# HABITAT CHARACTERIZATION AND PILOT REINTRODUCTION OF STAR CACTUS (*ASTROPHYTUM ASTERIAS*)

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by

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## ABSTRACT

HABITAT CHARACTERIZATION AND PILOT REINTRODUCTION OF STAR CACTUS (*ASTROPHYTUM ASTERIAS*)


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*Astrophytum asterias* is federally listed as endangered and in the United States is found only in Starr County, Texas. The species has a priority ranking of 2 by the United States Fish & Wildlife Service which means it has high recovery potential. One means to achieve recovery is by reintroduction. To establish a successful reintroduction, it is important to know the species' habitat. Therefore, this study characterized *A. asterias* habitat by conducting vegetation transects and collecting soil samples in 15 subpopulations. The top five plant species with greatest dominance included: *Varilla texana*, *Prosopis glandulosa*, *Acacia rigidula*, *Opuntia leptocaulis*, and *Castela erecta* subsp. *texana*. *Astrophytum asterias* has been found in the following soils: Catarina soils; Garceno clay loam; Jimenez-Quemado association; Maverick soils, eroded; Montell clay, saline; and Ramadero loam. Of the 15 subpopulations sampled, 9 were classified as saline-sodic; 2 saline; 2 sodic; and 3 non-saline, non-sodic. In this study a pilot reintroduction was also established to test the feasibility of reintroducing *A. asterias*. Seeds and seedlings were used as propagules for the pilot reintroduction. Four treatments were established: seeds planted in the spring; seedlings planted in the spring; seeds planted in the fall; seedlings planted in the fall. Each treatment consisted of 120 individuals. Overall less than 4% of the planted seeds produced seedlings. Seedling survivorship of the spring and fall treatments was 55% and 72.5%, respectively. Mortality of seedlings was due to desiccation, herbivory, infestation by weevils, burying by Mexican ground squirrel, and other miscellaneous and unknown causes. Twenty-eight candidate models were developed to assess the potential influence of season of planting; average state of the seedlings per subquadrat; environmental variables of monthly precipitation and average monthly ground temperature; average seedling diameter at the time of planting per subquadrat; and percent bare ground of each subquadrat on percent survivorship of the reintroduced seedlings. In addition to the 28 candidate models, a temporal model was also included which assessed passage of time as the only factor influencing survivorship. Of the model statements analyzed, the temporal model was the top model ($AIC_c$ = 1129.3094, $w_t$ = 0.99999854) indicating that of the factors analyzed, passage of time had the greatest influence on seedling survivorship. Based upon the research of this study, a draft reintroduction plan for *A. asterias* was developed to guide future reintroductions.

# CHAPTER I

## INTRODUCTION

*Astrophytum asterias* is a spineless cactus with a circular, disk- or dome-shaped body 5-15 cm wide and often flush with or just a few centimeters above the soil surface. The body is almost always divided into eight ribs of equal size with a line of evenly spaced wooly areoles running down the center of each rib (Poole, et al., 2007). The flowers are yellow with a red-orange throat (Damude and Poole, 1990). This species is a rare cactus of southern Texas and northern Mexico. *Astrophytum asterias* was listed as endangered under the Endangered Species Act on 18 October 1993 and by the state of Texas on 30 January 1997 (United States Fish & Wildlife Service (USFWS), 2003). As of 22 October 1987, *A. asterias* was also listed in Appendix I (species threatened with extinction) by CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora). The USFWS *A. asterias* recovery plan (2003) assigns the species a priority ranking of 2, which indicates *A. asterias* faces a high degree of threat, yet has high recovery potential. Recovery criteria include the maintenance or establishment of ten fully protected populations in the United States or Mexico. The populations must be fully protected, a minimum of 2,000 individuals each, and of an age class structure reflecting that the plants are reproducing and becoming naturally established (USFWS, 2003). To achieve this, surveys for new populations will continue by government agencies, non-government organizations, and researchers. If sufficient populations are not found, reintroduction of *A. asterias* is an acceptable step in the recovery of this species.

Reintroduction of rare plant species is a fairly new science and there is still much to learn about the procedure (Guerrant and Kaye, 2007). Based upon a 1990 USFWS report to Congress, nearly one-quarter of all USFWS recovery plans for plants call for reintroductions as a means to recover the species (Falk and Olwell, 1992). It is highly likely that the percentage is much higher today. Many plant reintroductions have been implemented with varying success (Turner, et al., 1969; Pavlik, et al., 1993; Mehrhoff, 1996; Obee and Cartica, 1997; Drayton and Primack, 2000; Kent, et al., 2000; Rowland and Maun, 2001; Maschinski, et al., 2004; Holl and Hayes, 2006; Maschinski and Duquesnel, 2006; Jõgar and Moora, 2008). The key to successful reintroductions is to know the organism of interest. For plants, knowing the species' current range (extant populations), reproductive biology, pollinators, genetic variability, and threats are all critical components to implementing a reintroduction. Reintroductions should occur within the current or historical range of the species taking into account spatial distribution of the current populations. For obligate outcrossing species the presence of effective pollinators is critical. Reintroductions should maintain or enhance genetic variability in and among populations and be conducted in areas where threats to the species have been minimized.

Choosing an appropriate site for reintroduction is crucial. When selecting potential reintroduction sites four categories of selection criteria should be considered: physical, biological, logistical, and historical (Fiedler and Laven, 1996). Abiotic factors such as geomorphic setting, landscape matrix, slope angle and aspect, albedo effect of the substrate, underlying geology, soil type, pH, and other soil-chemistry factors are crucial to site selection (Fiedler and Laven, 1996). Fiedler (1991) determined that the majority of mitigation-related introductions in California failed due to the inappropriateness of the soil characteristics at the receptor site. It is also important to understand the community composition and structure in which the species exists in order to maintain the diversity and natural processes of the species' habitat. Logistically the site must be readily accessible, afford a level of protection against anthropogenic effects, and ensure access to facilitate monitoring, research, and management (Fiedler and Laven, 1996).

There are other factors that are common to any reintroduction and must be considered before implementing one. These include choice of propagule type, number, and source; how and when they will be planted; if the site will be manipulated; if there will be supplemental care after establishment; etc. (Guerrant and Kaye, 2007). Any one of these things could be the deciding factor which leads to the success or failure of the reintroduction.

In 1993 when *A. asterias* was federally listed, there was only one known population in the U.S., in Starr County, Texas located on private property. There were also reports of *A. asterias* from Cameron, Hidalgo, and Zapata counties; however, none of those sites had been relocated (Damude and Poole, 1990). In Mexico, several populations were known from Tamaulipas and Nuevo León with verification surveys taking place in Tamaulipas (USFWS, 2003). At the initiation of the study described herein, there were nine known private properties in Starr County with extant subpopulations of *A. asterias* (Janssen et al., 2005). Seven of nine of these properties have multiple subpopulations or groupings of *A. asterias* (Fig. 1). Recent research in Mexico recognizes seven populations in Tamaulipas and two in Nuevo León with population numbers ranging from 10-704 (Martínez-Ávalos, et al., 2004).

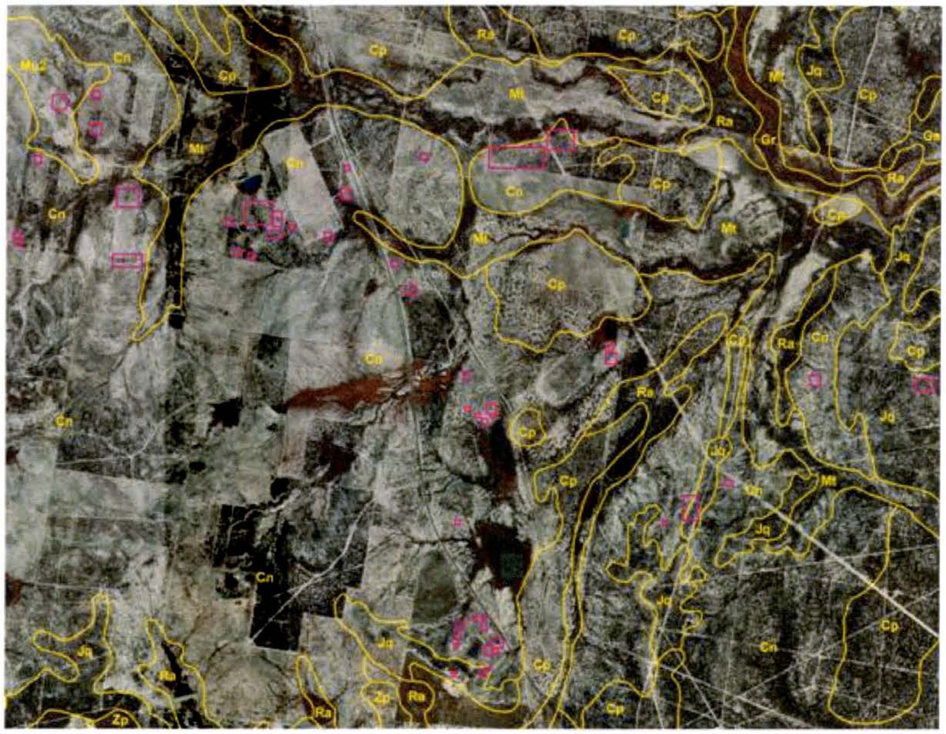


**Figure 1.** Depiction of subpopulations (pink polygons) of *A. asterias* across multiple soil types in Texas. Other soil types displayed: Copita fine sandy loam (Cp), Grulla clay (Gr), and Zapata (Zp).

The habitats in Texas and Mexico from which *A. asterias* is known differ slightly. Using the soil types as defined by Thompson, et al. (1972), the subpopulations of *A. asterias* in Texas are found predominantly on Catarina (Cn) soils; however, subpopulations also occur on Garceno clay loam (Ga); Jimenez-Quemado association (Jq); Montell clay, saline (Mt); Maverick soils, eroded (Mu2); and Ramadero loam (Ra) (Fig. 1). The underlying geology is of the Catahoula and Frio formations undivided and the Jackson Group (Bureau of Economic Geology, 1976). At the only protected site in the United States, Carr (2001, p. 3) described *A. asterias* as occurring in a "broad spectrum of habitats" from "gravel-veneered clay on very gently sloping footslopes just above valley flats" to "clay flats proper and on upper slopes and gravelly hilltops." In Texas some of the associated species of *A. asterias* include *Acacia rigidula*, *Billieturnera helleri*, *Bouteloua trifida*, *Castela erecta* subsp. *texana*, *Dyssodia pentachaeta*, *Echinocereus reichenbachii* var. *fitchii*, *Mammillaria heyderi*, *Monanthochloë littoralis*, *Opuntia leptocaulis*, *Prosopis glandulosa*, *P. reptans*, *Sporobolus airoides*, *Thelocactus bicolor*, *Varilla texana*, *Ziziphus obtusifolia*, and other common species of the Tamaulipan thornscrub (Damude and Poole, 1990; Carr, 2001; USFWS, 2003; Janssen, et al., 2005; Strong and Williamson, 2007; Blair and Williamson, 2008). Four of the nine populations in Mexico with the highest density of *A. asterias* inhabit two vegetation types: Tamaulipan thornscrub and Piedmont thornscrub (Martínez-Ávalos, et al., 2007). Tamaulipan thornscrub is found on well-drained sands and dominated by *Karwinskia humboldtiana*, *Parkinsonia texana*, *Schaefferia cuneifolia*, *Prosopis glandulosa*, *Porlieria angustifolia*, *Ziziphus obtusifolia* (Martínez-Ávalos, et al., 2007). As described by Martínez-Ávalos, et al. (2007), the soils of the Piedmont thornscrub are a mixture of sand and clay over limestone with dominant plant species including: *Acacia berlandieri*, *A. coulteri*, *A. rigidula*, *A. greggii*, *Astrocasia neurocarpa*, *Chloroleucon pallens*, *C. frutescens*, *Cordia boissieri*, *Castela tortuosa*, *Forestiera angustifolia*, *Fraxinus greggii*, *Gochnatia hypoleuca*, *Helietta parvifolia*, *Lycium berlandieri*, *Mimosa leucaenoides*, *Rhus virens*, *Neopringlea integrifolia*, and *Yucca filifera*.

Starr County, Texas is in the Tamaulipan Thornscrub ecoregion of the United States (The Nature Conservancy, 1999). According to the National Climatic Data Center

(2002), Rio Grande City, Starr County, Texas, received an average annual rainfall of 554 mm from 1971–2000. The 30-year monthly average rainfall was less than 50 mm for 8 months. Of these January, March, November, and December was less than 25 mm. Over this same 30-year period, the annual average maximum and minimum air temperatures recorded were 29.9ºC and 16.3ºC, respectively (National Climatic Data Center, 2002). For a total of 7 months, the 30-year monthly average maximum air temperature was >30ºC.

Cacti species often grow in environments with temperature extremes and limited rainfall. Laboratory and field experiments have shown that factors such as light, temperature, and water can influence the germination of cacti seeds and effect the establishment of seedlings (Nolasco, et al., 1996, 1997; Godínez-Alvarez and Valiente-Banuet, 1998; Leirana-Alcocer and Parra-Tabla, 1999; De la Barrera and Nobel, 2003; Flores-Martínez, et al., 2008; Martínez-Berdeja and Valverde, 2008). One strategy used by cacti to overcome harsh environmental conditions is to employ the protection afforded by another plant species (nurse plants) and objects such as rocks (Valiente-Banuet, et al., 1991; Suzán, et al., 1994; Mandujano, et al., 2002; Flores, et al., 2004; Peters, et al., 2008). Nurse plants/objects play key roles in the establishment and persistence of cacti species by reducing high temperatures and direct solar radiation (Nobel, 1980; Franco and Nobel, 1989; Valiente-Banuet and Ezcurra, 1991; Godínez-Alvarez, et al., 2005). Little is known regarding germination and seedling establishment of *A. asterias* in the wild. However, *A. asterias* has been observed under the shade of other plants, as well as in the open (Damude and Poole, 1990; pers. observ.). It will also retract into the soil during dry periods (Poole, et al., 2007; pers. observ.).

The *A. asterias* recovery plan outlines various tasks that must be accomplished to downlist this species. Many of these tasks provide the framework to create a reintroduction strategy for *A. asterias*. This study has three objectives which aim to meet some of the tasks outlined in the recovery plan: 1) conduct vegetation analyses within 15 subpopulations of *A. asterias* on the nine private properties known to have subpopulations of star cactus to characterize the current habitat and determine if there are differences in vegetation among the subpopulations; 2) conduct soil analyses within said subpopulations to determine average parameters of each soil type and ranges of variability; 3) establish a pilot reintroduction site to determine which propagule type (seeds or seedlings) has greatest success dependent upon season of planting (spring versus fall).

# CHAPTER II

## MATERIALS AND METHODS

### Vegetation transects

Prior to field work, I used several criteria to select 20 subpopulations in which to conduct vegetation and soil analyses. First, I overlaid 2000–2001 spatial data of known star cactus subpopulations on the USDA-NRCS Starr County Soil Survey data layer (Thompson, et al., 1972) to determine soil type of the subpopulations (Fig. 1). I also took into account the number of *A. asterias* located in previous surveys conducted by Texas Parks & Wildlife Department and The Nature Conservancy personnel, as well as the area of the subpopulation. I did this to ensure that the 20 subpopulations in total represented the variation in soil types in which *A. asterias* was known to occur and to ensure that the areas were estimated to support ≥15 individuals. Five subpopulations were excluded due to low numbers of *A. asterias*. Therefore, I conducted vegetation transects in 15 subpopulations (Fig. 2). I conducted the 15 vegetation surveys 13-17 March and 18-19 May 2006.

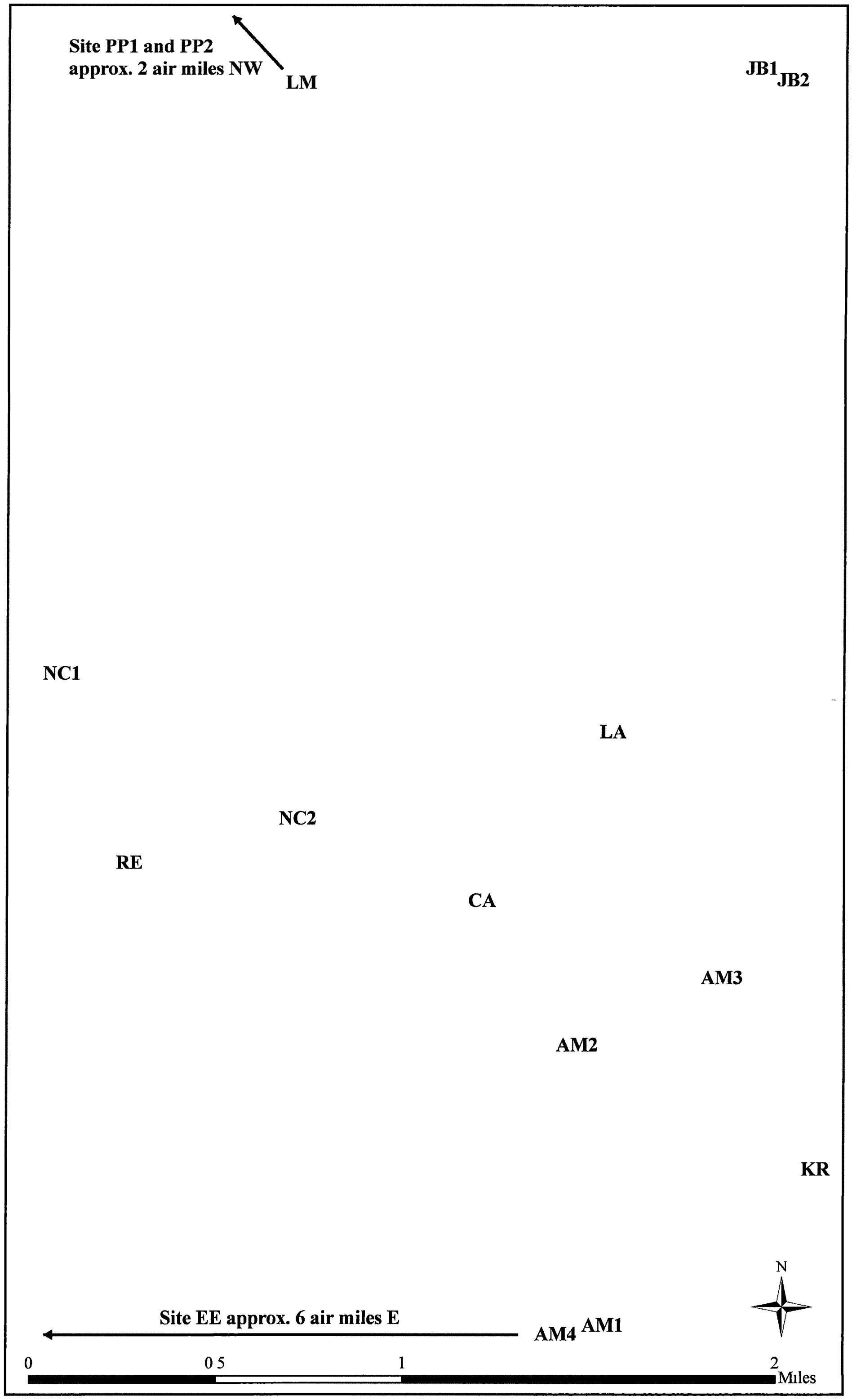



**Figure 2.** Spatial depiction of 13 of the 15 subpopulations in which vegetation transects were conducted and soil samples collected, March and May 2006. Also included is the reintroduction site (RE) at which a vegetation transect was conducted and a soil sample collected, March 2007.

The first step at each of the 15 subpopulations chosen for vegetation and soil analyses was to survey the area for *A. asterias* to confirm that a minimum of 15 individuals were in the area. I then conducted vegetation transects using the line-intercept method to document the plant species within the *A. asterias* subpopulations and determined percent dominance (cover) and percent relative dominance of these species (Brower, et al., 1990; Cox, 1996). In cases of overlapping canopies, I recorded intercepts of both overstory and understory plants. Nomenclature follows Jones et al. (1997).

Each vegetation transect was 75 m (three 25-m transects) and followed a stratified-random design. I set a 30-meter baseline at the edge of the subpopulation and ran it the length of the flagged individuals. Three 25-meter transects ran the width of the area (Fig. 3). A random numbers table was used to determine placement of the first 25-meter tape between 0 and 9 meters. I placed the two subsequent transects at 10 and 20 meters from the first randomly selected point along the baseline. A Trimble GeoExplorer 3 was used to take GPS coordinates at the baseline endpoints and the endpoints of the 25-m transects. I used the differentially corrected coordinates in ArcGIS to confirm the location of the vegetation transects to that of previously documented *A. asterias* subpopulations. The GPS coordinates can also be used to relocate the approximate location of the vegetation transects should future research be warranted. I also overlaid the vegetation transect endpoint coordinates on the Starr County soils data layer (Thompson, et al., 1972) to confirm in which soil type the vegetation transects were located.

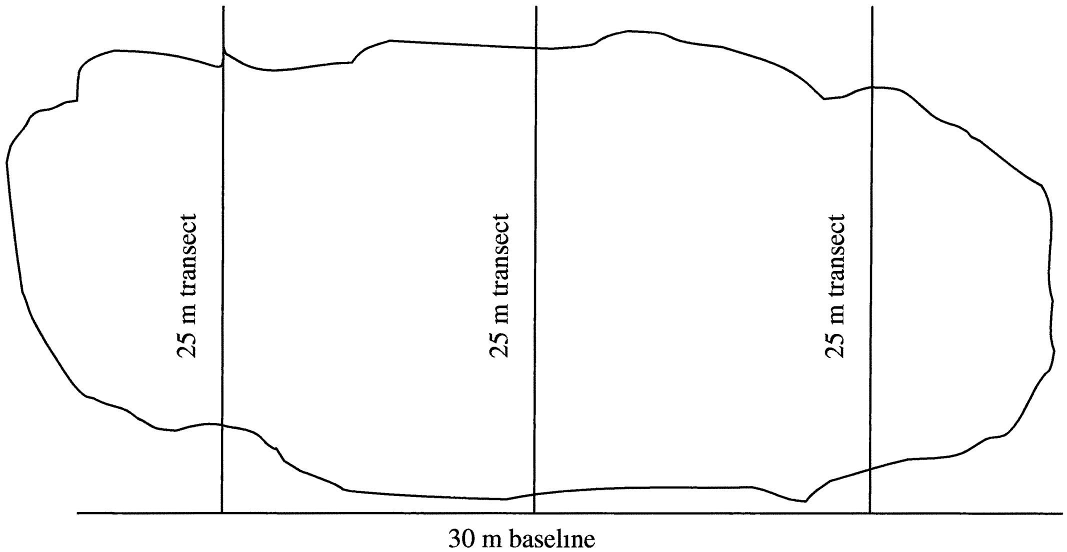



**Figure 3.** Polygon represents a subpopulation of *A. asterias* with individuals scattered throughout the area. Transects are located in a stratified-random design.

**Soil samples**

I collected soil samples within the 750 $m^2$ area of each of the 15 vegetation transects, according to the soil collecting guidelines of the Texas Cooperative Extension (TCE) Soil, Water & Forage Testing Laboratory (Provin and Pitt, 1999). Three holes were dug at haphazardly chosen locations within each 750 $m^2$ area to minimize differences that may exist within each area. The soil was collected within 0.5 meter of *A. asterias*. I pooled the three samples and sent the composite soil samples for each subpopulation to the TCE lab to determine pH, conductivity, and levels of nitrate ($NO_3$), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), sodium (Na), iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu). In addition to the routine analysis (buffered), detailed salinity tests (saturated paste extract) were also conducted for pH, conductivity, Na, K, Ca, Mg, soil adsorption ratio (SAR), and sodium saturation percentage (SSP). I calculated the average and range of values for each component of the soil analyses across the entire set of samples (subpopulations) as this information is not known for this species. At site JB1 a soil sample was collected in an area adjacent to the vegetation transect to compare the results of the soil analysis with the ranges of values for the soil analyses of the 15 vegetation transects. This area contained very little vegetation except for *Varilla texana* and no *A. asterias* were present in the area. I also classified the soils of each study site as saline, sodic, saline-sodic, or nonsaline, nonsodic based on the SAR and conductivity levels of the detailed salinity tests (Eynard, et al., 2006).

**Analysis of vegetation and soil data**

Principal component analysis (PCA) was performed using the thirteen parameters of the routine soil analyses of the 15 subpopulations and the pilot reintroduction site. The values of the soil parameters were z-score-transformed. The resulting loadings and plots were used to group similar sites. Plant species-soil relationships were investigated using canonical correspondence analysis (CCA) (Canoco 4.5; ter Braak, 1986; Palmer, 1993). Data analyzed included the 16 plant species with the greatest total intercept lengths for each of the 15 subpopulations and the reintroduction site, abundance of *A. asterias* within the three 2-m belt transects at each site (see Associated species section), and the thirteen parameters of the routine soil analyses of the 15 sites, as well as the pilot reintroduction site. All data were log transformed. A Monte Carlo permutation test using 1000 permutations was used to test for significance of the plant species-soil relationships.

