et al. 2008). As a plant matures, its ability to cope with the stress caused by environmental variability increases and mortality rates decrease (Nobel 1988).

In order to assess the response of *A. palmeri* to transplanting, I conducted an experiment with several hundred wild agaves, testing three primary variables: plant size, method of storage, and method of watering immediately upon transplanting. Between January 2009 and April 2010, wild agaves were removed from the field, stored in a shade house, replanted in an open, disturbed field site, and monitored for 9 months to assess post-replanting survivorship and growth. I designed the study with the intention of being applicable to a large-scale transplanting effort, and selected variables based on both the efficiency of human effort, as well as the physiological tolerance of the plants.

The primary variable of interest in this study is the long-term reproductive success of transplants. However, due to the long life span of the species (≤ 35 years) and the short time period available for this research, mortality was the only certain plant response to assess this variable. Agave researchers apply several measures to assess productivity, including dry weight and CO_2 uptake (Nobel 1984a, 1988, Nobel and Hartsock 1986b). Number of leaves has also been used as a non-destructive indicator of agave productivity (Nobel 1984a, 1988, Nobel and Meyer 1985, Nobel and Quero 1986), and was the primary variable I used to measure treatment effects, in addition to changes in leaf length (Nobel 1984a).

Long-term studies on a desert succulent, the saguaro cactus (*Carnegiea gigantea*), have documented a relatively predictable correlation between plant size and age, with some variation between localities based on climate and slope aspect (Pierson and Turner 1998). However, considerable variation in agave height and leaf length can result from heterogeneity in microsite conditions associated with temperature and light availability (Nobel 1988, Howell 1995). Like the saguaro, agaves lack growth rings to facilitate age estimation, however, little research has been conducted assessing alternate means to correlate age and morphology in agave species. For the purposes of this study, five size classes were created based on a plant's number of leaf whorls, which unfold radially in sequential courses from the center spike. Research by Nobel (1984a) has determined that the unfolding of leaves from the central spike is closely correlated to seasonal variation in water availability, temperature, and PAR. Thus, the addition of leaf whorls occurs over

time and is used here to delineate approximate age groups. Howell (1995) used a similar size class designation in a 5-year study on *A. palmeri* and found reasonable correlation with age in younger individuals.

Given the sensitivity of younger agaves to environmental variability, it should be expected that younger plants might respond differently to the stress of transplanting than older, mature plants. The removal, potting, transporting, storage, and replanting in an open, disturbed landscape may strongly affect the survivorship of young agaves. In contrast, older plants reportedly have a far greater adaptive ability to cope with environmental stresses (Nobel 1988), such as what might be associated with transplanting. Thus, I intended to include a range of ages in this study. I expected smaller (younger) agaves to have slower rates of growth and higher mortality than larger (older) plants. This is a crucial variable to test because the intended goal of a landscape-scale transplanting is to facilitate a functional, age-diverse cohort with the potential to provide relatively consistent inter-annual forage for *L. curasoae*.

The method of storage between salvage and replanting has considerable implications, both for project managers and for agave re-establishment, post transplanting. Large-scale, ground-moving projects commonly involve long delays, variable access to equipment and personnel, and unexpected changes in scheduling and project developments. If *A. palmeri* plants are to be moved and replanted in stages involving different seasons, shifting project plans, and delays in scheduling, the ability to store plants in between collection and replanting may be an important

tool in an effective transplanting effort. Plants range in size and can be large and awkward to maneuver, especially when potted in soil. Large plants potted in soil can weigh up to 45 kg (100 lbs) and wield over 1 m (3.3 ft) long stiff, serrated, spine-tipped leaves, making handling both inefficient and potentially hazardous. The adaptation of agaves to extreme temperatures and prolonged drought (Nobel 1988), may allow for some flexibility during transport and storage. I tested two storage treatments-- potted in soil and potted without soil (and covered with burlap). In this study, the latter 'bare' treatment made handling plants of all sizes far easier and more expedient through collection, transport, and replanting. In addition, bare root plants were notably more compact, facilitating the storage of hundreds of plants, and streamlining their maintenance. These amenities could prove valuable in a large transplanting effort involving thousands of plants, possibly decreasing the likelihood that large plants would be under-collected or heavily damaged due to the difficulty in handling them. However, the method of storage may strongly influence the ability of plants to recover from the stress of transplanting. I suspected that the bare root transplants would grow less and have higher mortality rates than the potted transplants due to the stress from lack of water, light, and nutrients from 6 months in storage.

Like other succulents, agaves have evolved to take advantage of small, infrequent amounts of rain. Research on *A. deserti* has shown that after 6 months of drought, plants responded to a pulse of water within 1-2 days with resumed stomatal opening and carbon fixation (North and Nobel 1998). Within 7-11 days,

plants renewed growth of main roots and generated new lateral superficial roots (North and Nobel 1998). Thus, even small and infrequent amounts of water can affect short- and longer-term agave growth. In a large-scale restoration context involving thousands of hectares, returning multiple times to water transplanted agaves on reclaimed slopes would require considerable effort that could be allocated to other restoration endeavors. I determined three methods for watering agaves on the day of planting when irrigating is most practical: a 90-day time-release gel irrigation supplement (water bound in cellulose gum), an 8 L (2 gal) pulse of water, and a dry treatment. Given the rapid response of agaves to available water, offering *A. palmeri* plants moisture directly after planting may instigate metabolic activity and renewed root growth, and afford those plants with a head start in adapting to their new environment. I expected the dry treatment plants to have the lowest growth and highest mortality.

The present study was intended to assess the response of *A. palmeri* to a suite of environmental variables that would likely be confronted in a large-scale transplantation effort. Size of individuals, method of storage, and water availability post-transplant are all treatments that managers would need to explicitly decide when designing a restoration strategy involving this species. The results to follow may offer some useful insights into the adaptability and tolerances of this important species.

Methods

The study site is located along the McCleary Canyon drainage in the rolling foothills of the northeastern Santa Rita Mountains, Pima County, Arizona (31°50’36”N, 110°45’00”W). The local vegetation is oak woodland and grama grassland with abundant *Quercus emoryi, Q. arizonica, Juniperus deppeana, Mimosa biuncifera, A. palmeri*, *Nolina microcarpa,* and *Bouteloua curtipendula*. Slopes face dominantly east, southeast, and northeast. Soils are classified as in the Mabray-Chiricahua Rock association, and are developed from conglomerates, mudstones, sandstones, and rock outcrops (Hargis and Harshbarger 1977, NRCS 2010). Soils are shallow and calcareous, and are primarily composed of very cobbly and very gravelly loams with good drainage. Local records indicate an annual average of 43 cm (17 in) of rainfall per year with more than half falling in the summer monsoon season (July-September) and the remainder falling in the winter, occasionally as snow (Sellers 1978, Tetra Tech 2009). Annual air temperatures generally range from -4-30°C (25-85°F) with over 100 days of frost (Sellers 1978, USDA 2006).

