FINAL REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. TX E-46-R

Endangered and Threatened Species Conservation

The Research and Recovery of Star Cactus (*Astrophytum asterias*)

Prepared by:

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TEXAS PARKS & WILDLIFE

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8 July 2010

FINAL REPORT

STATE: Texas **GRANT NUMBER:** TX E-46-R

GRANT TITLE: The Research and Recovery of Star Cactus (*Astrophytum asterias*)

REPORTING PERIOD: 9/01/03 to 7/31/10

OBJECTIVE(S):

To research the specific habitat requirements, demography, phenology, reproductive biology, and other field aspects of star cactus and apply findings to reintroduce, introduce and recover star cactus in Texas.

Segment Objectives:

1) Population Monitoring. Monthly monitoring at established monitoring plots, set up new plots as needed.
2) Habitat Analysis. A quantitative analysis of the vegetative composition of the habitat will be completed, along with a detailed soil analysis of the areas where star cactus is found.
3) Reproductive Biology Research. Research on the reproductive biology will be conducted both in the field and in the greenhouse to determine the mechanism which blocks self-fertilization of star cactus.
4) Surveys for Additional Extant Populations in Texas. Surveys to identify additional populations of star cactus in Texas will be accomplished. We will also identify landowners who may be willing to reintroduce star cactus to available suitable habitat on their properties.
5) Propagation of Star Cactus Individuals for Phase II Founding Populations. Seed representing all known genomes in Texas will be collected from private land sites using CPC guidelines, and transported to the Lady Bird Wildflower Research Center propagations.
6) Pilot Population Introduction. Experimental plots will be established on appropriate habitat to test the effects of timing of planting (spring, summer, fall, winter), to determine the most successful age classes, to determine the most successful placement (out in the open, under a nurse plant), along with many other important variables.
7) Pilot Population Monitoring. This population will be monitored for experimental and research purposes.

Significant Deviation:

None.

Summary Of Progress:

Please see Attachment A. File sent separately as pdf.

Location: Starr County, Texas.

Cost: ____**Not available at time of report**_________________________

Prepared by: _Craig Farquhar_____________ **Date:** _8 July 2010

Approved by: ______________________________ **Date:**____8 July 2010_____
C. Craig Farquhar

Final Report

The Research and Recovery of Star Cactus (*Astrophytum asterias*)

 # E-46WFR02

 # 126181

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TABLE OF CONTENTS

INTRODUCTION:

Star cactus (*Astrophytum asterias*) was considered one of the rarest and most imperiled listed plant species in the state of Texas at the onset of this study. Habitat destruction and years of over-collection by cactus enthusiasts led to the listing of this species as Endangered by the U. S. Fish and Wildlife Service (USFWS) on November 17, 1993 (U. S. Fish and Wildlife Service 1993). The state of Texas also listed the species as Endangered in 1997. Historically, star cactus was known from Cameron, Hidalgo, and Starr Counties in southern Texas. According to the Status Report on star cactus (Damude and Poole 1990), there were five known star cactus localities in Texas. When this research project began in 2003, only two of these localities were known to still exist in Texas. These two known extant sites were less than a mile apart in Starr County. Six small extant sites in Mexico [each of which has reportedly lost 50% of the individuals since 1998 (Martinez-Avalos 2002)] were also known.

Star cactus is protected in Mexico under legislation that prohibits the export of cacti. Star cactus is also listed in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (50 CFR 23.23) protecting the species from international trade. However, it is common knowledge that star cactus is prized worldwide, and is within the cactus collections of many an enthusiast from Japan to Czechoslovakia to Ireland. Some of these specimens were legally procured, some not.

Star cactus was given a Recovery Priority of 2 by the U. S. Fish and Wildlife Service (2003). Recovery designations range from 1 to 18 (1 being the highest). A designation of 2 indicates that star cactus has a high degree of threat yet a high recovery potential.

Recovery criteria include the maintenance or establishment of ten fully protected populations in the United States or Mexico (USFWS, 2003). 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). A rough minimum viable population estimate completed in 2000 by Kathryn Kennedy (at the time USFWS botanist for Texas), Jackie Poole and Dana Price (former botanist with Texas Parks and Wildlife Department's Wildlife Diversity Program) using Pavlik's criteria (Pavlik 1996), produced a somewhat lower estimate of about 1,300 individuals per population. To achieve the recovery criteria, surveys for new populations are needed. If sufficient populations are not found, reintroduction of *A. asterias* is an acceptable step in the recovery of this species.

Both survey and reintroduction efforts require comprehensive biological and ecological knowledge of the species. An understanding of suitable habitat is necessary to conduct surveys for wild populations. Knowledge of plant demography, environmental factors, and genetics is essential in the development of a reintroduction program (Friar et al., 2001). Since it is important when reintroducing a new population to create populations that closely mimic the characteristics of the naturally occurring population (Pavlik, 1996), it is critical to understand the population biology of the species. Although propagation techniques of star cactus are available on the web, studies of the species' biology were lacking in the literature when this study began. Studies of the habitat, population demography, phenology, and reproductive biology are vital to the recovery of this species in the wild and were urged in the Recovery Plan (U. S. Fish and Wildlife Service 2003). In this study we conducted research on habitat characteristics, demography, phenology, reproductive biology, and propagation. We investigated the impact of herbivory as a threat to the species. We also took a two-prong approach to recovery by both surveying for additional existing wild populations and testing feasibility of introduction through a pilot project.

Originally envisioned as a comprehensive, two-phased, eight-year project to research and recover star cactus in Texas, several years into the study it became apparent that the need to reintroduce multiple populations of star cactus might not be needed. Due to the discovery of many additional populations in Texas and the reports of larger populations in Mexico, we believe that a broad-scale population introduction of star cactus in Texas should be carefully reviewed at this time. This final report details results of March 2004 through June 2010.

OBJECTIVE:
Concomitant with surveys for new star cactus localities, research the specific habitat requirements, demography, phenology, reproductive biology, and other field aspects of star cactus and apply these data to on-the-ground recovery measures such as the augmentation of existing populations, the introduction of new populations, and private landowner conservation/protection plans, to recover star cactus in Texas.

EXPECTED RESULTS OR BENEFITS:
This project is, in essence, the implementation of the Recovery Plan for star cactus (U. S. Fish and Wildlife Service 2003). By creating partnerships with private landowners and involving other key groups (such as the Texas Nature Conservancy, Natural Resource Conservation Services staff and Soil and Water Board Members), we will develop enhanced education and knowledge, and dedicated on-the-ground conservation and protection of star cactus. Based upon the data garnered through the pilot reintroduction, as well as the other research associated with this Section 6 grant, a reintroduction plan for *A. asterias* was drafted in 2009. A finalized *A. asterias* reintroduction plan is included in this final report.

Hopefully, with success and continued support, this project can not only serve as a model for Mexico, but also a successful model of a rare plant recovery project on which other conservation biologists can rely.

PROJECT COMPONENTS:

1. Population Monitoring and Demographics
2. Herbivory Research
3. Habitat Analysis
4. Phenology and Reproductive Biology Research
5. Propagation of Star Cactus Individuals for Founding Populations
6. Establishment of a Pilot Reintroduction Site
7. Pilot Reintroduction Monitoring
8. Surveys for Additional Extant Populations in Texas

APPROACH AND RESULTS:

1) Population Monitoring and Demographics

Five monitoring plots were established in 2004 at two populations (Properties 2 and 7) to collect data necessary to determine demography, phenology, vulnerable life stages, and favorable times for establishment of individuals. All monitoring plots except one are permanent belt transects in which one meter square plots are monitored to one side of the transect along the transect's length. Each individual cactus within these transects is tagged and numbered. Four monitoring plots were established at Property 2 site and were used to track demography in addition to intensive reproductive studies during anthesis. Transect 1 is 25 meters in length, Transects 2.S and Transect 2.N are 20 meters in length, and Transect 4 is a cluster of plants (approximately 4 meters from the property's roadside fenceline) also used to detect potential poaching. Because Transects 1, 2N, 2S, and 4 were all within 550 meters of one another, the numbers were pooled (except in Table 1). A belt transect 25 meters in length was established at Property 7 for both intensive reproductive biology and demography.

Since many plants died in Transect 1 at Property 2 in 2005 [of 71 alive in March 2005, 35 (49%) remained in March 2006], two additional 25 meter transects were set up in 2006 at properties 8 and 9 for both reproductive biology and demography monitoring.

Data recorded in the monitoring regime included size class distribution (determined by plant diameter); whether or not an individual was protected by a "nurse plant" or in the open; vigor; color; positioning (flush, above or below ground; however, this character was dropped after March 2007 as being considered insignificant); natural recruitment and mortality; reproductive activity; presence of disease, herbivory, or other naturally occurring processes having a negative effect; and data revealing evidence of poaching. Data were recorded monthly March 2004-March 2007. However, phenological and reproductive capacity data were collected more frequently during the spring months. Starting March 2007, a biannual monitoring regime (spring and fall) began. Monitoring was done on an annual basis, beginning in March 2008, as the assumption that seedling recruitment could be most easily observed in the fall did not appear to be justified.

At the inception of the study there were 126 live individuals in transects on properties 2 and 7. With the addition of plots at properties 8 and 9 there were 156 plants as of March 2006. At the conclusion of the monitoring study in March 2009, 141 live individuals remained (Table 1).

Table 1. Number of individuals of *Astrophytum asterias* throughout transects, March 2004 to March 2009.

	Mar-04	Mar-05	Mar-06	Mar-07	Mar-08	Mar-09
Property 2 Transect 1	45	71	35	28	23	7
Property 2 Transect 2N	16	13	11	13	8	6
Property 2 Transect 2S	20	18	17	19	15	8
Property 2 Transect 4	9	11	13	14	11	11
Property 7 Transect	36	40	66	74	74	45
Property 8 Transect	—	—	44	50	43	11
Property 9 Transect	—	—	36	52	67	53

After six annual censuses (March 2004-2009), some demographic patterns became apparent. Three "reproductive/size" classes were established: <1.00 cm size class or "seedling", 1.00-4.00 cm size class or "juvenile" plants and >4.00 cm size class or "reproductive" plants. The terms "seedling", "juvenile", and "reproductive" are terms of convenience rather than strictly reflecting reproductive activity or germination events. The annual death rate across all size classes at all properties ranged from none (Property 2 – 2007, Property 8 – 2007, and Property 9 – 2007) to a high of 76% (Property 8 – 2009) (Figs. 1-4). The highest percent death rate was among the reproductive class, followed by the juvenile class. However, this differed among the properties as well as through time. Throughout the study, plants were not always relocated every month. These plants were recorded as missing, and after three years considered to be dead. Missing plants were more likely to be in the seedling or juvenile classes. At the conclusion of the monitoring study, all missing plants were considered to be dead. Seedling germination across all properties varied from none (Property 2 – 2007 & 2008, Property 8 – 2008, Property 9 – 2007, all properties - 2009) to a high of 45% (Property 7 – 2005). This high percentage was due to an extremely high germination event in July 2005 at the Property 7 transect. This one event of germination was the highest seen at any property in one month. Seedling germination during other years and/or at other properties is usually less than 10%.

In the following figures (1-4), standing stock represents the initial number of plants found in 2004 and the persisting plants from 2005-2008. In each year, there was a percentage of plants that was overlooked when they germinated and eventually were uncovered or discovered for various reasons. Pseudorecruits are any plants ≥ 1 cm in diameter that had not been previously discovered. Also, plants sometimes form new apices after apical meristem damage (fission). Each apex is recorded as a separate individual, and counted within pseudorecruits. Additionally, plants that were recorded as missing for one or two years are sometimes relocated. Rather than recalculating the data from previous years, these plants are added as pseudorecruits. Recruits are plants <1 cm in diameter.

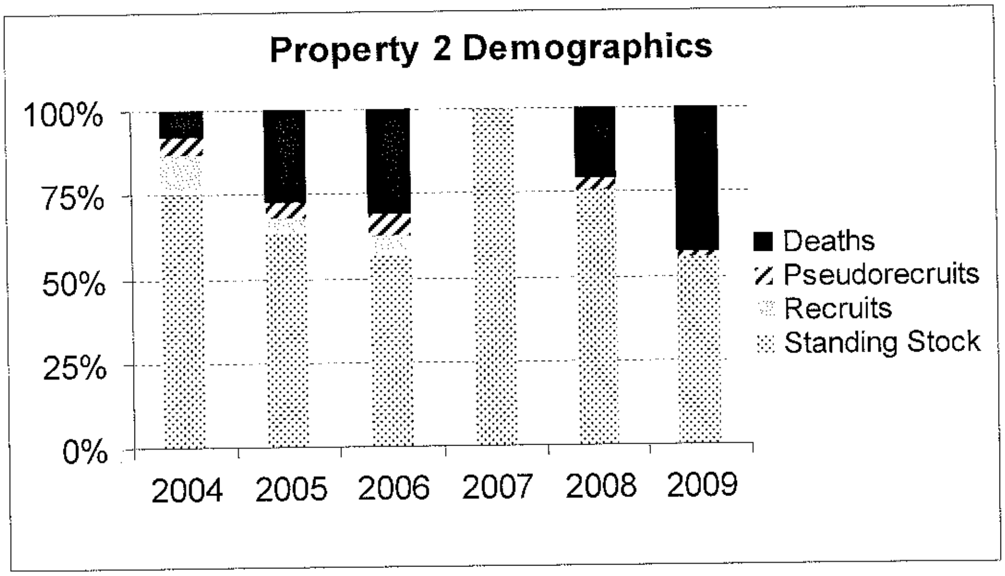


Figure 1. Standing stock, recruits, pseudorecruits, and deaths of *Astrophytum asterias* at Property 2 transects of each year from March 2004 to March 2009.

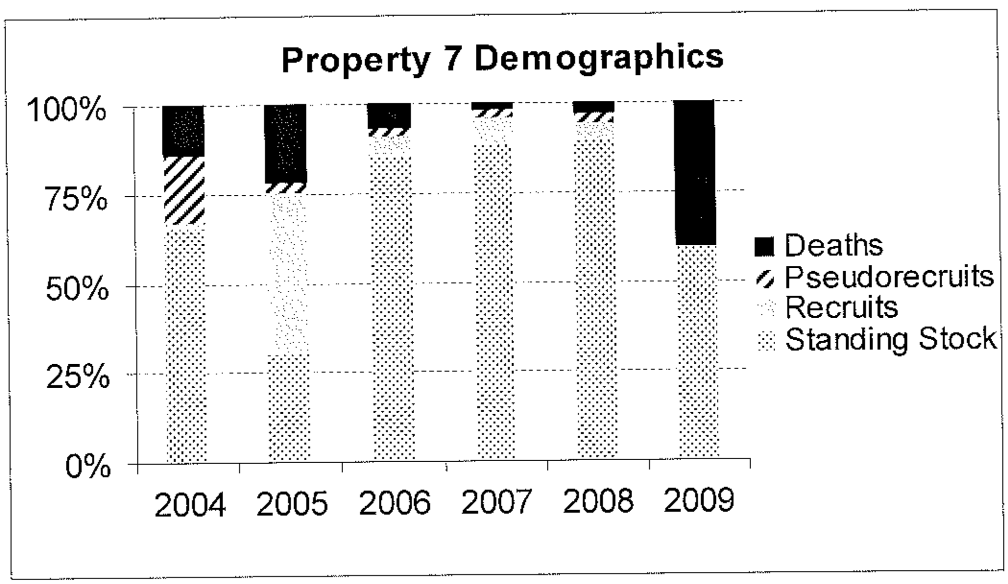


Figure 2. Standing stock, recruits, pseudorecruits, and deaths of *Astrophytum asterias* at Property 7 transect of each year from March 2004 to March 2009.