**Associated species**

I compiled a list of species associated with *A. asterias* at each of the 15 subpopulations by documenting the other plant species not intercepted by the vegetation transects but within a 2-m belt transect centered on each 25-m vegetation transect (hereafter referred to as 2-m belt transects). I combined these data to create a comprehensive associated species list to supplement the current knowledge regarding *A. asterias* plant associates. The *A. asterias* within this 150 $m^2$ area were flagged and counted to estimate density of *A. asterias* within each of the 15 subpopulations sampled. While I and a data recorder conducted the vegetation transect, another researcher surveyed and flagged *A. asterias* within each of the belt transects by making three passes the length of it. If additional *A. asterias* were observed within the belt transect by the data recorder and I, these were also flagged.

For each *A. asterias* located within the 2-m belt transects, I documented the presence of plant species directly overhead or immediately adjacent to the plant. When multiple species formed a canopy over *A. asterias* the species were documented collectively. If another plant was not directly overhead or immediately adjacent, I documented whether the surrounding area was bare ground (<25% rocks) or covered with rocks (≥25%). I calculated the percentages of *A. asterias* within each category (under/adjacent to other plants, bare ground, and rocks) to determine the frequencies of the three categories. The association of *A. asterias* to each category was evaluated using a single factor analysis of variance and Tukey's multiple comparison procedure. Data were log transformed as homoscedasticity was violated.

**Seedlings in cultivation**

Basic reproductive biology of star cactus had been minimally studied in Texas until Strong (2005) and Strong and Williamson (2007) conducted a breeding system and pollen-limitation experiment on *A. asterias* at The Nature Conservancy's (TNC) Las Estrellas Preserve in Starr County, Texas. Seeds from the above were planted 23 January 2005 and the seedlings have been maintained since in a greenhouse at the Lady Bird Johnson Wildflower Center, Austin, Texas.

In January 2006, I numbered the seedlings ($n$ = 682) individually and created diagrams of the seedlings in each of the 113 pots using write-on transparency film to document the position of each. This allowed each seedling to be tracked. I counted the cacti every two weeks beginning in January 2006 to document mortality. After 10 months mortality leveled-off, so I switched to counting them once a month. In January 2006, I randomly selected ~25% of the seedlings ($n$ = 170) and began measuring their diameters to analyze growth rate of *A. asterias* in cultivation. Individuals were measured monthly initially. I used a Mitutoyo digimatic caliper to take two diameter measurements perpendicular to one another which were averaged to obtain the monthly diameter. In October 2006, I switched to measuring them every two months as little change in the diameter was noted on a monthly basis. Therefore, I had a total of 18 measurements for each seedling over the 25-month study period. The exact date each seedling germinated was not known. Hence the seedlings were 352-367 days old when the first diameter was recorded in January 2006. The initial age of each seedling was considered 360 days which was the average age of the seedlings as of January 2006. The following five size classes (mm) were used to group the seedlings for each date of measurement: <4.00; 4.01-7.00; 7.01-10.00; 10.01-13.00; >13.00.

Of the 170 seedlings selected for measuring, 8 died and 53 were subsequently withdrawn to use in the pilot reintroduction. The diameter for one seedling was not recorded in one month of monitoring so this plant was removed from the growth rate analysis. Therefore diameters of 108 seedlings were used to evaluate the growth rate of *A. asterias* in cultivation. The 18 diameters of each seedling were plotted across time displaying a linear pattern. I determined whether the raw diameter data or the natural log transformed diameter data best represented the data over time using simple linear regression. I then used a likelihood ratio test to determine which linear mixed model (random variable = seedling; covariate = age of seedling) best represented the growth rate of *A. asterias* seedlings in cultivation (Fox, 2002). I compared the following linear mixed models: a model pooling seedling diameter data and two models blocking by individual. One of the blocked models allowed initial diameter (intercept) of each seedling to change while the other allowed initial diameter and growth rate (slope) of each individual to change. The best linear mixed model was then used to further evaluate the growth rate of *A. asterias* in cultivation. The age of each seedling was adjusted for the analysis by 359 days so that the day the first diameter was recorded was considered day 1.

**Pilot reintroduction site**

In 2007 I used 240 of the seedlings in cultivation to establish a pilot reintroduction at the TNC Las Estrellas Preserve. This property is a candidate for augmentation because *A. asterias* in two of four permanent demographic transects have been impacted by herbivory from desert cottontails (*Sylvilagus audubonii*) and possibly, Mexican ground squirrels (*Spermophilus mexicanus*) (Janssen, et al., 2005, 2008). The pilot reintroduction is not located near the demographic transects impacted by herbivory, but at the edge of another subpopulation within the preserve (Fig. 4). I also chose Las Estrellas as the pilot reintroduction site for the following reasons: 1) it is owned by a conservation agency and is highly likely to be maintained as such; 2) the predominant soil type of the preserve is Catarina soils which is a soil type that supports subpopulations of *A. asterias*. On 13 March 2007, I conducted vegetation transects and collected a soil sample, as previously described, at the pilot reintroduction site. I used seeds collected in April and May 2004 from the Las Estrellas Preserve, as well as the above mentioned seedlings propagated from Las Estrellas collected seeds as propagules.

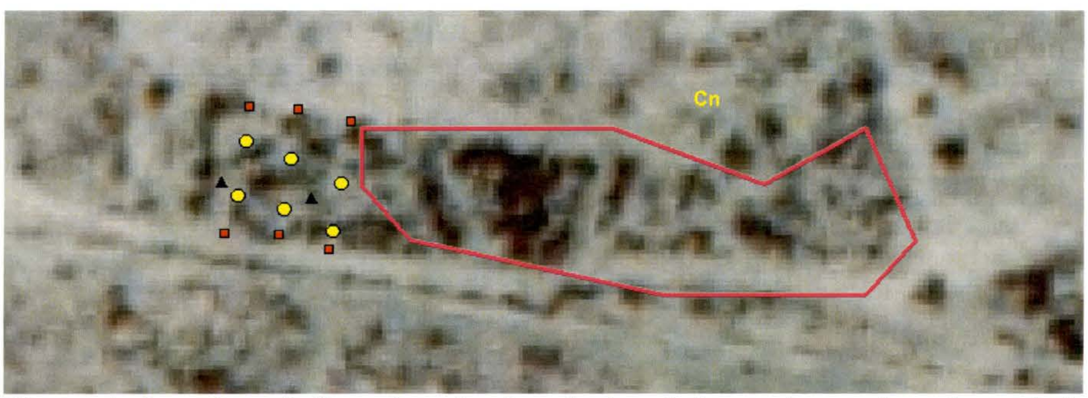



**Figure 4.** Location of pilot reintroduction site in relation to *A. asterias* subpopulation (pink polygon). Yellow circles = reintroduction quadrats (Q1-Q6); red squares = endpoints of vegetation transects; black triangles = weather stations; Cn = Catarina soils.

**Pilot reintroduction site design**

This is the first attempt to reintroduce *A. asterias*. It is well known in the horticultural trade that *Astrophytum* species, including *A. asterias*, are easily grown in cultivation (Higgins, 1960; Damude and Poole, 1990; Anderson et al., 1994). However, whether seeds or transplanted seedlings would survive best in the wild is not known. Therefore, I used both seeds and seedlings for the pilot reintroduction.

The pilot plot is a split-plot design. I located two 1-$m^2$ quadrats along each of the three 25-m transects of the vegetation transect for a total of 6 quadrats (Figs. 4, 5). I used a random numbers table to locate the first quadrat between 1 and 9 meters of the start of each transect. I located the second quadrat 10 m north of the first one. I centered the quadrat on the transect; however if this placement was not feasible, I then rotated the quadrat around this center point until a feasible placement was obtained. I considered the placement of the quadrat not feasible when: 1) 100% dense brush covered one or more of the subquadrats; 2) two or more of the planting rectangles were covered by shrub basal area; 3) a Mexican ground squirrel burrow was located within 1 m of the quadrat. I rotated the quadrat in this order: north, east, south, west, northwest, northeast, southeast, and southwest. I always kept an edge or corner of the quadrat touching the initial center point location. If I could not find a feasible placement, I moved the quadrat 50 cm north from its starting point and started the placement process again. I used 60d 6” (15.4 cm) nails in the corners of the quadrats to permanently locate them.

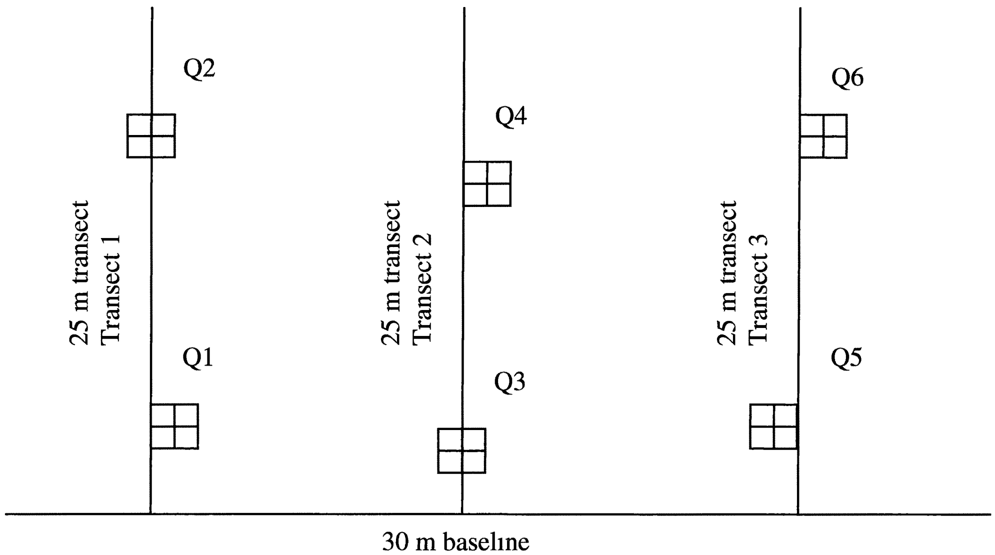



**Figure 5.** Stratified-random design of the 6 quadrats of the pilot reintroduction site.

Three planting grids following the example of Pavlik (1994) were constructed. Each grid is approximately 50 cm x 50 cm and consists of ¼" (0.6 cm) hardware cloth in a wooden frame. Twenty planting rectangles approximately 3.2 cm x 4.5 cm in size were cut into the hardware cloth creating a grid of 4 columns and 5 rows. I planted seeds and seedlings within the planting rectangles which allowed for equal spacing. I also used the grid to monitor the seeds and seedlings. I used 16d 6" (15.4 cm) nails to mark the corners of the subquadrats.

I subdivided each of the 6 quadrats into four 0.25 $m^2$ subquadrats. I randomly assigned one of four treatments to each subquadrat: a) 20 seeds planted in spring ($n$ = 120), b) 20 seedlings planted in the spring ($n$ = 120), c) 20 seeds planted in the fall ($n$ = 120), and d) 20 seedlings planted in the fall ($n$ = 120) (Fig. 6). I haphazardly chose the seeds for the spring and fall treatments from all seeds collected at the Las Estrellas Preserve which are permanently stored at the Desert Botanical Garden in Phoenix, Arizona (Center for Plant Conservation designated repository for *A. asterias*). I also randomly selected 240 of the seedlings in cultivation for the spring and fall treatments.

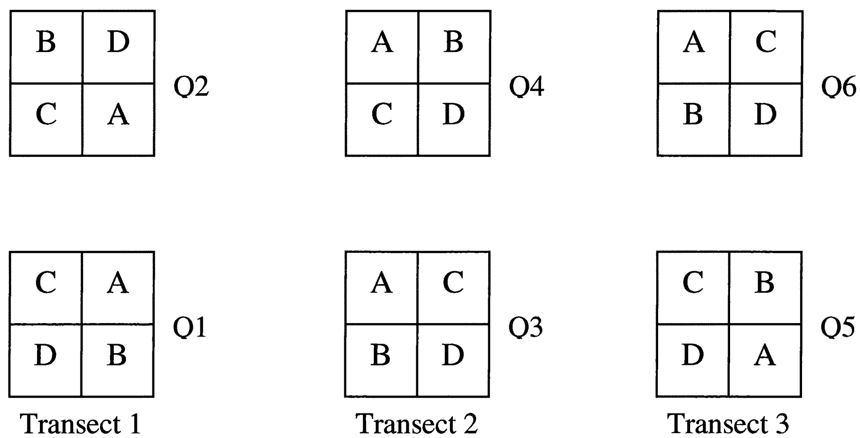



**Figure 6.** Randomly assigned treatments for each 0.25 $m^2$ subquadrat. A = seeds planted in the spring, B = seedlings planted in the spring, C = seeds planted in the fall, and D = seedlings planted in the fall.

**Planting methodology of reintroduced seeds and seedlings**

I developed a standardized methodology by which both treatments of seeds were planted. I inserted a 60d 6" (15.4 cm) nail approximately 1 cm into the ground to create a small divot in which I dropped the seed. Only one seed was planted per planting rectangle and it was left uncovered. If the rectangle was solid rocks, I did not create a divot, but dropped the seed amongst the rocks. I placed craft pins approximately 1 cm north of the seed location. Seeds were planted 14 March and 22 September 2007. At the time of planting, percent cover of each plant species, bare ground, and rocks within each subquadrat was also documented.

I also developed a set methodology for planting both treatments of seedlings. Approximately 6-10 days prior to planting the seedlings, they were removed from the greenhouse and housed out-of-doors in Starr County to acclimatize them. In the field I used a Mitutoyo digimatic caliper to measure the diameters of the seedlings prior to planting them. The seedlings were planted 19-20 April and 20-21 October 2007. The diameters of the seedlings planted in the spring averaged 8.78 ± 1.7 mm (±*SD*; range 4.96-13.50 mm). The average diameter of seedlings planted in the fall was 9.30 ± 2.1 mm (range 5.10-15.17 mm). I used average initial diameter of the seedlings for each subquadrat and season of planting in the model statements as factors possibly influencing seedling survivorship per subquadrat.

I planted approximately 20 seedlings at one time to limit the amount of time the seedlings were exposed. Initially I used a trowel to plant 40 of the spring seedlings. However, this disturbed too large of an area in and around the planting rectangle. A 3/8" x 12" (1.0 cm x 30.7 cm) slotted screwdriver was used to plant all other seedlings as it minimized disturbance. The screwdriver created a deeper, well-defined hole which allowed for straightening of the roots and overall easier planting. If the planting rectangle was rocky, I removed the rocks, planted the seedling and then replaced the rocks around or on the seedling. I gave each seedling ~3 mL of water and placed craft pins approximately 1-2 cm north of each seedling. At the time of planting, percent cover of plant species, bare ground, and rocks within each subquadrat was also documented. Percent bare ground was used in the model statements evaluating factors influencing seedling survivorship per subquadrat.

**Monitoring of pilot reintroduction**

I monitored each planting treatment two weeks after planting to document if a catastrophic loss had occurred. Thereafter, I collected presence/absence data for the seeds and seedlings every four weeks. I concluded data collection 1 June 2008 for the spring treatments and 15 November 2008 for the fall treatments. For spring and fall planted seeds, I documented whether the divot and/or seed was visible. I was able to see the seeds and/or divot at the two-week check-up for spring and fall planted seeds. However, by the first monthly monitoring of both seed plantings, it had rained which caused the divots to fill in. If the seeds germinated, I documented the month in which the seedling was first observed. At the conclusion of each 15-month study period of the seed treatments, the diameter of each seedling was recorded. Due to the low germination rate of planted seeds I did not perform any statistical analysis of these data.

For the spring and fall planted seedlings, I documented the state of each seedling: visible (≥75% of the seedling was visible); partially covered with dirt, leaves, rocks, etc. (<75% of the seedling was visible); covered with dirt, leaves, rocks, etc. (a sweep or two with a paintbrush or removal of the object(s) uncovered it); buried (digging was required to uncover it); uprooted; missing; or dead. I numerically coded the state of each seedling and used the average per subquadrat in the model statements as a factor possibly influencing seedling survivorship per subquadrat. I did not take diameter measurements of seedlings on a monthly basis as seedlings were flush with the soil surface or even buried. I feared that exposing the seedlings regularly would jeopardize their survival. I documented the cause of death when it could be determined to add to the known causes of mortality of this species.

At the end of each 14-month study period of the seedling treatments, the diameter of each seedling was recorded. The seedlings were grouped by the following five size classes (mm): <5.00, 5.00-8.00, 8.01-11.00, 11.01-14.00, >14.00. Differences in the final diameters of seedlings per subquadrat of the spring and fall treatments were evaluated using a single factor analysis of variance and Tukey's multiple comparison procedure. The growth rate of the reintroduced seedlings of the two planting treatments was evaluated using simple linear regressions. Differences in the final diameters of the seedlings for the spring and fall treatments were evaluated using a Student's t-test.

**Weather stations**

Two HOBO Micro Stations (Onset Computer Corporation, Pocasset, Massachusetts) were installed at the pilot reintroduction site in the vicinity of the reintroduction quadrats on 20 April 2007 to document rainfall, relative humidity, air and soil temperature, and soil moisture (Fig. 4). I installed three 12-bit temperature smart sensors to record soil temperature and three soil moisture smart sensors to document soil moisture at Q1, Q3, and Q6. One of the soil temperature sensors and all soil moisture sensors were destroyed by animals.

The micro stations were set to log data every 10 minutes and I downloaded the weather data on a monthly basis. The rainfall measurements were totaled for a monthly measurement. The air and ground temperature readings were averaged to obtain daily air and ground temperature. The daily temperatures were averaged to determine the monthly average air and ground temperatures. The daily maximum and minimum readings for air and ground temperature were also received as part of the output. I used a Student's t-test to determine if the difference between the monthly average ground temperatures of the two sensors was significant as one sensor was in the open and the other was at the edge of a shrub. Monthly rainfall and average monthly ground temperature were used in the model statements to evaluate factors influencing seedling survivorship per subquadrat. Weather data collected at the reintroduction site is summarized in Appendix A.

**Analysis of pilot reintroduction data**

Since few seeds of the pilot reintroduction germinated, data analysis focused on the reintroduced seedlings. I developed 28 candidate models to assess the potential influence of season of planting; average state of the seedling per subquadrat; environmental variables of monthly precipitation and average monthly ground temperature; average diameter of the seedlings at the time of planting per subquadrat; and percent bare ground of each subquadrat on percent survivorship of reintroduced *A. asterias* seedlings in each subquadrat (Table 1). Seedling survivorship may have also been affected simply by passage of time; therefore model 29 which evaluates the temporal aspect of survivorship was also included. A null model was also included (model 30) to demonstrate the validity of the factors evaluated in the other models.

**Table 1.** Candidate models used to assess potential influence of season of planting (sea); average state of the seedling per subquadrat (stat); environmental variables of monthly precipitation (rain) and average monthly ground temperature (gtemp); average initial diameters of seedlings per subquadrat (dm); and percent bare ground of each subquadrat (bare) on survivorship (surv) of the reintroduced *A. asterias* seedlings. A temporal (month) and null model (.) were also included.

| # | Model |
|---|---|
| 1 | surv = sea |
| 2 | surv = stat |
| 3 | surv = rain |
| 4 | surv = gtemp |
| 5 | surv = sea + dm |
| 6 | surv = sea + bare |
| 7 | surv = sea + stat |
| 8 | surv = sea + rain |
| 9 | surv = sea + gtemp |
| 10 | surv = stat + dm |
| 11 | surv = stat + bare |
| 12 | surv = stat + rain |
| 13 | surv = stat + gtemp |
| 14 | surv = rain + dm |
| 15 | surv = rain + bare |
| 16 | surv = rain + gtemp |
| 17 | surv = gtemp + dm |
| 18 | surv = gtemp + bare |
| 19 | surv = sea + rain + dm |
| 20 | surv = sea + stat + rain |
| 21 | surv = sea + stat + gtemp |
| 22 | surv = sea + stat + rain + gtemp |
| 23 | surv = rain + gtemp + dm |
| 24 | surv = rain + dm + bare |
| 25 | surv = stat + gtemp + bare |
| 26 | surv = stat + gtemp + dm |
| 27 | surv = stat + gtemp + dm + bare |
| 28 | surv = gtemp + dm + bare |
| 29 | surv = month |
| 30 | surv = (.) |

The models were evaluated using restricted maximum likelihood estimation of a linear mixed effects model with quadrat as the random variable. Sample size was the total number of observations across each 14-month study period of Q1-Q3, Q5, and Q6 of the spring planted treatment and Q1-Q6 of the fall planted treatment, $n$ = 154. Data for

Q4 of the spring treatment was not included as 95% of the seedlings were lost due to burrowing activity of a Mexican ground squirrel. I selected models based on the information-theoretic approach (Burnham and Anderson, 2002). A model fit the data well if it had a low Akaike information criterion corrected for small sample size ($AIC_c$) and a high Akaike weight (Burnham and Anderson, 2002).

# CHAPTER III

## RESULTS

### Analyses of soil data

The overlaying of the GPS coordinates of the vegetation transects confirmed that the soil samples and vegetation transects were conducted on the following soil types as defined in the Starr County soils data layer: 9 subpopulations in Catarina soils (Cn); 2 in Garceno clay loam (Ga); and one each in Jimenez-Quemado association (Jq); Maverick soils, eroded (Mu2); Montell clay, saline (Mt); and Ramadero loam (Ra). The reintroduction site was also established in Catarina soils.

The average pH of the 15 subpopulations was 8.3 with nitrate, phosphorus, and potassium levels averaging 10, 16, and 300 parts per million, respectively (Table 2). The routine soil analysis of the sample collected at the pilot reintroduction site fell within the ranges of the soil parameters of the other 15 samples, except for the levels of nitrate and magnesium which were lower (Table 2). The soil sample (Out) collected adjacent to the JB1 transect area, in an area of *Varilla texana*, but no *A. asterias*, tested slightly higher for conductivity, potassium, and manganese, but was within the ranges of all the other soil parameters of the 15 subpopulations. The results of the routine soil analyses of each site are shown in Appendix B, Table 12.

**Table 2.** Averages (*Avg*), standard deviations (*SD*), and ranges of soil parameters from the routine soil analyses of soil samples collected within the vegetation transects ($n$ = 15) and the results of said analyses for the samples collected at the pilot reintroduction site (RE) and adjacent to site JB1 (Out). Samples collected March, May 2006 and March 2007. Conductivity (Cnd) = µmho/cm; $NO_3$, P, K, Ca, Mg, S, Na, Fe, Zn, Mn, Cu = parts per million.

| | pH | Cnd | $NO_3$ | P | K | Ca | Mg | S | Na | Fe | Zn | Mn | Cu |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Avg*** | 8.3 | 2256 | 10 | 16 | 300 | 19,099 | 253 | 867 | 2,205 | 4.21 | 0.23 | 2.14 | 0.46 |
| ***SD*** | 0.35 | 1300.24 | 5.22 | 3.84 | 61.21 | 7147.42 | 64.27 | 1825.58 | 1397.38 | 1.21 | 0.04 | 0.56 | 0.16 |
| **Low** | 7.8 | 231 | 7 | 9 | 176 | 9,852 | 176 | 35 | 240 | 2.13 | 0.14 | 1.04 | 0.18 |
| **High** | 9.0 | 4,641 | 28 | 21 | 386 | 35,901 | 382 | 6,143 | 4,530 | 6.30 | 0.32 | 3.54 | 0.72 |
| | | | | | | | | | | | | | |
| **RE** | 8.3 | 586 | 3 | 19 | 231 | 12,010 | 152 | 69 | 835 | 2.57 | 0.21 | 2.16 | 0.19 |
| **Out** | 8.2 | 4,748 | 7 | 13 | 493 | 13,557 | 197 | 4,352 | 3,186 | 5.83 | 0.27 | 4.81 | 0.32 |

The average pH of the 15 subpopulations as determined by the detailed salinity test was 7.5 (Appendix B, Table 13). Using sodium adsorption ratio (SAR) and conductivity levels, sites AM2, CA, EE, JB1, JB2, KR, LM, NC2, and PP2 are classified as saline-sodic soils (Table 3). Sites AM4 and NC1 are sodic while sites LA and PP1 are saline. Sites AM1, AM3, and RE were nonsaline, nonsodic. Textural analyses were conducted on four of the samples (EE, NC1, NC2, and RE). Sites EE, NC1, and NC2 had 22-26% sand and silt and ≥ 50% clay, thereby classifying them as clay soils. Site RE was classified as a clay loam with 40% silt, 32% sand, and 28% clay.