Collection

In the winter of 2009, five collection plots with dense populations of *A. palmeri* were identified, each with similar vegetation and elevation [1550-1600 m (5100-5250 ft)]. Plots were approximately 4000 m^2 (13000 ft^2) in size, and were within 3 km (2 mi) of each other. Initially, only the soil storage treatment was planned, and all methods that follow describe the sampling for that treatment, until

the bare storage treatment is introduced. Approximately 40 plants were selected in each plot. In addition to relatively steep and undulating terrain, soils in the study area are very dense and layered with rock fragments. A preliminary excavation revealed the challenge of unearthing plants by hand in a reasonable timeframe and without significant damage to the plant roots and shoots. Therefore, in an effort to standardize collection procedures, plants were not selected randomly, but were chosen from areas where access by a backhoe excavator was possible. In using a backhoe excavator, root balls remained intact, no discernable damage was recorded to any of the collected individuals, and many more individuals were collected than would have been possible with hand shovels. Therefore, plants in areas isolated by vertical drainages or dense tree-dominated vegetation were excluded from sampling.

Five size classes were determined based on the number of leaf whorls. Whorls (classes 1-5) were measured by laying a straight-edge across the center of each plant, and counting the number of sequential leaves radiating from the central spike, so that one leaf whorl was labeled size class 1, two whorls, size class 2, etc. (Howell 1995).

Approximately 10 individuals from each size class were selected in each plot, resulting in about 40 plants per size class. A minimum of 10 m (33 ft) between individuals was maintained in order to minimize the possibility of collecting genetic clones, however, plants collected for the bare root treatment did not follow this rule. Location, plant height and width, slope aspect and inclination, proximity to

neighboring agave plants, canopy cover by other plants, surface cover (gravel, cobble, soil, and organic matter), and dominant vegetation were documented for each plant. To facilitate orientation upon replanting, an ID number was written on the southernmost leaf with a Sharpie permanent marker and the leaf tip was marked with orange paint. A painted and flagged wooden lathe was placed on the south side of the individual, and photographs were taken for reference.

Plants were collected from the field between 6-12 Jan 2009. Timing for collection was based on access to property and equipment, as well as when soils would be sufficiently moist to avert damage to root systems. Creating significant damage to root systems unevenly between individuals could have potentially skewed treatment results. Plants were carefully unearthed with a backhoe excavator [46 cm (18 in) bucket], and were planted into 19, 38, or 57 L (5, 10, or 15 gal) nursery pots. A small number of plants on apparently inaccessible ravines and slopes were excavated carefully by hand, using shovels (about 10% of the sampled individuals). Because *A. palmeri* often grows in loose colonies (Gentry 1982), it was not uncommon for plants neighboring the target individual to be unearthed. I decided to collect these individuals also, marking them with the target individual number plus a sub letter. These individuals became the bare root treatment specimens. They were tapped free of soil among their roots and deposited into 19 or 38 L (5 or 10 gal) nursery pots without soil. Although these plants are potentially related to the target individuals by seed or cloning, and the post hoc design is far less than ideal, I believe the promise of using such a convenient technique for

transplanting warranted testing. In total, 199 plants were collected for the soil treatment and another 188 plants were collected for the bare treatment.

Storage

Soil and bare root treatment plants were transported to Tucson, Arizona, and stored in a greenhouse with the ceiling and wall panels removed and replaced by shade cloth at the Campus Agricultural Center from 6 Jan-14 Jul 2009. A shade house was used in this experiment because managers attempting any large-scale transplanting effort involving several hundred or more plants would likely store them in an informal, open-air, shaded structure, rather than a climate-controlled facility. Soil treatment plants were placed on concrete slabs, 0.6 m (2 ft) apart. These plants were watered to field capacity once per month until temperatures warmed in April, at which time were watered every 2 weeks (Irish and Irish 2000). Bare root plants remained in empty pots, were lined into rows, and entire plants (roots and shoots) were covered with 2 sheets of burlap cloth that was moistened every two weeks. In July 2009, prior to replanting, I conducted an inventory of each plant in the shade house, and recorded a qualitative assessment of general appearance (good, fair, poor, dead) based on the number of dead leaves, as well as leaf color, thickness, and wrinkling. Photographs were taken of each plant.

Transplanting

In the summer of 2009, a 2400 m^2 (0.6 acre) transplanting plot was prepared on a moderate east-facing slope in the McCleary Canyon study area. Vegetation was removed with a bulldozer, and the soil surface was raked with a ripper to a depth of 15-30 cm (6-12 in) to simulate disturbance. Three distinct sites (north, center, and south) were delineated within the transplanting plot based on soil textural differences. Soil samples were collected at 0-20 cm (0-8 in) and 20-40 cm (8-16 in) in the profile from two locations in each site and were sent to a laboratory for testing. Planting locations were marked with pin flags, and were triangulated in a diamond pattern along the slope contour at 1 m (3.3 ft) intervals.

Transporting and planting occurred from 12-17 Jul 2009, in an effort to coincide with the summer monsoon. A 46 cm (18 in) auger on a skid steer drilled 64 cm (25 in) deep holes at each pre-marked planting location. Holes that did not meet the depth requirements for larger plants due to substrate obstructions were deepened manually with a digging bar. Individual plants, treatments, and planting locations were completely randomized, although due to different numbers of size classes, there were uneven numbers of treatments and replications within and between sites, resulting in an unbalanced randomized block design. Locating plants large enough to be classified size class 5 (the largest category in the experiment), but small enough to be potted, transported, stored, and replanted in the same method as the other plants in the study was challenging, resulting in far fewer of this size category relative to the other size classes. Additionally, because the bare

root plants had a high mortality rate in storage, there were fewer plants of this treatment than of the soil treatment.

Plants were reoriented to their original southern side in order to minimize the uneven application of stress between plants from unaccustomed sun exposure (Irish and Irish 2000). Care was taken to settle the base of each plant to ground level so that planting depth and method were standardized. Three watering treatments were used: a 90-day time-release gel pack (model DWP-TG, DriWater, Santa Rosa, CA) buried with gel directly against the root ball on the south side of the agave, 8 L (2 gal) of water slowly applied directly over the rosette for maximum local infiltration and minimal run-off, and a dry treatment. A total of 93 plants in 24 treatment combinations (2-5 replications) were planted in the south site, 100 plants of 27 treatment combinations (2-5 replications) in the center site, and 84 plants of 24 treatment combinations (2-5 replications) in the north site. A Taylor 2710N rain gauge (Taylor Precision Products, Oak Brook, IL) was installed in the center of each of the three sites, and was manually checked on a monthly basis.

The first post-transplant inventory was conducted in mid-August 2009. A leaf on both the north and south sides of each plant was marked with a Sharpie permanent marker and the length measured. Photographs of each plant were taken. Additional assessments included: mortalities, height of the central spike, number of leaves, number of ramets, a qualitative plant assessment regarding discoloration and desiccation characteristics, and the number of dead leaves. A second and final

inventory was conducted in April 2010, and measurements and photographs were repeated.