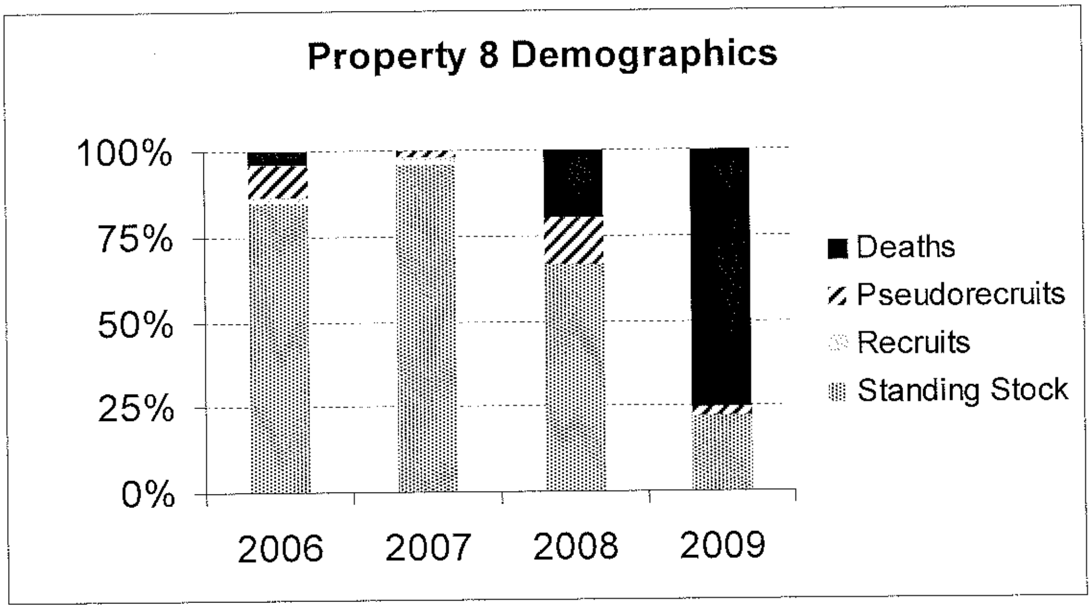


Figure 3. Standing stock, recruits, pseudorecruits, and deaths of *Astrophytum asterias* at Property 8 transect of each year from March 2006 to March 2009.

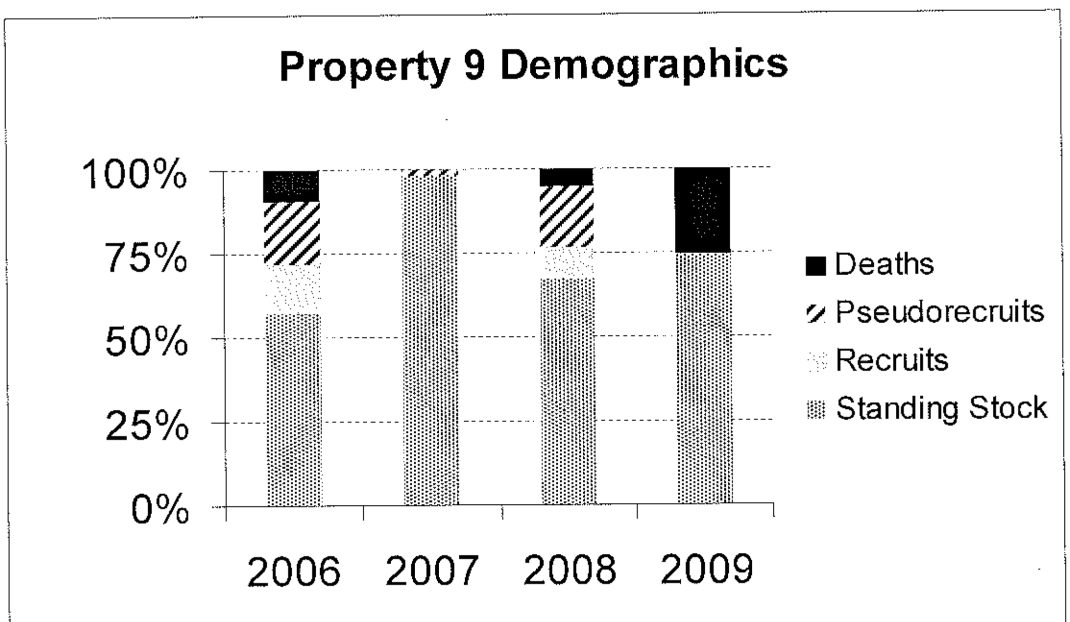


Figure 4. Standing stock, recruits, pseudorecruits, and deaths of *Astrophytum asterias* at Property 9 transect of each year from March 2006 to March 2009.

Diameter of plants located within transects was recorded in the spring of each year or when the plant was discovered. Three size classes, similar to the "reproductive/size" classes were chosen: seedling (<1.00 cm), juvenile (1.00-4.00 cm), and reproductive (>4.00 cm). The relative frequency of size classes of plants for every year for all properties was calculated (Figs. 5-8).

The number of individuals within each property and year does not necessarily match the number of individuals per property/transect as shown in Table 1 because the size class distributions take into account all individuals that existed in the transect during a particular year while the number of individuals per property/transect are the number of live individuals as of that date.

Property 2 (Fig. 5) experienced an overall decrease in population. Where initially the plants numbered 116 individuals, the March survey in 2009 resulted in only 32 plants. Property 2 is experiencing very little seedling establishment (none in 2007-2009), whereas the juvenile and reproductive size classes are remaining more or less steady over the last four years (the increase in the juvenile class in 2009 is a result of increased mortality in the reproductive class). This trend, where the smaller size class (seedling) represents less of the population than the larger size classes (reproductive and juvenile) generally indicates a population in or near stasis. Little establishment is not uncommon in long-lived species, and recruitment events may be more than 10 years apart without any noticeable change in population structure (Harper 1977).

Even within property 2, which consists of four transects one-third of a mile apart, there was a large difference in the death rate between the transects. In late 2005 most of the deaths occurred at one belt transect where over 50% of the cacti were lost due to herbivory. A third of a mile away some plants died and some plants germinated, but overall the number of individual cacti stayed the same. In 2009 there was substantial mortality in 3 of the 4 transects. Across the landscape there is definite variability within the population structure.

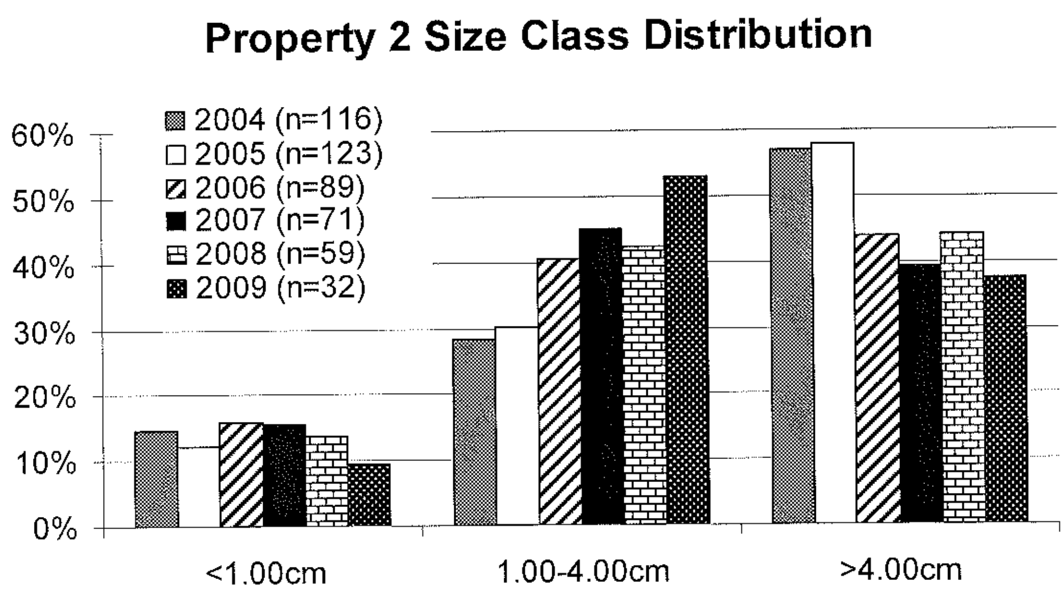


Figure 5. Size class distribution of *Astrophytum asterias* within transects at Property 2, March 2004, 2005, 2006, 2007, 2008, 2009.

At Property 7 (Fig. 6), the total number of cacti increased from 2004 to 2005. From 2006-2008, the population slightly decreased but more or less remained stable. Most of the increase was due to a large germination event that occurred primarily around one plant in 2005. A substantial increase in mortality decreased the population by 40% in 2009. A larger percentage of seedlings generally indicates a growing trend in any population. However, for the Cactaceae, seedling survivorship plays a significant role in population growth since it tends to be low and variable (Nobel 2002). Of the 28 seedlings recorded in July and August 2006, only 10 were found in March 2009. The population structure has changed overall during the six years of the study. Whereas in 2004, the seedling size class accounted for less than 10% of the population, in 2009, the seedling size class accounted for 36% of the population. The reverse has occurred for the reproductive size class plants, which went from 40% of the population to 27%.

Property 8 (Fig. 7) has suffered a more dramatic loss of individuals than any other transect. At inception of the transect in March 2006, there were 44 plants and an additional 6 plants were found by the end of 2006. However, by March 2009, only 11 individuals were left, all in the larger size classes.

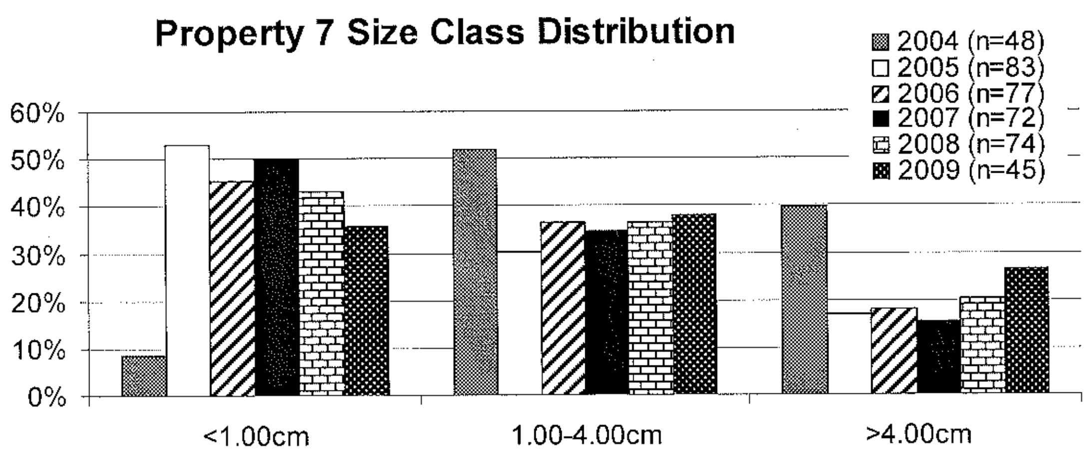


Figure 6. Size class distribution of *Astrophytum asterias* within transects at Property 7, March 2004, 2005, 2006, 2007, 2008, 2009.

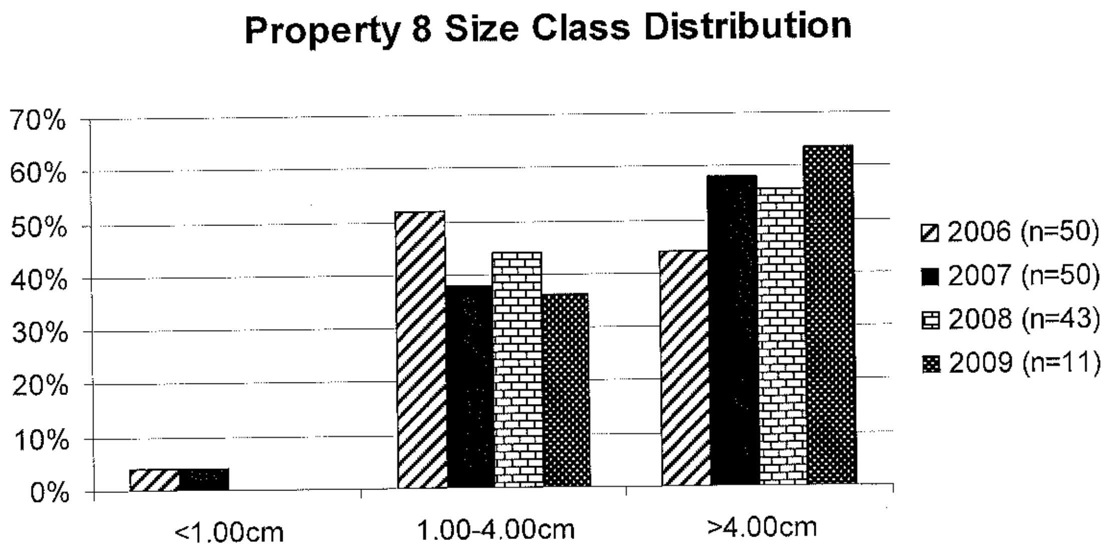


Figure 7. Size class distribution of *Astrophytum asterias* within transects at Property 8, March 2006, 2007, 2008, 2009.

The numbers of individuals at the Property 9 transect (Fig. 8) has remained more stable than the other transects. However, the distribution of individuals among the size classes has changed over the four years the transect was monitored. The seedling size class has decreased, due to an almost equal amount of mortality and diameter increase to the juvenile size class, while the juvenile size class has doubled and the reproductive size class remained more or less constant.

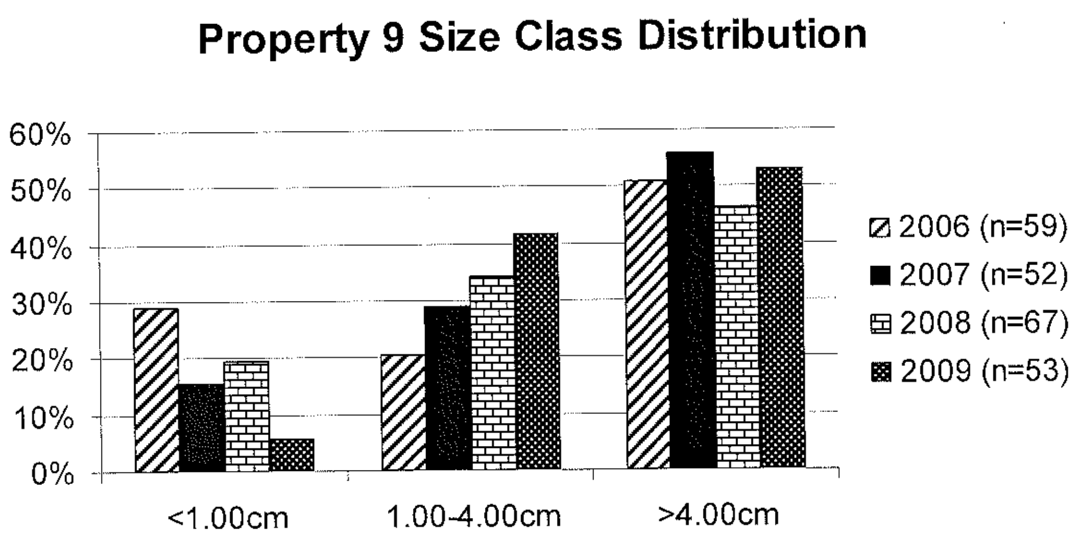


Figure 8. Size class distribution of *Astrophytum asterias* within transects at Property 9, March 2006, 2007, 2008, 2009.

The diameter range for all plants at all transects over all years was between 2.6 and 101.9 mm. The mean diameters for properties 2 and 7 decreased from 2004 to 2009 (Table 2). At Property 2 this was largely because of a total decrease in population due to herbivores killing larger cacti and leaving the smaller ones alone. At Property 7, the decrease in diameter was largely due to an increase in smaller cacti due to a mass germination event in one month mostly around one individual cactus. Mean diameters at properties 8 and 9 increased from 2006 to 2009. Although there was over 75% mortality in 2009 at Property 8, over half the remaining individuals are greater than 50 cm in diameter.

Table 2. Mean diameter (mm) of *Astrophytum asterias* within transects at Properties 2, 7, 8 and 9, March 2004 - March 2009.

Property	2004	2005	2006	2007	2008	2009
2	41.51	43.80	36.67	34.75	37.42	38.01
7	33.12	29.78	28.16	25.39	21.78	24.68
8	-	-	38.96	43.37	42.66	46.56
9	-	-	37.21	38.30	38.92	45.44

Annual growth rate was calculated by averaging the growth rate among all living individuals for 2004-2009 at Properties 2 and 7, and for 2006-2009 at Properties 8 and 9. Only individuals that survived all five years at Properties 2 and 7 (n=34) or all three years at Properties 8 and 9 (n=44) were used in this analysis. Growth rate fluctuates yearly among the same plants (Fig. 9).

Growth rate for each of the five years (between March 2004 and March 2009) was averaged to determine an overall total growth rate for all *A. asterias*. This assumes a constant average annual growth rate among all size classes and results in a rate of 2.71 mm/year. This indicates that it takes 15 years for *A. asterias* to reach reproductive maturity (ca. 4 cm). When mean diameter growth rate is analyzed according to different size classes, the rate ranges between a negative growth rate (-0.85mm/yr) for the largest size class of 80.01-90.00 mm and 3.65 mm/yr for the 50.01-60.00 mm size class (Table 3). Using a calculation dependent upon size class growth rate, it would take *A. asterias* 25 years to become reproductive.