**Table 3.** Classification of sites according to sodium adsorption ratio (SAR) and conductivity levels of the detailed salinity tests of soil samples collected March, May 2006 and March 2007.

| Classification | Soil Type | SAR | Conductivity | Site Name |
|---|---|---|---|---|
| saline | Cn | 9.23 | 6.00 | LA |
| saline | Ga | 7.42 | 6.51 | PP1 |
| sodic | Cn | 31.69 | 0.88 | AM4 |
| sodic | Mu2 | 18.89 | 3.91 | NC1 |
| saline-sodic | Cn | 48.15 | 13.81 | CA |
| saline-sodic | Cn | 33.94 | 17.29 | AM2 |
| saline-sodic | Cn | 24.57 | 6.53 | JB1 |
| saline-sodic | Cn | 21.51 | 5.50 | EE |
| saline-sodic | Cn | 18.94 | 8.03 | NC2 |
| saline-sodic | Ga | 18.74 | 7.40 | PP2 |
| saline-sodic | Jq | 39.91 | 8.78 | KR |
| saline-sodic | Mt | 78.66 | 15.66 | LM |
| saline-sodic | Ra | 28.70 | 4.99 | JB2 |
| nonsaline, nonsodic | Cn | 8.34 | 1.80 | RE |
| nonsaline, nonsodic | Cn | 2.24 | 0.95 | AM1 |
| nonsaline, nonsodic | Cn | 1.25 | 0.87 | AM3 |

Principal component axes I, II, and III (PC I, PC II, PC III) in total explained 65% of the variation in soil parameters among the 16 sites (includes the reintroduction site). PC I explained 34% of the variation and represented a conductivity and copper gradient (Fig. 7). The saline and saline-sodic sites had the strongest positive loadings on PC I. These sites had the highest levels of conductivity. Saline-sodic sites also tended to have higher levels of copper and nitrate. The nonsaline, nonsodic and sodic sites had the strongest negative loadings on PC I. These sites had the lowest levels of conductivity. A majority of these sites also had low levels of copper. The nonsaline, nonsodic sites also had low levels of nitrate while the sodic soils also had low levels of manganese. Total variation explained by PC II was 18%. It represented a pH, sodium, phosphorus, and zinc gradient (Fig. 7). The saline-sodic sites had the highest levels of sodium with many of these sites also having low levels of zinc. The saline sites had low pH as well as low levels of sodium. PC III explained 13% of the total variation and represented an iron, calcium, phosphorus, and manganese gradient (Fig. 7). The saline sites as well as a majority of the saline-sodic sites had high levels of iron while the nonsaline, nonsodic and sodic sites had low levels. Many of the saline-sodic sites also had low levels of calcium and high levels of phosphorus. A majority of the nonsaline, nonsodic sites also had high levels of phosphorus.

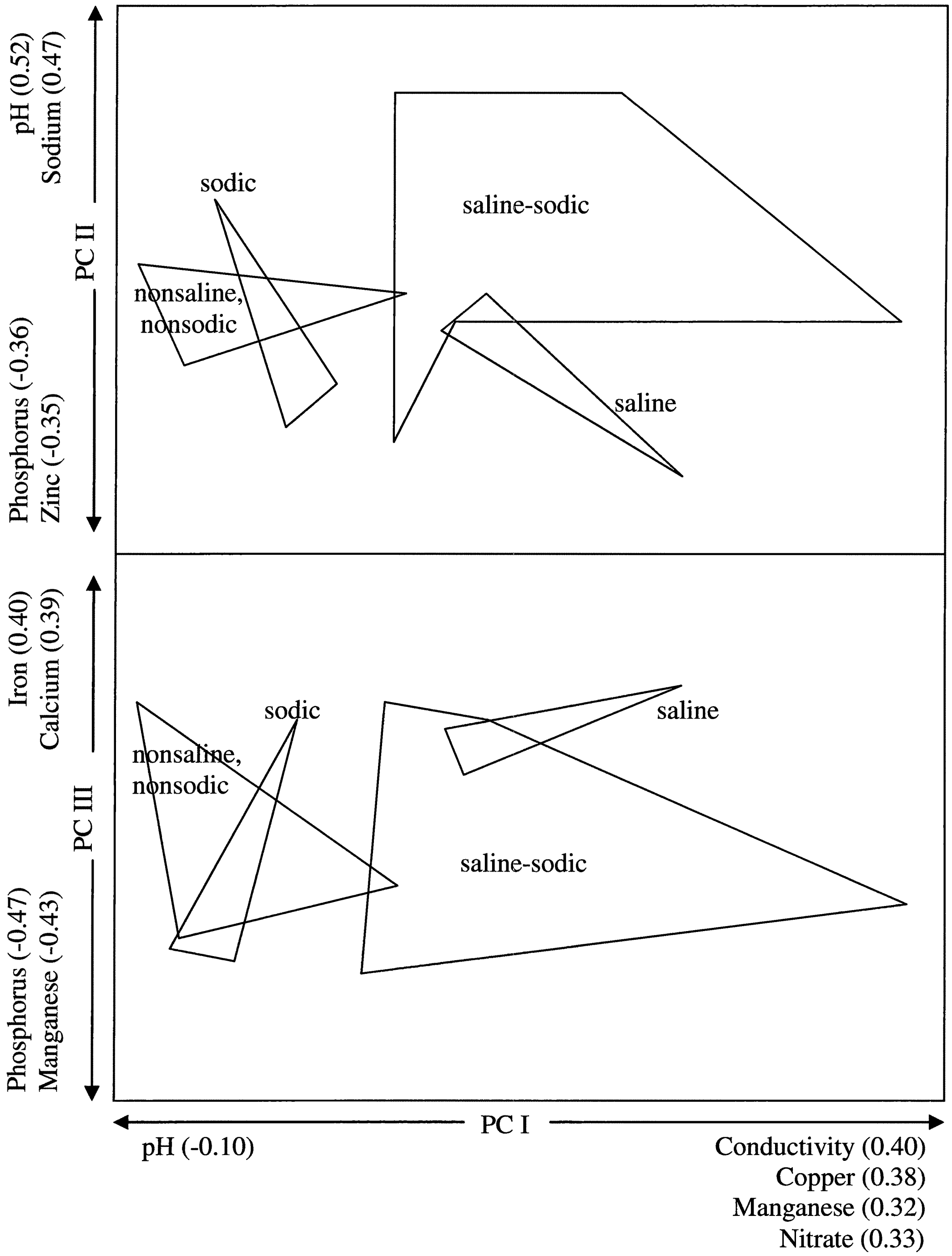



**Figure 7.** PCA soil parameters plot of PC axes I, II, and III for the 16 vegetation transects (includes the reintroduction site).

**Analyses of vegetation data**

Of the 15 vegetation transects in subpopulations of *A. asterias*, only three had total vegetative dominance over 50%. Site NC1 had the highest total vegetative dominance of 57.15% while site JB2 had the lowest at 20.99%. *Varilla texana* was the most dominant species (11.6%) and accounted for over one-quarter (27.8%) of the relative dominance for all sites (Table 4). This species was intercepted in 12 of the 15 vegetation transects. *Prosopis glandulosa* accounted for nearly 15% of the relative dominance for all sites, had a dominance of 6.1%, and was also intercepted at 12 sites. *Acacia rigidula* accounted for 12.5% of the relative dominance, had a dominance of 5.2%, and was intercepted at 9 of the 15 vegetation transects. The only other species with over 10% relative dominance for all sites was *Opuntia leptocaulis* which was intercepted at 13 sites and had a dominance of 4.4%. The top ten species with the greatest dominance and relative dominance within the 15 vegetation transects are listed in Table 4. A complete list of species intercepted by the 15 vegetation transects along with the dominance and relative dominance of each species is shown in Appendix C Table 14.

**Table 4.** Ten species with the greatest dominance and relative dominance within the 15 vegetation transects conducted March and May 2006.

| Species | Dominance (%) | Relative Dominance (%) |
|---|---|---|
| *Varilla texana* | 11.6 | 27.8 |
| *Prosopis glandulosa* | 6.1 | 14.5 |
| *Acacia rigidula* | 5.2 | 12.5 |
| *Opuntia leptocaulis* | 4.4 | 10.5 |
| *Castela erecta* subsp. *texana* | 1.7 | 4.1 |
| *Ziziphus obtusifolia* var. *obtusifolia* | 1.6 | 3.9 |
| *Suaeda conferta* | 1.2 | 2.8 |
| *Parkinsonia texana* var. *macra* | 1.2 | 2.8 |
| *Monanthochloë littoralis* | 1.0 | 2.4 |
| *Xylothamia palmeri* | 0.9 | 2.0 |

*Varilla texana* was the dominant species at 8 of the 15 vegetation transects. *Prosopis glandulosa* was the dominant at 3 sites as was *Acacia rigidula*. *Suaeda conferta* was the dominant species at one site. Appendix C Table 15 lists the species and dominance values of each by site. Ten additional plant species not intercepted by the 15 vegetation transects, but documented within the 2-m belt transects across the sites are shown in Table 5. Sixty-nine plant species comprise the comprehensive list of species associated with *A. asterias* as documented in the 15 vegetation transects and the 2-m belt transects across all sites (Appendix C Table 16).

**Table 5.** List of species not intercepted by the 15 vegetation transects but documented within the 2-m belt transects across the 15 sites.

| | |
|---|---|
| *Chloris* sp. | *Ibervillea lindheimeri* |
| *Cissus incisa* | *Leucophyllum frutescens* var. *frutescens* |
| *Condalia hookeri* | *Mammillaria sphaerica* |
| *Coryphantha macromeris* var. *runyonii* | *Manfreda longiflora* |
| *Cuscuta* sp. | *Salvia ballotiflora* |

A vegetation transect was also conducted at the pilot reintroduction site (RE). Total vegetative dominance at the site was 47.41%. The species with the greatest dominance included *Castela erecta* subsp. *texana* (15.47%), *Acacia rigidula* (6.75%), and *Ziziphus obtusifolia* var. *obtusifolia* (5.51%). All species intercepted and dominance of each are shown in Appendix C Table 15. Species intercepted by the vegetation transect or documented within the three 2-m belt transects at the pilot reintroduction site which were not documented in the other 15 vegetation transects are listed in Table 6.

**Table 6.** List of species documented at the pilot reintroduction site which were not documented in the other 15 vegetation transects.

| | |
|---|---|
| *Acourtia runcinata* | *Eragrostis* sp. |
| *Chamaesaracha conoides* | *Glandularia vercunda* |
| *Condalia spathulata* | *Verbena* sp. |
| *Dyssodia tenuiloba* var. *treculii* | *Yucca treculeana* |
| *Ephedra antisyphilitica* | *Zanthoxylum fagara* |

**Cannonical correspondence analysis of soils and vegetation data**

Soil parameters of the routine soil analyses explained 45% of the variation in vegetation within the 16 vegetation transects (includes reintroduction site). The Monte Carlo permutation test was not significant ($P = 0.44$). Eight of the 16 species analyzed in the canonical correspondence analysis (CCA) were clustered around the intersection of the CCA I and CCA II axes indicating no preferential association with a particular soil parameter (Fig. 8). These species were each recorded at 12 or more sites. *Varilla texana* was the dominant species at 6 of the 9 saline-sodic sites. *Prosopis glandulosa* was also ranked as one of the top three dominants at 6 of the 9 saline-sodic sites. *Acacia rigidula* was the dominant species at 2 of the 3 nonsaline, nonsodic sites as well as one of the sodic sites. *Suaeda conferta* was documented at only four sites, but all of these were saline-sodic.

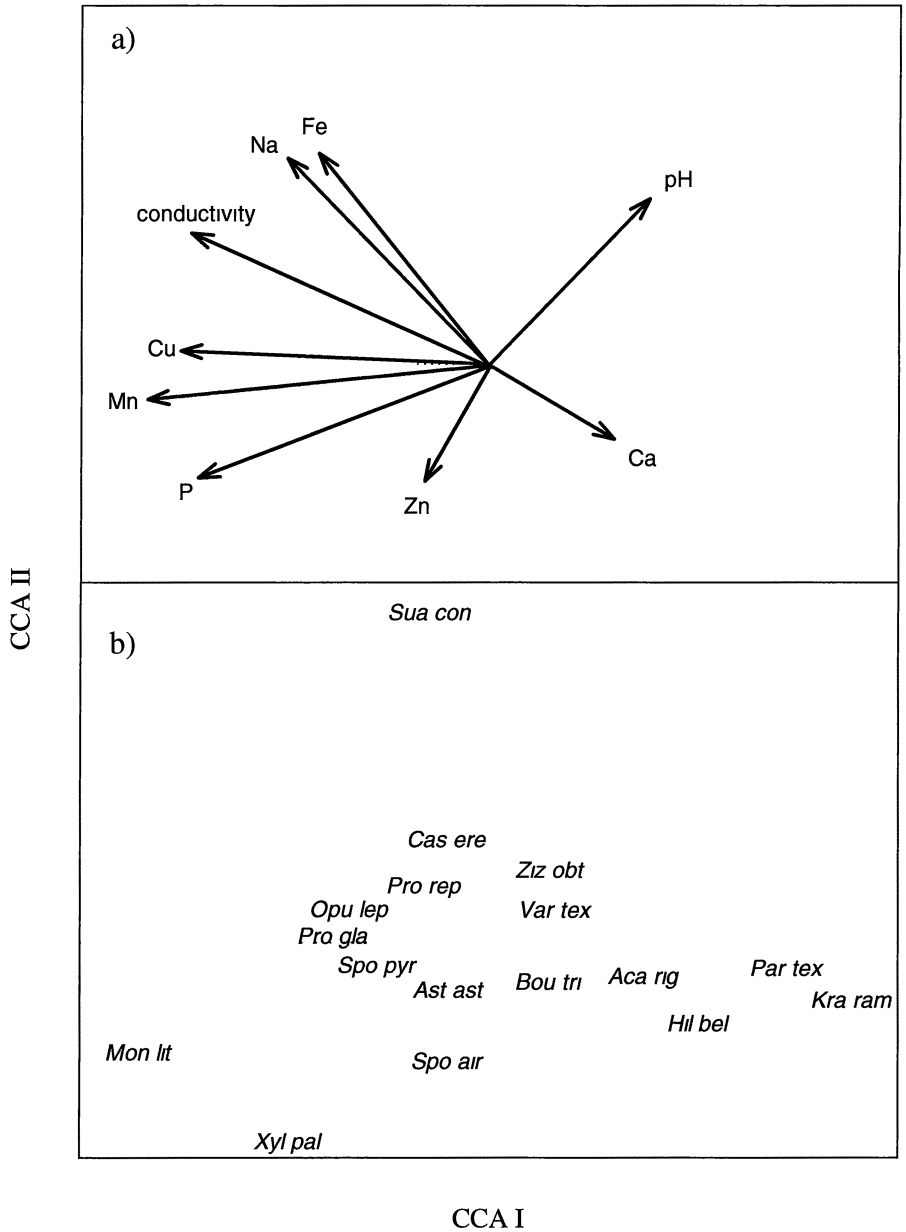



**Figure 8.** CCA biplot of (a) soil parameters and (b) 16 plant species with greatest dominance from the 16 vegetation transects (includes the reintroduction site). Species names are the first three letters of scientific binomial. Ten of the species are listed in Table 4; others are *Bou tri* = *Bouteloua trifida*; *Hil bel* = *Hilaria belangeri* var. *belangeri*; *Kra ram* = *Krameria ramosissima*; *Pro rep* = *Prosopis reptans* var. *cinerascens*; *Spo air* = *Sporobolus airoides* var. *airoides*; *Spo pyr* = *S. pyramidatus*; *Ast ast* = abundance of *A. asterias* within the three 2-m belt transects at each site.

*Acacia rigidula* and *Hilaria belangeri* var. *belangeri* were recorded at 10 and 8 sites, respectively, and showed a positive association with CCA I (Fig. 8). The sites generally had a high pH and low iron levels. The sites with *A. rigidula* also had low conductivity while the sites with *H. belangeri* var. *belangeri* were low in sodium and high in calcium. *Krameria ramosissima*, *Parkinsonia texana* var. *macra*, *Sporobolus airoides* subsp. *airoides*, *Xylothamia palmeri*, *Monanthochloë littoralis*, and *Suaeda conferta* had strong associations with the CCA axes and were each recorded at 6 or fewer sites (Fig. 8). *Krameria ramosissima* and *P. texana* var. *macra* were at sites with low conductivity and levels of iron, as well as high levels of calcium. Sites with *P. texana* var. *macra* also had a high pH. Sites in which *S. airoides* subsp. *airoides* was documented were generally high in levels of zinc and low in levels of iron as well as having a relatively low pH. *Xylothamia palmeri* was observed at sites with a low pH and high levels of zinc. Sites with *M. littoralis* were high in phosphorus and copper and generally had a low pH and high conductivity. *Suaeda conferta* was documented at sites with high levels of iron and sodium, as well as low levels of zinc.

### Density of *Astrophytum asterias*

A total of 294 *A. asterias* were counted in the 2-m belt transects across the 15 vegetation transects. The abundance of *A. asterias* was also clustered around the intersection of the CCA I and CCA II axes indicating no preferential association with a particular soil parameter (Fig. 8). Sites CA and EE which had the highest density of *A. asterias* were saline-sodic followed by sites LA and PP1 which were saline (Table 7). Site NC1 had the fifth highest density and was sodic. The nonsaline, nonsodic sites had some of the lowest densities (Table 7). At three sites no *A. asterias* were observed within the 150 $m^2$ area. At site RE three *A. asterias* were documented within one of the 2-m belt transects. Density of *A. asterias* was not calculated for site RE as it was intentionally located at the edge of a known subpopulation and therefore, would not constitute a valid measurement.

**Table 7.** Number and density of *A. asterias* documented in the three 2-m belt transects of the 15 vegetation transect sites, March and May 2006.

| **Site** | **# *A. asterias*** | **density/m²** |
|---|---|---|
| CA | 64 | 0.43 |
| EE | 59 | 0.39 |
| LA | 39 | 0.26 |
| PP1 | 35 | 0.23 |
| NC1 | 31 | 0.21 |
| AM4 | 16 | 0.11 |
| PP2 | 16 | 0.11 |
| AM2 | 13 | 0.09 |
| NC2 | 12 | 0.08 |
| LM | 5 | 0.03 |
| JB1 | 2 | 0.01 |
| KR | 2 | 0.01 |
| AM1 | 0 | 0.00 |
| AM3 | 0 | 0.00 |
| JB2 | 0 | 0.00 |

**Analysis of directly associated species**

Of the 294 *A. asterias* documented in the 2-m belt transects, 81% had a plant directly overhead or immediately adjacent. Another 12.2% of the *A. asterias* documented were found in rocky areas with no associated plants, followed by an additional 6.8% that were in open, bare areas with no rocks or plants (Table 8). The analysis of variance was significant indicating an association of *A. asterias* with one or more of the categories examined ($F$ = 7.36; $P$ = 0.003; $df$ = 2). The Tukey's multiple comparison procedure showed that the number of *A. asterias* associated with plants was significant compared to the number observed in rocky or bare areas (plant-bare confidence intervals: lower = 0.3637 and upper = 2.9535; rocks-plant confidence intervals: lower = -2.6651 and upper = -0.3009). The difference in the number of *A. asterias* observed in rocky areas compared to bare areas was not significant (confidence intervals: lower = -1.2231 and upper = 1.5742). *Varilla texana* alone accounted for ~24% of the plants documented (Table 8). Nearly 40% of all plants overhead or immediately adjacent, singly or in combination, consisted of *V. texana*. Plant species documented directly overhead or immediately adjacent to an *A. asterias* are included in Table 9. Appendix C Table 17 contains a complete list of plant species/object(s) singly or in combination along with the percentage of *A. asterias* associated with each. At the reintroduction site, two of the three *A. asterias* were associated with rocks; the other was in an open, bare area.

**Table 8.** The ten most documented plant species/object(s) overhead or immediately adjacent to *A. asterias* and percent occurrence within the 2-m belt transects across the 15 vegetation transect sites, March and May 2006. More than one plant species/object in a row indicates a combination.

| Plant species/object(s) | Percent |
|---|---|
| *Varilla texana* | 23.8 |
| rock(s) (no plant) | 12.2 |
| bare ground (no plant) | 6.8 |
| *Monanthochloë littoralis* | 5.1 |
| *Prosopis glandulosa, M. littoralis* | 3.4 |
| *V. texana*, rocks | 3.4 |
| *Opuntia leptocaulis* | 3.1 |
| *Thelocactus bicolor* var. *bicolor*, rocks | 2.7 |
| *V. texana, O. leptocaulis* | 2.4 |
| *V. texana, P. glandulosa* | 2.4 |

**Table 9.** Species documented directly overhead or immediately adjacent to an *A. asterias*.

| | |
|---|---|
| *Acacia rigidula* | *Parkinsonia texana* var. *macra* |
| *Billieturnera helleri* | *Pennisetum ciliare* var. *ciliare* |
| *Bouteloua trifida* | *Prosopis glandulosa* |
| *Castela erecta* subsp. *texana* | *P. reptans* var. *cinerascens* |
| *Gutierrezia texana* | *Setaria* sp. |
| *Hilaria belangeri* var. *belangeri* | *Sporobolus airoides* subsp. *airoides* |
| *Isocoma coronopifolia* | *S. pyramidatus* |
| *Jatropha dioica* | *Suaeda conferta* |
| *Krameria ramosissima* | *Thelocactus bicolor* var. *bicolor* |
| *Monanthochloë littoralis* | *T. setispinus* |
| *Opuntia engelmannii* var. *lindheimeri* | *Tiquilia canescens* var. *canescens* |
| *O. leptocaulis* | *Varilla texana* |
| *Panicum* sp. | *Ziziphus obtusifolia* var. *obtusifolia* |
| *Pappophorum bicolor* | |

**Analysis of data for seedlings in cultivation**

At the onset of monitoring *A. asterias* seedlings in cultivation in January 2006, there were 681 seedlings. During the 25-month study period 36 died. *Astrophytum asterias* in cultivation displayed a myriad of colors including various shades of green, brown, red, and orange. Often the seedlings were a combination of these colors, such as brown-green or having red ribs with green grooves. It was difficult to observe mortality of seedlings. Sometimes the black, rotting body could be found pulled several centimeters below the soil surface or a shriveled body remained while other times no trace of the seedling was evident.

When initially measured in January 2006, 87% of the seedlings ($n$ = 108) were in the 4.01-7.00 mm size class (Fig. 9). At the end of the 25-month study period 50% of the seedlings ($n$ = 108) were in the 7.01-10.00 mm size class with another 38% in the 10.01-13.00 mm size class (Fig. 9). The simple linear regression of raw seedling diameter data ($r^2 = 0.48$, $P < 0.0001$; $n$ = 108) fit the data better than that of the log transformed data ($r^2$

= 0.45, $P < 0.0001$; $n = 108$). The likelihood ratio tests of the seedling diameter models were significant ($P < 0.001$). The best fit model indicated that a regression allowing both the initial diameter (intercept) of the seedlings and the growth rate per day (slope) to change was warranted. This regression accounted for 85.7% of the variation in final diameter of the seedlings. The initial diameter of seedlings was not correlated with growth rate (confidence intervals: lower = -0.0993 and upper = 0.2526). The largest estimated initial diameter of 9.6840 mm was 2.5 times larger than the smallest of 3.7830 mm with 75% of the initial diameters being <6.47 mm (Fig. 10). The largest estimated growth rate of 0.0165 mm/day (6.02 mm/year) was nearly 8 times larger than the smallest estimated growth rate of 0.0021 mm/day (0.77 mm/year) with 75% of the estimated growth rates being <0.0080 mm/day (2.92 mm/year) (Fig. 10).