Analysis

Mortality results were pooled within treatments and across sites because numbers were relatively low. Fisher's exact test of independence was used to test if mortality was significant between treatments (Fisher 1922).

Due to small sample sizes, the unbalanced numbers of replicates and treatment combinations, and the post-hoc, non-random nature of the bare treatment imposition, non-parametric tests were used for analyzing statistical significance among treatments. To assess plant response, three measurement variables were selected for analysis: north leaf length, south leaf length, and number of leaves. These variables were calculated using the following equation:

$$\Delta = \frac{Sp - Su}{Su} \text{ x } 100$$

where Sp is the spring 2010 measurement, Su is the summer 2009 measurement, and Δ is the proportional change in growth. Plant growth measurements were only analyzed for plants and leaves that survived for the entire monitoring period.

Each site was analyzed separately. All 27-treatment combinations [5 size classes, 2 storage methods, 3 water treatments (size class 4 in bare treatments was absent)] were assigned an identification number, and this code was used as the explanatory variable in all analyses. Kruskal-Wallis tests were used to assess

significance with each response variable (Kruskal and Wallis 1952). Only data from plants and leaves that survived for the entire monitoring period were included in growth response analyses.

Statistix 9.0 software was used for analysis and enabled pairwise comparisons of means. Due to the increased conservatism of the test when making multiple comparisons, several variables were significant in the primary model, but no discernable treatment differences were detected at the 0.05-0.2 alpha level in mean comparisons. For this reason, I re-ran the analyses pooling all data within each of the following treatments: water treatment (dry, gel, water), size class (1, 2, 3, 4, 5), and storage treatment (bare root, soil). With the pooled data, I re-ran Kruskall-Wallis tests for each respective treatment, within each site. Tests were considered significant at $P \leq 0.05$. Descriptive statistics were also compiled and arithmetic means were reported alongside mean ranks.

Results

Sites

The north site was was bordered on its north and east sides by *Juniperus deppeana*, *Quercus arizonica*, and *Q. emoryi* woodlands. Soils there were very to extremely gravelly and cobbly (40-65%), and were sandiest of the three sites (Table 2.1). In contrast, the south site had been an open, grassland prior to mechanical clearing. The soil was gravelly, but had the lowest gravel content (10-30%) with very few cobbles (Table 2.1). The central site was intermediate in gravel and cobble

content (10-35%) (Table 2.1). Soils in all sites had a visible accumulation of carbonates within the profile.

Table 2.1. Composition of the fine earth fraction (<2 mm) for 0-40 cm (0-16 in) depth of profile, averaged between 2 soil pits within each site at the study plot in the Santa Rita Mountains, Pima County, Arizona.

SITE	SAND (%)	SILT (%)	CLAY (%)
North	45.5	19.5	35
Center	32.5	26	41.5
South	32	31	37

There were few differences in micronutrient content between sites. Magnesium appeared to be lower in the north site (885 ppm) compared to the south and central sites (1400 and 1496 ppm, respectively), and copper concentrations were higher on the north site (83 ppm) compared to the south and central sites (9 ppm).

Temperatures recorded between Jul 2009-Apr 2010 at a weather station within 5 km (3 mi) from the transplanting plot showed a monthly mean range between 6.6-25.2°C (43.9-77.4°F). The mean temperatures for the summer, fall, and winter seasons (as defined in this study) are shown in Table 2.2.

Table 2.2. Mean temperatures and total precipitation (averaged between sites) from 14 Jul 2009-14 Apr 2010, at the study plot in the northeast Santa Rita Mountains, Pima County, Arizona.

SEASON	Mean temperature (C)			Total precipitation (mm)
	Min	Max	Ave	
Summer (Jul-Sep)	19.2	28.8	24.1	140
Fall (Oct-Nov)	9.8	19.8	14.7	0
Winter (Dec-Feb)	2.5	11.4	6.8	129
Spring (Mar-Apr)	6.1	16.6	11.6	96
AVERAGE	9.7	19.3	14.6	365 TOTAL

Prior to replanting on 14 Jul 2009, the plot had received 48 mm (1.9 in) of precipitation. After planting, cumulative totals for the study period (14 Jul 2009-14 Apr 2010) indicated that the north site received 377 mm (14.8 in), the central site 364 mm (14.3 in), and the south site 354 mm (13.9 in). An across-site average of 38% total precipitation occurred during the summer growing season (mid-July through early September) (Table 2.2). Including July rainfall prior to replanting, this is between 18-37% below the long-term average seasonal precipitation for the area of 230-300 mm (9-12 in) (Sellers 1978). No rain occurred from mid-September to the end of November. Winter/spring (Dec-Apr) rainfall comprised 62% of the precipitation receive during the study period, and its levels were well above the long-term average (Sellers 1978) (Table 2.2).

Mortality

While in storage, 96% of soil treatment plants were classified as in "good" condition, with few dead leaves, and minimal leaf discoloration, thinness, or wrinkling. Additionally, only two soil treatment plants from two size classes died, resulting in a 1% mortality rate for this treatment prior to planting in the field. A size class 5 plant flowered and later died; it is unclear if that plant would have flowered if undisturbed, or if it flowered in response to the stress of transplanting. A size class 1 plant also died, although two ramets appeared in the pot while in storage and were included in the replanting effort. In contrast, during this time only 4% of bare root plants were classified as in "good" condition. Additionally, 31% of bare root plants died while in storage, the majority of which were small (75% size class 1, 22% size class 2, 3% size class 3). In total, 45% of all the size class 1 plants collected for the bare root treatment died while in storage, in addition to 28% of all size class 2 and 7% of all size class 3 plants.

After replanting, overall mortality was low. By the study's conclusion in mid-April 2010, 12 of 277 transplants had died, resulting in a 4% overall post-replanting mortality rate. Results from Fisher's exact test of independence for each of the pooled treatments (water, storage, and size class) indicate that only storage treatment appeared to have a significant effect on transplant mortality (2-tail P= 0.002). Across all sites, 11% post-replanting mortality was observed in bare root transplants, compared to 2% of soil treatment transplants.

Plant growth

Number of leaves

The differences in proportional change (Δ) in number of leaves among treatments was significant in each site (Addendum: Tables 1-3). In the north site, Kruskal-Wallis tests indicate the Δ in number of leaves [H=43.2, df=23, *P*=0.007] was greatest in bare root treatments, particularly of smaller size classes (Tables 2.3, 2.4; Addendum: Table 1).

Table 2.3. Proportional change in number of leaves pooled by size class treatment for each site in the study plot between 14 Jul 2009-14 Apr 2010, in the northeastern Santa Rita Mountains, Pima County, Arizona.