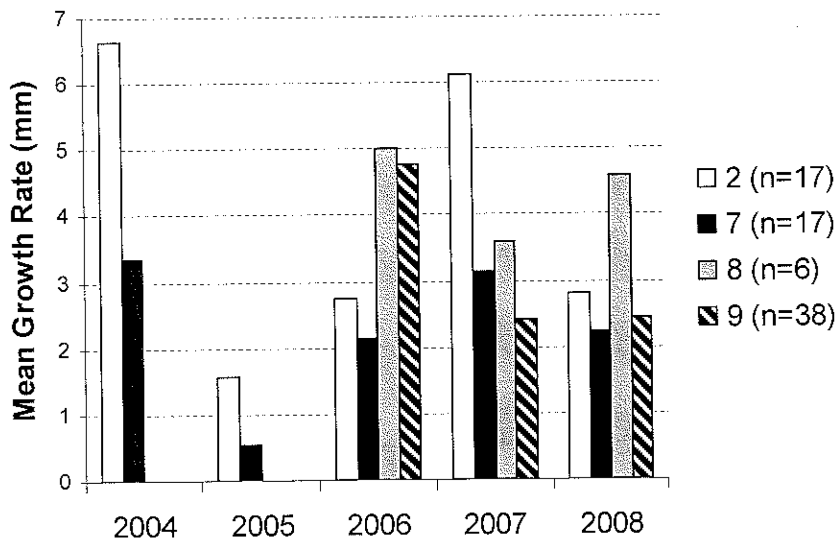


Figure 9. Mean annual growth rate (mm) of *Astrophytum asterias* within transects at Properties 2, 7, 8, and 9, March 2004-2005, March 2005-2006, March 2006-2007, March 2007-2008, and March 2008-2009.

Table 3. Mean diameter growth rate (mm) of *Astrophytum asterias* within transects at Property 2 and 7, March 2004-March 2007.

Diameter Class (mm)	0.01-10.00	10.01-20.00	20.01-30.00	30.01-40.00	40.01-50.00	50.01-60.00	60.01-70.00	70.01-80.00	80.01-90.00
Mean Diameter Growth Rate (mm)	0.92	2.03	1.61	3.35	1.88	3.65	1.99	1.26	-.0.85

2) Herbivory Research

In one of the first quantitative assessments regarding the efficacy of recovery plans, Clark et al. (2002) identified that threats to species had received insufficient attention in recovery plans. For *Astrophytum asterias* identified threats include habitat destruction, over-collection by cactus enthusiasts, inadequate regulatory protection, reduction of genetic variability through diminution of population size, and disease or predation (USFWS 2003). Although disease and predation were identified as potential threats, the 2003 recovery plan states "Disease or predation, which although not evidenced at the time of listing, may be having deleterious effects, as herbivory by rodents has been reported in Texas". Recent studies conducted in Mexico have confirmed that herbivory poses a serious threat to populations of *A. asterias* (Martínez-Ávalos et al. 2007). Although Martínez-Ávalos et al. (2007) documented impacts of herbivory on populations of *A. asterias,* anecdotal data collected from populations in Texas indicated that the demographic impacts of herbivory as well as those species responsible for herbivory-induced mortality differed among Texas populations. Thus, the purpose of this study was to 1) document the species of herbivores posing a threat to *A. asterias* in Texas 2) examine rates of mortality among populations of *A. asterias* in Texas and 3) relate herbivore-induced mortality to environmental variables and life-stages of *A. asterias.* In addition, we sought to compare our results gathered from populations of *A. asterias* in Texas to the results of Martínez-Ávalos et al. (2007) generated from Mexican populations. In order to assess herbivore-induced mortality in Texas populations of *A. asterias,* we employed two methods: motion-sensor cameras and demographic quadrat surveys.

Motion-Sensor Camera Surveys

Trailmaster® motion-sensor cameras were used to examine which mammalian predators pose a threat to *A. asterias* and to identify the variety of herbivores which consume or damage *A. asterias.* Initial camera surveys were conducted on Property 2 and Property 4 in March of 2006 and after successful documentation of mammalian herbivory, an additional 8 cameras were added to the two properties for a total of 10 cameras. Cameras required constant upkeep and maintenance, and were all permanently removed in July 2007 due to extensive damage of the units by rodents. From March 2006 – July 2007, a total of 277 photographs documenting 27 species including 5 potential herbivores were recorded (Table 4). 119 photographs (43%) were of birds. The two most common herbivores included desert cottontails *Sylvilagus audubonii* with 74 photographs (26.7%) and Mexican ground squirrels *Spermophilus mexicanus* with 39 photographs (14.1%). Other mammals accounted for 10% of the photographs taken.

Table 4. Number of individual species recorded with Trailmaster® cameras at sites with star cactus, March 2006 – July 2007.

Common Name	Number Photographed
Birds	
Bewick's Wren	1
Warbler sp.	1
Wren sp.	1
Cactus Wren	2
Long-billed Thrasher	2
Common Ground Dove	3
Pyrrholuxia	3
Curve-billed Thrasher	4
White-crowned sparrow	4
Meadowlark sp.	5
Bird sp.	6
Scaled Quail	11
Greater Roadrunner	16
Mourning Dove	17
Northern Mockingbird	18
Black-throated Sparrow	25
Reptiles/Amphibians	
Bullsnake	1
Anuran sp.	1
Reticulate Collared Lizard	4
Texas Spiny Lizard	4
Invertebrates	
Walking Stick	1
Moth sp.	1
Tarantula	2
Mammals	
Bobcat	1
Coyote	1
Southern Plains Woodrat	3
Raccoon	3
White-footed Mouse	6
Cow	14
Mexican Ground Squirrel	39
Desert Cottontail	74

Demographic Quadrat Surveys

Five 10,000-m^2 quadrats were established and surveyed for density of potential herbivores and cacti. Quadrats were established around populations of cacti on Properties 2, 4, 7, 8, and 11, and all quadrats were in place and surveyed by July 2007. Quadrats were systematically surveyed using a minimum of four people in a grid-like fashion, and all cacti were marked with orange pin flags. Sign of Mexican ground squirrels (*Spermophilus mexicanus*), cottontail spp. (*Sylvilagus* spp.), and southern plains woodrats (*Neotoma micropus*), were identified and marked for an index of relative abundance of each species at all five sites (Table 5). Results of sign surveys indicate differences exist in density among sites of these three mammals. Population estimates for quadrats ranged from 150-487 *A. asterias* (Table 5).

Table 5. Results of initial 10,000 m^2 quadrat surveys for herbivore sign and *A. asterias* density on 5 properties, March – July 2007.

Quadrat	Total # Cacti	# Cacti with Herbivory	# Woodrat Middens	# Ground Squirrel Holes	# Rabbit sign
Property 2	150	20	20	34	18
Property 4	445	50	9	26	14
Property 7	487	9	0	22	3
Property 8	338	12	18	26	22
Property 11	248	5	18	19	6

Using previously collected data from the 7 demographic monitoring transects (Section 1) surveyed on Properties 2, 7, 8, and 9 (Table 6), we attempted to estimate effects of climatic conditions on levels of herbivory. Using a multiple linear regression with total monthly precipitation, mean maximum, mean minimum temperatures, and mean monthly temperatures as independent variables and the total number of cacti with herbivory per month as the dependent variable. No significant correlation existed between number of cacti with herbivory and the degree of rainfall or temperatures for the four properties ($r^2 = 0.06$, $P = 0.333$). Despite the lack of significant correlation, extensive levels of herbivory were documented disproportionately among the sites (Table 6).

Table 6. Results of mortality due to herbivory and other factors on *Astrophytum asterias* along 7 transects, 2005-2007.

Transect	Total # of Cacti	Total Deaths	Deaths Due to Herbivory	% Mortality	% Mortality Due to Herbivory
Property 2, Transect 1	94	58	42	61.7%	72.4%
Property 2, Transect 2N	18	4	2	22.2%	50.0%
Property 2, Transect 2S	22	3	1	13.6%	33.3%
Property 2, Transect 4	16	2	0	12.5%	0.0%
Property 7	64	12	7	18.8%	58.3%
Property 8	51	2	1	3.9%	50.0%
Property 9	59	5	1	8.5%	20.0%

Beginning in July 2007 previously marked populations of cacti contained within the demographic quadrats on properties 2, 4, 7, 8 and 11 were resurveyed for new herbivory and mortality. All cacti marked with pin flags were inspected for new herbivory or other causes of mortality and if herbivory was detected the orange pin flag was replaced with a yellow pin flag. Surveys were scheduled for every other month with the most recent survey conducted in September 2009.

In February 2008, each quadrat was resurveyed to measure and mark individual cacti based on diameter. Ten different colors of pin flags (Table 7) were used to distinguish between cacti categorized in 1 cm size classes. Cacti were measured with drafting circle stencils and metric rulers, and classified into one of ten classes (Table 7). Previous cacti mortalities sizes were estimated when possible. After February 2008 all mortalities were recorded related to size of the individual cactus.

Table 7. Size class categories for star cactus marked within each quadrat.

Flag Color	Size Class
Dark Red	≤ 1 cm
Dark Orange	> 1 cm to 2 cm
Neon Red	> 2 cm to 3 cm
Neon Blue	> 3 cm to 4 cm
White	> 4 cm to 5 cm
Neon Pink	> 5 cm to 6 cm
Dark Blue	> 6 cm to 7 cm
Yellow	> 7 cm to 8 cm
Neon Orange	> 8 cm to 9 cm
Neon Green	> 9 cm

Results regarding the distribution for size classes among each quadrat are presented in figures 10-14. In addition, mortalities of individual size classes are presented in relation to total numbers of each size class.

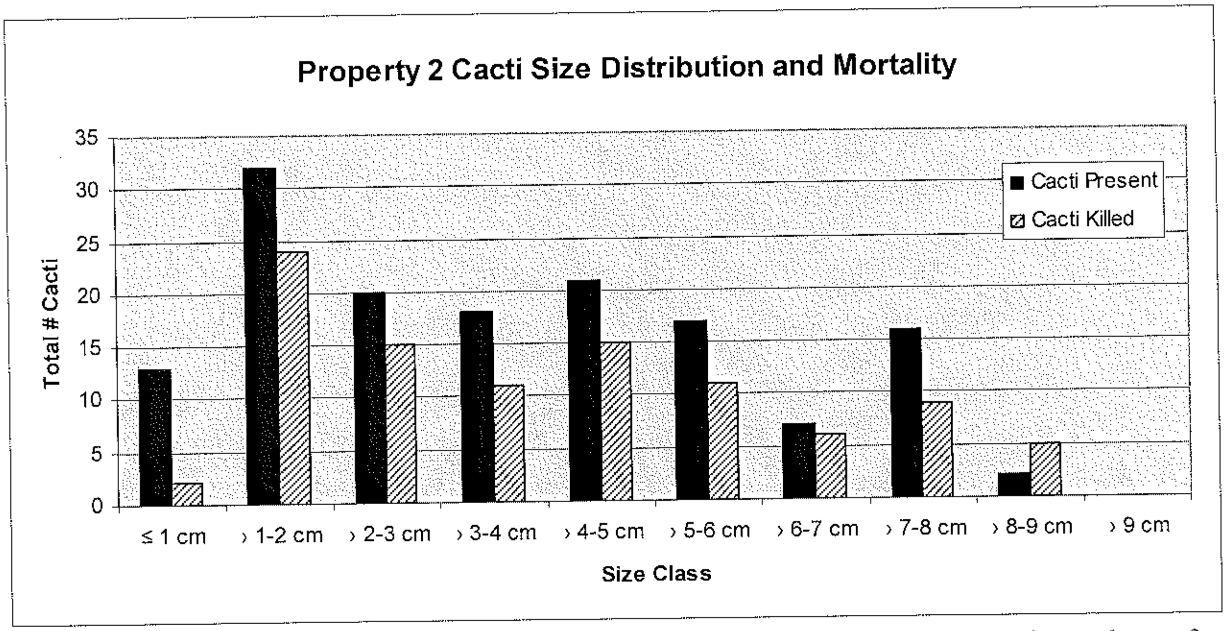


Figure 10. Size class distribution of cacti marked within Property 2 quadrat with numbers of individual deaths per size class indicated, July 2007 – September 2009.

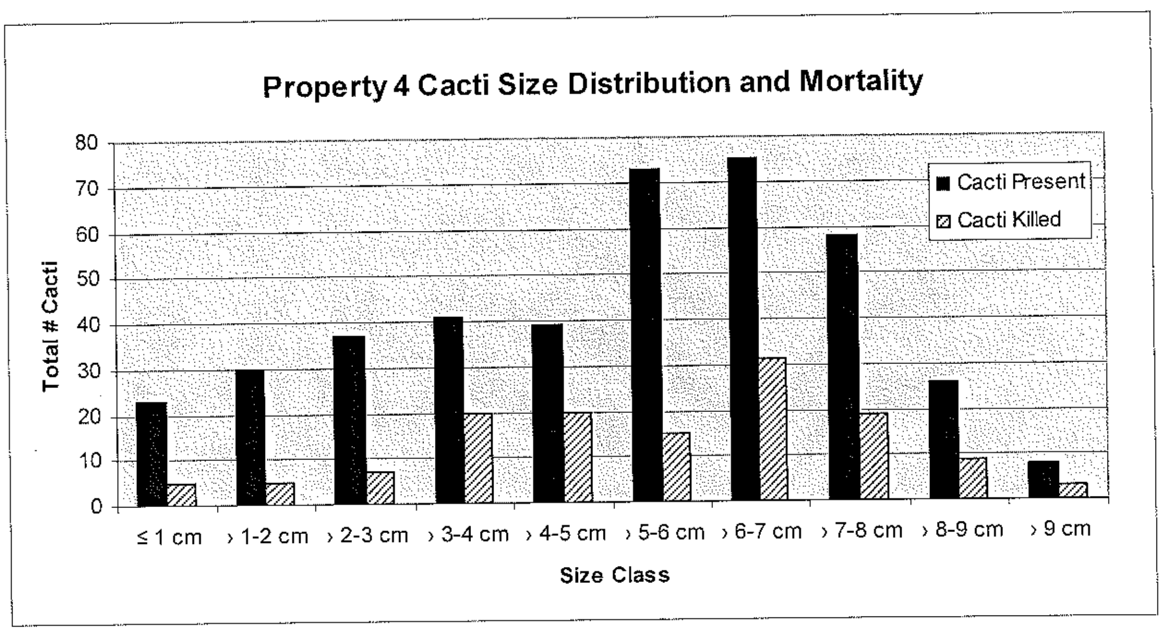


Figure 11. Size class distribution of cacti marked within Property 4 quadrat with numbers of individual deaths per size class indicated, July 2007 – September 2009.

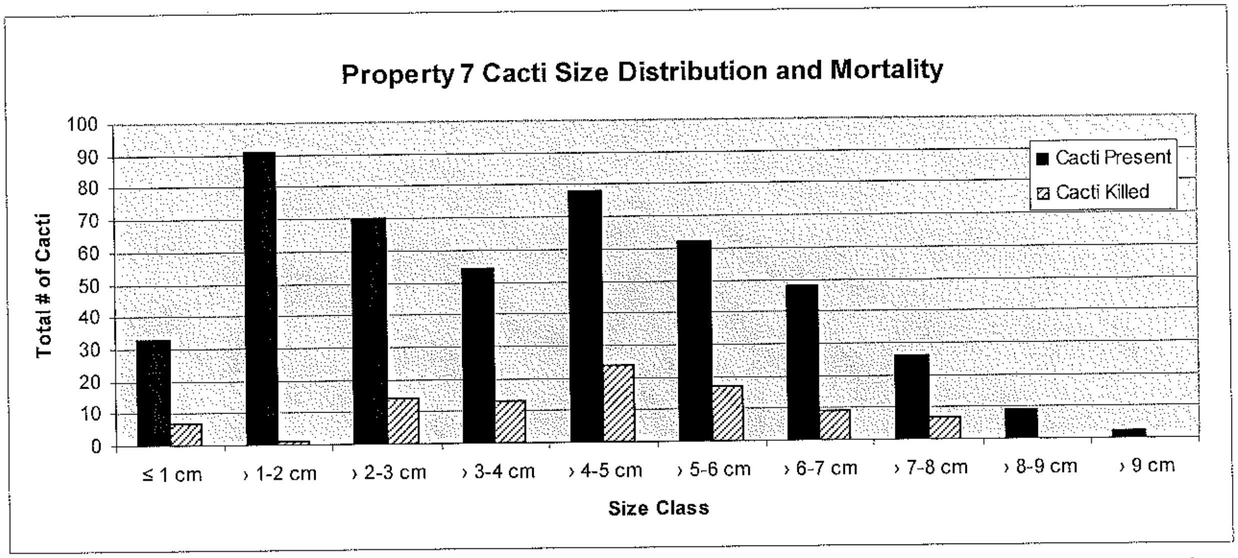


Figure 12. Size class distribution of cacti marked within Property 7 quadrat with numbers of individual deaths per size class indicated, July 2007 – September 2009.