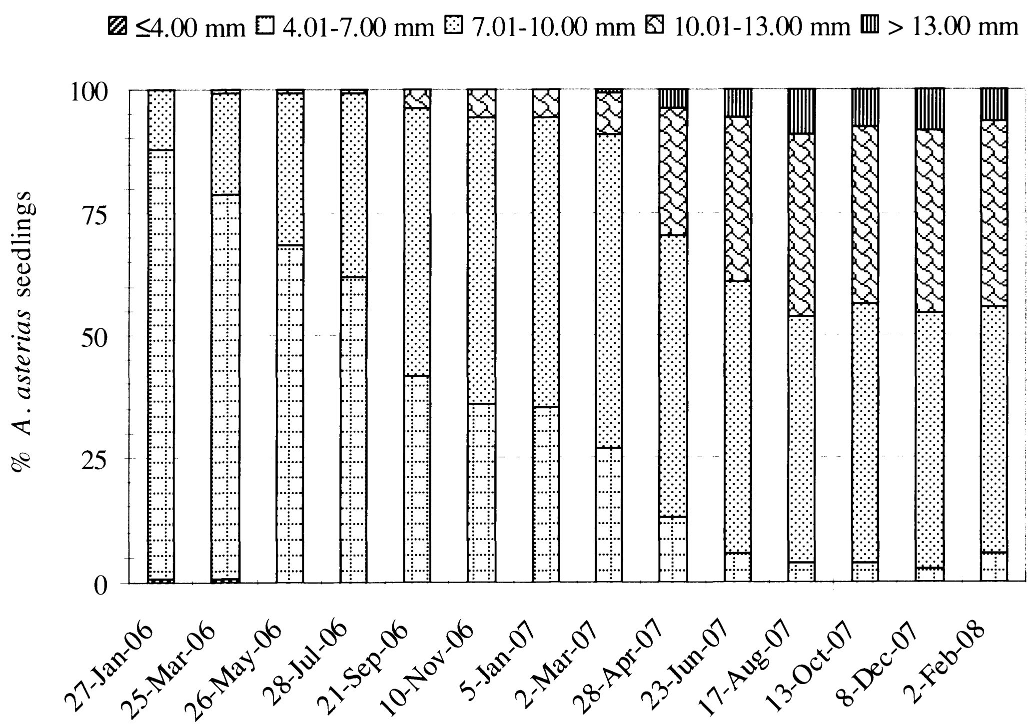



**Figure 9.** Size classes of 108 seedlings in cultivation across the 25-month study period.

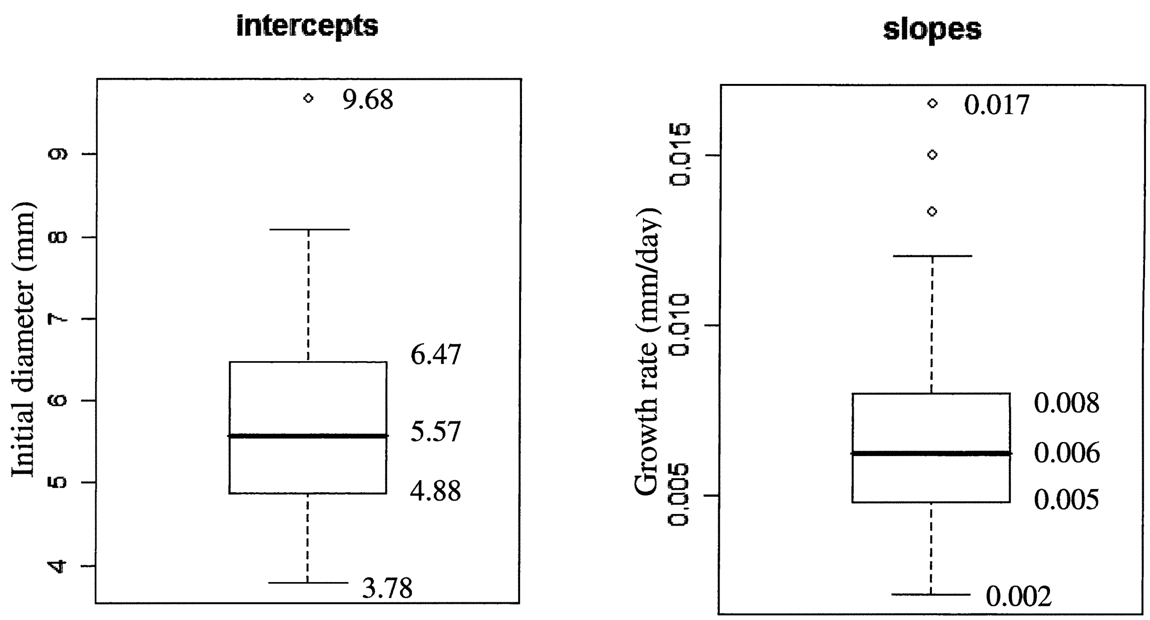



**Figure 10.** Box plots of the 108 coefficients estimating initial diameters (intercepts) and growth rates (slopes) of the cultivated seedlings.

**Fate of reintroduced seeds**

The subquadrats receiving spring planted seeds had greater percentages of bare ground than the subquadrats in the fall (Fig. 11). Despite the wetter than normal months of June and July, the amount of vegetation within the fall subquadrats did not increase. However, the fall subquadrats contained greater percentages of soil crust and rock than the spring subquadrats.

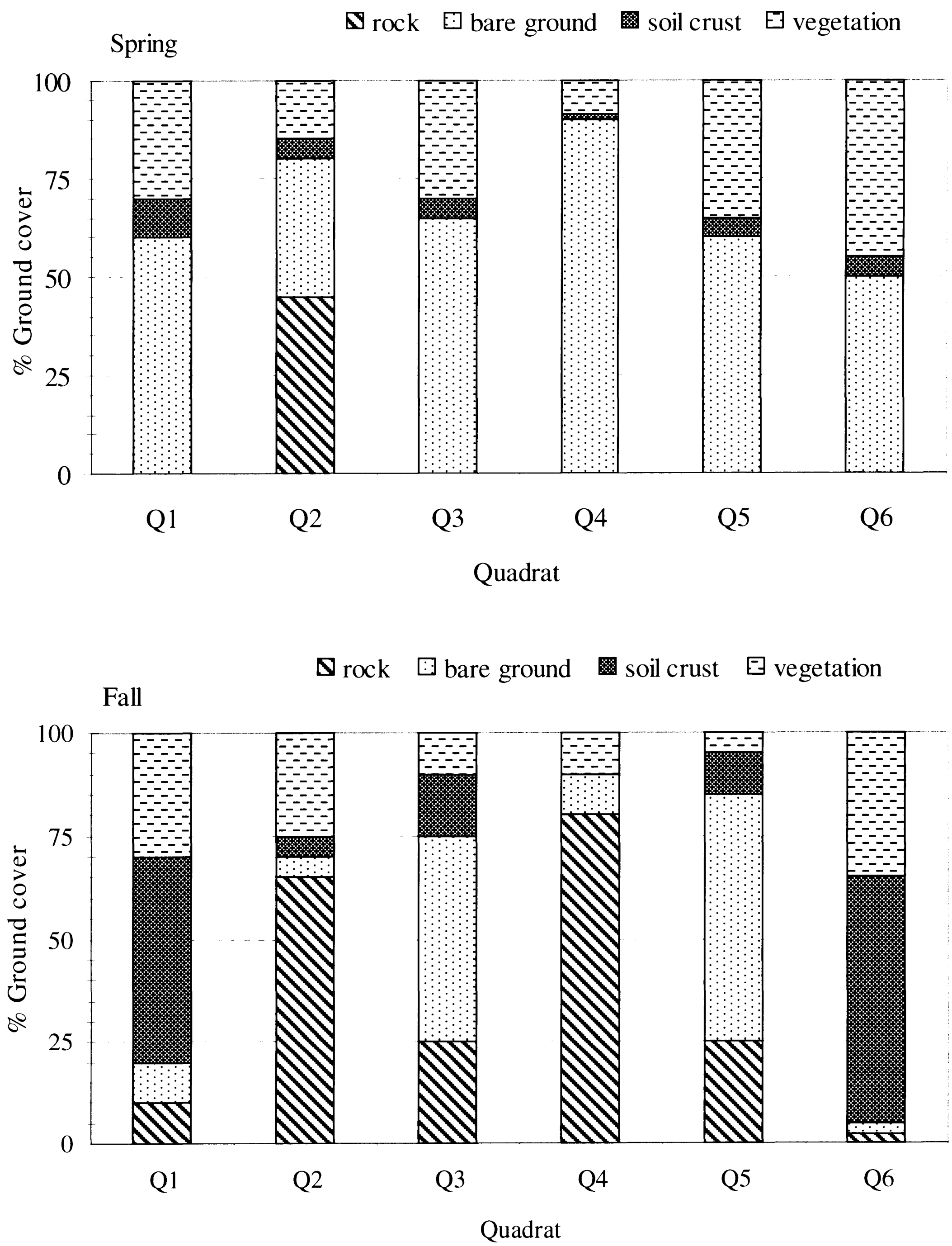



**Figure 11.** Percent rock, bare ground, soil crust, and vegetation of each subquadrat when seeds were planted, March and September 2007.

Of the 120 seeds planted in the spring, five produced seedlings. At the end of the spring planting study period (June 2008) four of the five seedlings were alive. Four of the 120 seeds planted in the fall produced seedlings (Table 10). Monitoring of fall planted seeds concluded November 2008 and all four seedlings of this treatment were alive.

**Table 10.** Date planted and first observed, quadrat, and final diameter of the seedlings from *A. asterias* seeds planted in spring and fall at the end of the respective study periods, June and November 2008.

| Planted | Date first observed | Quadrat | Diameter (mm) |
|---|---|---|---|
| 14 March 2007 | 22 September 2007 | Q3 | 3.47 |
| 14 March 2007 | 22 September 2007 | Q3 | 3.56 |
| 14 March 2007 | 22 September 2007 | Q5 | dead |
| 14 March 2007 | 15 December 2007 | Q6 | 4.23 |
| 14 March 2007 | 15 December 2007 | Q6 | 3.51 |
| 22 September 2007 | 2 August 2008 | Q3 | 3.82 |
| 22 September 2007 | 2 August 2008 | Q5 | 3.38 |
| 22 September 2007 | 23 August 2008 | Q6 | 4.24 |
| 22 September 2007 | 20 September 2008 | Q6 | 3.98 |

## Fate of reintroduced seedlings

There were minimal differences between measurements of ground cover at the beginning and ending of the study periods for each treatment (Fig. 12). The amount of soil crust documented for spring planted seedlings was higher at the conclusion of the study period (Fig. 12). The opposite was true for fall planted seedlings (Fig. 12).

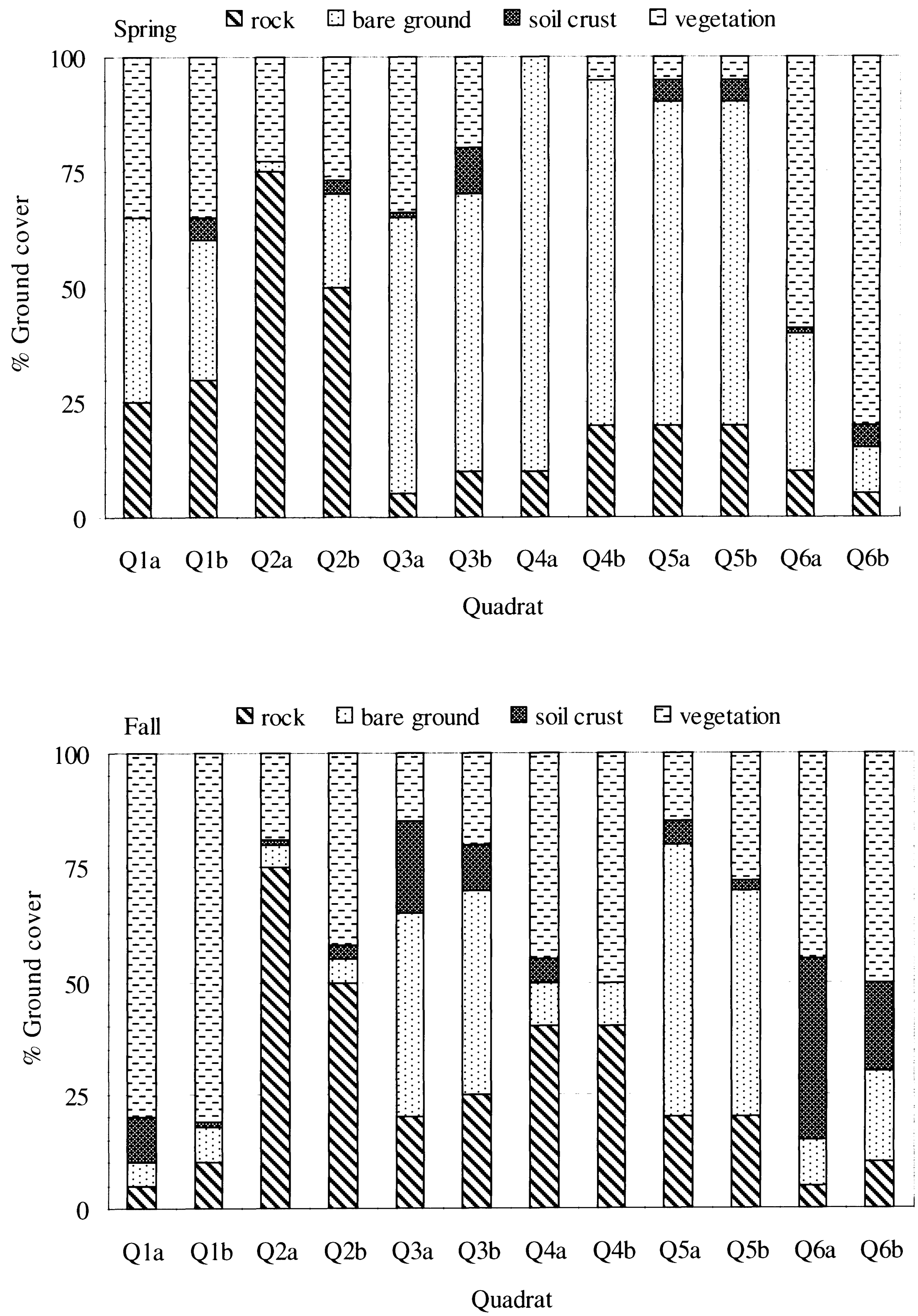



**Figure 12.** Percent rock, bare ground, soil crust, and vegetation of each subquadrat when seedlings were planted April and October 2007 (Q1a, Q2a, etc.) and at the end of the respective study periods, June and November 2008 (Q1b, Q2b, etc.).

A total of 66 *A. asterias* seedlings (55.0%) of the spring planted treatment survived the 14-month study period (Fig. 13). The majority of spring planted seedlings in the Q4 subquadrat were lost due to a Mexican ground squirrel (*Spermophilus mexicanus*). Removal of this subquadrat from the percent survivorship increases the spring survivorship to 65.0% (Fig. 13). A total of 87 (72.5%) survived from the fall treatment (Fig. 13). The number of seedlings surviving per quadrat for spring planted seedlings ranged from 1-16 (Fig. 14). For the fall planted seedlings, the number of seedlings surviving per subquadrat ranged from 12-19 (Fig. 14).

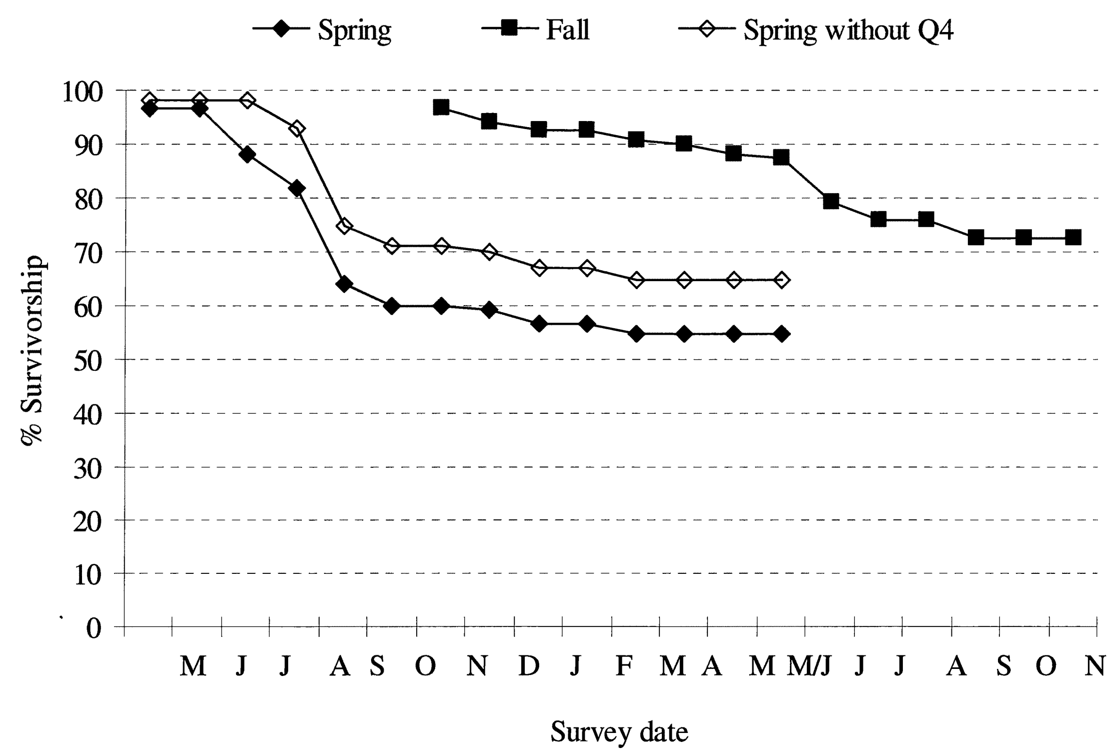



**Figure 13.** Percent survivorship per month of the seedlings ($n$ = 120) planted April and October 2007. The "spring without Q4" line is the survivorship of seedlings planted in the spring without the 20 seedlings lost in Q4.

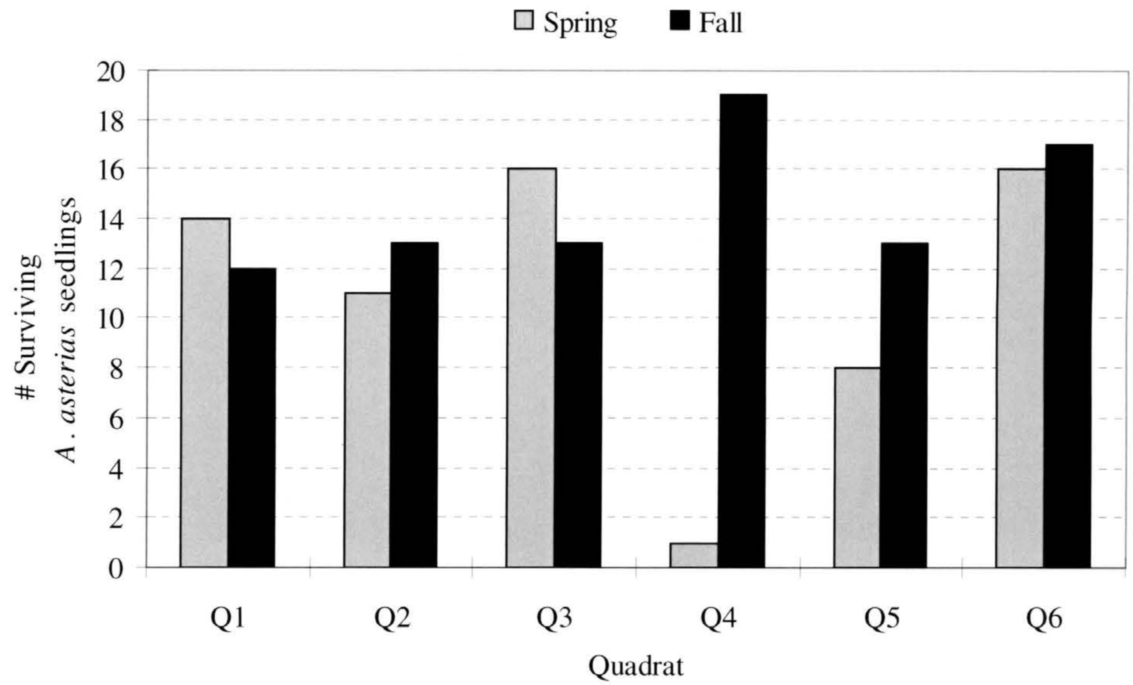



**Figure 14.** Number of *A. asterias* seedlings per quadrat out of 240 planted in the spring and fall that were alive at the end of the 14-month study periods.

Causes of mortality included burrowing activity by Mexican ground squirrel, desiccation, herbivory, infestation by weevils, and other causes (Fig. 15). Seedlings were classified as dead when body piece(s) could be identified as *A. asterias*. The category "other" includes seedlings which were soft, uprooted, or otherwise damaged that eventually died. The "missing" category represents seedlings not relocated at the end of the study periods and for purposes of data analysis, missing seedlings were assumed dead (Fig. 15).

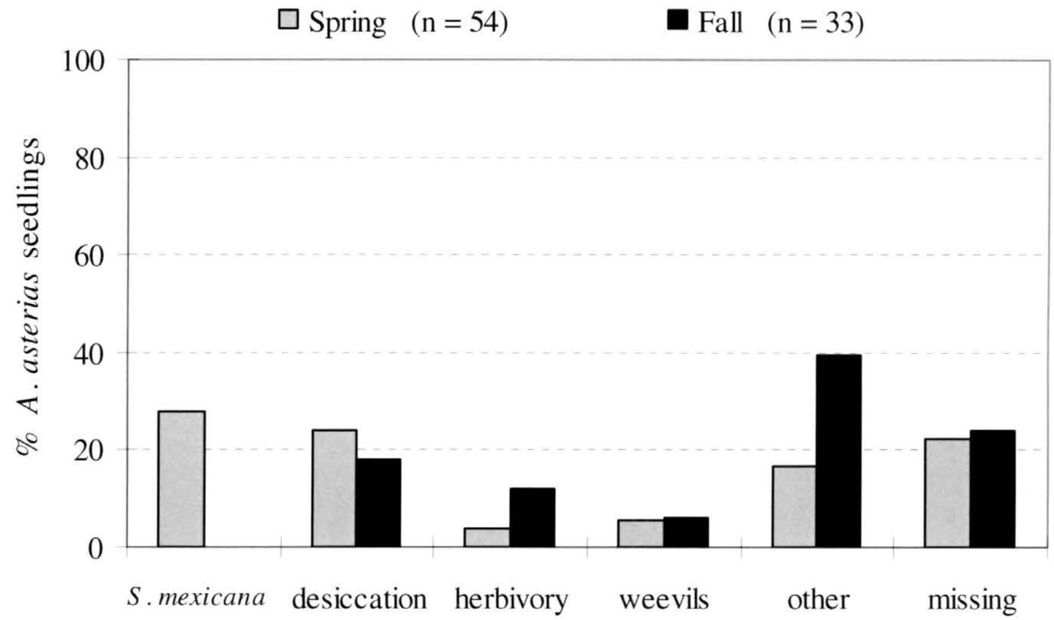
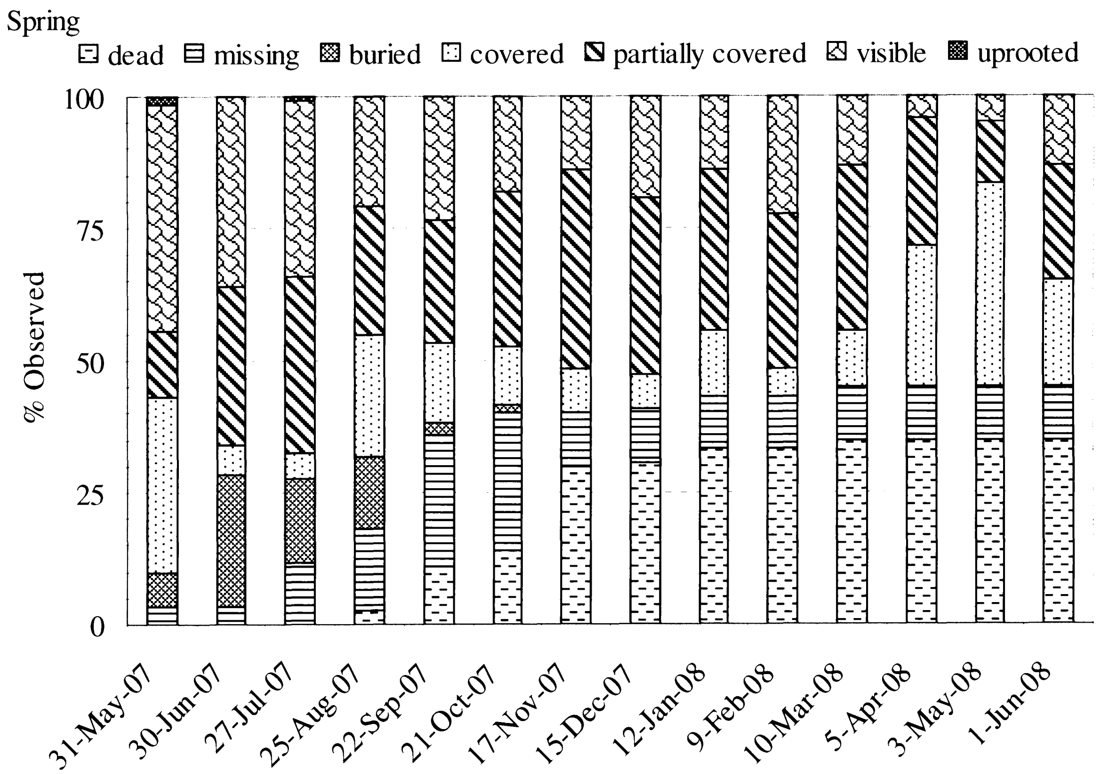



**Figure 15.** Causes of mortality for reintroduced *A. asterias* seedlings.

Nineteen of 20 seedlings planted in the spring in Q4 were lost due to burrowing activity of a Mexican ground squirrel. Desiccation accounted for 22% of the total deaths (Fig. 15). A total of six seedlings died from herbivory as evidenced by teeth marks. Another impact noted, that could possibly be due to rodents, is uprooting of the seedlings. Twenty of the fall planted seedlings were uprooted at least once. Of these only nine were alive at the end of the study period. Two died as a direct result of uprooting. Fifty-nine percent of the uprooting events occurred in November 2007 with over half of the uprootings (52%) occurring in Q3. One seedling in Q6 was still alive at the end of the study period despite being uprooted in November 2007, February, March, and April 2008. When seedlings were uprooted, I replanted them and gave each ~3 mL of water.