Size class treatment	Mean rank	Sample size (N)	Δ Number of leaves (%) ◆
NORTH SITE			
1	47.4	19	43.3 **A**
2	43.3	18	21.3 **A B**
3	39.9	19	58.3 **A B**
4	30.6	15	12.6 **A B**
5	17.4	6	6.9 **B**
CENTER SITE			
2	61.4	25	76.0 **A**
1	60.2	22	30.6 **A B**
3	44.7	23	36.9 **A B C**
4	34.1	15	11.5 **B C**
5	29.8	12	1.6 **C**
SOUTH SITE			
2	61.0	24	78.8 **A**
1	55.8	21	65.6 **A**
3	50.2	25	52.3 **A**
4	14.9	15	12.7 **B**
5	12.3	6	10.4 **B**

◆ Arithmetic means followed by different letters are significantly different (*P*=0.05) in like sites based on Kruskal-Wallis test.

The center site (H=43.9, df=26, P=0.016) showed similar trends in Δ in number of leaves, as did the south site (H=69.8, df=23, P<0.001) (Tables 2.3, 2.4; Addendum: Tables 1, 2).

Table 2.4. Proportional change in number of leaves pooled by storage treatment for each site in the study plot between 14 Jul 2009-14 Apr 2010, in the northeastern Santa Rita Mountains, Pima County, Arizona.

Storage treatment	Mean rank	Sample size (N)	Δ Number of leaves (%) ⬩
NORTH SITE			
bare	59.1	14	60.3 **A**
soil	34.5	63	27.0 **B**
CENTER SITE			
bare	63.2	31	80.8 **A**
soil	42.3	66	16.2 **B**
SOUTH SITE			
bare	75.1	25	121.4 **A**
soil	35	66	26.7 **B**

⬩ Arithmetic means followed by different letters are significantly different (*P*=0.05) in like sites based on Kruskal-Wallis test.

All tests indicate that smaller size classes (1, 2) and bare root treatments were associated with a greater Δ in the number of leaves added, when compared to larger size classes and soil treatments. Water treatments did not significantly affect Δ in number of leaves, and when water treatment data was pooled, Δ in number of leaves was statistically comparable between sites.

Leaf length

In all sites, the Δ in north leaf lengths was significantly different between unpooled treatments (Addendum: Tables 4-6). In the north site (H=39.5, df=23,

P=0.017), the greatest documented Δ in north leaf growth occurred primarily in bare root treatments and smaller size classes (Tables 2.5, 2.6; Addendum: Table 4). The center site (H=41.4, df=26, *P*=0.028) and the south site (H=46.9, df=23, *P*=0.002) followed similar trends (Tables 2.5, 2.6; Addendum: Tables 5, 6). In pooled water treatment data, there were no significant differences among treatments associated with Δ in north leaf length, in any site.

Table 2.5. Proportional change in north leaf lengths pooled by size class treatment for each site in the study plot between 14 Jul 2009-14 Apr 2010, in the northeastern Santa Rita Mountains, Pima County, Arizona.

Size class	Mean rank	Sample size (N)	Δ North leaf length (%) ◆
NORTH SITE			
1	52.6	17	8.2 **A**
2	43.8	18	5.1 **A B**
3	35.4	19	2.9 **A B**
5	24.5	6	1.4 **A B**
4	23.1	15	1.2 **B**
CENTER SITE			
1	65.9	22	14.3 **A**
2	49.9	22	9.0 **A B**
3	40.3	21	3.4 **B**
4	33.4	15	1.8 **B**
5	25.0	11	0.8 **B**
SOUTH SITE			
2	60.7	24	11.8 **A**
1	53.8	20	14.2 **A B**
3	37.8	25	5.5 **B**
5	32.9	6	2.7 **A B C**
4	27.5	15	1.4 **C**

◆ Arithmetic means followed by different letters are significantly different (*P*=0.05) in like sites based on Kruskal-Wallis test.

Table 2.6. Proportional change in north leaf length pooled by storage treatment for each site in the study plot between 14 Jul 2009-14 Apr 2010, in the northeastern Santa Rita Mountains, Pima County, Arizona.

Storage treatment	Mean rank	Sample size (N)	Δ North leaf length (%) ⬩
NORTH SITE			
bare	54.6	12	9.0 **A**
soil	34.8	63	3.2 **B**
SOUTH SITE			
bare	54.6	24	14.0 **A**
soil	42.2	66	6.2 **B**

⬩ Arithmetic means followed by different letters are significantly different (*P*=0.05) in like sites based on Kruskal-Wallis test.

The Δ in south leaf length was significantly different among treatments only in the center site (H=42.29, df=26, P=0.023). Again, smaller size classes and bare root treatments appeared to have significantly greater positive changes in leaf length than larger size classes and soil treatments (Tables 2.7, 2.8; Addendum: Table 7). Water treatment had no discernable effect on the Δ in south leaf length, and when pooled in analyses, differences among treatments were not significant in any site.

Table 2.7. Proportional change in south leaf length pooled by size class treatment for each site in the study plot between 14 Jul 2009-14 Apr 2010, in the northeastern Santa Rita Mountains, Pima County, Arizona.

Size class	Mean rank	Sample size (N)	Δ South leaf length (%) ⬩
CENTER SITE			
1	60.6	21	7.4 **A**
2	50.5	24	2.9 **A B**
4	38.7	14	0.1 **A B**
5	37.8	10	-0.1 **A B**
3	35.1	22	0.1 **B**

⬩ Arithmetic means followed by different letters are significantly different (*P*=0.05) based on Kruskal-Wallis test.

Table 2.8. Proportional change in south leaf length pooled by storage treatment for each site in the study plot between 14 Jul 2009-14 Apr 2010, in the northeastern Santa Rita Mountains, Pima County, Arizona.

Storage treatment	Mean rank	Sample size (N)	Δ South leaf length (%) ◆
CENTER SITE			
bare	61.5	27	7.5 **A**
soil	39.5	64	0.5 **B**

◆ Arithmetic means followed by different letters are significantly different (*P*=0.05) based on Kruskal-Wallis test.

Discussion

This experiment investigated the response of 277 field-grown *A. palmeri* plants from 5 size classes to a transplantion effort involving salvaging, storing for 6 months, and replanting in a disturbed area. Treatment variables were selected based on what might commonly be employed in a mitigation effort involving this species. Overall, results from the 9-month monitoring period suggest that *A. palmeri* responded well to transplanting, with only 4% post-replanting mortality. Findings from this study prompt some useful insights about the life history of *A. palmeri*, and the adaptive ability of this species to respond to the stress of transplanting.

Within the period of monitoring, results between sites were very similar, suggesting little actual difference between them. However, it may take a longer time period to observe significant plant response to site-associated differences. One notable outlier in soil characteristics that may have a longer-term impact on agave response was the high level of copper (mean 83 ppm) in all four of the north site soil samples. Work by Nobel and Berry (1985) assessing elemental effects on *A. deserti*

seedlings grown in sand-culture has shown that seedlings were fairly resistant to heavy metals, however, a Hoagland solution with neutral pH containing 20 ppm of copper decreased the dry weight of exposed seedlings by half. The levels of copper in soil samples from the north site, also of generally neutral pH according to laboratory analyses, appear to be greater than four times that.