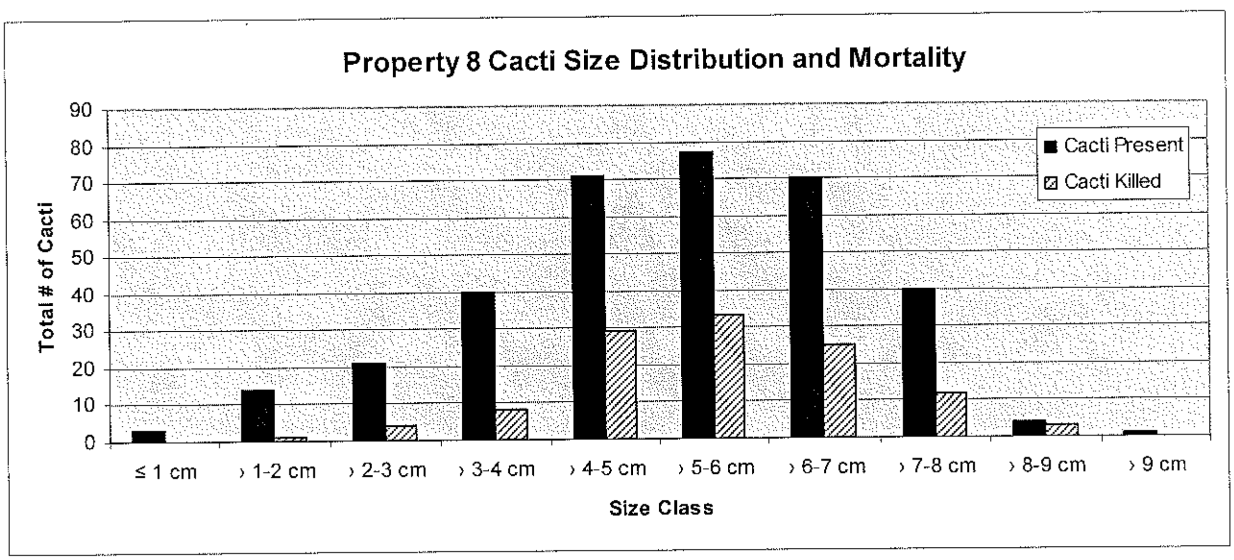


Figure 13. Size class distribution of cacti marked within Property 8 quadrat with numbers of individual deaths per size class indicated, July 2007 – September 2009.

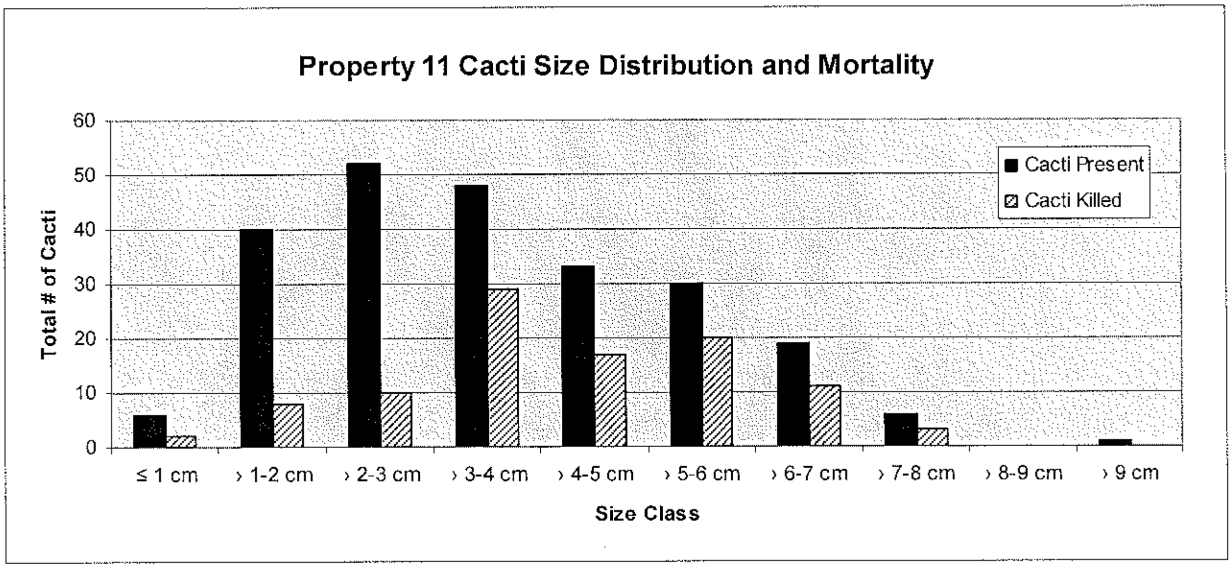


Figure 14. Size class distribution of cacti marked within Property 11 quadrat with numbers of individual deaths per size class indicated, July 2007 – September 2009.

During the mortality surveys (July 2007 – September 2009) three major causes of death were recorded among star cacti: mammalian herbivory, rot or fungal infection, and insect induced mortality associated with infestations by *Moneilema armatum* (Coleoptera: Cerambycidae) (Ferguson and Williamson 2009). Quadrat results for overall mortality are shown in Table 8. Assessment of cause of death was often difficult, especially considering infestations of *M. armatum* lead to similar rotting conditions found from fungal infections. Thus, only plants with definitive evidence of insect activity (larva or pupal chambers present or chewed out burrows in cacti flesh) were categorized as *M. armatum* deaths. All other unrecognizable causes of death were placed in the unknown category.

Insect Mortality

Moneilema armatum induced mortality was highest in the months of September, October, November, and December, accounting for 82% of the 56 recorded deaths from this insect (Fig. 15). Out of 527 mortalities of *A. asterias* among two populations in Mexico, 105 were attributable to cerambycid beetle larva, accounting for 19.9% of all mortality recorded (Martínez-Ávalos et al. 2007). In comparison, our study covered five populations and recorded a total of 644 deaths, of which only 37 were confidently attributable to infestation with the cerambycid beetle larva *Moneilema armatum*. Another 19 instances of mortality were likely caused by *M. armatum* larva but no definitive evidence was obtained from those cacti. Thus, 8.7% of all deaths in our study were attributable to *M. armatum* herbivory, almost half the amount reported in Martínez-Ávalos et al. (2007). This difference in percent mortality could be due to differences in how deaths associated with cerambycid larva were identified. Either way, it appears that cerambycid larva, at least of the genus *Moneilema*, do pose a threat to individual *A. asterias.*

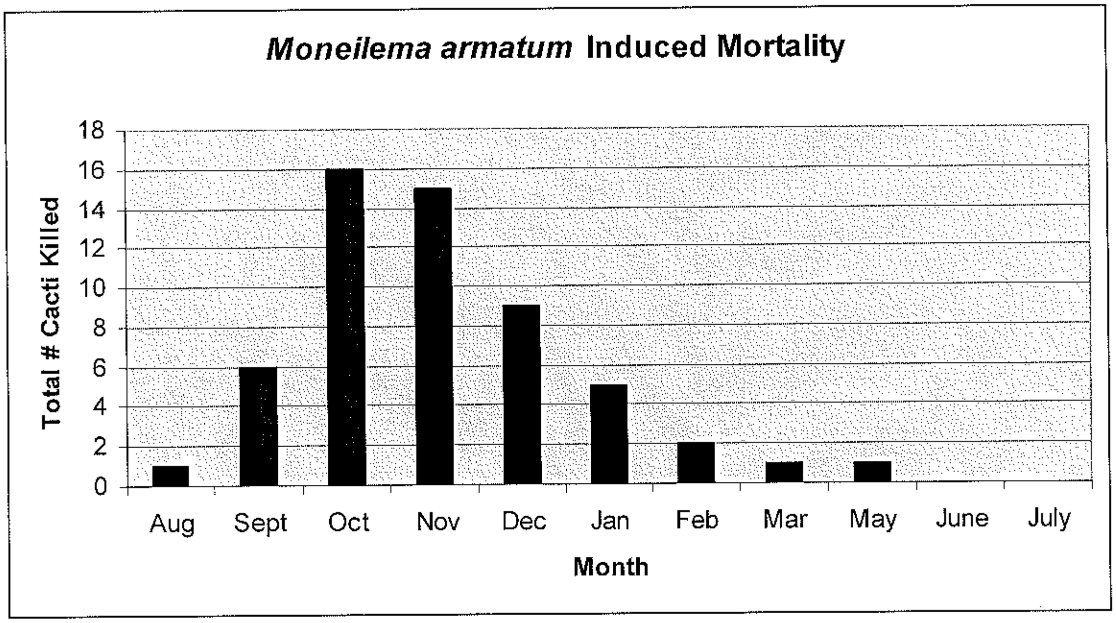


Figure 15. Deaths of star cactus caused by the cerambycid beetle *Moneilema armatum* recorded among all 5 quadrats July 2007 – September 2009.

Mammalian Herbivory

From September 2007 – September 2009 percent mortality associated with mammalian herbivory accounted for over half of total mortality recorded for all 5 quadrats ranging from 63.6% for Property 4 to 81.5% for Property 11 (Table 8). Although July 2007 had a high level of mammalian herbivory, this could be an artifact of accumulation of herbivory prior to the initiation of monitoring of the quadrats, and thus this data is only presented in Figure 16. No distinction between old herbivory and fresh herbivory was made during the initial survey, leading to the potential inclusion of plants with herbivory that did not originate in the month of July. The majority of herbivory, post-initial survey, was recorded in December 2008 and February 2009 (Fig. 16). Out of 644 recorded deaths of *A. asterias*, 463 (71.9%) were attributable to mammalian herbivores. Although data from the motion-sensor camera surveys indicate that *Sylvilagus audubonii* poses the greatest threat to individual *A. asterias* other potential herbivores were recorded causing damage to *A. asterias.* The southern plains woodrat *Neotoma micropus* caused damage to over 15 plants on Property 2 as assessed by proximity to runs leading into and out of woodrat middens and the presence of woodrat sized fecal pellets next to consumed cacti. Besides this site, no other sight had definitive woodrat induced damage to *A. asterias.* In addition, it appeared that the hispid cotton rat *Sigmodon hispidus* was involved in the deaths of over 50 individual plants on Property 7 in December 2008. The identification of damage attributable to this rodent species was based on the presence of fecal pellets consistent in size and shape with that of *S. hispidus*, presence of epidermal shavings at each of the consumed cactus, and the high density of cotton rats and cotton rat runways through the grass observed on this site at this time. It does appear that rodents tend to shave the epidermal layer off prior to consuming cactus flesh. This pattern in conjunction with the presence of small fecal pellets inside or adjacent to the killed cactus is often quite distinguishable by damage caused by lagomorphs, where the entire cactus is dished out to the base of the apical meristem and no shavings of the epidermis or feces are found in proximity to the plant. Despite this difference, identification of the exact species responsible for the death of individual plants would be almost impossible to discern without the assistance of video monitoring or forensic DNA evidence of some sort.

Table 8. Percentage of mortality among cacti within each of the five monitored quadrats broken down by of cause of death (COD) September 2007 – September 2009.

Quadrat	Total # of Cacti	Total Deaths	COD Mammalian Herbivory	COD Rotten	COD Unknown	COD *Moneilema*	COD Dessication	% Mortality	% Mortali Due to Herbivo
Property 2	146	115	83	2	25	3	2	78.8%	72.1%
Property 4	410	162	103	4	31	23	1	39.5%	63.6%
Property 7	473	121	86	4	24	3	4	25.6%	71.1%
Property 8	341	127	94	6	22	5	0	37.2%	74.0%
Property 11	236	119	97	1	18	3	0	50.4%	81.5%

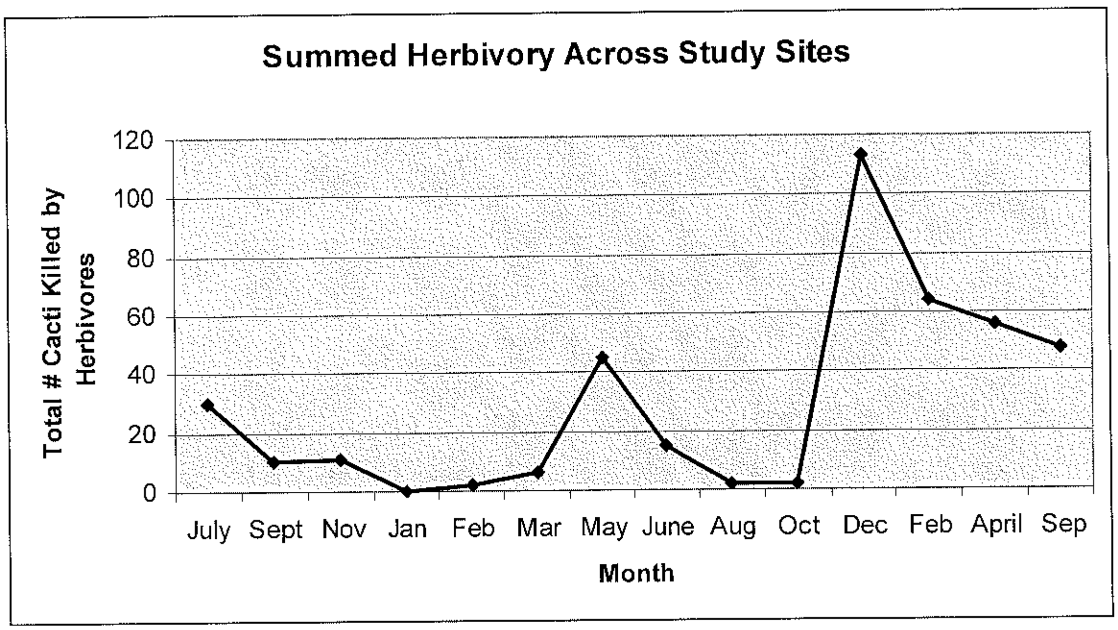


Figure 16. Herbivory summed across all 5 quadrats during July 2007 – September 2009 monitoring period.

In general, it appears that mammalian herbivory and insect damage by cerambycid larva pose a serious threat to populations of *A. asterias* in Texas. Our study identified a broader range of mammalian predators than previously reported (Martínez-Ávalos et al. 2007) including: *S. audubonii, N. micropus, S. hispidus,* and *S. mexicanus*. All monitored populations experienced some level of mortality associated with these herbivores. In some instances, mortality associated with mammalian herbivory accounted for over 80%. Analyzing these data with weather data collected from the study sites will allow us to examine weather patterns in relation to herbivory intensity. If patterns in herbivory do exist, and can be predicted by local weather conditions such as rainfall or temperature, managers may be able to take action to prevent high levels of mortality among populations of *A. asterias*. Since herbivory by both mammals and insects seems to be a major cause of mortality, covering individual plants with some type of exclusion device may provide the best way to prevent mortality of existing stock of *A. asterias*.

Of particular concern is the fact that the only protected property, Property 2, has experienced the greatest mortality rate, with a loss of 78.8% of the original population within the monitored quadrat. In fact, during the last survey conducted in September 2009, only 11 *A. asterias* were found to exist within the quadrat boundaries for this population. Our data clearly indicate that herbivory and other sources of mortality pose serious threats to existing populations of *A. asterias* and patterns of herbivory are not constant across a temporal scale. A more in depth analysis of the data collected should enable us to draw inferences about these patterns in an attempt to understand the population dynamics of this endangered plant.

3) Habitat Analysis

This study has two 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.