Weevil infestation accounted for 6% of the total deaths (Fig. 15). In January 2008, I collected two seedlings (one each planted in the spring and fall) containing larvae; these died before identification could be made. In March 2008, I collected three more seedlings (two planted in the spring and one in the fall) which contained larvae. After approximately one month two adult weevils emerged. I preserved the specimens but have not had them identified. All confirmed seedling deaths due to weevils were located in Q5.

The reintroduced seedlings also displayed a myriad of colors, but not to the extent of the seedlings in cultivation. Most often the reintroduced seedlings were either brown or green in color. Brown was most often associated with seedlings that were exposed or when it was drier. If seedlings were covered with dirt or by an object they would often be a shade of green. I once observed a seedling which had a thin layer of soil on one half of it; this side was green while the exposed half was brown. The seedlings would also retract below the soil surface and often be covered by soil when precipitation was limited. When ample moisture was available the seedlings would be green, plump, and easily visible. A summary of the state (visible, covered, buried, etc.) of the seedlings by month for the spring and fall treatments is provided in Fig. 16.



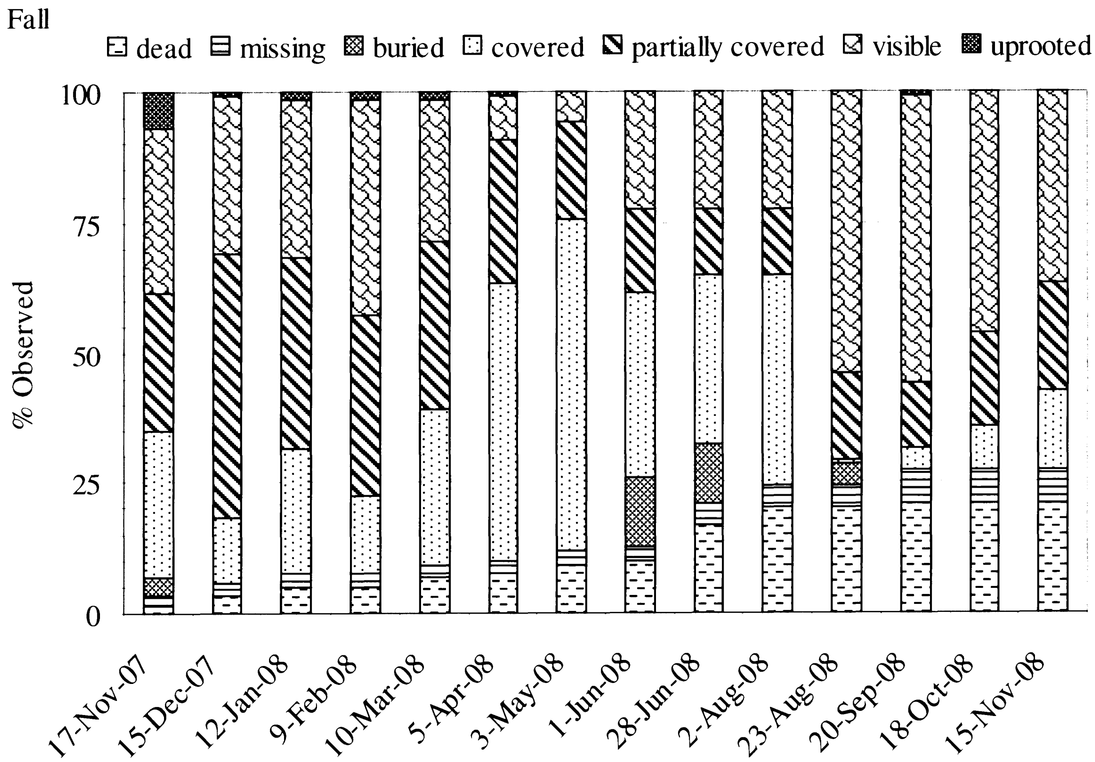




**Figure 16.** Percent of spring and fall planted seedlings observed in the various states per month of monitoring.

At the end of the 14-month study period, average diameter of the spring planted seedlings had increased from 8.78 mm at planting to 10.40 ± 2.0 mm (range 6.43-14.92 mm). Fifty-six of the 66 seedlings alive at the end of the spring study period had diameters ranging from 8.01-14.00 mm (Fig. 17). The Q2 subquadrat ($n$ = 11) had the smallest average final diameter of 9.53 while the Q5 subquadrat ($n$ = 8) had the largest average diameter at 11.07 mm. However, the differences in final diameters per subquadrat of the spring planted seedlings were not significant ($F$ = 1.32, $P$ = 0.2729, $df$ = 4). Ten of the 66 spring planted seedlings alive at the end of the study period decreased in diameter. Five of the 10 lost >1.00 mm in diameter with the greatest loss being 3.07 mm. The other seedlings showed an increase in diameter ranging from 0.02-4.37 mm. Thirty-three seedlings showed an increase in diameter by more than 2.00 mm. Four of these had an increase in diameter over 3.00 mm. The simple linear regression of final diameter of spring planted seedlings on initial diameter was significant ($r^2$ = 0.47, $P$ < 0.0001, $n$ = 66; Fig. 18). Over the 14-month study period, the diameters of reintroduced seedlings increased by 0.8358 mm (Fig. 18). This equates to an estimated growth rate of 0.73 mm/year.

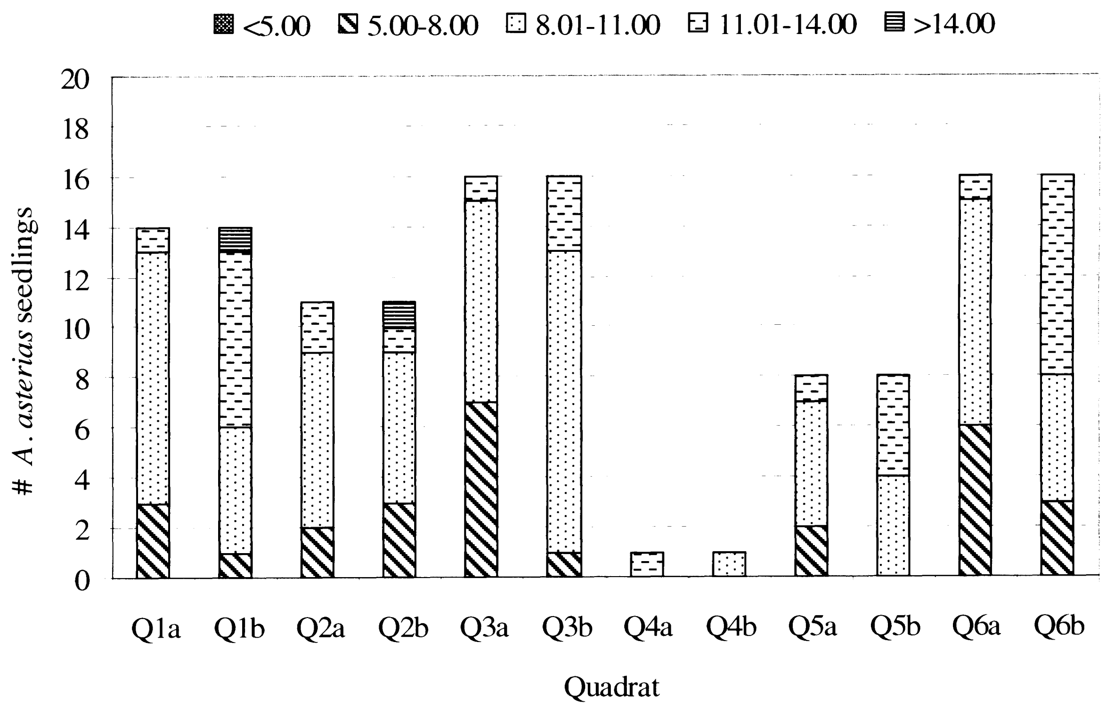



**Figure 17.** Size classes per quadrat of the 66 seedlings when planted in April 2007 (Q1a, Q2a, etc.) and at the end of the study period, June 2008 (Q1b, Q2b, etc.).

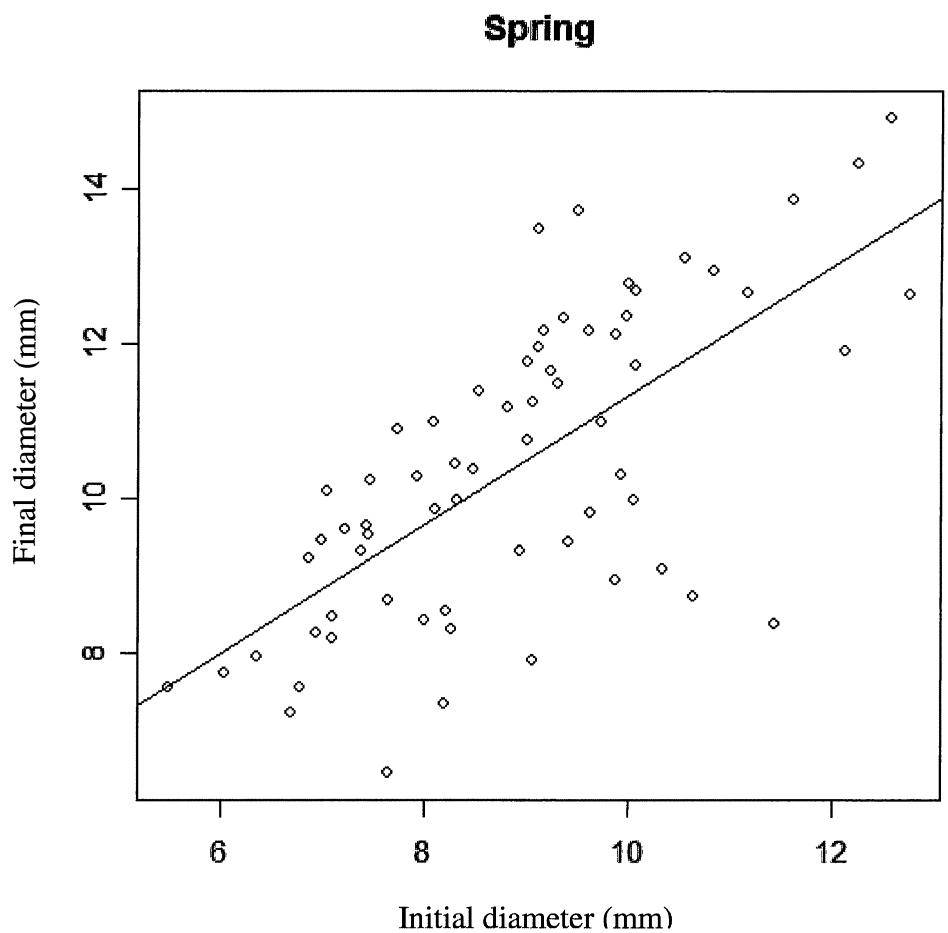



**Figure 18.** Linear regression of final diameter of spring planted seedlings on initial diameter ($r^2 = 0.47$, $P < 0.0001$, $n = 66$; $y = 2.9676 + 0.8358x$).

At the end of the 14-month study period, average diameter of the fall planted seedlings had increased from 9.30 mm at planting to 11.31 ± 2.6 mm (range 6.67-18.45 mm). Sixty-seven of the 87 seedlings alive at the end of the fall study period had diameters ranging from 8.01-14.00 mm (Fig. 19). The subquadrat in Q2 had the smallest average final diameter of 9.68 mm while the largest average final diameter of 12.71 mm was in the Q4 subquadrat. The differences in final diameters per subquadrat of Q2 and Q4 were significant ($F = 2.59$, $P = 0.0319$, $df = 5$; Q4 - Q2 confidence intervals: lower =

0.4646 and upper = 5.5938). The differences in final diameters per subquadrat of the other quadrats were not significant. Of the 87 fall planted seedlings, 6 decreased in diameter size but by <1.00 mm. The other seedlings increased in diameter from 0.15-6.18 mm. Thirty-six of the seedlings increased by >2.00 mm with 15 increasing by >3.00 mm. The simple linear regression of final diameter of fall planted seedlings on initial diameter was significant ($r^2 = 0.71$, $P < 0.0001$, $n = 87$; Fig. 20). Over the 14-month study period, the diameters of reintroduced seedlings increased by 1.0752 mm (Fig. 20). This equates to an estimated growth rate of 0.99 mm/year. The difference in final diameters of the spring and fall treatments was significant ($t = -2.41$, $P = 0.0173$, $df = 151$).

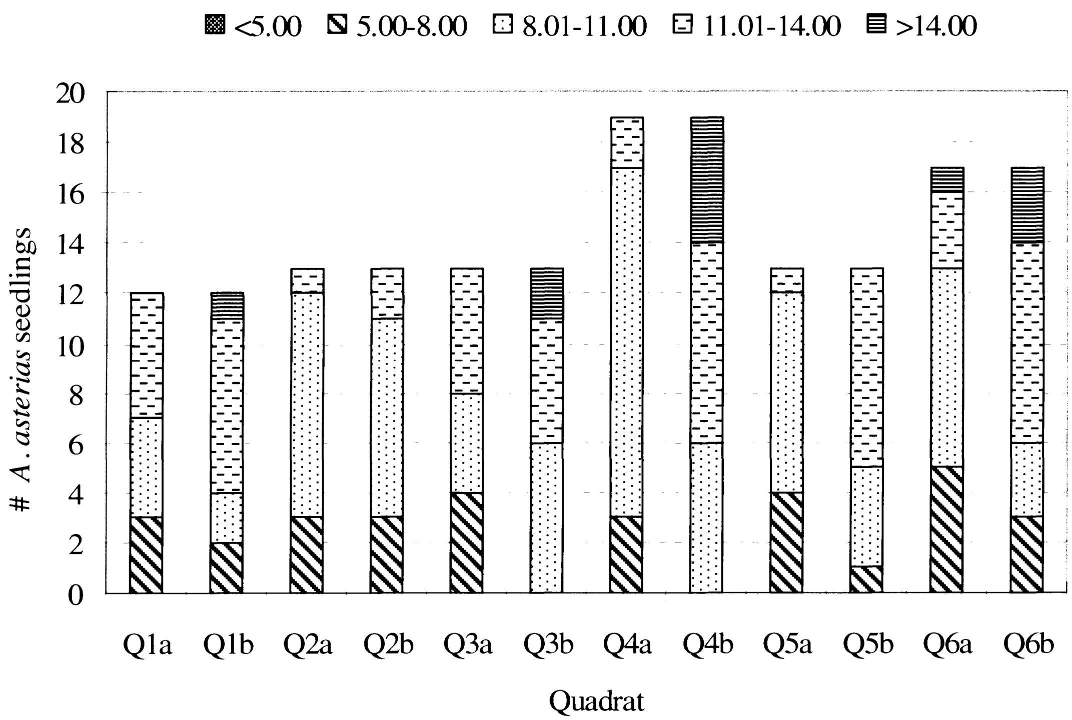



**Figure 19.** Size classes per quadrat of the 87 seedlings when planted in October 2007 (Q1a, Q2a, etc.) and at the end of the study period, November 2008 (Q1b, Q2b, etc.).

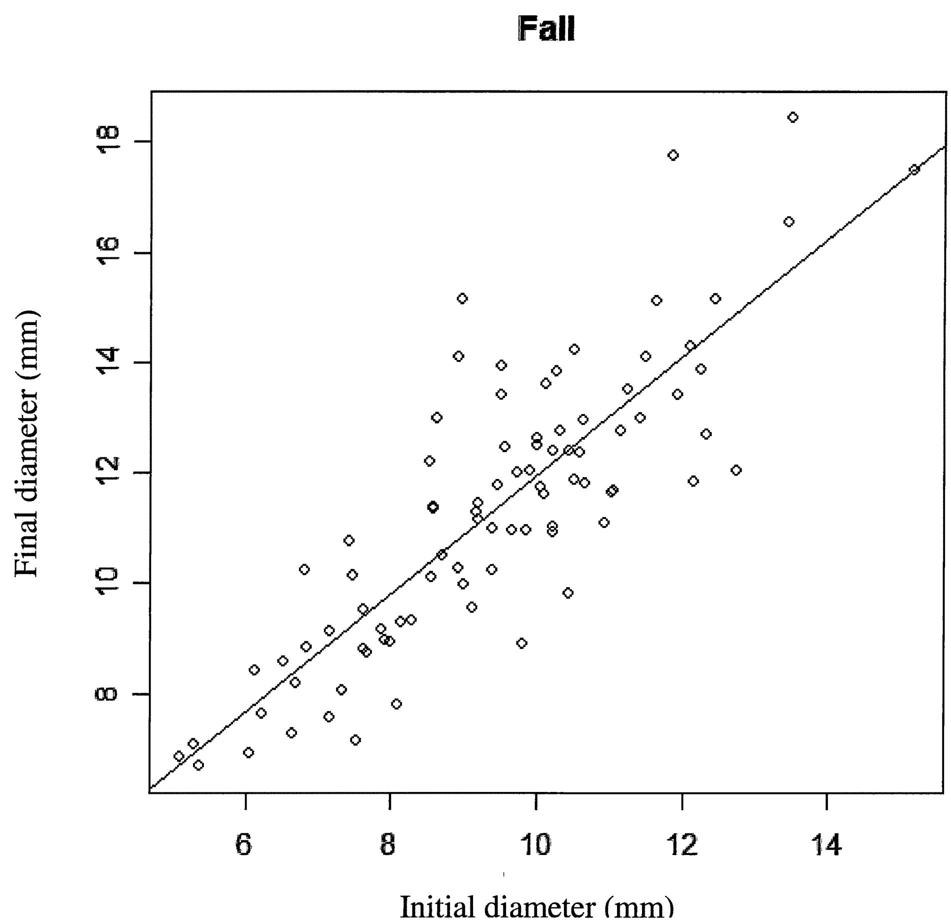



**Figure 20.** Linear regression of final diameter of fall planted seedlings on initial diameter ($r^2$ = 0.71, $P$ <0.0001, $n$ = 87; $y$ = 1.1987 + 1.0752$x$).

**Factors influencing seedling survivorship**

The difference between the monthly average ground temperatures as recorded by the two ground temperature sensors was not significant ($t$ = -0.485; $P$ = 0.6304, $df$ = 38). Therefore, only ground temperatures as recorded by the sensor at Q3 were analyzed in the model statements. The top 10 models with the smallest Akaike information criterion corrected for small sample size ($AIC_c$) and the highest Akaike weight ($w_i$) as well as other model selection statistics of log likelihood ($LL$), the number of parameters of each model ($K$) and the difference between model $AIC_c$ and $AIC_c$ value of the best model ($\Delta_i$) are shown in Table 11. The temporal model (# 29) was the top model of the candidate model set ($AIC_c$ = 1129.3094, $w_i$ = 0.99999854) having ~100% probability of being the best model. Of the factors analyzed, passage of time had the greatest influence on survivorship of the reintroduced seedlings.

**Table 11.** Summary of the ten models with the smallest Akaike information criterion corrected for small sample size ($AIC_c$) and largest Akaike weight ($w_i$) that sought to explain factors influencing seedling survivorship per subquadrat. Also included are other model selection statistics of log likelihood (*LL*), number of parameters of each model (*K*), and the difference between the model $AIC_c$ and $AIC_c$ value of the best model ($\Delta_i$).

| # | Model | *K* | *LL* | $AIC_c$ | $\Delta_i$ | $w_i$ |
|---|---|---|---|---|---|---|
| 29 | surv = month | 16 | -546.6693 | 1129.3094 | 0.0000 | 0.99999854 |
| 5 | surv = sea + dm | 5 | -619.5885 | 1249.5824 | 120.2730 | <0.00000001 |
| 19 | surv = sea + rain + dm | 6 | -622.8114 | 1258.1942 | 128.8848 | <0.00000001 |
| 7 | surv = sea + stat | 5 | -624.4781 | 1259.3616 | 130.0522 | <0.00000001 |
| 27 | surv = stat + gtemp + dm + bare | 7 | -622.9130 | 1260.5931 | 131.2837 | <0.00000001 |
| 10 | surv = stat + dm | 5 | -626.0478 | 1262.5010 | 133.1916 | <0.00000001 |
| 21 | surv = sea + stat + gtemp | 6 | -625.1386 | 1262.8486 | 133.5392 | <0.00000001 |
| 1 | surv = sea | 4 | -627.5109 | 1263.2903 | 133.9809 | <0.00000001 |
| 28 | surv = gtemp + dm + bare | 6 | -625.5156 | 1263.6026 | 134.2932 | <0.00000001 |
| 11 | surv = stat + bare | 5 | -627.7610 | 1265.9274 | 136.6180 | <0.00000001 |

# CHAPTER IV

## DISCUSSION

Reintroduction projects have multiple purposes and each will be unique due to the species involved, but there are common underlying elements to many, if not all, reintroductions (Guerrant and Kaye, 2007). When choosing a reintroduction site biotic and abiotic factors must be taken into consideration as well as logistical and historical criteria (Fiedler and Laven, 1996). For plants common factors include propagule type, source of propagules, season of planting, site preparation, post-planting care, etc. (Guerrant and Kaye, 2007). Decisions regarding the aforementioned factors could determine the success of any reintroduction project.

Pavlik (1996) addresses two forms of success regarding reintroductions: biological and project. Biological success can be measured at the individual and population levels (Pavlik, 1996). The biological success of reintroductions can only be determined by following the fate and performance of individual plants through time (Pavlik, 1996). Thus long-term monitoring of the reintroduced *A. asterias* seedlings is necessary.

Project success has broader implications and includes more than biological success. A project may be considered a success due to the knowledge contributed regarding the specific taxon, even if the biological aspect fails (Pavlik, 1996). At a minimum, the pilot reintroduction of *A. asterias* has achieved some project success.

I was able to 1) provide quantitative data regarding community composition and basic edaphic parameters; 2) demonstrate that *A. asterias* occurs in association with other plants more often than rocky or open, bare areas; 3) demonstrate that seedlings are a better choice for propagule type; 4) determine that seedlings planted in the fall had a higher growth rate than spring planted seedlings; 5) determine that seedlings should be planted at least 3 m from active *Spermophilus mexicanus* burrows; and 6) develop a planting and monitoring methodology. Based upon this study, a draft reintroduction plan for *A. asterias* was developed (Appendix D) as required by Federal Register 65(183):56916-22 (USFWS, 2000).

In Texas, researchers often choose survey sites for *A. asterias* based on the presence of *Varilla texana* in gravelly soils (pers. observ.). *Varilla texana* is a halophytic plant and the highest densities of *A. asterias* observed in my study were at sites with soils classified as saline-sodic, saline, and sodic. *Varilla texana* also had the greatest relative dominance at the 15 sites. However, this species was not the dominant at four sites and it was not documented at three other sites. *Astrophytum asterias* is also found in nonsaline, nonsodic soils. Thus, concentrating survey efforts in areas with *V. texana* is not an unsound approach; however, survey effort must be of equal magnitude across habitats with species composition and edaphic parameters similar to the 15 sites of this study. Less than 28% of the known *A. asterias* subpopulations were evaluated in my study. If more subpopulations are discovered in areas without *V. texana*, further evaluation of these areas could provide guidance for future survey efforts as well as additional knowledge regarding *A. asterias* habitat.