Water treatment

The water treatments in this experiment were selected with the intention of minimizing time and logistics required for implementation. The ability of other agave species to rapidly respond to a pulse of water with new root growth and immediate water uptake (Nobel and Cui 1991, Nobel 1988, North and Nobel 1998), suggests that water availability, even on a single occasion, has the potential to have a dramatic impact on the survivorship and growth of transplants. My hypothesis was that immediate water availability upon replanting in elevated summer temperatures when the shock from transplanting may be greatest (Irish and Irish 2000), would minimize mortality and enable plants to better recover from the stress of storage, thus promoting transplant growth and survival.

Contrary to expectations, the water treatments had no significant effects. A possible explanation for this lack of response is that replanting occurred at the beginning of the summer monsoon period. Although rainfall was below the long-term average during the summer of 2009, 140 mm (5.5 in) fell on the study plot that season, half of which occurred within a month after planting (Table 2.2). This

rainfall may have overridden the affect of water treatments, suggesting that supplemental irrigation may not be critical for plant survivorship when transplanting coincides with periods of sufficient precipitation. If planting had occurred during a more climatically unfavorable period, for example before the warm temperatures and dry weather of the spring season, the application of these water treatments may have had a more important role in influencing mortality and growth.

Size class treatment

There were 5 plant size classes, intended to represent 5 relative age groups. With smaller volume to surface ratios and less developed root systems, younger plants are known to be more sensitive to extremes in temperature and moisture availability (Jordan and Nobel 1979, Nobel 1988). Incredibly low survivor rates are testament to this vulnerability, as only one in 1.2 million seeds is estimated to reach maturity (Nobel 1977, Gentry 1982). Young plants are thought to require a nurse plant or rock to provide protection from unfavorable environmental variation (Jordan and Nobel 1979, Gentry 1982). The open plot where replanting occurred did not offer any such protection to young plants. Thus, I hypothesized that size class would have a significant effect on mortality and plant growth, wherein the substantial reserves and more resilient physiology of larger plants would allow them to have both a greater Δ in growth and a lower rate of mortality.

Size class was associated with a significant effect on plant growth, however, smaller classes (1-2) consistently showed a greater Δ in growth, both in the number of leaves added and in the length of leaves, across all treatment combinations and in all sites (Tables 2.3, 2.5, 2.7), and size class did not significant affect mortality. There were some problems comparing the measurement variables between size classes, based on the physiologically different way that smaller and larger agave plants grow (described in more detail below). Although this issue affects the inference of the results, the trends still maintain that smaller plants grew proportionally more than larger plants in the 9-month monitoring period. Research investigating CO_2 uptake in agaves has shown that several species perform C_3 photosynthesis as seedlings, switching to CAM months or years later (Jordan and Nobel 1979, Nobel 1988, Nobel 2010). The C_3 metabolic strategy allows faster growth during early life stages when plants are most susceptible to temperature extremes, water stress, and herbivory. This ability is contingent on ample water availability, which is also requisite for germination and seedling establishment (Jordan and Nobel 1979, Nobel 1988). Although it remains untested in *A. palmeri* seedlings, it is possible that above average rainfall during the winter 2009-2010 period allowed smaller plants to make use of this strategy, resulting in rapid growth.

Although their response was not as resoundingly positive for the time period monitored, larger plants (size class 4-5 and greater) have substantially more water holding capacity than smaller plants, and are known to successfully endure long-term variability in temperature extremes and available resources (Nobel 1976,

Woodhouse et al. 1983, Nobel 1988). Further, their more extensive root systems, as well as their greater water-gathering leaf surface area, allow them to take better advantage of highly variable moisture, characteristic to the region (Jordan and Nobel 1984, Nobel 1988, North and Nobel 1998). Thus, although larger plants may have a slower growth response, they are arguably more stable in a long-term timeframe, provided that they are collected with minimal damage to root and shoot systems.

Storage treatment

I tested two methods for storing agave plants previous to replanting: potted in soil, and potted without soil with bare roots exposed (and entire plants covered in burlap). Other than being moistened every two weeks, bare treatment plants had minimal exposure to water and light and likely endured more extreme drought conditions with very low metabolic activity, relative to the soil treatment plants. I had thought that bare root plants would have greater mortality and less proportional growth than soil treatment plants due to the stress incurred from 6 months with little access to water, light, and nutrients.

Storage treatment did have a significant effect on both survivorship and growth response. While bare root plants were associated with significant mortality while in storage and after replanting at the study plot, they also had the most positive plant response with the greatest Δ in number of leaves and in length of leaf, across all sites after replanting (Tables 2.4, 2.6, 2.8). There are several stress-

mitigating mechanisms agaves have been shown to use that may have contributed to the overall survivorship and growth of bare treatment plants. A study by Nobel and Sanderson (1984) investigating the hydraulic conductivity of *A. deserti* roots during times of drought discovered that the cellular make-up of agave roots act much like a rectifier—allowing roots to rapidly uptake water from the soil when it becomes available, but greatly restricting water loss when the soil is dry. Moreover, as drying increases, soil constricts and roots are prone to shrink, creating an air gap which further restricts hydraulic conductivity (Nobel and Cui 1991). Water from the fleshy shoot at the base of the rosette is exported to the roots to keep them alive and receptive to soil moisture when it becomes available (North and Nobel 1998). *A. deserti* seedlings have been shown to survive up to 70% water loss (Jordan and Nobel 1979). As young plants grow older and larger they develop the capacity to store more water per unit surface area, thus able to tolerate longer droughts with up to 95% plant water loss before irreversible cellular damage (Nobel 1988, 1994).

In addition to conserving water in a drying substrate, agave plants have adapted to maximizing their uptake of water when it becomes available. The radial arrangement of imbricated leaves funnels water directly to the core shoot where primary root growth is generated (Gentry 1982, North and Nobel 1998). *A. deserti* has been shown to rapidly respond to a pulse of water by growing so-called "rain roots", up to 5 mm (0.2 in) in length within 6 hours after wetting (Nobel 1988). After 5 months of drought, watering caused the growth of new roots and the elongation of existing roots within 7-11 days (North and Nobel 1998). In addition,

leaves resumed stomatal opening within 12 hours (Nobel 1976) and recovered nearly 30% of their original thickness within 24 hours (Schulte and Nobel 1989). Given that bare treatment plants were under considerable stress upon planting, it is possible that drought-mitigating strategies such as these may have played a role in re-establishment.

To frame the context of significant growth in bare treatment plants it is important to consider their poor condition and reduced size (in leaf number) prior to replanting. Because of leaf die-off during the storage period, many size class 1 bare root plants had only 1-2 leaves in the summer 2009 inventory and subsequently grew an additional 2-3 leaves by spring 2010, yielding over 100% proportional change. In comparison, soil treatment plants were generally in very good shape upon replanting. For these individuals, a size class 1 plant may have started with 3-4 leaves in summer 2009, and subsequently added 2-3 leaves, resulting in a less dramatic proportional change in growth.