Several criteria were used to select subpopulations in which to conduct vegetation and soil analyses. First, 2000-2001 spatial data were overlaid on USDA-NRCS Starr County Soil Survey maps (Natural Resources Conservation Service 2005). Based on the soil type, number of *A. asterias*, and area of the subpopulation possible areas in which to conduct vegetation sampling were chosen. Subpopulations of star cactus have been found predominantly in Catarina soils. In 2006 when the habitat analysis was conducted, subpopulations of star cactus were also found in Montell clay, saline; Garceno clay loam; Jimenez-Quemado association; Maverick soils, eroded; and Ramadero loam. Nine vegetation transects were conducted in Catarina soils; two each in Garceno clay loam and Jimenez-Quemado association; and one each in Maverick soils, eroded; Montell clay, saline; and Ramadero loam. After the vegetation transects were conducted, a new subpopulation of *A. asterias* was found in Copita fine sandy loam. Vegetation and soil analyses were also conducted at the reintroduction site (RE). The data for the reintroduction site are also included in this section.

Initially, a reconnaissance of the chosen area was completed and *A. asterias* flagged. The number of *A. asterias* in the areas where sampling was conducted ranged from 11 to 283. The line-intercept method was used to document the plant species within the *A. asterias* subpopulations and determine percent dominance (cover) and percent relative dominance of these species (Brower et al. 1990, Cox 1996). In cases of overlapping canopies, both overstory and understory plants were recorded. Each vegetation transect was 75 meters (three 25-meter transects) and followed a stratified-random design. Due to the small, island-like areas of *A. asterias*, a 30-meter baseline was used. This baseline was situated along the length of the *A. asterias* subpopulation and the three 25-m transects ran the width of the area. A random numbers table was used to determine placement of the first 25-m transect between 0 and 9 meters. The two subsequent transects were placed at 10 and 20 meters from the first. 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) were also recorded to compile a comprehensive associated species list. The number of *A. asterias* within the three 2-m belt transects (150 m^2) of each site was recorded to estimate density. For each star cactus in the 150 m^2 area, the presence of a plant species directly overhead or immediately adjacent to the plant was documented. When multiple species formed a canopy over *A. asterias* the species were documented collectively. If another plant was not directly overhead or immediately adjacent, the immediate area around the star cactus was documented as bare ground (<25% rocks) or covered with rocks (≥25% rocks).

Soil samples were collected within the 750 m^2 area of each of the 15 vegetation transects, according to the soil collecting guidelines of the Texas Cooperative Extension Service (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*. The three samples were pooled and the composite soil samples for each subpopulation were sent 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). At one of the sites a soil sample was collected, following the same guidelines as outlined above, in an area adjacent to the vegetation transect. This area contained very little vegetation except for *Varilla texana* and no *A. asterias* were present in the area.

Analyses of Vegetation Data

Of the 15 vegetation transects in subpopulations of *A. asterias*, only three had total vegetative dominance over 50%. Site NC1 (Property 2) had the highest total vegetative dominance of 57.15% while site JB2 (Property 5) 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 9). 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 9. 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 A, Table A.1.

Table 9. 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 A, Table A.2 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 10. 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 A, Table A.3).

Table 10. 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*

Total vegetative dominance at the reintroduction 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 A, Table A.2. 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 11.

Table 11. 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*

Analyses of Soil Data

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 12). The routine soil analysis of the sample collected at the pilot reintroduction site (RE; Property 2) 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 12). The soil sample (Out) collected adjacent to the JB1 (Property 5) 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 A, Table A.4.

Table 12. 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; Property 2) and adjacent to site JB1 (Out; Property 5). 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 A, Table A.5). Using sodium adsorption ratio (SAR) and conductivity levels, sites AM2 (Property 1), CA (Property 9), EE (Property 7), JB1 (Property 5), JB2 (Property 5), KR (Property 3), LM (Property 6), NC2 (Property 2), and PP2 (Property 8) are classified as saline-sodic soils (Table 13). Sites AM4 (Property 1) and NC1 (Property 2) are sodic while sites LA (Property 4) and PP1 (Property 8) are saline. Sites AM1 (Property 1), AM3 (Property 1), and RE (Property 2) 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 13. 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 analysis (PCA) was performed using the thirteen parameters of the routine soil analyses of the 15 subpopulations and the pilot reintroduction site (16 sites in total). The values of the soil parameters were z-score-transformed. The resulting loadings and plots were used to group similar sites. 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. PC I explained 34% of the variation and represented a conductivity and copper gradient (Fig. 17). 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. 17). 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. 17). 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.

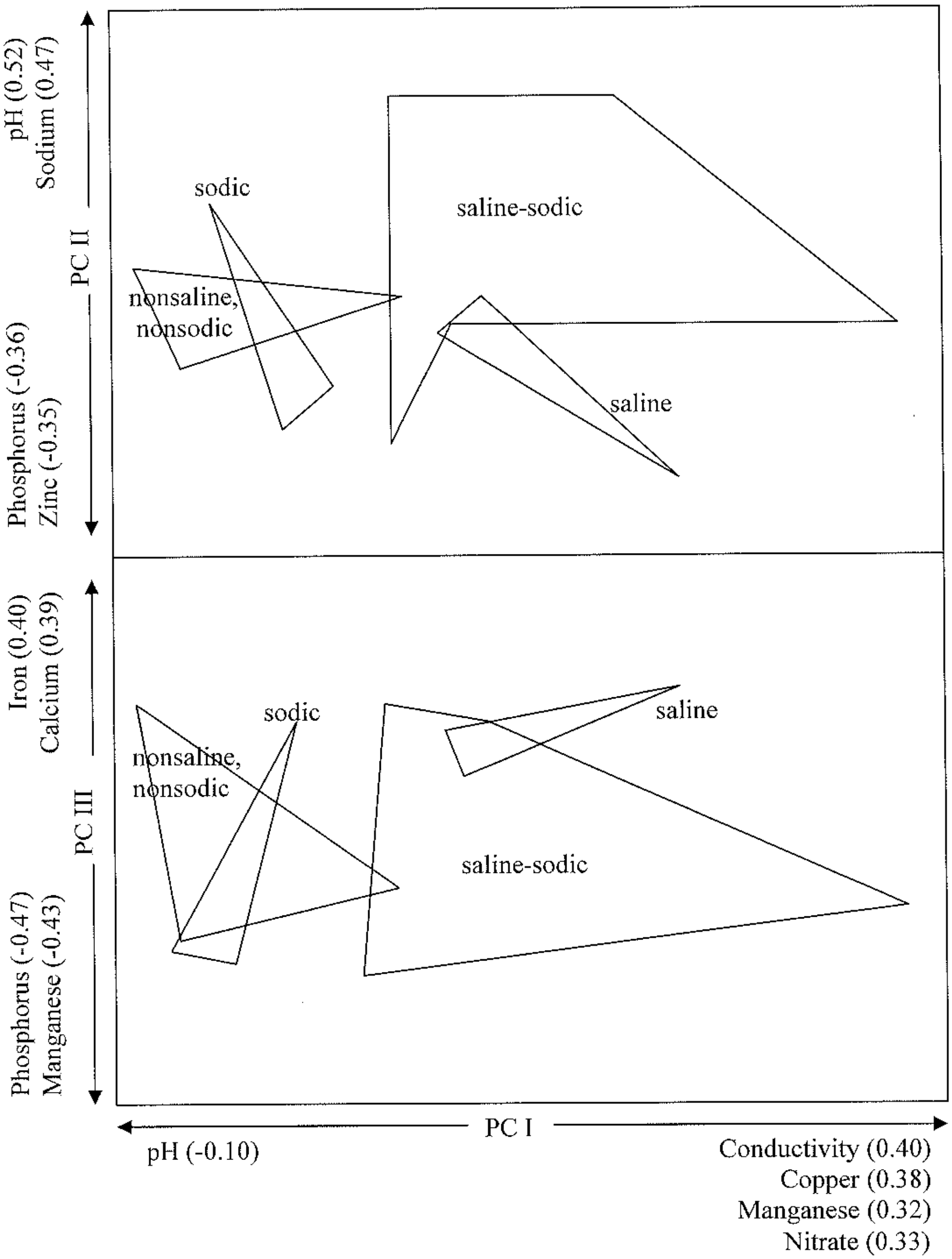


Figure 17. PCA soil parameters plot of PC axes I, II, and III for the 16 vegetation transects (includes the reintroduction site).

Analysis of Soils and Vegetation Data

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 Density of *Astrophytum asterias* section), and the thirteen parameters of the routine soil analyses of the 15 sites, as well as the pilot reintroduction site. Soil parameters of the routine soil analyses explained 45% of the variation in vegetation within the 16 vegetation transects. Eight of the 16 species analyzed in the CCA were clustered around the intersection of the CCA I and CCA II axes indicating no preferential association with a particular soil parameter (Fig. 18). 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.

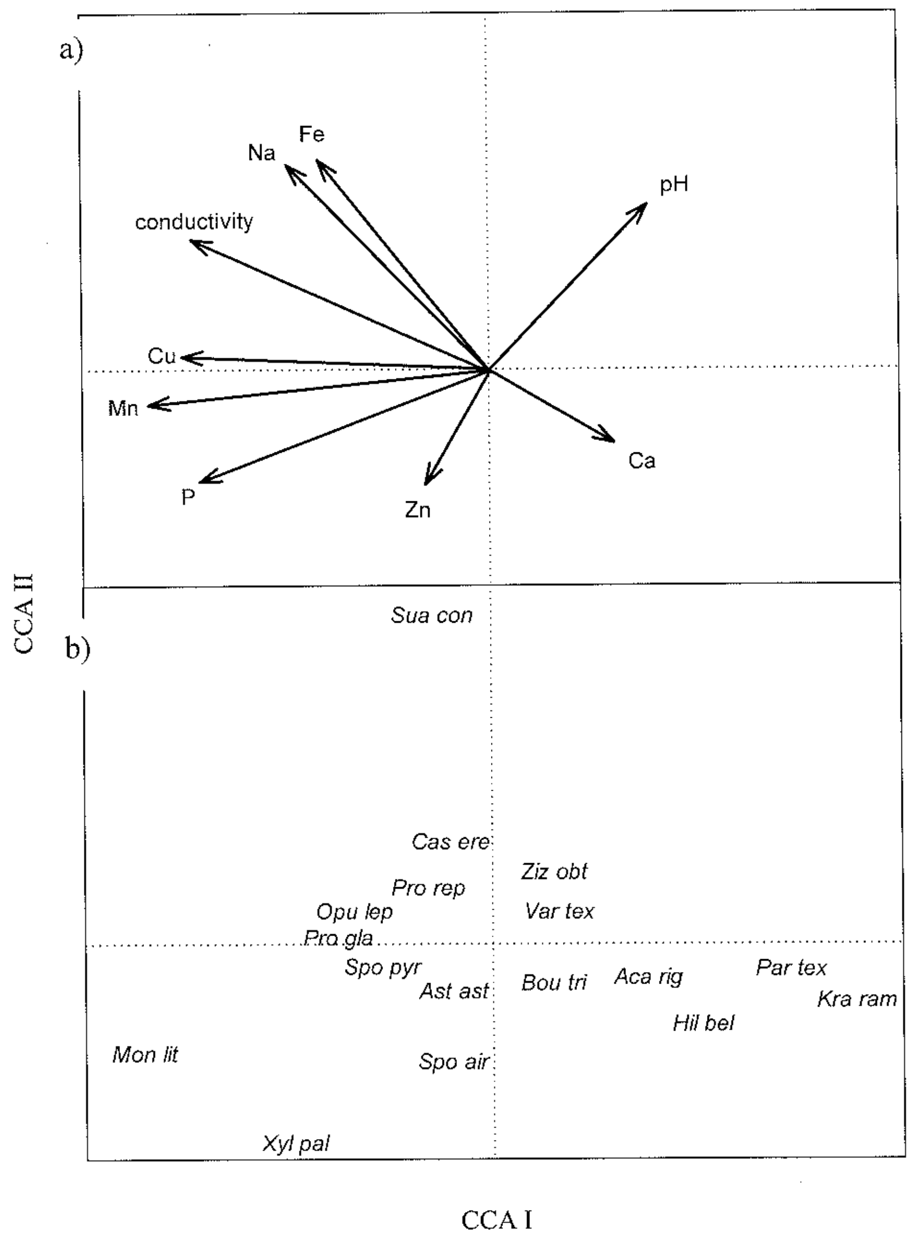


Figure 18. CCA biplot of (a) soil parameters and (b) 16 plant species with greatest dominance from the 16 vegetation transects (includes the reintroduction site). Species names are the first three letters of scientific binomial. Ten of the species are listed in Table 9; others are *Bou tri* = *Bouteloua 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. 18). 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. 18). *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. 18). Sites CA (Property 9) and EE (Property 7) which had the highest density of *A. asterias* were saline-sodic followed by sites LA (Property 4) and PP1 (Property 8) which were saline (Table 14). Site NC1 (Property 2) had the fifth highest density and was sodic. The nonsaline, nonsodic sites had some of the lowest densities (Table 14). At three sites no *A. asterias* were observed within the 150 m^2 area. At site RE (Property 2) three *A. asterias* were documented within one of the 2-m belt transects. Density of *A. asterias* was not calculated for site RE (Property 2) as it was intentionally located at the edge of a known subpopulation and therefore, would not constitute a valid measurement.

Table 14. Number and density of *A. asterias* documented in the three 2-m belt transects of the 15 vegetation transect sites, March and May 2006. Sites AM1, AM2, AM3 = Property 1; CA = Property 9; EE = Property 7; JB1, JB2 = Property 5; KR = Property 3; LA = Property 4; LM = Property 6; NC1, NC2 = Property 2; PP1, PP2 = Property 8.

Site	**# *A. asterias***	**density/m^2**
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 15). The association of *A. asterias* to each category was evaluated using a single factor analysis of variance (ANOVA) and Tukey's multiple comparison procedure. Data were log transformed as homoscedasticity was violated. The ANOVA 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 15). 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 16. Appendix A, Table A.6 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 15. 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 16. 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	

4) Phenology and Reproductive Biology Research

The purpose of this study is to provide fundamental information about the reproductive biology of *A. asterias* to support recovery efforts. The objective of this study is to answer three questions: (1) What are the phenological patterns in *A. asterias*? (2) What is the breeding system of *A. asterias*? (3) How do the findings affect the conservation strategies for *A. asterias*?

Phenology

From 2004 to 2007 phenological and reproductive capacity data were recorded at Property 2 and 7. Monitoring at both properties lasted throughout the year though data were collected more frequently in the spring of each year and only monthly the rest of the year (Table 17). From June 2006, the monthly monitoring occurred until the spring of 2007 when, beginning in March, monitoring was reduced to a biannual collection period.
In spring of 2006, two more transects were added on two additional properties (Property 8 and 9). These new transects were added to offset deaths occurring in the five previously established transects on properties 2 and 7, and were established in areas undiscovered before 2006.

Table 17. Spring phenological monitoring periods for Properties 2, 7, 8, and 9 from March 2004 through April 2006.

	Property 2	Property 7	Property 8	Property 9
2004	Mar 9 - Apr 29 *	Mar 11- Apr 29 +	N/A	N/A
2005	Mar 17 - May 11 +	Mar 17 - May 11 +	N/A	N/A
2006	Mar 13 - Apr 26 ‡	Mar 13 - Apr 26 ‡	Mar 13 - Apr 26 ‡	Mar 13 - Apr 26 ‡

Data collection time intervals: * Daily; +Every 3rd day; ‡Average of every 4th day

Anthesis began each year in mid-March (Fig. 19). There is an obvious peak in fruiting from April-July. Within this flowering period there was a few day period in the first week of April on Property 2 where a great number of flowers bloomed. In the first week of April in 2004 and 2005, respectively 39% (38/98) and 61% (33/54) of the total flowers for the year bloomed at Property 2. This peak in flowering was not as obvious or as consistent for Property 2 in 2006 where multiple peaks were observed in March and April.

On Property 7, an obvious peak was observed but did not always occur in synchronicity with Property 2 (Fig. 20). In 2004 43% (9/21) and in 2006 44% (4/9) of the flowering occurred in the last week of April. In 2005, 66% (4/6) of the flowering occurred in the first week of April.

Although 6.5 miles away from Property 7, 44% (4/9) of Property 8's flowering also occurred in the last week of April 2006.

Additionally, even though Property 9 is a mile from Property 2 and therefore closest of the four properties, 35% (7/20) of the flowering observed in 2006 occurred on one day in the third week of March. Throughout all of the flowering seasons and transects, smaller secondary flowering peaks were observed into July. There seems to be a synchronous bloom cycle that occurs among small geographically similar areas but not necessarily within a whole population.