*Varilla texana* was most commonly documented in direct association with *A. asterias*. However, *A. asterias* is also found beneath the shade of other species. Many cacti species are known to benefit from nurse plant associations (Franco and Nobel, 1989; Valiente-Banuet and Ezcurra, 1991; Leirana-Alcocer and Parra-Tabla, 1999; Flores, et al., 2004; Godínez-Alvarez, et al., 2005; Cervera, et al., 2006). However, nurse plants can have negative effects too such as reducing photosynthetic photon flux which can decrease seedling growth rate (Franco and Nobel, 1989) and increasing competition for limited nutrients (summarized by Valiente-Banuet and Godínez-Alvarez, 2002). In Mexico, Martínez-Ávalos (2003) showed that *A. asterias* are randomly distributed under shrubs shorter than 1 m and *Castela erecta* subsp. *texana* was documented as the most frequent nurse plant with *A. asterias* tending to be distributed on the northern side of shrubs. However, no research has been conducted in Texas regarding the costs and benefits to *A. asterias* of being associated with other plants. Strong (2005) observed that *A. asterias* which were deep within a nurse plant and received minimal direct light lacked reproductive structures despite having diameters larger than the average reproductive size of *A. asterias* of her study. The Q4 subquadrat of reintroduced seedlings planted in the fall was located in and around a *V. texana* clump. This is the lone subquadrat of the spring and fall treatments which had 95% survivorship, with one seedling lost to herbivory. When designing future reintroduction experiments, it may be valuable to evaluate the importance of *V. texana* to the establishment of *A. asterias* seedlings. It is also possible that the importance of *V. texana* and other star cactus plant associates vary with the life stage of *A. asterias*.

Mandujano, et al. (2002) evaluated the spatial distribution of three species of globose cacti, *Mammillaria carnea*, *M. haageana* and *Coryphantha pallida*, and found that more individuals were associated with nurse plants than bare areas. My study determined that more individuals of *A. asterias* were also associated with other plants than bare or rocky areas. Similar to *M. carnea*, *M. haageana*, and *C. pallida* which have been observed under the canopy of nurse shrubs and in open areas (Mandujano, et al., 2002), *A. asterias* is also observed to occupy bare areas. Two factors that Mandujano, et al. (2002) presented but did not evaluate regarding why cacti can survive in bare areas included the possibility that: 1) projected shade from nearby plants could ameliorate the harsh environmental conditions for a short period during the day; 2) annuals may also emerge and act as an ephemeral nurse plant during times when conditions are favorable for seedling establishment. These are two plausible reasons why *A. asterias* survives in open areas. All 15 vegetation transects had less than 58% vegetative cover with 12 sites having less than 50%. Often the vegetation is in clumps leaving interstices of varying size. Included in the clumps of vegetation are various shrub species that are 1 m or taller which can provide projected shade for *A. asterias*. Secondly, emergence of annuals adjacent to the reintroduced seedlings was also observed throughout the study period. These too could provide temporary shade and a respite from harsh environmental conditions.

Many factors including but not limited to water availability, temperature, light, and seed age can affect the germination of cacti seeds (Nolasco, et al., 1996; Godínez-Alvarez and Valiente-Banuet, 1998; Flores, et al., 2005; Cervera, et al., 2006; Flores, et al., 2006). *A. asterias* will germinate in a laboratory setting (Maiti, et al., 2002; Strong, 2005). However, germination in the wild occurs sporadically and seedling recruitment is low (pers. observ.; Janssen, et al., 2008). According to Steenbergh and Lowe (1969), *Carnegiea gigantea* seedlings emerged after 2 or more precipitation events ranging from 21-85 mm occurring in a 5-day period. Godínez-Alvarez, et al. (2005) speculated that no seeds of *Stenocereus stellatus* germinated in their experiment because rainfall events were <20 mm and occurred more than 4 days apart. Of the spring planted seeds, three seedlings were noted in September 2007 with two more noted in December 2007. There was one 6-day period (28 June-3 July 2007) which had a total of 166.31 mm with 3 days having >23 mm. June-September 2007, there were five other 2 to 6-day periods where rainfall was >31 mm. Of the fall planted seeds, three seedlings were first observed in August 2008 with one more in September 2008. Nearly 90 mm of precipitation was documented in an 11-day period from 29 June-9 July 2008; however, the daily totals were each <16 mm. This was followed by another 152 mm of precipitation on 23-24 July. In August 2008 two significant rainfall events (162 and 112 mm) were documented in a three and 11-day period, respectively. During the 11-day period only two daily totals were >21 mm each. It may be possible to tie *A. asterias* germination to rainfall events of a specific magnitude, but that would require monitoring in a shorter time frame than monthly as was done in this study.

Cacti seeds of a number of species germinate in a wide range of temperatures; however, the optimal temperature for germination is 20-30°C (Valiente-Banuet and Godínez-Alvarez, 2002). Maiti, et al. (2002) and Strong (2005) germinated *A. asterias* at 25-30°C using growth chambers. Rojas-Aréchiga and Vázquez-Yanes (2000) summarized temperature effect on cacti seed germination and found that: 1) temperature extremes >28°C do not favor germination; and 2) as temperature increases, time to complete germination decreases (such is the case with *A. myriostigma*). Nolasco, et al. (1996) found that for the cactus *Pachycereus pringlei*, seeds were capable of germinating after being exposed for 2 hours at 70°C; however, after 22 days of exposure at this temperature germination was inhibited. Daws, et al. (2007) suggest that the ability of seeds of desert succulents to withstand extreme temperatures is related to the maximum annual temperature of their environment. The average monthly air temperature at the reintroduction site for 11 months of the 18-month study period was in the optimal range for cacti seed germination. In total the average monthly ground temperature and maximum air and ground temperatures of the reintroduction site exceeded 28°C for 17 months of the 18-month study period.

Laboratory germination experiments of some cacti seeds have demonstrated that seed age affects germination rate. De la Barrera and Nobel (2003) found that *Stenocereus queretaroensis* seeds which had been in storage for 12-28 months had an average germination of 85%, but seeds stored for 40 months had a germination rate around 65%. Flores, et al. (2005) determined that for two species of *Turbinicarpus* fresh seeds had higher germination values than old seeds. Flores-Martínez, et al. (2008) also noted that germination decreased as seed age increased for *Mammillaria huitzilopochtli*. The *A. asterias* seeds used by Strong (2005) for the laboratory germination experiment were <12 months old and resulted in ~75% germination. The seeds planted at the reintroduction site were collected in April and May 2004 and thus were approximately 3 and 3.5 years old at the time of planting in the spring and fall 2007, respectively.

Cacti seeds are also known to vary in the amount and type of light required to germinate. Flores, et al. (2006) determined that 11 cacti species of the Chihuahuan Desert, Mexico were positively photoblastic (require light to germinate). However, some species such as *Pachycereus pringlei* and *Stenocereus thurberi* do not require light to germinate (Nolasco et al., 1996, 1997). Still other species such as *Mammillaria gaumeri* exhibit a higher germination rate under lower light (receiving 20% of the total photosynthetic photon flux density as opposed to 50%) (Cervera, et al., 2006). Light sensitivity of *A. asterias* seeds has not been studied. While collecting a soil sample for one study site, three *A. asterias* seedlings (<10 mm in size) were found buried approximately 1-3 cm under rocks and soil. This may indicate that seeds can germinate underground or it may mean that the seedlings were covered by shifting of the soil and rocks. Another plausible explanation is that the seeds which produced the aforementioned seedlings received sufficient light and germinated in crevices adjacent to rocks.

Peters, et al. (2008) demonstrated that nurse rocks are more important than nurse plants in determining the distribution and establishment of *Mammillaria* spp. in the Tehuacán Valley, Mexico. Munguía-Rosas and Sosa (2008) also determined that rock cavities were important in protecting the seeds of *Pilosocereus leucocephalus* from predation. Many of the *A. asterias* subpopulations in Texas are found in soils with a gravel component; therefore, rocks may provide a microhabitat conducive to the germination of *A. asterias* seed and establishment of seedlings.

Rivera-Aguilar, et al. (2005) suggested that biological soil crusts have a positive effect on the seed germination of *Mimosa luisana* and *Myrtillocactus geometrizans* in the

Tehuacán Valley, Mexico. Soil crusts were documented within the subquadrats in which *A. asterias* seeds were planted. The percentages of ground covered in each subquadrat by soil crusts ranged from 1-60%. There are multiple factors that influence the effect of soil crusts on germination of plant species (Rivera-Aguilar, et al., 2005). While it is possible that soil crusts could play a role in the germination and establishment of *A. asterias*, no information is available on this from my study.

There is much to be learned regarding germination of *A. asterias* seeds in the wild. Despite having a few rainfall events of the magnitude described by Steenbergh and Lowe (1969) and optimal temperatures for inducing germination of cacti seeds (Valiente-Banuet and Godínez-Alvarez, 2002), planting *A. asterias* seeds in the pilot reintroduction was unsuccessful with <4% producing seedlings, regardless of timing of planting. The effect of high air and ground temperatures on *A. asterias* seed viability is not known. However, the seeds planted in the spring and fall that produced seedlings were exposed to high air and ground temperatures for 6-9 and 11-12 months, respectively. Seed age may have also played a role in the low germination of *A. asterias* seeds of the pilot reintroduction as the effect of seed age on germination rate has not been studied for this species. The ability of *A. asterias* to form a seed bank is also not known. It is possible that the seeds planted in the pilot reintroduction have formed a seed bank and may germinate given the right environmental factor or combination of factors necessary to trigger germination.

Insight regarding germination of *A. asterias* seeds and establishment of seedlings could be garnered through dispersal studies which have not been conducted for *A. asterias*. Damude and Poole (1990) report an observation made by Poole on 28 April

1988 of disintegrating fruits leaving the majority of the seeds piled on top of the plant, although a few seeds had started to slide down the grooves. Blair and Williamson (2008) also reported observing *A. asterias* seeds on top of and within a few centimeters of the adult plant. I too have observed this on several occasions (Fig. 21). Bregman (1988) described forms of seed dispersal in Cactaceae and included the genus *Astrophytum* as possibly dispersing by water (hydrochory) due to its seed structure. The apex of *A. asterias* is often concave which would allow for the collection of water. If rainfall is sufficient the water could flow down the grooves dispersing the seeds. If rainfall occurs with enough force, the seeds may also be ejected from the surface of the plant.

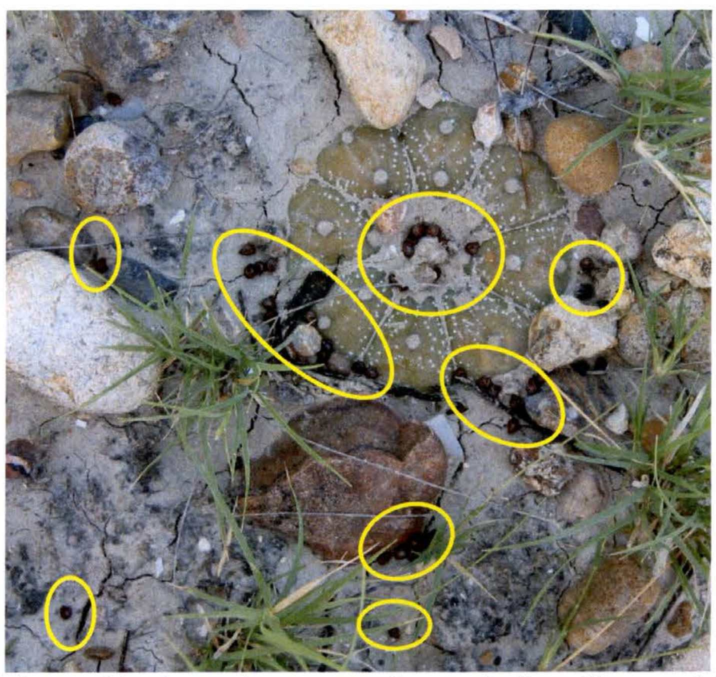


**Figure 21.** *Astrophytum asterias* seeds (in yellow ovals) dispersed on and around an adult plant, 2 August 2008.

Researchers have frequently evaluated the benefits of nurse plants; effect of varying levels of solar radiation and soil moisture; and impact of herbivores on the establishment and growth of cacti seedlings (Steenbergh and Lowe, 1969; Leirana-Alcocer and Parra-Tabla, 1999; Flores, et al., 2004; Godínez-Alvarez, et al., 2005; Cervera, et al., 2006; Flores-Martínez, et al., 2008; Martínez-Berdeja and Valverde, 2008). Often this research is conducted using seedlings less than a year old and in some cases as young as one week. Seedling survivorship has subsequently varied from 0-85% in studies varying in length from 78-210 days (Leirana-Alcocer and Parra-Tabla, 1999; Godínez-Alvarez, et al., 2005; Cervera, et al., 2006; Flores-Martínez, et al., 2008; Martínez-Berdeja and Valverde, 2008). Of the factors analyzed in the study reported herein, only passage of time had a significant effect on seedling survivorship. A likely explanation for the failure to detect the effects of other factors is the age of the reintroduced seedlings. The reintroduced seedlings were over 2-years old. Keeping them in a controlled environment until this age may have allowed them to develop sufficient roots and body size to withstand the harsh environment into which they were introduced. However, it is plausible that there is a maximum age at which seedlings in cultivation can be successfully reintroduced. Rearing in a controlled environment for too long may increase the plants susceptibility to high temperatures and minimal precipitation.

In cultivation, the growth rate of *A. asterias* was estimated to be 0.77-6.02 mm/year. The estimated growth rate of the reintroduced seedlings was 0.73 and 1.08 mm/year for the spring and fall treatments, respectively. As expected the field estimated growth rates are at the low end of the growth rates of *A. asterias* in the controlled environment. Janssen, et al. (2008) estimated that *A. asterias* reaches reproductive maturity at 40 mm. Using the growth rates from the field and assuming a linear growth rate, the reintroduced seedlings are projected to take 36-55 years to reach reproductive maturity. However, Janssen, et al. (2008) also calculated the mean annual growth rates of *A. asterias* by size classes in 5 demographic transects over a 4-year period and demonstrated that growth rate varies by size class. The mean annual growth rates ranged from -0.85 mm for the 80.01-90.00 mm size class to 3.65 mm for the 50.01-60.00 mm size class (Janssen, et al., 2008). Mean annual growth rate also varied from year to year at each site (Janssen, et al., 2008). The growth rates of both greenhouse-grown and wild plants demonstrate that *A. asterias* has a variable growth rate. However, it is a slow growth rate indicating that star cactus may take years to reach reproductive maturity.

*Astrophytum asterias* exhibits characteristics (slow-growth rate, obligate outcrosser, low flower production, low fruit and seed set) that compared to other cacti may limit population growth (present study; Strong, 2005). This species also faces threats of both natural and human origin. Martínez-Ávalos, et al. (2007) documented mortality of *A. asterias* in Mexico due to herbivory by Mexican ground squirrels, a plant pathogen (*Phytophthora infestans*), and a cerambicid beetle (species unidentified). Janssen, et al. (2005, 2008) and Ferguson and Williamson (in press) documented mortality of *A. asterias* in Texas due to herbivory by desert cottontails (*Sylvilagus audubonii*) and possibly Mexican ground squirrels, fungal infection, and a cerambicid beetle (*Moneilema armatum*). In Mexico and Texas mortality by the aforementioned causes occurs in all size classes.

At the pilot reintroduction site burrowing activity by a Mexican ground squirrel caused the loss of 95% of the seedlings planted in Q4 of the spring treatment. In March 2007 when the pilot site was established, a Mexican ground squirrel burrow was approximately 2-3 meters SE of Q4. I thought this was sufficient distance to safely establish the quadrat. After heavy rains in June and early July 2007, the burrow collapsed. A new burrow was then constructed within 0.5 m of the subquadrat containing spring planted seedlings. The burrow was active for several months. The greatest disturbance to the subquadrat was from addition of soil as the ground squirrel excavated and maintained the burrow.

A new insect predator of *A. asterias* seedlings was documented in my study: weevils tentatively identified to the genus *Gerstaeckeria*. Other seedlings died due to uprooting and herbivory by unknown fauna, desiccation, and possibly rotting. Preliminary research shows no correlation between mammalian herbivory of naturally established subpopulations and climatic conditions (Janssen, et al., 2008).

Anthropogenic threats listed in the *A. asterias* recovery plan (USFWS, 2003) included habitat destruction/modification and over-collection by cactus enthusiasts. *Astrophytum asterias* faces similar threats in Mexico (Martínez-Ávalos, et al., 2004). These threats are still relevant today. Land in Starr County, Texas is still being rootplowed and converted to non-native, forage grasses, in particular, buffelgrass (*Pennisetum ciliare* var. *ciliare*). Collection of *A. asterias* is hard to document, but is still assumed to be of significance. Incidental collection of *A. asterias* by licensed peyote distributors is known to occur. Peyote harvest in Texas has fluctuated around 2,000,000 buttons, so even an incidental harvest rate of 0.1% has profound implications for *A. asterias* numbers (Terry, 2005). The most recent (fall 2008-spring 2009) threats to *A. asterias* subpopulations in Texas have been from two 100 $mi^2$ seismic projects. Impacts occurred but were minimal due to coordination between the gas companies and a private consultant knowledgeable of star cactus subpopulations (Janssen, et al., 2008). However, oil and gas exploration and development remains a potential threat. The human population of Starr County was estimated at 62,249 for 2008, up 16.1% from April, 2000 (United States Census Bureau, 2009). Rio Grande City, Texas is sprawling in the direction of *A. asterias* subpopulations. The proposed US 83 Roma/Rio Grande City Bypass (4-lane tollway from FM 650 to FM 1430), is a project that could potentially have direct, indirect, and cumulative impacts to *A. asterias* subpopulations and habitat depending on the chosen alignment (Texas Sunset Advisory Commission, 2009).

Due to the intrinsic biological characteristics of *A. asterias* and the aforementioned threats, the outlook for star cactus is uncertain. Although this study demonstrates that reintroduction of *A. asterias* is possible, true conservation of this species can only occur through protection of subpopulations and more importantly preservation of its habitat and associated ecological processes. Hobbs (2007) points out that it is important to collect and propagate plants, but what benefit is achieved if the plants are simply reintroduced to the same degrading environment? If proper habitat can be protected and/or restored, it is highly likely that more reintroductions of *A. asterias* will be necessary to maintain its presence in the Tamaulipan Thornscrub ecoregion. Unfortunately, time is of the essence due to the threats facing *A. asterias* and only time will tell if this reintroduction can be deemed a 'successful' step in the conservation of *A. asterias*.

# APPENDIX A

WEATHER DATA SUMMARY

The total amount of rainfall recorded at the reintroduction site from 20 April 2007 to 14 November 2008 was 1,270.7 mm. The highest monthly totals of rainfall were 280.6, 226.1, 168.0, and 143.4 mm for August 2008, July 2008, July 2007, and June 2007, respectively (Fig. 22). During this 18-month span, there were 8 months for which <25 mm of precipitation was recorded. For 4 of the 10 months <10 mm of rainfall was recorded and two of those months (December 2007 and March 2008) no precipitation was recorded (Fig. 22). The rainfall as totaled for each of the monitoring periods (four-week periods which didn't equate to calendar months) was analyzed in the model statements. The average monthly air temperatures recorded at the reintroduction site did not differ much from the 30-year average monthly air temperatures for Rio Grande City, Starr County, Texas approximately 8 air miles southeast of the site. The average monthly air temperature at the reintroduction site was approximately 3°C higher in December 2007 and February 2008 than the 30-year average for those months in Rio Grande City (Fig. 22). It was also more than 2°C lower in July 2007 and July, August, and September 2008 at the reintroduction site than the 30-year average for those same months in Rio Grande City.

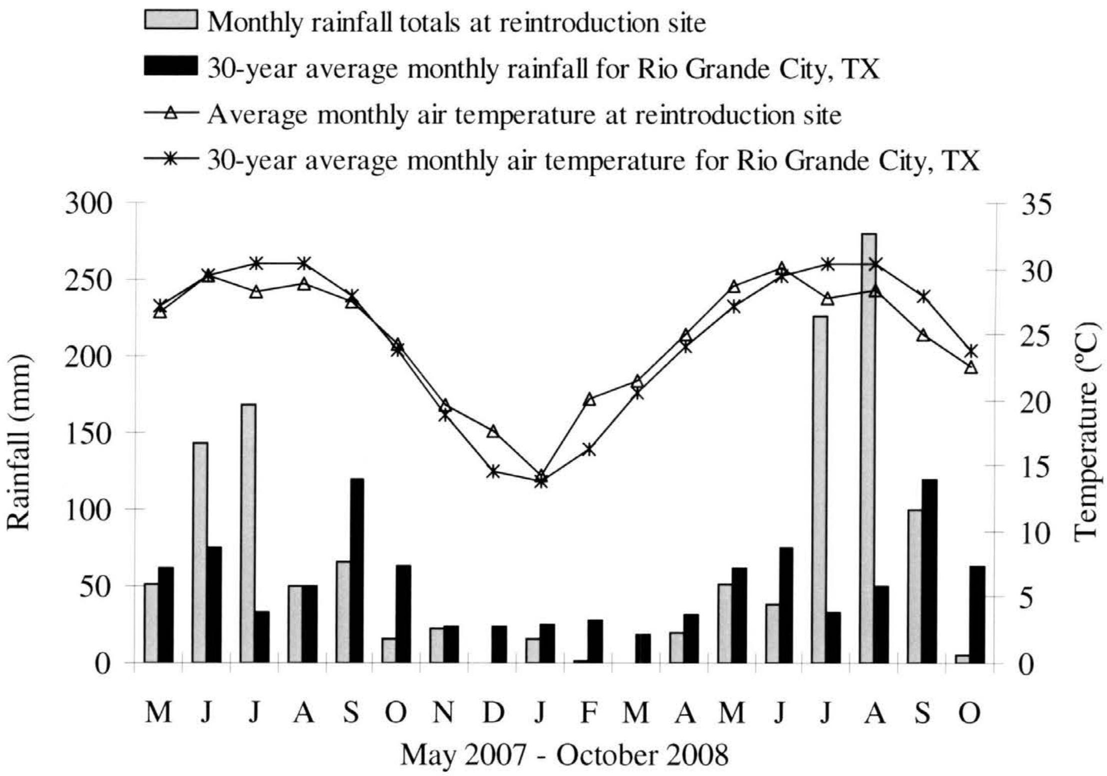



**Figure 22.** Monthly rainfall and average monthly air temperature at the reintroduction site and the average monthly rainfall and air temperature for Rio Grande City, Starr County, Texas, 1971-2000 (National Climatic Data Center, 2002).

The highest monthly average air temperature during the study period was 30.2°C and 29.4 °C in the months of June 2008 and 2007, respectively (Fig 23). Of the 575 days for which air temperature data were recorded, nearly 60% of the days had a daily average air temperature of 25-32°C. For a total of 75 days the daily average air temperature was >30°C. The highest daily average air temperature of 31.9°C was recorded on 19 June 2007. The maximum daily air temperature was ≥35°C for a total of 197 days of which 7 days it was ≥40°C. On 23 May 2008 the highest maximum daily air temperature of 41.7°C was recorded. January 2008 had the coldest monthly average air temperature of 14.2°C (Fig. 23). For a total of 14 of the 575 days, the daily average air temperature was

<10°C. The daily average air temperature dropped below 5°C on only one day, 18 January 2008 and was 4.8°C. The minimum daily air temperature was <5°C for a total of 27 days of which 6 days it dropped below freezing. The single lowest minimum daily air temperature of -2.4°C was recorded on 3 January 2008.