Soil treatment plants, in addition to low mortality (2% post-replant), had consistent but lesser Δ in number and length of leaves (Tables 2.4, 2.6, 2.8). After reviewing the repeated photographs I collected during each phase of the experiment, I have concluded that this unexpected result of milder response in plant growth when compared to stressed bare treatment plants is largely a result of when measurements were collected. Soil treatment plants had been watered to field capacity once a month from January-April 2009, and then bimonthly through late spring until replanting. My irrigation strategy was intended to provide the basic

water required to keep plants alive, but in retrospect, the amount and frequency of irrigation was likely ample water to facilitate growth in this drought-adapted species. Thus, by the July 2009 planting period, these soil treatment plants had already been growing for some time, likely since warmer weather began in April 2009. It was evident from photos and notes that these plants had added multiple leaves and had grown larger while in storage. Additionally, root systems appeared to be much better developed than they had been after salvaging, another indication of their growth while in storage. Thus, soil treatment plants had already been growing before being replanted in the field and the post-replanting monitoring period did not accurately capture their full, seasonal growth response.

A one-year experiment on a resilient, arid-adapted, long-lived succulent prompted many challenges. The ultimate variable of interest in experimental plants is their long-term reproductive success. I struggled with determining how to apply another definition of "success" given time restrictions, and experimented with several physiological measurements including chlorophyll fluorescence and tissue moisture sampling. I decided to assess plant growth, with the intention to reveal if plants had a generally positive or negative response to applied treatment combinations. However, because these measures were only made after replanting and not previous to plant collection, an accurate depiction of the total response of plants from collection to post-transplanting was limited. Further, in reviewing the results I have discovered that there are a few problems with the variables that I selected, particularly when comparing plants between size classes. Both the

proportional change (Δ) in leaf length, as well as the proportional change (Δ) in number of leaves were difficult measurements to apply between size classes, due to the different way that small and large agaves grow.

Agave leaves can be active a long time—up to 15 years (Gentry 1982)—but do not continuously grow for that entire period. Above-ground meristimatic growth tissue is located in the stem at the base of the center core spike, from which plants grow taller and leaves longer (Nobel 1988). Over time, as leaves unfold from the spike, older leaves descend toward the base of the rosette and eventually senesce (Woodhouse et al. 1980). Thus the leaves closer to the spike are younger and will grow more than the older leaves at the base of the rosette. In a small size class 1 plant with only 3-4 leaves, all the leaves are relatively young and close to the core spike, and would be expected to have more proportional growth than the 5+ year old leaf lower in the rosette of an larger size class 5 plant. That is, in fact, what I discovered (Tables 2.5, 2.7). Comparing the growth measurements of smaller plants to larger plants would prompt one to think that smaller plants are responding "better" to treatments because their proportional leaf growth was greater. This conclusion is misleading, however, as the explanation is instead more closely attributable to physiological growth differences based on leaf age.

An additional concern involving leaf measurements also occurred in similar-sized plants. Without considering the possibility of variable growth rates, I predominately elected to measure leaves lower in the rosette on the south side that had been previously marked with an ID before collection. In contrast, northern

leaves were generally selected high in the rosette and closer to the core spike and were likely younger. Results from each site indicate that there were significant differences between treatments in proportional north leaf growth, while only the center site showed differences in proportional growth for the southern leaves. It is possible that these results are attributable to the proximity of leaves to the core spike.

Recommendations

Re-establishing *A. palmeri* in areas of large-scale disturbance will most likely involve two methods: seeding and transplanting. In the wild, agave seedlings are episodically recruited, requiring several consecutive years of above average rainfall in order to both germinate and establish (Gentry 1972, Nobel 1977, Jordan and Nobel 1979). Young plants are much more vulnerable to succumbing from extremes in temperature, water availability, and herbivory, and have been shown to be exceedingly rare in the wild (Gentry 1972, Nobel 1977, Jordan and Nobel 1979). In an associated greenhouse experiment I conducted (Chapter 1) investigating the early life stages of *A. palmeri* within a disturbance scenario specific to this field site, I tested seedling emergence and establishment to several critical environmental variables including amount of irrigation, presence or absence of shade, soil surface mulches, and soil type (Turner et al. 1966, Steenbergh and Lowe 1969, Jordan and Nobel 1979). In 5.5 months I documented only 28% overall emergence in this experiment in which several important variables that cause seedling mortality were

absent, like freezing temperatures, long-term water variability, competition for resources, and herbivory. Results from this experiment showed that shaded seedlings were associated with over three times the emergence and almost two times the size of unshaded seedlings, indicating that shade is a crucial component to the early life history of *A. palmeri* (Chapter 1). This suggests that totally open, homogeneous, disturbed landscapes would not provide adequate growing conditions for seedlings. Further, any plants that did survive would represent a single-aged cohort that would not flower for up to three decades, providing a considerable temporal gap in the foraging needs for multiple generations of *L. curasoae*. Therefore, it is critical that seeding is not the only effort made to restore *A. palmeri* in a disturbed landscape, both because it is unreliable and potentially ineffectual, and also because it would not offer a persistent, functional relationship with the endangered species of concern at the site. Thus, transplanting of variously-aged *A. palmeri* plants may be essential to an effective restoration strategy in this setting.

In the present study, plants appeared to establish well following transplanting, indicating that there may be a good probability of successful reestablishment in a disturbed landscape. An important caveat, however, is that this was a short study on a long-lived plant. Further monitoring is required to determine how plants will respond long-term. This note is particularly important because the year of study had above-average winter precipitation, which may have positively skewed response. Collecting and replanting plants during different

seasons than attempted in this experiment, or during periods with less rainfall could have very different results in plant growth and mortality. In addition, the disturbance scenario that was created at the study plot involved removal of above-ground vegetation and a superficial disturbance of the soil from 15-30 cm (6-12 in). However, soil profiles remained largely intact, were rich in clay, and high in micronutrients. Many native plants returned within a few months, having sprouted from roots or emerged from seeds. How *A. palmeri* would respond to a transplanting effort in areas of profound disturbance, as would be expected in a mining reclamation scenario, is still unknown.

In reviewing the results, three issues emerged as most prominent in affecting the outcomes of this experiment, and should be primary considerations to managers considering an *A. palmeri* transplanting in this area: season of replanting, storage methods, and incorporation of multiple size classes.

Seasonal variation in temperature and water availability is a driving factor in plant stress, affecting both survivorship and growth (Osmond et al. 1987). Research has shown that agaves are well adapted to periods of drought, and capable of responding rapidly to moisture when it becomes available (Nobel 1976, Nobel and Sanderson 1984, Nobel 1988, North and Nobel 1998). In the present study, it appears that the considerable effort and cost required to apply water to transplants may not be required for their establishment if they receive sufficient rainfall. Thus, transplanting should be timed to coincide with seasonal precipitation whenever possible.