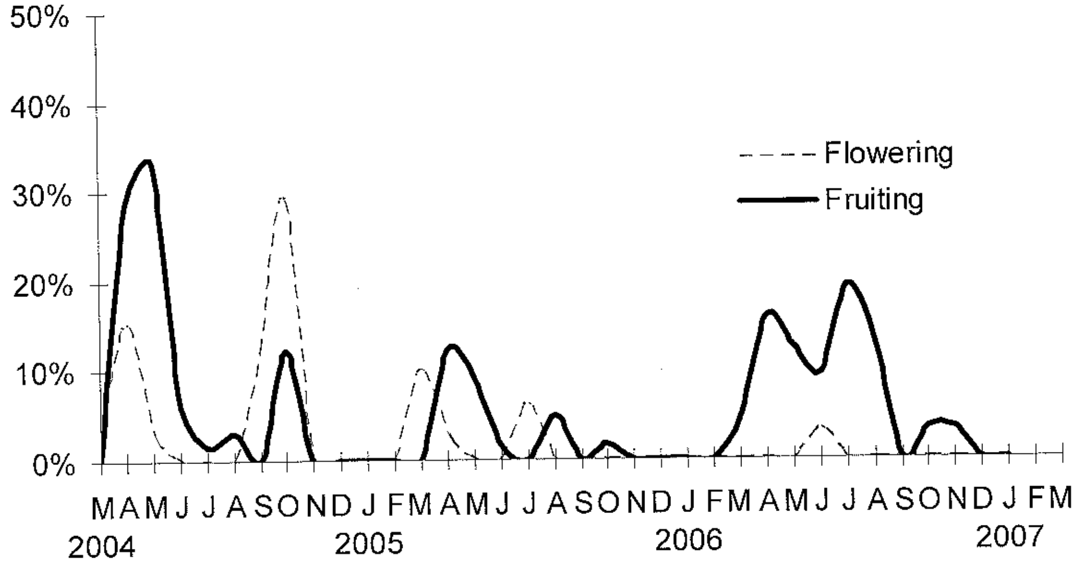


Figure 19. Percent flowering and fruiting individuals at Property 2 from March 2004 to March 2007.

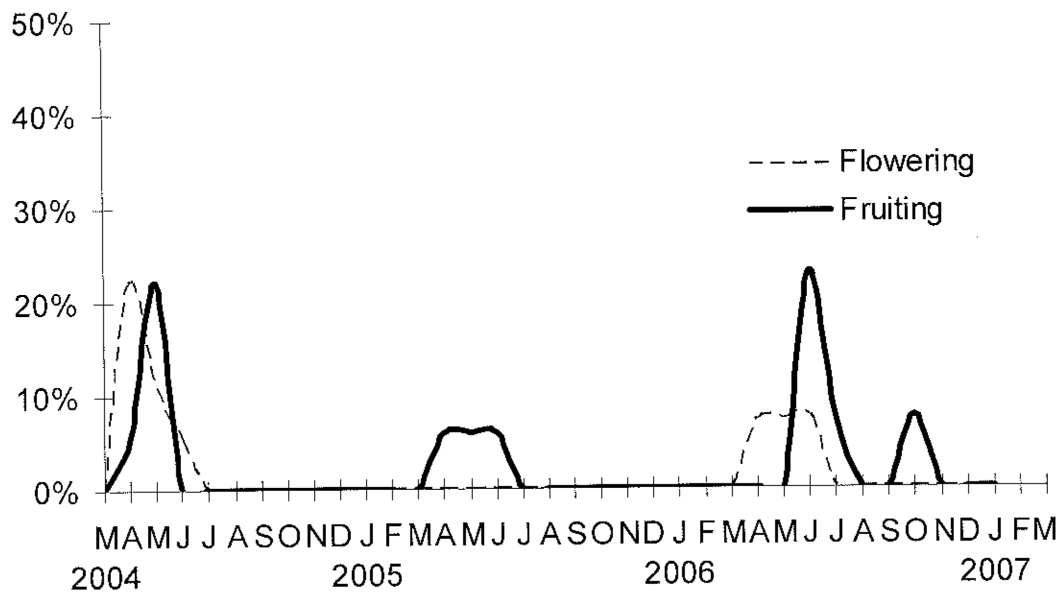


Figure 20. Percent flowering and fruiting individuals at Property 7 from March 2004 to March 2007.

Buds were first observed in February of all years and bud formation persisted into August or September. However, buds present later in the year occurred in smaller numbers compared to the spring buds. The timing of the bud production and anthesis is interesting since February and March follow a relatively long period of little rain. At the Rio Grande City NOAA weather station, March is the first month in five months that has on average much above an inch of rain. It has been shown that in several southwestern cacti, flowering is little influenced by water availability (Nobel 2002). This is not terribly surprising considering the water storage capacity of cactus stems.

Monthly monitoring misses blooms. Monthly data entries show fruits even though flowers were not seen the previous month. This is due to flowering and fruiting lengths; flowers last 1-3 days whereas fruits can last a couple of weeks. Since not all blooms are seen, the best gauge of reproductive output for a population is fruit presence.
Fruiting can occur from March to October depending on various environmental factors between years and properties. However, there is still a definite peak of fruiting in the late spring to early summer.

Research on the reproductive biology of star cactus was conducted at Property 2 and Property 4 (located within a mile of each other). Field studies included:

- documentation of timing of floral and fruit development
- documentation of fruit set and seed production in the field
- determination of the breeding system
- determination of the presence of pollen limitation
- determination of the optimum outcrossing distance
- observation and collection of insect visitors
- test pollinator effectiveness
- documentation of associate cacti insect visitors

Timing of floral and fruit development

Most flowers in 2004 opened two consecutive days or for a single day; however, some flowers opened for a three-day period (Fig. 21). This information could not be assessed for 2005 and 2006 due to the discontinuous collection of data.

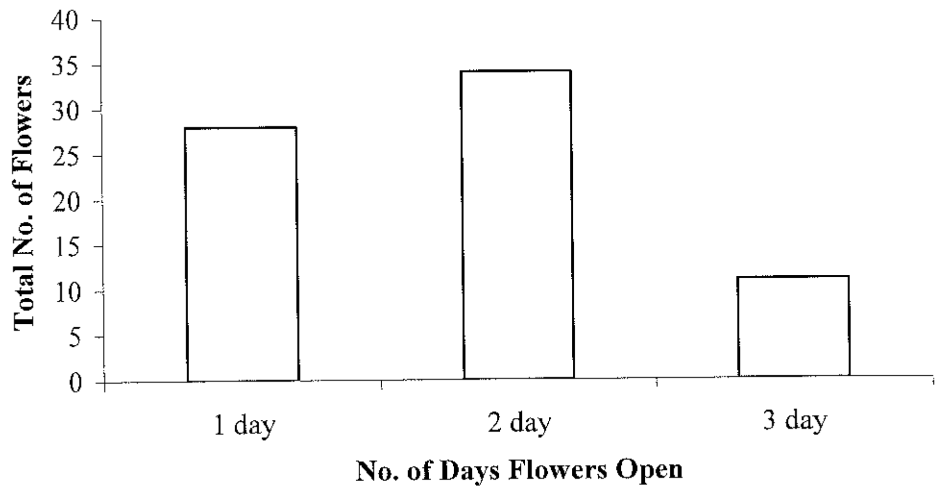


Figure 21. Period of time a flower opens during anthesis. Data collected from 98 *Astrophytum asterias* flowers within the Transects 1, 2N and 2S in Property 2 population, spring 2004.

The average reproductive capacity changed at Property 2 (Transects 1, 2N and 2S) and Property 7 from 2004 to 2006 (Fig. 22). In 2004, plants monitored (n=86; this number for all following properties was derived from plants that lived through a majority of the flowering season) in the Property 2 population produced a total of 98 flowers of which 42 developed into fruits, giving a 42.9% fruit set. Plants monitored (n=40) in the Property 7 population produced a total of 23 flowers of which 10 developed into fruits, giving a 43.5% fruit set. In 2005, plants monitored (n=105) in the Property 2 population produced a total of 54 flowers of which 19 developed into fruits, giving a 35.2% fruit set. Plants monitored (n=40) in the Property 7 population produced a total of 6 flowers of which 4 developed into fruits, giving a 66.7% fruit set. In 2006, plants monitored (n=32) in the Property 2 population produced a total of 26 flowers of which 16 developed into fruits, giving a 61.5% fruit set. Plants monitored (n=43) in the Property 7 population produced a total of 10 flowers of which 9 developed into fruits, giving a 90.0% fruit set.

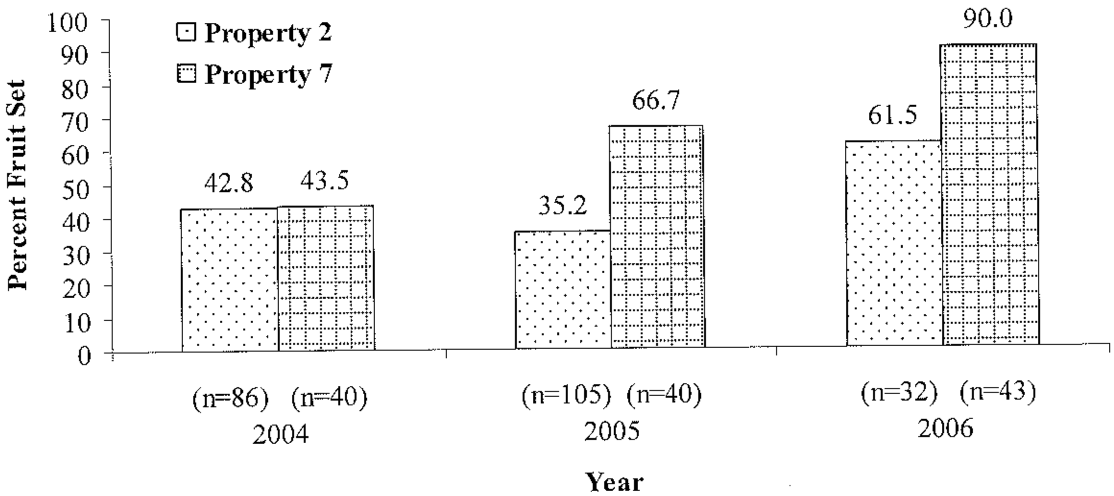


Figure 22. Percent fruit set of *Astrophytum asterias* recorded at Property 2 and Property 7 transects from 2004 to 2006 (number of plants monitored in parentheses).

The two new transects established in 2006 were not analyzed for differences between mean number or total numbers of flowers and fruit produced since only one year of data exists. However, plants monitored (n=44) in the Property 8 population produced a total of 8 flowers of which 7 developed into fruits, giving a 87.5% fruit set (Fig. 23) and plants monitored (n=55) in the Property 9 population produced a total of 20 flowers of which 8 developed into fruits, giving a 40.0% fruit set (Fig. 23).

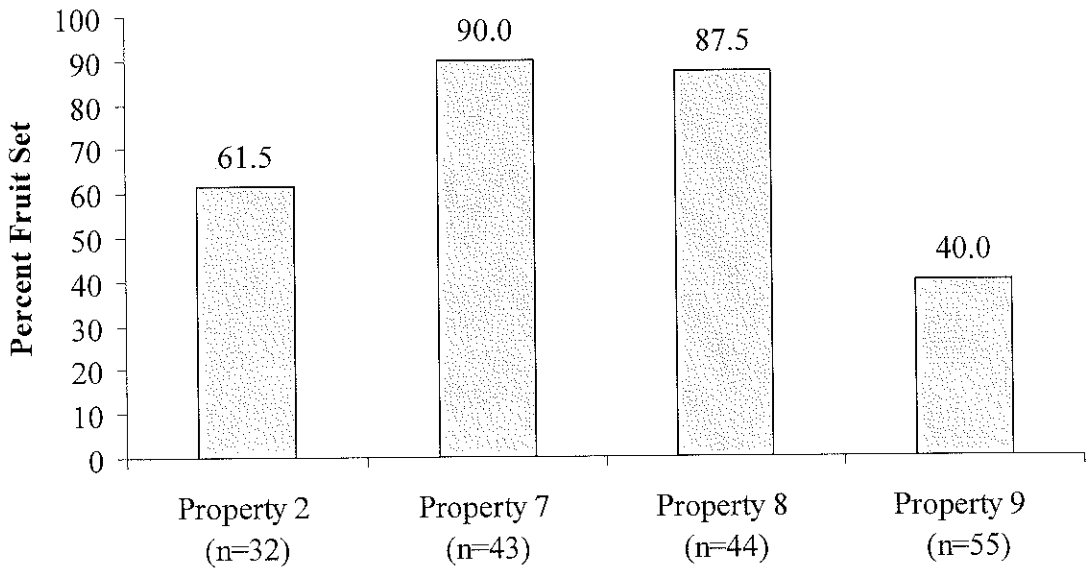


Figure 23. Percent fruit set of *Astrophytum asterias* recorded at Property 2, Property 7, Property 8 and Property 9 transects in March, April and May 2006 (number of plants monitored in parentheses).

Fruit and Seed Production

Flowers and fruit of 19 plants from all three transects at Property 2 were used for a comparison in 2004, 2005, and 2006 (one plant at Transect 1, five plants at Transect 2N and 13 plants at Transect 2S). No significant difference was detected between years in flower or fruit number at Property 2 (Figs. 24, 25). Similarly, an analysis was conducted on 11 plants from Property 7 for comparison of flowers and fruit among the three years and again no significant differences were detected (Figs. 26, 27). Results of a Single Factor Repeated Measures ANOVA for number of total flowers produced by each plant at Property 2 show no significant difference between years ($p=0.0615$; F-value=3.71990; df=37). The same was found for total number of fruit produced by each plant at Property 2 ($p=0.5706$; F-value=0.32743; df=37) and total number of flowers ($p=0.2288$; F-value=1.53659; df=21) and fruit ($p=0.1237$; F-value=2.57143; df=21) produced by each plant at Property 7 among years.

The mean number of flowers/plant ranged from 1 to 2 in 2004, 2005 and 2006 (Figs. 25, 27). Mean number of fruit/plant ranged from 0 to 1 in 2004, 2005 and 2006 (Figs. 25, 27). An average of seven days elapsed between the last day a flower was open and the onset of fruit set in 2004. However, period of time to set fruit was variable (range of 3-14 days) and determining if fruit was actually set was problematic at times.

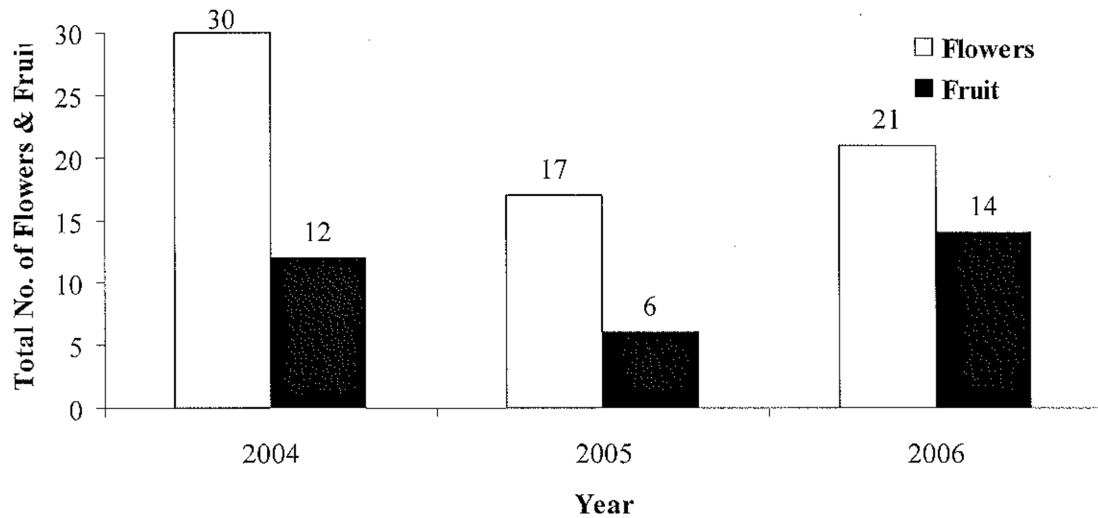


Figure 24. Total number of flowers and fruit produced by all plants in Transects 1, 2.N and 2.S at Property 2 in 2004, 2005 and 2006.