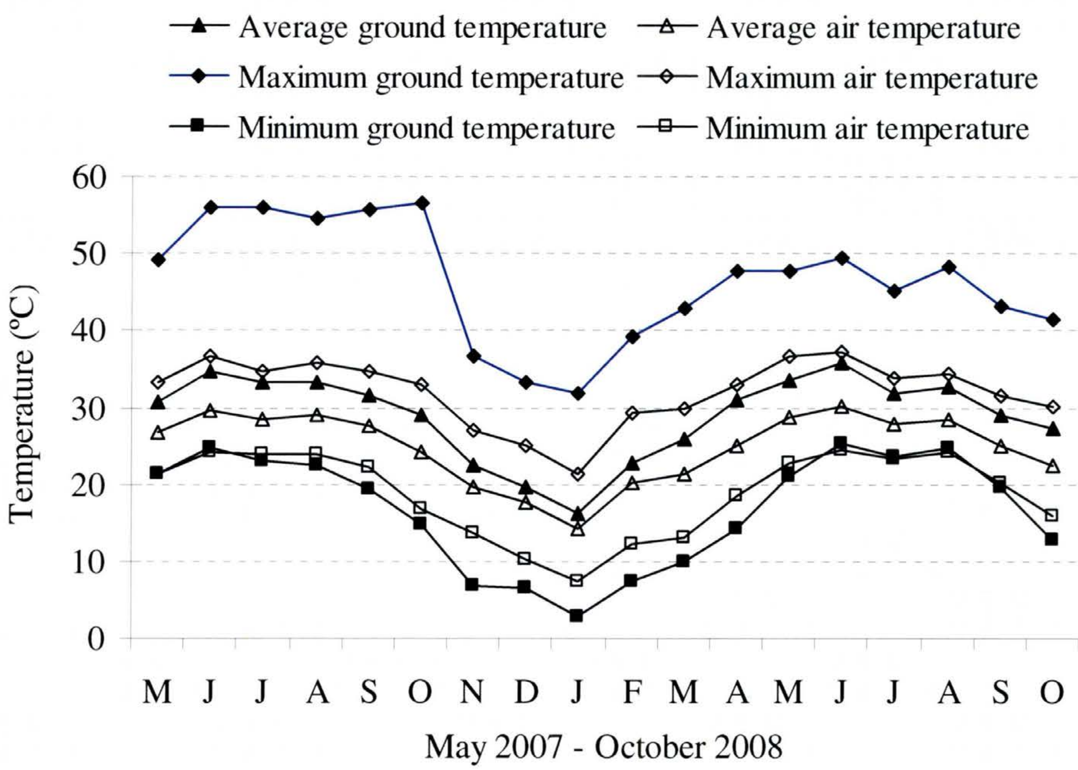



**Figure 23.** Average, maximum, and minimum monthly air and ground temperatures at the reintroduction site.

The highest monthly average ground temperature of 35.9°C was recorded for June 2008 (Fig. 23). Of the 554 days for which ground temperature data were recorded, 48.7% of the days had a daily average ground temperature of ≥30°C; 98 of which it was >35°C. The highest daily average ground temperature of 39.2°C was recorded on 16 June 2007. The maximum daily ground temperature was ≥40°C for a total of 281 days. For

22% of those days, the maximum daily ground temperature was >50ºC with 7 of these days ≥55ºC. The highest maximum ground temperature of 56.7ºC was recorded on 1 October 2007. The lowest monthly average ground temperature of 16.3ºC was recorded for January 2008 (Fig. 23). For only a total of 2 days, the daily average ground temperature dropped below 10ºC. The lowest daily average ground temperature of 7.9ºC was recorded 18 January 2008. The minimum daily ground temperature was <10ºC for 21 days; only 2 of which it was <5ºC. The minimum ground temperature of 2.7ºC was recorded 20 January 2008.

Initially I had difficulty with the soil moisture probes and soil temperature sensors as they were dislodged by animals on a monthly basis. Rain also caused soil to settle and expose them. As of October 2007 all soil moisture probes had been destroyed by animals. Therefore, no soil moisture data were available for analysis. The soil temperature sensor by Q1 was also destroyed by an animal. However, soil temperature sensors at Q3 and Q6 survived despite being dislodged on multiple occasions. After the last soil moisture probe was destroyed, the soil temperature sensors were never dislodged. The difference between the monthly average ground temperatures as recorded by the two ground temperature sensors was not significant ($t = -0.485$; $P = 0.6304$, $df = 38$). Therefore, only ground temperatures as recorded by the sensor at Q3 were discussed above.

## APPENDIX B

SOILS DATA

**Table 12.** Routine soil analysis results of soil samples collected within the 15 vegetation transects, pilot reintroduction site (RE), and one sample (Out) collected adjacent to site JB1. Samples collected March, May 2006 and March 2007. Conductivity (cnd) = µmho/cm; No$_3$, P, K, Ca, Mg, S, Na, Fe, Zn, Mn, Cu = parts per million.

| Site | pH | cnd | $NO_3$ | P | K | Ca | Mg | S | Na | Fe | Zn | Mn | Cu |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AM1 | 8.4 | 361 | 8 | 10 | 342 | 22,680 | 213 | 67 | 330 | 2.26 | 0.20 | 1.04 | 0.18 |
| AM2 | 8.0 | 4,641 | 28 | 21 | 386 | 27,732 | 269 | 1,484 | 4,048 | 3.50 | 0.27 | 3.54 | 0.60 |
| AM3 | 7.8 | 231 | 8 | 20 | 273 | 16,791 | 197 | 44 | 240 | 3.84 | 0.26 | 2.54 | 0.42 |
| AM4 | 7.9 | 459 | 10 | 14 | 226 | 35,901 | 278 | 62 | 292 | 3.79 | 0.25 | 1.73 | 0.25 |
| CA | 8.8 | 3,082 | 15 | 16 | 316 | 16,690 | 382 | 155 | 4,530 | 5.07 | 0.24 | 2.26 | 0.63 |
| EE | 8.5 | 2,023 | 9 | 20 | 204 | 15,179 | 308 | 79 | 2,254 | 2.13 | 0.22 | 2.16 | 0.39 |
| JB1 | 8.3 | 2,982 | 8 | 12 | 358 | 13,876 | 230 | 166 | 3,109 | 6.30 | 0.23 | 1.92 | 0.33 |
| JB2 | 8.4 | 2,212 | 7 | 18 | 286 | 18,468 | 191 | 121 | 3,195 | 5.42 | 0.21 | 1.99 | 0.52 |
| KR | 9.0 | 2,897 | 11 | 9 | 294 | 25,695 | 178 | 127 | 3,524 | 5.31 | 0.21 | 1.82 | 0.37 |
| LA | 7.9 | 3,292 | 9 | 14 | 347 | 17,363 | 176 | 4,225 | 1,424 | 4.48 | 0.20 | 2.25 | 0.36 |
| LM | 8.1 | 3,729 | 9 | 18 | 363 | 9,852 | 232 | 100 | 3,463 | 4.30 | 0.24 | 2.87 | 0.47 |
| NC1 | 8.7 | 1,121 | 9 | 16 | 176 | 15,041 | 201 | 35 | 1,750 | 2.71 | 0.14 | 1.99 | 0.39 |
| NC2 | 8.2 | 1,582 | 9 | 18 | 329 | 10,158 | 313 | 51 | 2,073 | 3.95 | 0.26 | 2.24 | 0.61 |
| PP1 | 8.1 | 2,880 | 8 | 21 | 330 | 25,954 | 288 | 6,143 | 1,023 | 4.68 | 0.32 | 1.79 | 0.72 |
| PP2 | 8.2 | 2,348 | 8 | 14 | 273 | 15,107 | 346 | 139 | 1,824 | 5.35 | 0.24 | 1.96 | 0.68 |
| **Avg** | **8.3** | **2,256.0** | **10.4** | **16** | **300** | **19,099** | **253** | **867** | **2,205** | **4.21** | **0.23** | **2.14** | **0.46** |
| **Low** | 7.8 | 231 | 7 | 9 | 176 | 9,852 | 176 | 35 | 240 | 2.13 | 0.14 | 1.04 | 0.18 |
| **High** | 9.0 | 4,641 | 28 | 21 | 386 | 35,901 | 382 | 6,143 | 4,530 | 6.30 | 0.32 | 3.54 | 0.72 |
| | | | | | | | | | | | | | |
| RE | 8.3 | 586 | 3 | 19 | 231 | 12,010 | 152 | 69 | 835 | 2.57 | 0.21 | 2.16 | 0.19 |
| Out | 8.2 | 4,748 | 7 | 13 | 493 | 13,557 | 197 | 4,352 | 3,186 | 5.83 | 0.27 | 4.81 | 0.32 |

**Table 13.** Detailed salinity test results of soil samples collected within the 15 vegetation transects, the pilot reintroduction site, and one sample (Out) collected adjacent to site JB1. Samples collected March, May 2006 and March 2007. Conductivity (cnd) = mmhos/cm; Na, K, Ca, Mg = parts per million; SAR = sodium absorption ratio; SSP = sodium saturation percentage.

| Site | pH | cnd | Na | K | Ca | Mg | SAR | SSP |
|---|---|---|---|---|---|---|---|---|
| AM1 | 7.3 | 0.95 | 96 | 10 | 128 | 7 | 2.24 | 36.66 |
| AM2 | 7.4 | 17.29 | 3,545 | 41 | 749 | 48 | 33.94 | 78.45 |
| AM3 | 7.3 | 0.87 | 57 | 12 | 145 | 8 | 1.25 | 23.22 |
| AM4 | 7.4 | 0.88 | 3,005 | 35 | 625 | 35 | 31.69 | 78.90 |
| CA | 7.9 | 13.81 | 3,024 | 21 | 250 | 30 | 48.15 | 89.48 |
| EE | 7.1 | 5.50 | 989 | 11 | 139 | 13 | 21.51 | 83.86 |
| JB1 | 7.7 | 6.53 | 1,298 | 19 | 197 | 9 | 24.57 | 83.63 |
| JB2 | 7.8 | 4.99 | 1,055 | 10 | 96 | 4 | 28.70 | 89.52 |
| KR | 7.8 | 8.78 | 1,921 | 17 | 161 | 9 | 39.91 | 90.08 |
| LA | 7.5 | 6.00 | 895 | 22 | 680 | 20 | 9.23 | 51.87 |
| LM | 7.6 | 15.66 | 3,005 | 43 | 63 | 29 | 78.66 | 95.18 |
| NC1 | 7.6 | 3.91 | 662 | 8 | 85 | 5 | 18.89 | 85.57 |
| NC2 | 7.2 | 8.03 | 1,335 | 17 | 336 | 25 | 18.94 | 75.11 |
| PP1 | 7.3 | 6.51 | 807 | 24 | 828 | 43 | 7.42 | 43.58 |
| PP2 | 7.8 | 7.40 | 1,333 | 19 | 333 | 31 | 18.74 | 74.70 |
| **Avg** | **7.5** | **7.14** | **1,535** | **21** | **321** | **21** | **25.59** | **71.99** |
| **Low** | 7.1 | 0.87 | 57 | 8 | 63 | 4 | 1.25 | 23.22 |
| **High** | 7.9 | 17.29 | 3,545 | 43 | 828 | 48 | 78.66 | 95.18 |
| | | | | | | | | |
| RE | 7.4 | 1.80 | 331 | 13 | 113 | 4 | 8.34 | 69.57 |
| Out | 7.5 | 13.73 | 2,494 | 45 | 938 | 30 | 21.87 | 68.28 |

# APPENDIX C

VEGETATION DATA

**Table 14.** Dominance and relative dominance of plant species intercepted by the 15 vegetation transects conducted March and May 2006.

| Species | Dominance (%) | Relative Dominance (%) |
|---|---|---|
| *Varilla texana* | 11.6 | 27.8 |
| *Prosopis glandulosa* | 6.1 | 14.5 |
| *Acacia rigidula* | 5.2 | 12.5 |
| *Opuntia leptocaulis* | 4.4 | 10.5 |
| *Castela erecta* subsp. *texana* | 1.7 | 4.1 |
| *Ziziphus obtusifolia* var. *obtusifolia* | 1.6 | 3.9 |
| *Suaeda conferta* | 1.2 | 2.8 |
| *Parkinsonia texana* var. *macra* | 1.2 | 2.8 |
| *Monanthochloë littoralis* | 1.0 | 2.4 |
| *Xylothamia palmeri* | 0.9 | 2.0 |
| *Krameria ramosissima* | 0.7 | 1.8 |
| *Bouteloua trifida* | 0.6 | 1.5 |
| *Sporobolus airoides* subsp. *airoides* | 0.6 | 1.4 |
| *Hilaria belangeri* var. *belangeri* | 0.4 | 1.0 |
| *Prosopis reptans* var. *cinerascens* | 0.4 | 0.9 |
| *Gutierrezia texana* | 0.4 | 0.9 |
| *Sporobolus pyramidatus* | 0.4 | 0.9 |
| *Lycium berlandieri* var. *berlandieri* | 0.3 | 0.8 |
| *Opuntia engelmannii* var. *lindheimeri* | 0.3 | 0.7 |
| *Pennisetum ciliare* var. *ciliare* | 0.3 | 0.6 |
| *Pappophorum bicolor* | 0.2 | 0.5 |
| *Billieturnera helleri* | 0.2 | 0.5 |
| *Jatropha dioica* | 0.2 | 0.5 |
| *Tiquilia canescens* var. *canescens* | 0.2 | 0.4 |
| *Setaria* sp. | 0.2 | 0.4 |
| *Karwinskia humboldtiana* | 0.1 | 0.3 |
| *Isocoma coronopifolia* | 0.1 | 0.3 |
| *Echinocereus enneacanthus* | 0.1 | 0.3 |
| *Schaefferia cuneifolia* | 0.1 | 0.2 |
| *Thelocactus setispinus* | 0.1 | 0.2 |
| *Guajacum angustifolium* | 0.1 | 0.2 |
| *Celtis pallida* | 0.1 | 0.2 |

Dominance and relative dominance was ≤0.1% for the following species: *Acleisanthes longiflora*, *A. obtusa*, *Ancistrocactus sheerii*, *Argythamnia* sp., *Astrophytum asterias*, *Atriplex acanthocarpa*, *A. texana*, *Coryphantha robertii*, *Cynanchum* sp., *Desmanthus virgatus* var. *depressus*, *Echinocactus texensis*, *Echinocereus berlandieri*, *E. reichenbachii* var. *fitchii*, *Ferocactus hamatacanthus*, *Forestiera angustifolia*, *Koeberlinia spinosa* var. *spinosa*, *Leptochloa* sp., *Lophophora williamsii*, *Mammillaria heyderi*, *Matelea sagittifolia*, *Opuntia schottii*, *Opuntia* sp. (seedling), *Panicum* sp., *Polygala glandulosa*, *Ruellia* sp., *Thelocactus bicolor* var. *bicolor*, *Wilcoxia poselgeri*, and *Yucca treculeana*.

**Table 15.** Species intercepted and dominance values (%) of each by study site.

| **Study Site** | **AM1** | **AM2** | **AM3** | **AM4** | **CA** | **EE** | **JB1** | **JB2** | **KR** | **LA** | **LM** | **NC1** | **NC2** | **PP1** | **PP2** | **RE** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Species** | | | | | | | | | | | | | | | | |
| *Acacia rigidula* | 7.73 | | 18.92 | 19.72 | 3.80 | 2.49 | 4.35 | | 9.85 | | | 3.45 | 8.39 | | | 6.75 |
| *Acleisanthes longiflora* | 0.03 | | | | 0.03 | 0.01 | | | | | | | | | | |
| *Acleisanthes obtusa* | | | | | | | | | | | | | 0.27 | | | 0.07 |
| *Ancistrocactus sheerii* | | | | 0.03 | | | | | | | | | | | | |
| *Argythamnia* sp. | | | | | | 0.01 | | | | | | | | | | |
| *Astrophytum asterias* | | 0.07 | | 0.03 | 0.17 | 0.11 | 0.05 | | | | | 0.13 | | 0.03 | 0.16 | |
| *Atriplex acanthocarpa* | | | | | 0.01 | | | | | | | | | | | |
| *Atriplex texana* | | | | | | | | 0.28 | | | | | | | | |
| *Billieturnera helleri* | 0.20 | 1.33 | 0.51 | 0.03 | 0.29 | 0.04 | 0.43 | | | | 0.03 | | | 0.15 | 0.28 | 0.21 |
| *Bouteloua trifida* | 1.59 | 0.16 | 0.31 | 1.65 | | 0.67 | 0.32 | 0.52 | 0.19 | 0.53 | | 0.64 | 0.07 | 1.01 | 1.95 | 3.69 |
| *Castela erecta* subsp. *texana* | | 0.21 | | 2.47 | 4.09 | | 3.24 | 0.56 | 1.96 | 4.89 | 3.75 | | 3.31 | 0.57 | 0.95 | 15.47 |
| *Celtis pallida* | | | | | | | 0.01 | 0.49 | | | 0.60 | | | | | |
| *Coryphantha robertii* | | | | | 0.03 | | | | | | | | | | | |
| *Cynanchum* sp. | | | | | | | | | | | | | 0.07 | | | 0.04 |
| *Desmanthus virgatus* var. *depressus* | | | | | | | 0.05 | | | | | | | | | |
| *Dyssodia tenuiloba* var. *treculii* | | | | | | | | | | | | | | | | 0.24 |
| *Echinocactus texensis* | 0.16 | | | | | | | | | | | | | | | |
| *Echinocereus berlandieri* | | | | | | | | | | | 0.12 | | | | | |
| *Echinocereus enneacanthus* | | | | | | 0.03 | | | | | 1.88 | | | | | |
| *Echinocereus reichenbachii* var. *fitchii* | 0.07 | | 0.01 | 0.05 | 0.03 | 0.03 | | | | | 0.09 | | | 0.09 | | |
| *Ephedra antisyphilitica* | | | | | | | | | | | | | | | | 1.35 |
| *Eragrostis* sp. | | | | | | | | | | | | | | | | 0.04 |
| *Ferocactus hamatacanthus* | | | | | | 0.03 | | | | | | | | | | |
| *Forestiera angustifolia* | | | | | | | 0.64 | | | | | | | | | 0.75 |
| *Guajacum angustifolium* | | | | | | 0.01 | 0.04 | | 0.92 | 0.13 | | 0.24 | | | | 1.11 |
| *Gutierrezia texana* | | 4.07 | | | | | | | | | | | | 1.08 | 0.56 | |

**Table 15-Continued.** Species intercepted and dominance values (%) of each by study site.

| Study Site | AM1 | AM2 | AM3 | AM4 | CA | EE | JB1 | JB2 | KR | LA | LM | NC1 | NC2 | PP1 | PP2 | RE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Species** | | | | | | | | | | | | | | | | |
| *Hilaria belangeri* var. *belangeri* | 1.89 | | | 0.69 | 0.12 | | | | 0.03 | 0.15 | | 3.17 | 0.23 | | | 0.05 |
| *Isocoma coronopifolia* | | | | | | 1.64 | | | | | | 0.24 | 0.08 | | | |
| *Jatropha dioica* | | 0.05 | | | 2.19 | 0.68 | | | 0.01 | | | | | | | |
| *Karwinskia humboldtiana* | | | 0.04 | 1.31 | | | | | 0.19 | | | 0.45 | | | | 0.96 |
| *Krameria ramosissima* | 1.33 | | | 7.23 | | | | | 0.19 | | | | 2.47 | | | |
| *Koeberlinia spinosa* var. *spinosa* | | | | | | | | | | 0.57 | | | | | | |
| *Leptochloa* sp. | | | | | | | | | | 0.16 | | | | | | |
| *Lophophora williamsii* | | | | | | | 0.04 | | | | | | | | | |
| *Lycium berlandieri* var. *berlandieri* | | | | | 2.81 | 1.47 | | | | | | 0.01 | | 0.68 | | |
| *Mammillaria heyderi* | | | | | 0.07 | 0.05 | | 0.08 | | | | 0.08 | | | | |
| *Matelea sagittifolia* | | | | | | | | | | | 0.03 | | | | | |
| *Monanthochloë littoralis* | | 6.71 | | | | | | | | | | | | 3.75 | 4.31 | |
| *Opuntia engelmannii* var. *lindheimeri* | | 2.05 | | 1.19 | 0.17 | | | | | | | 0.51 | | | 0.44 | 0.27 |
| *Opuntia leptocaulis* | | 4.17 | 1.95 | 0.16 | 6.00 | 6.45 | 2.44 | 3.75 | | 9.67 | 11.51 | 7.31 | 3.00 | 6.59 | 2.68 | 0.03 |
| *Opuntia schottii* | | | 0.01 | 0.04 | | | 0.04 | | | | | | 0.03 | 0.03 | | 0.01 |
| *Opuntia* sp. *(seedling)* | | | | | | | | | 0.01 | | | | | | | |
| *Panicum* sp. | | | | | | | | | | | | 0.49 | | | | |
| *Pappophorum bicolor* | | | | | | 1.80 | | | 0.25 | 0.71 | | | 0.51 | 0.16 | | |
| *Parkinsonia texana* var. *macra* | | | | 8.21 | | | | | 0.67 | | | 8.40 | | | | |
| *Pennisetum ciliare* var. *ciliare* | | | 0.32 | | | 0.19 | | | 0.64 | | | | 2.01 | 0.73 | | |
| *Polygala glandulosa* | | | | 0.07 | | | | | | | | | | | | |
| *Prosopis glandulosa* | | 13.47 | 1.88 | | 4.95 | 3.05 | 3.68 | 1.92 | | 13.00 | 12.53 | 3.29 | 2.79 | 20.75 | 9.77 | 2.35 |

**Table 15-Continued.** Species intercepted and dominance values (%) of each by study site.

| Study Site | AM1 | AM2 | AM3 | AM4 | CA | EE | JB1 | JB2 | KR | LA | LM | NC1 | NC2 | PP1 | PP2 | RE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Species** | | | | | | | | | | | | | | | | |
| *Prosopis reptans* var. *cinerascens* | 0.11 | 0.09 | 0.20 | | 0.43 | | 1.13 | 1.91 | 0.05 | 0.03 | | 0.45 | 0.04 | 0.65 | 0.73 | 0.05 |
| *Ruellia* sp. | | | | | | 0.07 | | | | | | | | | | |
| *Schaefferia cuneifolia* | | 0.13 | | | | | | 0.03 | 0.19 | 0.49 | 0.53 | | 0.17 | | | 0.20 |
| *Setaria* sp. | | | | | | 0.53 | | 0.43 | | | | 0.55 | 0.73 | 0.33 | 0.09 | 0.09 |
| *Sporobolus airoides* subsp. *airoides* | | 0.35 | 0.48 | | | | 0.16 | | | | | 0.59 | 7.16 | | | 2.52 |
| *Sporobolus pyramidatus* | | 0.79 | 0.80 | | 0.28 | 0.49 | 0.55 | 0.33 | 0.11 | 0.24 | | 0.88 | | 0.28 | 0.77 | 0.96 |
| *Suaeda conferta* | | | | | | | 0.13 | 8.83 | 2.88 | | 5.77 | | | | | |
| *Thelocactus bicolor* var. *bicolor* | | 0.05 | | 0.11 | 0.12 | 0.39 | 0.08 | | | | | | 0.05 | | 0.07 | |
| *Thelocactus setispinus* | | 0.15 | | | 0.61 | | | | | 0.12 | 0.28 | 0.05 | | 0.28 | | |
| *Tiquilia canescens* var. *canescens* | 1.04 | | 0.03 | 0.28 | | 0.12 | 0.05 | 0.09 | | 0.01 | | 1.16 | | | | 1.47 |
| *Varilla texana* | 2.84 | | | 5.41 | 18.80 | 11.83 | 12.36 | | 25.33 | 14.43 | 2.35 | 21.27 | 17.92 | 19.13 | 23.08 | 2.55 |
| *Wilcoxia poselgeri* | | | | | | | | | | | 0.03 | | | | | |
| *Xylothamia palmeri* | | 1.25 | 8.80 | | | | | | | 2.76 | | | | | | |
| *Yucca treculeana* | | | | | | | | | | | 0.05 | | | | | 0.69 |
| *Ziziphus obtusifolia* var. *obtusifolia* | 0.48 | | 1.93 | 0.29 | 0.96 | | 4.67 | 1.77 | 1.25 | | 1.12 | 3.77 | 6.51 | 0.04 | 1.49 | 5.51 |

**Table 16.** Comprehensive list of associated plant species of *A. asterias* per the 15 vegetation transects and additional species documented within the 2-m belt transects across all study sites, March and May 2006.

| | |
|---|---|
| *Acacia rigidula* | *Leptochloa* sp. |
| *Acleisanthes longiflora* | *Leucophyllum frutescens* var. *frutescens* |
| *Acleisanthes obtusa* | *Lophophora williamsii* |
| *Ancistrocactus sheerii* | *Lycium berlandieri* var. *berlandieri* |
| *Argythamnia* sp. | *Mammillaria heyderi* |
| *Astrophytum asterias* | *Mammillaria sphaerica* |
| *Atriplex acanthocarpa* | *Manfreda longiflora* |
| *Atriplex texana* | *Matelea sagittifolia* |
| *Billieturnera helleri* | *Monanthochloë littoralis* |
| *Bouteloua trifida* | *Opuntia engelmannii* var. *lindheimeri* |
| *Castela erecta* subsp. *texana* | *Opuntia leptocaulis* |
| *Celtis pallida* | *Opuntia schottii* |
| *Chloris* sp. | *Opuntia* sp. (seedling) |
| *Cissus incisa* | *Panicum* sp. |
| *Condalia hookeri* | *Pappophorum bicolor* |
| *Coryphantha macromeris* var. *runyonii* | *Parkinsonia texana* var. *macra* |
| *Coryphantha robertii* | *Pennisetum ciliare* var. *ciliare* |
| *Cuscuta* sp. | *Polygala glandulosa* |
| *Cynanchum* sp. | *Prosopis glandulosa* |
| *Desmanthus virgatus* var. *depressus* | *Prosopis reptans* var. *cinerascens* |
| *Echinocactus texensis* | *Ruellia* sp. |
| *Echinocereus berlandieri* | *Salvia ballotiflora* |
| *Echinocereus enneacanthus* | *Schaefferia cuneifolia* |
| *Echinocereus reichenbachii* var. *fitchii* | *Setaria* sp. |
| *Ferocactus hamatacanthus* | *Sporobolus airoides* subsp. *airoides* |
| *Forestiera angustifolia* | *Sporobolus pyramidatus* |
| *Guajacum angustifolium* | *Suaeda conferta* |
| *Gutierrezia texana* | *Thelocactus bicolor* var. *bicolor* |
| *Hilaria belangeri* var. *belangeri* | *Thelocactus setispinus* |
| *Ibervillea lindheimeri* | *Tiquilia canescens* var. *canescens* |
| *Isocoma coronopifolia* | *Varilla texana* |
| *Jatropha dioica* | *Wilcoxia poselgeri* |
| *Karwinskia humboldtiana* | *Xylothamia palmeri* |
| *Koeberlinia spinosa* var. *spinosa* | *Yucca treculeana* |
| *Krameria ramosissima* | *Ziziphus obtusifolia* var. *obtusifolia* |