Drought tolerance of agave may also provide some flexibility during a transplanting effort if replanting is delayed and plants must be stored. Depending on the length of storage, and the season of replanting, different strategies may be more effective. Bare treating transplants is a highly convenient option, particularly for very large plants which are perilously awkward to handle, but crucial to incorporate into a restoration plan. Because mortality was high (31%) in the bare root treatment over the 7-month storage period, I would recommend this method as more suitable for shorter periods of time, and/or for larger plants which had no mortality while in storage. Soil treatment plants of all sizes had virtually no mortality while in storage and appeared to be in good condition upon replanting. These plants were probably more resilient and better adjusted upon replanting and may have been less negatively affected by a poor precipitation season than the bare treated plants whose reserves were almost certainly more depleted. Thus, the soil treatment method would be expected to have significantly less mortality, both while in storage and after replanting, and may be the most secure method for storing plants for long time periods—especially smaller plants.

Present study results indicate that multiple size classes may be re-established in disturbed areas successfully. Transplanting larger plants (size class 4-5 and larger) has the potential to provide more immediate forage for *L. curasoe*, as well as expand a re-established population over time through dispersed seeds and vegetative ramets—a crucial link to a self-maintained, functional population. In addition, these plants had low mortalities in both soil and bare treatments, and

appeared to readily establish after replanting. In the context of this experiment, smaller plants (size class 1-2) also transplanted with good success. However, it has been shown that young succulents are highly susceptible to mortality by extreme temperatures, drought, and herbivory, and may require facilitative relationships to survive to maturity (Turner et al. 1966, Jordan and Nobel 1979, Gentry 1982, Peters et al. 2008). The relative success of the smaller plants may be largely attributable to above average rainfall after replanting, with almost a year's-worth of precipitation deposited in under 7 months. Further monitoring of these transplants is essential to evaluating their adaptive response. It may prove to be important to transplant smaller plants into a heterogeneous landscape—below existing perennial vegetation, near other agaves, adjacent to vegetative debris, or neighboring large rocks—in order for them to successfully adapt to extreme environmental conditions.

In conclusion, the potential of eliminating significant critical foraging areas for *L. curasoae* would demand that a multi-age cohort of established plants be restored to the landscape. Research by Ober et al. (2005) suggests that reducing *A. palmeri* numbers could adversely affect *L. curasoae* populations by forcing them to travel longer distances for foraging and roosting sites, and by increasing competition for a limited food supply. Because *A. palmeri* tends to grow in loose colonies (Gentry 1982), large-scale disturbance can impact large numbers of plants, as would occur at the study site, with unknown ramifications for this endangered migratory bat. Agaves have been shown to be adaptable to temperature and water

variability and to be tolerant to prolonged stress. Thus transplanting is a promising option for restoration, and may be imperative to the functional re-establishment of the species, if habitat disturbance cannot be avoided.

Addendum

Table 1. Ranking of the proportional change in number of leaves for all treatment combinations in the north site at the study plot in the northeastern Santa Rita Mountains, Pima County, Arizona, between 14 Jul 2009-14 Apr 2010 [Kruskal-Wallis, H=43.2, df=23, P=0.007].

NORTH SITE					
Mean rank	**Sample size (N)**	**Water treatment**	**Storage treatment**	**Size class treatment**	**Δ Number of leaves (%)**
74	2	gel	bare	1	183.3
72	1	gel	bare	3	66.7
70	2	dry	bare	1	53.3
62.5	2	water	bare	1	60.0
61.8	5	dry	soil	1	30.9
61.5	1	dry	bare	2	25.0
53.3	2	gel	bare	2	44.6
52.3	2	dry	bare	3	20.1
51.9	5	dry	soil	2	24.0
51.1	5	water	soil	3	173.9
43	1	water	bare	2	16.7
40.4	5	gel	soil	2	17.8
39.1	5	gel	soil	4	15.9
34.3	2	dry	soil	5	14.6
31.5	5	water	soil	4	12.9
30.2	5	gel	soil	3	11.7
29.8	5	dry	soil	3	11.9
28	4	gel	soil	1	11.6
27	1	water	bare	3	12.5
26.5	4	water	soil	2	11.0
21.3	5	dry	soil	4	8.9
16.9	4	water	soil	1	7.1
13.5	2	gel	soil	5	5.0
4.5	2	water	soil	5	1.0

Table 2. Ranking of the proportional change in number of leaves for all treatment combinations in the center site at the study plot in the northeastern Santa Rita Mountains, Pima County, Arizona, between 14 Jul 2009-14 Apr 2010 [Kruskal-Wallis, H=43.9, df=26, P=0.016].

CENTER SITE					
Mean rank	**Sample size (N)**	**Water treatment**	**Storage treatment**	**Size class treatment**	**Δ Number of leaves (%)**
88.7	3	dry	bare	1	66.7
85.5	3	water	bare	1	54.2
82	3	gel	bare	2	61.3
75.8	2	gel	bare	3	33.6
74.8	3	water	bare	2	145.8
66.4	5	dry	soil	2	26.0
64.3	3	dry	bare	2	285.7
58.8	3	water	bare	3	176.2
57.9	5	dry	soil	3	21.8
57.5	5	dry	soil	1	24.1
53	5	gel	soil	1	20.9
52.7	5	water	soil	2	27.5
50.2	3	dry	bare	3	20.8
45.5	2	dry	bare	5	15.3
44.8	5	water	soil	1	18.8
42.8	5	gel	soil	2	15.6
42.3	2	gel	bare	1	11.7
41.5	2	gel	bare	5	15.3
38.6	5	gel	soil	4	14.0
35	2	dry	soil	5	11.3
32.9	5	water	soil	4	10.9
32.8	2	gel	soil	5	10.6
30.7	5	dry	soil	4	9.7
26.2	5	gel	soil	3	8.5
25.9	5	water	soil	3	7.9
18.3	2	water	bare	5	-40.3
5.5	2	water	soil	5	-2.4

Table 3. Ranking of the proportional change in number of leaves for all treatment combinations in the south site at the study plot in the northeastern Santa Rita Mountains, Pima County, Arizona, between 14 Jul 2009-14 Apr 2010 [Kruskal-Wallis, H=69.8, df=23, P<0.001].