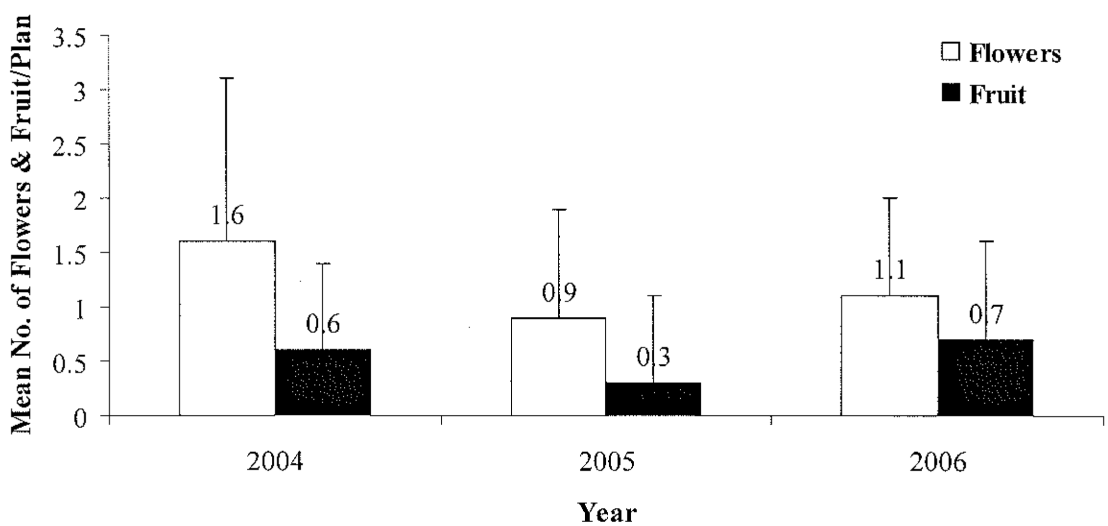


Figure 25. Mean number of flowers and fruit produced by all plants in three transects at Property 2 in 2004, 2005 and 2006.

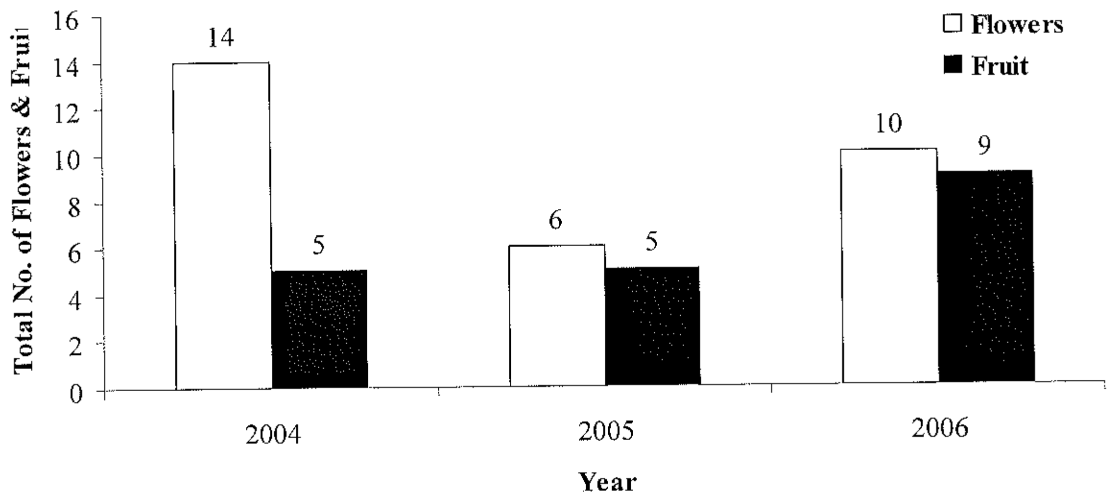


Figure 26. Total number of flowers and fruit produced by all plants in the transect at Property 7 in 2004, 2005 and 2006.

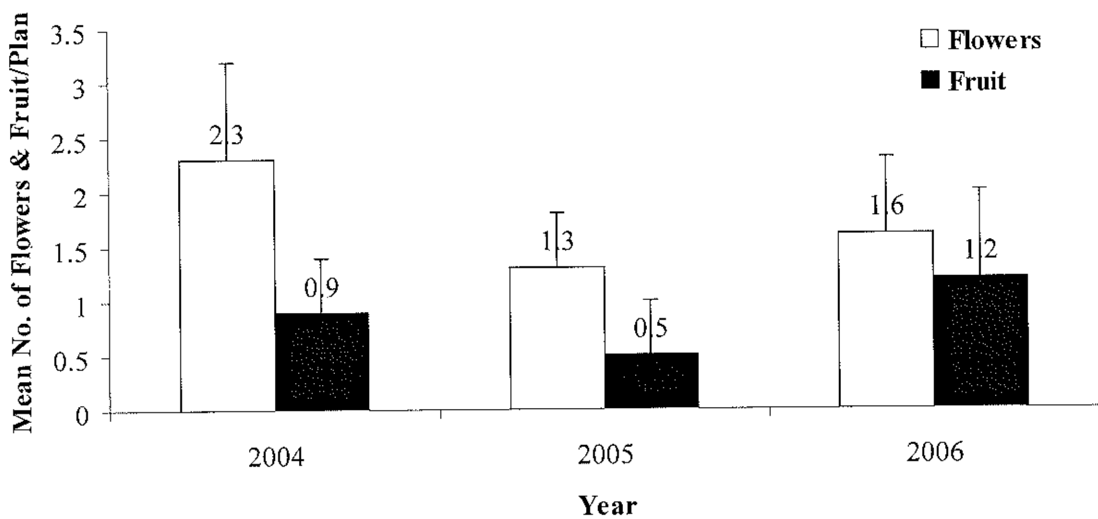


Figure 27. Mean number of flowers and fruit produced by all plants in the transect at the Property 7 in 2004, 2005 and 2006.

A correlation was run to examine the relationship between plant diameter and flower production using 98 individuals in the Property 2 population (Fig. 28). Individual plants monitored daily from March 9 – April 29, 2004 were scored based on production of flowers. Individuals that produced flowers during this time period were given a value of one, while individuals that did not produce flowers were given a value of zero. The mean diameter of non-reproductive individuals (n=61) was 21.7mm, with a range of 3.59-70.45mm. The mean diameter of reproductive individuals (n=48) was 61.1mm, with a range of 35.4-96.13mm. A Spearman's Correlation revealed a moderately strong relationship between plant size class (based on diameter) and maturation to a reproductive stage (Fig. 28). Several of the non-reproductive individuals that had attained large diameters may not have flowered due to shading rather than due to juvenile life history stage. Other factors that may result in mature individuals not flowering include poor plant vigor, limited nutrient availability, and limited availability of soil water.

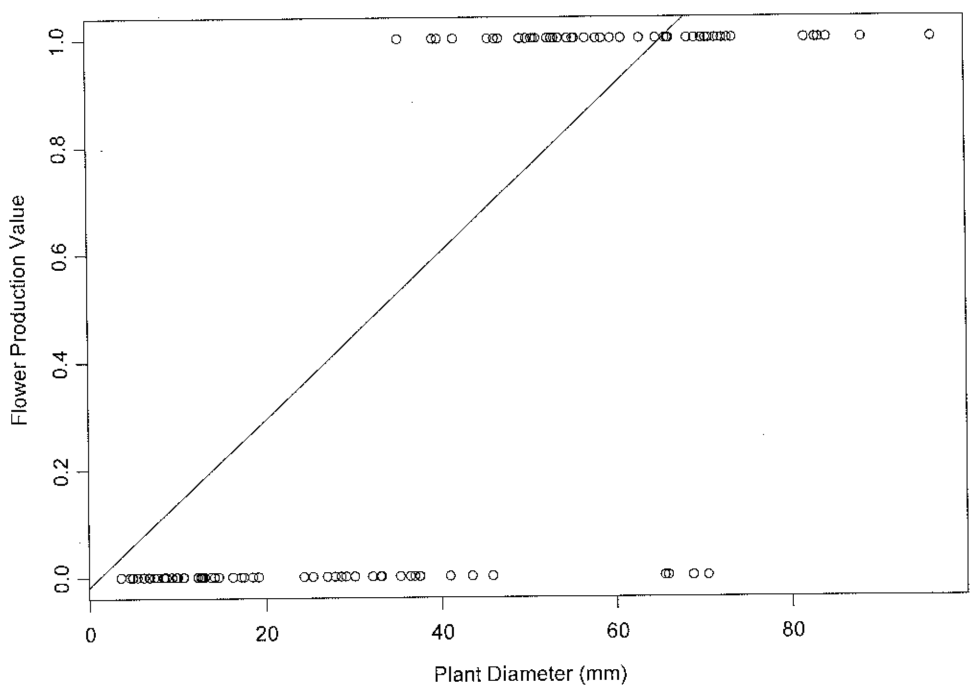


Figure 28. Correlation of flowering vs. plant diameter of *Astrophytum asterias* individuals within transects at the Property 2 population, March 9 – April 29, 2004. Plants observed to produce flowers are given a value of one, those not observed to produce flowers are given a value of zero (Spearman's Correlation; r^2=0.78; P<0.000001).

Reproductive Biology

Breeding system

The breeding system was experimentally examined. There were four treatments and a control. Treatments included facilitated autogamy (plants were bagged and flowers were self-pollinated by hand), non-facilitated autogamy (plants were bagged and flowers were allowed to passively self-pollinate), facilitated emasculated xenogamy (plants were bagged, stamens were removed and flowers were cross-pollinated), and geitonogamy (flowers were bagged and pollinated using pollen from another flower on the same individual plant). Controls were not manipulated and left to open pollinate. Tanglefoot was applied at the base of bags to prevent crawling insects from visiting flowers. The number of fruit set in each treatment and the control were recorded. The number of seed set per fruit was also recorded.

Fruit and seed only resulted from controls and the xenogamy treatment (Fig. 29). These results show that the species is an obligate outcrosser. Seed set results show that only facilitated emasculated xenogamous crosses are significantly different from all other treatments (Fig. 30). The significantly greater seed set resulting in the experimental outcrossing performed by hand vs. the controls left to open pollinate may be correlated to pollinator limitation or to environmental factors affecting pollinator behavior. Further studies are needed to make definitive conclusions.

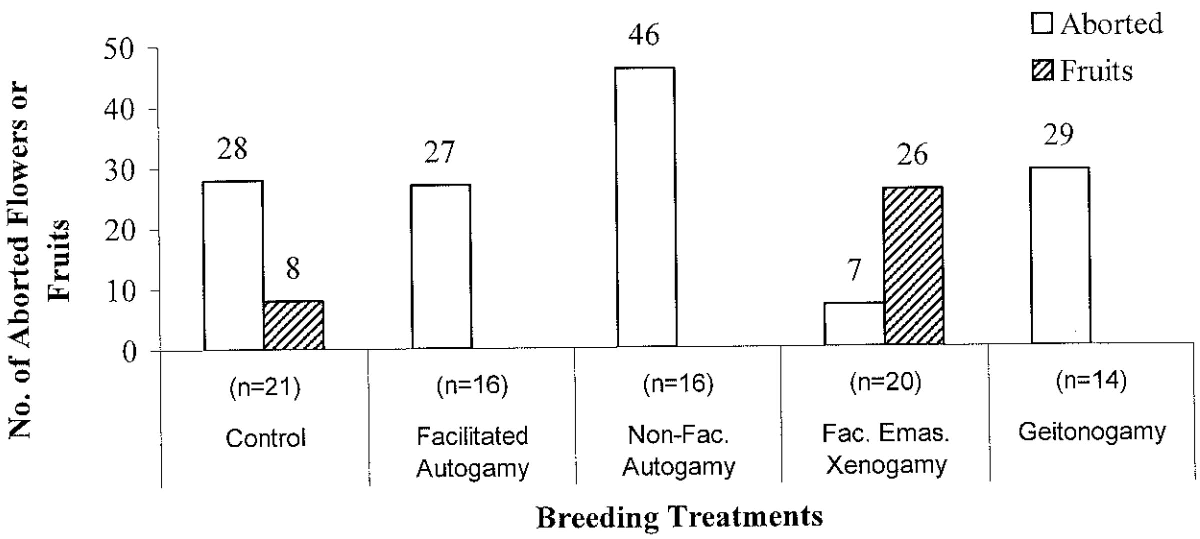


Figure 29. Number of aborted flowers and fruits resulting from facilitated and non-facilitated autogamous treatments, facilitated emasculated xenogamous treatment, geitonogamous crosses, and controls of *Astrophytum asterias* plants within the Property 2 population, spring 2004 (sample size in parentheses).

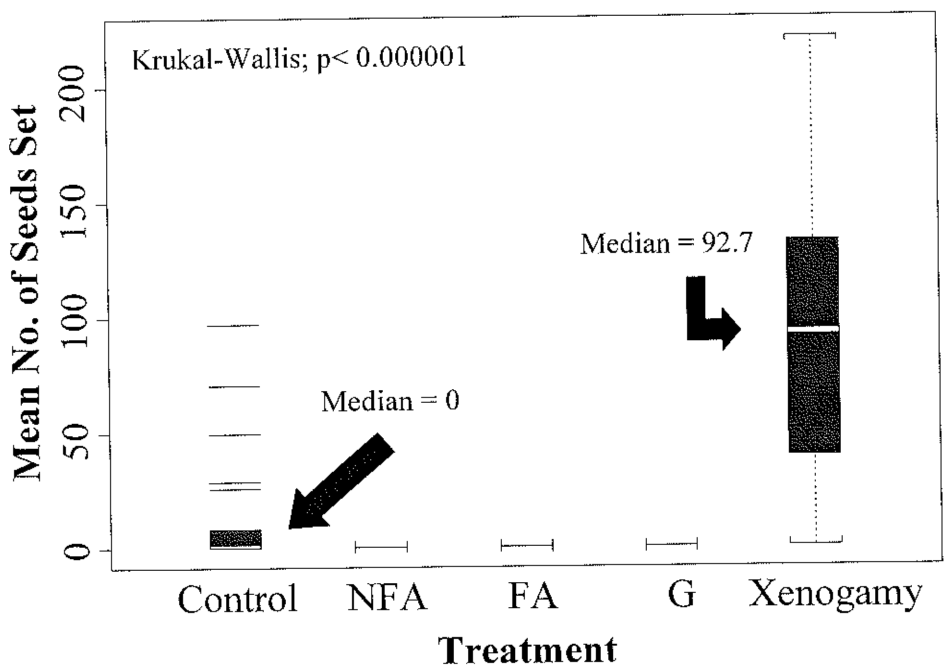


Figure 30. Median seed set/plant of *Astrophytum asterias* plants in facilitated (FA) and non-facilitated autogamous treatments (NFA), facilitated xenogamous treatment (Xenogamy), geitonogamous (G) crosses, and controls within Property 2 population, spring 2004. Statistical difference only exists for xenogamy treatment compared to all other treatments (Kruskal-Wallis; $p<0.000001$).

Pollinator limitation

An experiment was conducted to assess the extent of pollinator limitation. Seed set in two flowers on an individual plant (n=20 plants in Property 2 population) were compared. One flower served as the control and was not manipulated. The other flower was hand pollinated with pollen from another individual in the population (xenogamous cross). Neither flower was bagged, so both were available for pollinator visitation. Not only did the quantity of fruits from hand-pollinated crosses significantly increase compared to controls (Fig. 31), but the quality of those crosses was significantly different and fruits set more seeds than controls (Fig. 32). These data suggest that pollen limitation results in a lower seed set than the plant is capable of producing. Pollen limitation, therefore, may place constraints on fecundity.

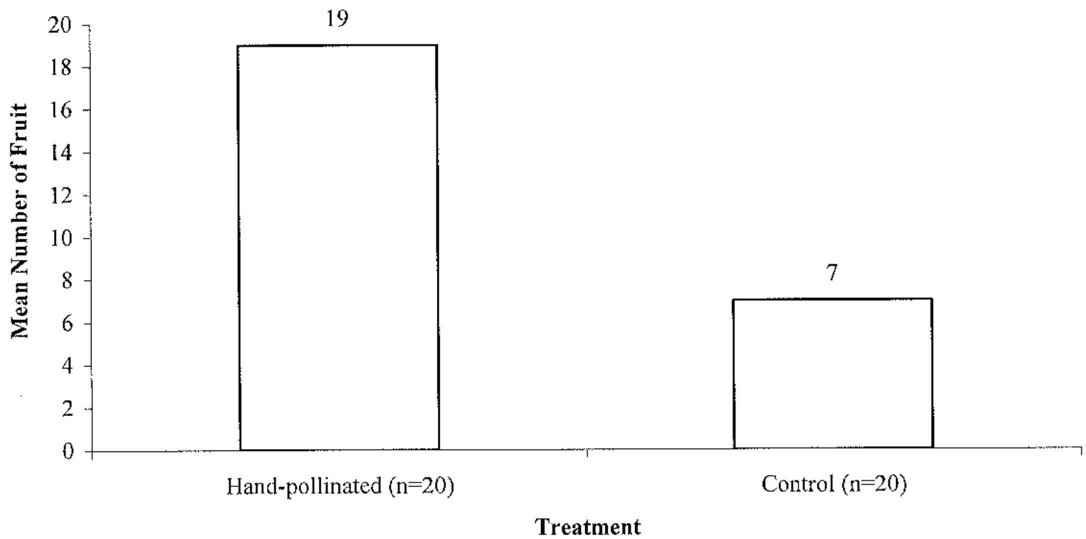


Figure 31. Comparison of fruit set by individual plants (n=20 plants) in which one flower was open pollinated (control) and one flower was experimentally outcrossed by hand (facilitated xenogamy) (Wilcoxon Signed Rank Test; p=0.0004).