**Table 17.** Percent plant species/object(s) documented directly overhead or immediately adjacent to the *A. asterias* within the 2-m belt transects across the 15 study sites. More than one plant species/object in a row indicates a combination.

| **Plant species/object(s)** | **Percent** |
|---|---|
| *Varilla texana* | 23.8 |
| rock(s) (no nurse plant) | 12.2 |
| bare ground (no nurse plant) | 6.8 |
| *Monanthochloë littoralis* | 5.1 |
| *Prosopis glandulosa*, *M. littoralis* | 3.4 |
| *Varilla texana*, rocks | 3.4 |
| *Opuntia leptocaulis* | 3.1 |
| *Thelocactus bicolor* var. *bicolor*, rocks | 2.7 |
| *Varilla texana*, *Opuntia leptocaulis* | 2.4 |
| *V. texana*, *Prosopis glandulosa* | 2.4 |
| *Monanthochloë littoralis*, rocks | 2.0 |
| *Varilla texana*, *Opuntia leptocaulis*, *Prosopis glandulosa* | 1.7 |
| *Acacia rigidula*, *Bouteloua trifida* | 1.4 |
| *Krameria ramosissima* | 1.4 |
| *Opuntia leptocaulis*, *Monanthochloë littoralis* | 1.4 |
| *O. leptocaulis*, *Prosopis glandulosa* | 1.4 |
| *O. leptocaulis*, rock | 1.4 |
| *Prosopis glandulosa* | 1.4 |
| *Ziziphus obtusifolia* var. *obtusifolia* | 1.4 |
| *Acacia rigidula* | 1.0 |
| *Prosopis glandulosa*, rock(s) | 1.0 |
| *Isocoma coronopifolia* | <1.0 |
| *I. coronopifolia*, rocks | <1.0 |
| *Jatropha dioica*, rocks | <1.0 |
| *Setaria* sp. | <1.0 |
| *Sporobolus pyramidatus* | <1.0 |
| *Suaeda conferta* | <1.0 |
| *Thelocactus bicolor* var. *bicolor* | <1.0 |
| *Varilla texana, Hilaria belangeri* var. *belangeri* | <1.0 |
| *V. texana*, *Opuntia leptocaulis*, *Castela erecta* subsp. *texana* | <1.0 |
| *V. texana*, *Prosopis glandulosa*, *Monanthochloë littoralis* | <1.0 |
| *Acacia rgidula*, *Tiquilia canescens* var. *canescens* | <1.0 |
| *A. rigidula*, *Hilaria belangeri* var. *belangeri* | <1.0 |
| *A. rigidula*, *Opuntia engelmannii* var. *lindheimeri*, *Krameria ramosissima* | <1.0 |
| *Bouteloua trifida*, rocks | <1.0 |
| *Castela erecta* subsp. *texana* | <1.0 |
| *Thelocactus setispinus* | <1.0 |
| *Jatropha dioica* | <1.0 |
| *Monanthochloë littoralis*, *Prosopis reptans* var. *cinerascens*, rocks | <1.0 |

**Table 17-Continued.** Percent plant species/object(s) documented directly overhead or immediately adjacent to the *A. asterias* observed in the 2-m belt transects across the 15 study sites. More than one plant species/object in a row indicates a combination.

| Plant species/object(s) | Percent |
|---|---|
| *Opuntia leptocaulis*, *Isocoma coronopifolia* | <1.0 |
| *O. leptocaulis*, *Prosopis glandulosa*, *Pappophorum bicolor* | <1.0 |
| *P. bicolor* | <1.0 |
| *P. bicolor*, rock | <1.0 |
| *Parkinsonia texana* var. *macra* | <1.0 |
| *P. texana* var. *macra*, *Panicum* sp. | <1.0 |
| *Pennisetum ciliare* var. *ciliare*, rocks | <1.0 |
| *Prosopis glandulosa*, *Castela erecta* subsp. *texana* | <1.0 |
| *P. glandulosa*, *Monanthochloë littoralis*, *Thelocactus setispinus* | <1.0 |
| *Setaria* sp., *Jatropha dioica* | <1.0 |
| *Setaria* sp., rocks | <1.0 |
| *Sporobolos airoides* subsp. *airoides*, *Prosopis glandulosa* | <1.0 |
| *Sporobolus pyramidatus*, *Prosopis reptans* var. *cinerascens* | <1.0 |
| *Thelocactus bicolor* var. *bicolor*, *Jatropha dioica* | <1.0 |
| *T. bicolor* var. *bicolor*, *Tiquilia canescens* var. *canescens*, rocks | <1.0 |
| *T. canescens* var. *canescens*, rocks | <1.0 |
| *Varilla texana, Acacia rigidula*, *Opuntia leptocaulis* | <1.0 |
| *V. texana*, *Billieturnera helleri* | <1.0 |
| *V. texana*, *B. helleri*, *Prosopis glandulosa* | <1.0 |
| *V. texana*, *B. helleri*, *P. glandulosa*, *Thelocactus setispinus* | <1.0 |
| *V. texana*, *Castela erecta* subsp. *texana* | <1.0 |
| *V. texana*, *Monanthochloë littoralis* | <1.0 |
| *V. texana, Parkinsonia texana* var. *macra*, rocks | <1.0 |
| *V. texana*, *Prosopis glandulosa*, *Gutierrezia texana* | <1.0 |
| *V. texana*, *P. glandulosa*, *Pappophorum bicolor* | <1.0 |
| *V. texana*, *P. glandulosa*, *P. bicolor*, *Monanthochloë littoralis* | <1.0 |

# APPENDIX D

*ASTROPHYTUM ASTERIAS* REINTRODUCTION PLAN (DRAFT)

# *Astrophytum asterias* Reintroduction Plan
**Prepared by Sandy Birnbaum**
**June 2009**

## Introduction

*Astrophytum asterias* was listed endangered under the Endangered Species Act on 18 October 1993 and by the state of Texas on 30 January 1997. As of 22 October 1987, *A. asterias* is also listed in Appendix I by CITES. When *A. asterias* was federally listed, there was only one known population in Starr County, Texas on private property. There were also reports of *A. asterias* from Cameron, Hidalgo, and Zapata counties, but none of those sites had been relocated (Damude and Poole, 1990). In Mexico, several populations were known from Tamaulipas and Nuevo León (U. S. Fish & Wildlife Service (USFWS), 2003).

Using the soil types as defined in the Soil Survey of Starr County, Texas (Thompson, et al., 1972), the subpopulations of *A. asterias* are found predominantly on Catarina soils; however, subpopulations also occur on Garceno clay loam; Jimenez-Quemado association; Montell clay, saline; Maverick soils, eroded; and Ramadero loam. The underlying geology is of the Catahoula and Frio formations undivided and the Jackson Group (Bureau of Economic Geology, 1976). Dominant species of 15 subpopulations surveyed in 2006 included: *Varilla texana*, *Prosopis glandulosa*, *Acacia rigidula*, *Opuntia leptocaulis*, *Castela erecta* subsp. *texana*, *Ziziphus obtusifolia* var. *obtusifolia*, *Suaeda conferta*, *Parkinsonia texana* var. *macra*, *Monanthochloë littoralis*, *Xylothamia palmeri*, *Krameria ramosissima*, *Bouteloua trifida*, *Sporobolus airoides* subsp. *airoides*, *Hilaria belangeri* var. *belangeri*, *Prosopis reptans* var. *cinerascens*, *Gutierrezia texana*, *Sporobolus pyramidatus*, *Lycium berlandieri* var. *berlandieri*, *Opuntia engelmannii* var. *lindheimeri*, *Pappophorum* *bicolor*, *Billieturnera helleri*, *Jatropha dioica*, *Tiquilia canescens* var. *canescens*, and other common species of the Tamaulipan thornscrub (Birnbaum, 2009).

*Astrophytum asterias* is an obligate outcrosser (Strong and Williamson, 2007) with a slow growth rate (Janssen, et al., 2008; Birnbaum, 2009), has low flower production, and low fruit and seed set compared to other cacti (Strong, 2005) which could be limiting factors to population growth. It also faces many threats natural and human in origin. Mortality of *A. asterias* due to herbivory by *Sylvilagus audubonii* and possibly *Spermophilus mexicanus*, fungal infection, a cerambicid beetle (*Moneilema armatum*), and a weevil (tentatively identified to the genus *Gerstaeckeria*) has been documented in Texas (Janssen, et al., 2008; Birnbaum, 2009; Ferguson and Williamson, in press). Martínez-Ávalos, et al. (2007) have documented similar threats in Mexico. Anthropogenic threats to *A. asterias* included habitat destruction/modification and over-collection by cactus enthusiasts (USFWS, 2003). Land in Starr County, Texas is still being rootplowed and converted to non-native, forage grasses, in particular, buffelgrass (*Pennisetum ciliare* var. *ciliare*). Collection of *A. asterias* is hard to document, but is still assumed to be of significance. Peyote harvest in Texas has fluctuated around 2,000,000 buttons, so even an incidental harvest rate of 0.1% has profound implications for *A. asterias* numbers (Terry, 2005). Other threats include gas exploration (seismic surveys) and urbanization/sprawl.

*Astrophytum asterias* is assigned a priority ranking of 2 by the USFWS (2003), which indicates it faces a high degree of threat, yet has high recovery potential. The recovery criteria as outlined by the recovery plan include maintaining or establishing "ten fully protected, self-sustaining (i.e. a minimum of 2,000 individuals) populations of star cactus in the United States or Mexico on Federal lands, voluntary State lands, voluntary private lands, or a combination, within the geographical and historical areas known to support the species" (USFWS, 2003). To achieve this, surveys for new subpopulations will continue by government agencies, non-government organizations, researchers, etc. However, if sufficient subpopulations are not found, reintroduction of *A. asterias* is an acceptable step in the recovery of this species.

Currently there are 16 properties in a 29.2 square mile area of Starr County with ~3,548 *A. asterias* (Janssen, et al., 2008). Recent research in Mexico recognizes seven populations in Tamaulipas and two in Nuevo León with population numbers ranging from 10-704 (Martínez-Ávalos, et al., 2004). Birnbaum (2009) established a pilot reintroduction of *A. asterias* by planting 120 seeds in March 2007, 120 seedlings in April 2007, 120 seeds in September 2007, and 120 seedlings in October 2007 at the Texas Chapter of The Nature Conservancy's Las Estrellas Preserve in Starr County. Of the 240 seeds planted only 9 produced a seedling. As of March 2009, 8 of the 9 seedlings which germinated from reintroduced seeds were alive. A total of 58% of the 240 seedlings that were reintroduced have survived.

**Objective(s)**

The reintroduction project should be a well-designed experiment to further the biological knowledge of *A. asterias* which in turn can guide future management and conservation decisions. Project objectives can be developed using the objectives and recovery criteria as outlined in the *A. asterias* recovery plan.

**Location and selection of reintroduction sites**

The first consideration in site selection is land ownership. A reintroduction site must be on a property where long-term protection can be ensured. This includes ease of access for long-term monitoring. Reintroduction should also occur near extant subpopulations of *A. asterias* in Starr County and expand outward since this species is an obligate outcrosser. Preliminary research regarding *A. asterias* pollen dispersal showed that 80% of recipient plants were within 30 m of the source plant; the single longest dispersal event recorded was 142 m (Blair, 2007). If a reintroduction is implemented away from an existing subpopulations of *A. asterias*, the number of introduced plants must have the proper age structure and sufficient numbers to attract pollinators. Adequate numbers of other spring blooming cacti in the area will also help to attract pollinators.

Sites selected should have one of the following soil types: Catarina soils; Garceno clay loam; Jimenez-Quemado association; Montell clay, saline; Maverick soils, eroded; or Ramadero loam. Vegetation transects should be conducted using a standard methodology (e.g. line-intercept) and soil samples (see Provin and Pitt, 1999) collected and analyzed for each site prior to reintroduction. The edaphic parameters should be within the ranges as listed in Table 1 (Birnbaum, 2009). A complete list of associated species in order of dominance as documented in 15 vegetation transects are provided in the appendix (Birnbaum, 2009). The dominant species at the reintroduction site should be on this list. The vegetation should also contain interstices of varying sizes as vegetation coverage within the 15 transects ranged from 21-57% (Birnbaum, 2009).

**Table 1.** Averages (*Avg*), standard deviations (*SD*), and ranges of soil parameters from routine soil analyses of soil samples collected within the vegetation transects ($n$ = 15) and the results of said analyses for the sample collected at the pilot reintroduction site (RE). Samples collected March, May 2006 and March 2007. Conductivity (Cnd) = µmho/cm; $NO_3$, P, K, Ca, Mg, S, Na, Fe, Zn, Mn, Cu = parts per million.

| | pH | Cnd | $NO_3$ | P | K | Ca | Mg | S | Na | Fe | Zn | Mn | Cu |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Avg*** | 8.3 | 2,256 | 10 | 16 | 300 | 19,099 | 253 | 867 | 2,205 | 4.21 | 0.23 | 2.14 | 0.46 |
| ***SD*** | 0.35 | 1300.24 | 5.22 | 3.84 | 61.21 | 7147.42 | 64.27 | 1825.58 | 1397.38 | 1.21 | 0.04 | 0.56 | 0.16 |
| **Low** | 7.8 | 231 | 7 | 9 | 176 | 9,852 | 176 | 35 | 240 | 2.13 | 0.14 | 1.04 | 0.18 |
| **High** | 9.0 | 4,641 | 28 | 21 | 386 | 35,901 | 382 | 6,143 | 4,530 | 6.30 | 0.32 | 3.54 | 0.72 |
| | | | | | | | | | | | | | |
| **RE** | 8.3 | 586 | 3 | 19 | 231 | 12,010 | 152 | 69 | 835 | 2.57 | 0.21 | 2.16 | 0.19 |

**Genetics** (Terry, 2005)

In 2005, 94 individuals of *A. asterias* were sampled (tepal collected) from four subpopulations on three properties (Fig. 1). The specifics regarding DNA extraction procedure and microsatellite development are in Terry (2005). Most of the subpopulations sampled were surprisingly healthy in terms of levels of heterozygosity and genetic diversity. However, current small effective population size is a concern even in the largest of the subpopulations sampled. Property 7 showed a high degree of homozygosity at several loci and a moderate degree of drift away from the mean allele frequencies of all four subpopulations combined. Therefore, this property should not be used as a propagule source for reintroductions. Property 2a and Property 4 subpopulations have the highest levels of heterozygosity of the subpopulations sampled. These are the best source of propagules for future reintroductions. As more subpopulations are found, further genetic work is needed to determine best propagule source.

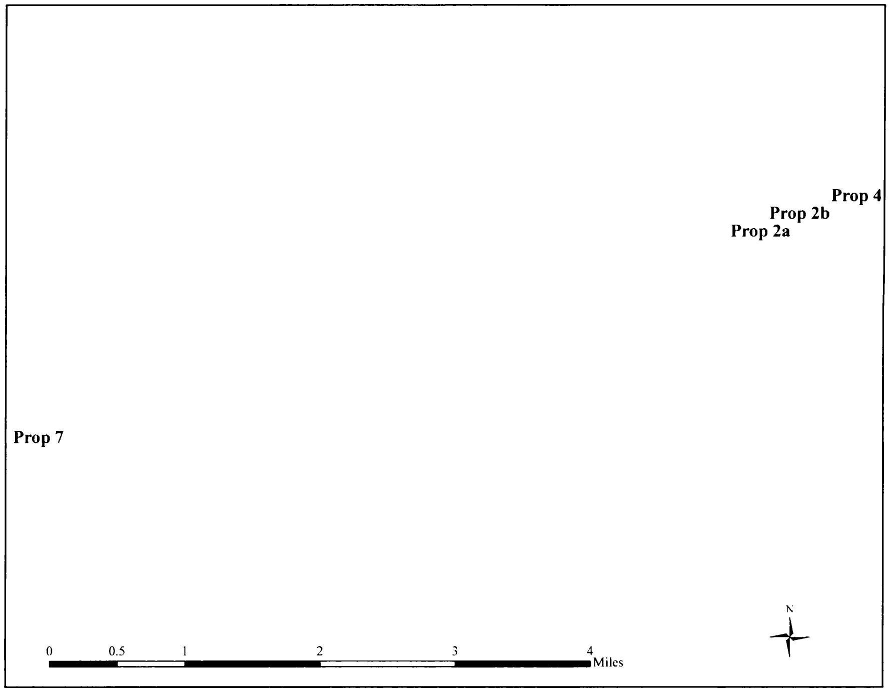



**Figure 1.** Location of the four subpopulations sampled on three properties. Property numbers correspond to those used in the Section 6 reports by Janssen, et al., 2005, 2008.

## Propagation

Seedlings are the preferred propagule for reintroduction as <4% of the seeds planted in the pilot reintroduction germinated (Birnbaum, 2009). Seed for propagation can be obtained from the Desert Botanical Garden, Phoenix, AZ (the Center for Plant Conservation (CPC) designated seed repository for *A. asterias*). If seeds will be collected from the field, follow the CPC guidelines for seed collection (see CPC, 1991). *A. asterias* has a slow growth rate; this must be calculated in the timeframe of the reintroduction project. The seedlings planted at the pilot reintroduction site were over 2-years old and had an average diameter of 9.04±1.9 mm (±*SD*; range of 4.96-15.17 mm) (Birnbaum, 2009). Propagation techniques (propagation medium, temperature and light settings, etc.) are provided in Maiti, et al. (2002), Strong (2005), and Strong and Williamson (2007).

The seedlings were maintained in a metal, free standing, rigid frame style gable greenhouse covered with glass at the Lady Bird Johnson Wildflower Center, Austin, Texas. In the fall/winter the thermostat was set at 50°F at night and 75°F during the day. During the spring/summer the thermostat was set at 60°F at night and 80°F during the day. Greenlight brand Neem Oil was used every two weeks to control insects. Care should be taken to ensure that the propagated plants are free of insects before reintroduced to the wild. If the cacti are grown in a greenhouse there will need to be a longer hardening off period (4-5 weeks), gradually increasing the amount of ultraviolet light exposure. If the seedlings are grown outside in 50% shade, less hardening off time is necessary. Depending on the objectives of the reintroduction, propagation may need to be staggered over several months/years to achieve proper age/size class structure.

**Planting procedures**

Basic planting procedures are provided by Birnbaum (2009). These may need to be modified depending on the size of the reintroduced plants. Regardless of plant size, they should be marked in some way (e.g. aluminum tags, craft pins) for monitoring. The number of plants reintroduced will depend on the objectives of the study. The pilot reintroduction used a total of 240 seedlings; 120 planted in April and 120 in October. The growth rate of the fall planted seedlings was significantly larger than the growth rate of the spring planted seedlings (Birnbaum, 2009). Sufficient numbers should be planted to allow for statistical analysis of the data and as a bet-hedging technique against a catastrophic mortality event. The objectives of the reintroduction project will further guide decisions regarding time of planting, microsite selection, site preparation/maintenance, etc. Obtaining rainfall data from the National Climatic Data Center for the years prior to the reintroduction may aid in deciding when to plant.

### Monitoring

The objectives of the reintroduction project will ultimately guide the monitoring protocol. At a minimum, monitoring should occur monthly to document presence/absence of the reintroduced plants. Assigning a unique number to each plant will allow tracking of individuals through time. Monitoring protocol should be documented such that it can be carried out in perpetuity. The layout of the reintroduction site should be permanently marked and GPS coordinates collected. A long-term monitoring plan should be designed at the inception of the reintroduction project.

### Management

Currently no known management techniques are required for *A. asterias*. However, rootplowing and other intensive ground disturbance land management techniques should not be used in *A. asterias* habitat. Vegetation cover was documented as <60% in vegetation transects conducted in 2006 (Birnbaum, 2009). Therefore, monitoring of sites for increases in vegetative cover is advisable. Reintroduction sites should be monitored for invasive species, especially buffelgrass.

### Other requirements

Before reintroduction occurs, a thorough survey of the site and surrounding area should be conducted. Document the location of natural and reintroduced subpopulations with a GPS unit. Reintroductions should be coordinated with the USFWS Corpus Christi Ecological Services Field Office and the Wildlife Diversity Program of Texas Parks & Wildlife Department, Austin, Texas. Lastly, document the reintroduction in the CPC's reintroduction database which can be accessed from their website.

**Documents, articles, and books used to compose *A. asterias* reintroduction plan and in general are useful in planning reintroductions:**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**Useful websites**

Center For Plant Conservation (CPC): http://www.centerforplantconservation.org/

CPC reintroduction database:
http://www.centerforplantconservation.org/reintroduction/MN_ReintroductionEntrance.asp

Desert Botanical Garden: http://www.dbg.org/

## Appendix

Dominance and relative dominance of the species intercepted in the 15 vegetation transects conducted in 2006 at nine private properties.

| Species | Dominance | Relative Dominance |
|---|---|---|
| *Varilla texana* | 11.6 | 27.8 |
| *Prosopis glandulosa* | 6.1 | 14.5 |
| *Acacia rigidula* | 5.2 | 12.5 |
| *Opuntia leptocaulis* | 4.4 | 10.5 |
| *Castela erecta* subsp. *texana* | 1.7 | 4.1 |
| *Ziziphus obtusifolia* var. *obtusifolia* | 1.6 | 3.9 |
| *Suaeda conferta* | 1.2 | 2.8 |
| *Parkinsonia texana* var. *macra* | 1.2 | 2.8 |
| *Monanthochloë littoralis* | 1.0 | 2.4 |
| *Xylothamia palmeri* | 0.9 | 2.0 |
| *Krameria ramosissima* | 0.7 | 1.8 |
| *Bouteloua trifida* | 0.6 | 1.5 |
| *Sporobolus airoides* subsp. *airoides* | 0.6 | 1.4 |
| *Hilaria belangeri* var. *belangeri* | 0.4 | 1.0 |
| *Prosopis reptans* var. *cinerascens* | 0.4 | 0.9 |
| *Gutierrezia texana* | 0.4 | 0.9 |
| *Sporobolus pyramidatus* | 0.4 | 0.9 |
| *Lycium berlandieri* var. *berlandieri* | 0.3 | 0.8 |
| *Opuntia engelmannii* var. *lindheimeri* | 0.3 | 0.7 |
| *Pennisetum ciliare* var. *ciliare* | 0.3 | 0.6 |
| *Pappophorum bicolor* | 0.2 | 0.5 |
| *Billieturnera helleri* | 0.2 | 0.5 |
| *Jatropha dioica* | 0.2 | 0.5 |
| *Tiquilia canescens* var. *canescens* | 0.2 | 0.4 |
| *Setaria* sp. | 0.2 | 0.4 |
| *Karwinskia humboldtiana* | 0.1 | 0.3 |
| *Isocoma coronopifolia* | 0.1 | 0.3 |
| *Echinocereus enneacanthus* | 0.1 | 0.3 |
| *Schaefferia cuneifolia* | 0.1 | 0.2 |
| *Thelocactus setispinus* | 0.1 | 0.2 |
| *Guajacum angustifolium* | 0.1 | 0.2 |
| *Celtis pallida* | 0.1 | 0.2 |

Dominance and relative dominance was ≤0.1% for the following species: *Acleisanthes longiflora*, *A. obtusa*, *Ancistrocactus sheerii*, *Argythamnia* sp., *Astrophytum asterias*, *Atriplex acanthocarpa*, *A. texana*, *Coryphantha robertii*, *Cynanchum* sp., *Desmanthus virgatus* var. *depressus*, *Echinocactus texensis*, *Echinocereus berlandieri*, *E. reichenbachii* var. *fitchii*, *Ferocactus hamatacanthus*, *Forestiera angustifolia*, *Koeberlinia spinosa* var. *spinosa*, *Leptochloa* sp., *Lophophora williamsii*, *Mammillaria heyderi*, *Matelea sagittifolia*, *Opuntia schottii*, *Opuntia* sp. (seedling), *Panicum* sp., *Polygala glandulosa*, *Ruellia* sp., *Thelocactus bicolor* var. *bicolor*, *Wilcoxia poselgeri*, and *Yucca treculeana*.

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