SOUTH SITE					
Mean rank	**Sample size (N)**	**Water treatment**	**Storage treatment**	**Size class treatment**	**Δ Number of leaves (%)**
87	2	dry	bare	2	150.0
82.7	3	gel	bare	1	191.7
82.5	3	water	bare	1	100.0
79.8	3	water	bare	2	102.4
76.8	3	dry	bare	3	115.6
70.3	3	water	bare	3	64.2
67.7	3	gel	bare	2	235.0
66.7	3	gel	bare	3	67.1
61.8	2	dry	bare	1	54.2
58.7	5	water	soil	2	44.1
53.1	5	dry	soil	2	37.8
53.1	5	water	soil	1	39.9
45.8	5	water	soil	3	34.9
45.3	5	gel	soil	2	32.3
42.6	5	gel	soil	3	29.9
42.4	5	dry	soil	1	37.2
37.4	5	gel	soil	1	27.9
24.1	5	dry	soil	3	18.4
22.5	2	gel	soil	5	16.1
17	5	dry	soil	4	14.4
15.8	5	gel	soil	4	13.7
11.8	5	water	soil	4	10.0
11.5	2	water	soil	5	11.4
2.8	2	dry	soil	5	3.5

Table 4. Ranking of the proportional change in north leaf length for all treatment combinations in the north site at the study plot in the northeastern Santa Rita Mountains, Pima County, Arizona, between 14 Jul 2009-14 Apr 2010 [Kruskal-Wallis, H=39.5, df=23, P=0.017].

NORTH SITE					
Mean rank	**Sample size (N)**	**Water treatment**	**Storage treatment**	**Size class treatment**	**Δ North leaf length (%)**
75	1	dry	bare	1	25.0
72	1	gel	bare	1	16.7
70.5	2	gel	bare	2	14.5
65	2	water	bare	1	8.6
64	1	water	bare	2	8.0
59.5	1	water	bare	3	6.3
56.5	1	gel	bare	3	5.1
53	4	water	soil	1	5.5
50.9	5	dry	soil	1	9.4
48.8	5	dry	soil	2	4.6
45.5	4	water	soil	2	5.5
41.3	5	dry	soil	3	4.1
39.5	5	water	soil	3	3.4
37.9	4	gel	soil	1	2.9
33.5	2	water	soil	5	2.5
31.2	5	gel	soil	2	2.5
28.3	2	dry	bare	3	1.9
27.8	5	dry	soil	4	1.7
23.4	5	gel	soil	4	1.3
23.3	2	dry	soil	5	1.2
19.4	5	gel	soil	3	0.5
18.1	5	water	soil	4	0.7
16.8	2	gel	soil	5	0.6
1	1	dry	bare	2	-3.1

Table 5. Rank of proportional change in north leaf lengths for all treatment combinations in the center site at the study plot in the northeastern Santa Rita Mountains, Pima County, Arizona, between 14 Jul 2009-14 Apr 2010 [Kruskal-Wallis, H=41.4, df=26, P=0.028].

CENTER SITE					
Mean rank	**Sample size (N)**	**Water treatment**	**Storage treatment**	**Size class treatment**	**Δ North leaf length (%)**
86.2	3	water	bare	1	31.3
76.5	1	water	bare	3	4.0
73.8	3	dry	bare	1	22.3
73.4	5	dry	soil	1	14.6
68.5	1	dry	bare	2	4.7
65.6	5	water	soil	1	12.2
65.2	3	gel	bare	2	28.1
62.8	2	water	bare	2	11.1
56.9	5	gel	soil	1	7.3
51.8	5	water	soil	2	7.7
50.5	1	water	bare	5	2.2
45.9	5	water	soil	3	4.5
44.5	3	dry	bare	3	4.0
41.8	2	gel	bare	3	4.2
41.1	5	dry	soil	4	2.7
41.1	5	dry	soil	2	4.3
38.8	5	gel	soil	2	2.5
36.2	5	water	soil	4	2.0
32.9	5	dry	soil	3	1.8
31.7	5	gel	soil	3	1.6
29.8	2	water	soil	5	1.3
28.5	2	gel	bare	1	-1.4
24.5	2	gel	soil	5	0.7
22.8	5	gel	soil	4	0.5
21	2	dry	bare	5	0.1
18.5	2	gel	bare	5	0.0
18.5	2	dry	soil	5	0.0

Table 6. Ranking of proportional change in north leaf length for all treatment combinations in the south site at the study plot in the northeastern Santa Rita Mountains, Pima County, Arizona, between 14 Jul 2009-14 Apr 2010 [Kruskal-Wallis, H=46.9, df=23, P=0.002].

SOUTH SITE					
Mean rank	**Sample size (N)**	**Water treatment**	**Storage treatment**	**Size class treatment**	**Δ North leaf length (%)**
75.7	3	gel	bare	2	27.9
75.7	3	water	bare	1	36.3
74.8	5	gel	soil	1	20.7
74.2	3	water	bare	2	15.7
70.3	2	dry	bare	1	12.9
65.9	5	water	soil	2	10.7
64.4	5	gel	soil	2	11.8
58.7	3	dry	bare	3	13.1
49.3	3	gel	bare	3	11.7
46.8	5	dry	soil	2	5.2
45.1	5	dry	soil	1	6.5
42	5	water	soil	1	11.4
40	2	gel	soil	5	4.1
38.6	5	gel	soil	3	3.4
35.3	5	dry	soil	4	3.3
33.7	3	water	bare	3	3.0
33.3	2	water	soil	5	2.7
30.5	2	dry	bare	2	2.3
29.8	5	dry	soil	3	2.4
27.8	5	water	soil	3	2.1
25.5	2	dry	soil	5	1.3
25.3	5	gel	soil	4	0.4
21.8	5	water	soil	4	0.4
3.3	2	gel	bare	1	-8.1

Table 7. Ranking of proportional change in south leaf length for all treatment combinations in the center site at the study plot in the northeastern Santa Rita Mountains, Pima County, Arizona, between 14 Jul 2009-14 Apr 2010 [Kruskal-Wallis, H=42.29, df=26, P=0.023].

CENTER SITE					
Mean rank	**Sample size (N)**	**Water treatment**	**Storage treatment**	**Size class treatment**	**Δ South leaf length (%)**
90	1	gel	bare	1	21.4
84.5	3	dry	bare	2	13.2
82.7	3	water	bare	1	27.4
78.7	3	dry	bare	1	7.9
78	2	water	bare	2	8.7
66.5	1	water	bare	5	1.6
65.2	5	water	soil	1	8.9
58	1	water	soil	5	1.0
57.7	3	gel	bare	2	2.2
54.4	4	gel	soil	4	1.5
46.4	5	gel	soil	1	1.9
44.5	2	gel	bare	5	0.6
44.5	2	water	bare	3	1.5
41.8	5	water	soil	2	0.2
40.3	5	dry	soil	1	-3.9
39.7	5	gel	soil	3	0.2
38.3	3	dry	bare	3	0.4
38	5	gel	soil	2	0.2
36.5	2	dry	bare	5	0.1
36	5	dry	soil	2	0.0
36	5	dry	soil	4	0.0
36	2	dry	soil	5	0.0
36	2	gel	bare	3	0.0
31	5	dry	soil	3	-0.4
28.9	5	water	soil	4	-1.2
28.4	5	water	soil	3	-0.5
9.5	2	gel	soil	5	-3.6

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