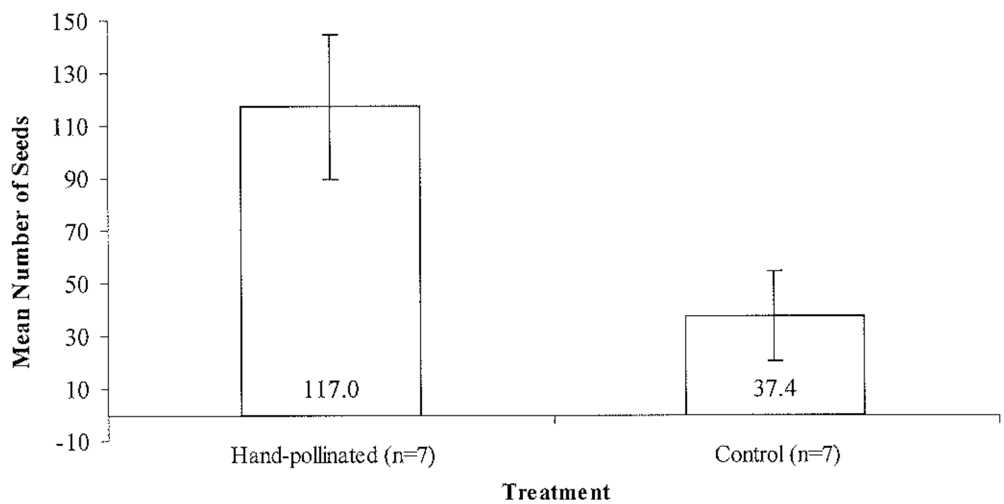


Figure 32. Comparison of seed set by individual plants (n=7) in which one flower was pollinated (control) and one flower was experimentally outcrossed by hand. Graph shows only plants that resulted in fruit for both the control and hand-pollination (Single Factor Repeated Measures ANOVA; p=0.0499).

Outcrossing distance

The distance between the plant serving as the pollen donor and the hand-pollinated plant was determined in the xenogamous crosses. These data were compared to determine if there is a relationship between distance and seed set. Several studies have analyzed the relationship between seed set and proximity of individuals to demonstrate that shorter distances result in fewer seeds set (Waser and Price 1983, Schemske and Pautler 1984). Distances ranged from 1 meter to approximately 200 meters. Regression analysis of these data show that distance of parental plants has no significant effect on seed set of maternal parent in 2004 (p-value is 0.1427; r^2=0.122). However, distances classes were clustered around 1-15 meters (n=14) with three crosses conducted on plants 20-30 meters apart and only two crosses over 200 meters.

In 2006, the relationship between outcrossing distance and seed set was further examined in a larger study involving 104 crosses. Plants were opportunistically chosen according to distance between pollen donor and recipient plants. Distances classes (and sample sizes) were: 0-10.9 meters (n=26), 11.0-30.0 meters (n=26), ~140-175 meters (n=26), ~6440 meters (n=26). The distance class of ~140-175 meters represents crosses made across a population located on one property. The furthest distance class of ~6440 meters (~4.0 miles) represents crosses between Property 8 and Property 4 in Starr County. Crosses were conducted on four different days with sufficient blooms to conduct complete sets of treatments on both properties. Results show that there is a significant difference between distance and seed set; the date crosses were made and seed set; and distance, seed set, and cross dates (Table 18).

Table 18. Fruit set and average seed set for outcrossing distance treatments conducted March 30, April 3, April 15 and April 25, 2006 at Property 8 and Property 4.

Distance Class (m)	**0-9.9**	**10.0-30.0**	**~140-175**	**~6440**
Fruit Set	50.0%	65.4%	57.7%	61.5%
Average Seed Set	27.5	38.3	30.4	23.3
p-value of seed set and distance	<0.000000001			
p-value of seeds set and date crossed	<0.000000001			
p-value of distance, seed set, and date crossed	0.0514			

Insect visitors

The purpose of this study is to determine the effectiveness and importance of various floral visitors to the pollination of *A. asterias*. This study specifically examines female fecundity; male reproductive success (exporting pollen, fertilizing ovules) is not examined. The following questions are addressed: (1) What are the visitation frequencies of various insect species to flowers of *A. asterias*? (2) What visit and visitor characteristics effected fruit set and seed set? (3) Do floral visitors differ in their effectiveness in causing pollination and what characteristics of pollinators are correlated with effectiveness? And (4) based on their visitation frequency and pollinator effectiveness, which pollinators are most important to the pollination of *A. asterias*? The answers to these questions will be used to inform management officials working to recover *A. asterias*.

A preliminary investigation of pollination biology was conducted in 2004 at Property 2. Floral visitors were collected and identified. Two orders of insects were collected: Coleoptera and Hymenoptera. Insects belonging to the Coleoptera order that have been identified to genus or species include: *Carpophilus* sp. (n=1), *Euphoria kerni* (Haldeman) (n=3) and *Acmaeodera* sp. (n=4). Insects belonging to the Hymenoptera have been identified to genus or species and include: *Macrotera lobata*, (n=5), *Lassioglossum* sp. (n=2), and *Osmia subfasciata* (n=1).

A more in-depth investigation of pollination biology was conducted in 2005 at a population found in 2004 (Property 4). Each day flowers were open, insect visitation was observed for 10 minutes at each flower. An attempt was made to capture all visitors. Date, time of day, visitor description, and floral organ of contact were recorded. All uncaptured visitors were recorded, a description given, and noted as not captured. Flowers were observed open between 10:00am and 7:00pm. Twenty species of insects, four orders, and 276 individuals were observed visiting star cactus within a 3,130-minute observation period. Twelve hymenopteran (n=81), six coleopteran (n=126), one formicid (n=75), and one syrphid (n=2) species were observed over a 52 day period (Fig. 33 & Appendix B). Flowering occurred on only 11 of the 52 days of observation.

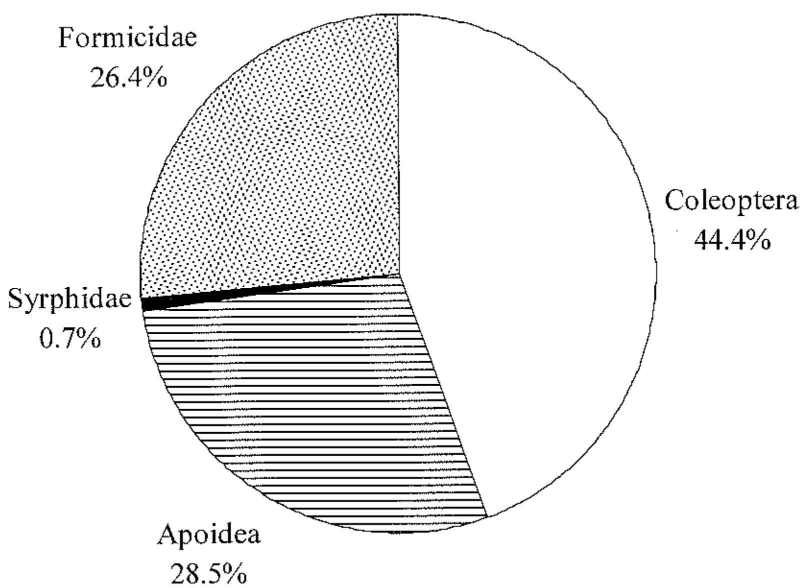


Figure 33. Percentage of total individuals of insect taxa visiting star cactus March 17 – May 7, 2005 at Property 4.

Over all flowers observed, the Apoidea had a visitation rate of 0.51/10 minute period or 3.06 bees/hour. Two species, *Macrotera lobata* (Timberlake) and *Ashmeadiella maxima* (Michener), made up 40.0% of all bees visiting star cactus (Fig. 34). Fifteen bee species accounted for 27.1% of the visits. Unidentified bees (32.9%) made the remaining visits.

Macrotera lobata and *Diadasia rinconis* are cactus specialists and all other bees are generalists (J. Neff, pers. comm.). Most of the bees are common in south Texas except for *Macrotera lobata* and *Dianthidium discors* which are rare in collections (J. Neff, pers. comm.). Common species like *Macrotera lobata* may add more toward pollination just in sheer numbers compared to rarer bee species which are better at pollinating (Jennersten and Morse 1991); however, Thomson and Thomson (1992) caution that an area saturated in common yet ineffective pollinators might never equal the quality of an effective pollinator.

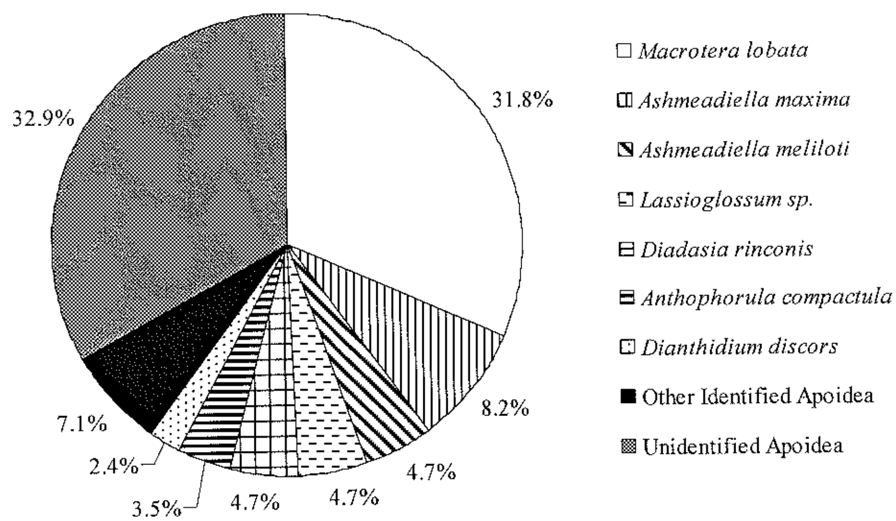


Figure 34. Percentage of total individuals of Apoidea visiting star cactus March 17 – May 7, 2005 at Property 4.

Rate of peak visitation for bees was between 12:00 and 2:00pm with a secondary peak between 5:00 and 6:00pm. Rate of peak visitation was 0.58/hour for *Macrotera lobata* between 12:00 and 2:00pm (Fig. 35). *Ashmeadiella meliloti, Ashmeadiella maxima* and *Diadasia rinconis* account for identified bee visits during the hours between 4:00 and 7:00pm (Fig. 35). Throughout the whole season *Macrotera lobata* had the highest visitation rate at 1.1/hour (Fig. 36). All other bee visitors ranged between 0.47/hour and 0.05/hour (Fig. 36).

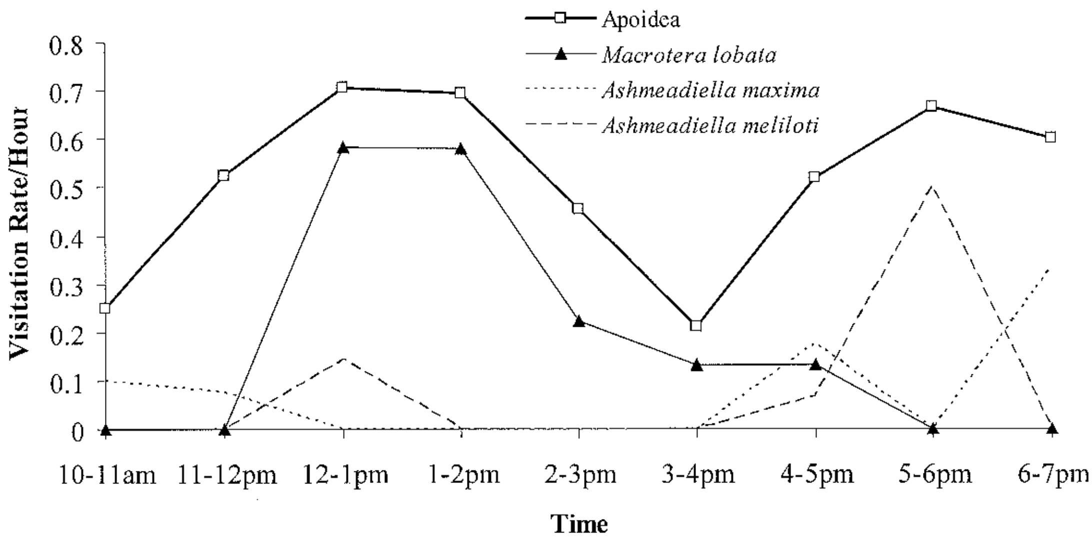


Figure 35. Mean hourly visitation rates of Apoidea and *Macrotera lobata* between March 17 – May 7, 2005 at Property 4.

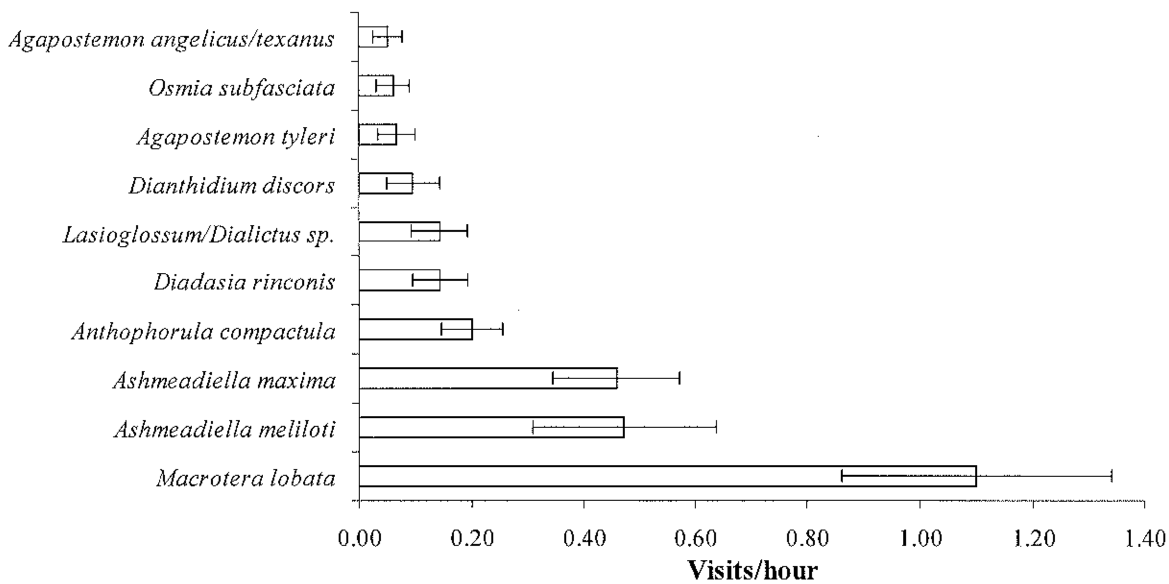


Figure 36. Mean visitation rate/hour of Apoidea between March 17 – May 7, 2005 at Property 4 (SD=1).

Bees appear to be the effective pollinator of star cactus. Other insects, including beetles, ants, and syrphid flies, do not appear to be effective pollinators of star cactus, and hence were not included in the previous figures. Due to minimal movement between flowers and lack of contact with the stigma in the flower, beetles were considered ineffective as star cactus pollinators. A similar observation has been made in other cacti species by several researchers (Grant and Grant

1979, Parfitt and Pickett 1980, McFarland et al. 1989). Ants, although a frequent visitor (27% of all visitors), were also considered ineffective due to distances traveled between flowers (>1 meter). It has been shown in other studies that ants secrete antibacterial and antifungal substances which have been shown to interrupt pollen germination and pollen-tube growth (Beattie et al. 1984, 1985). Since syrphids accounted for only 0.7% of all visits to star cactus, they were considered ineffective pollinators.

Pollinator effectiveness

Pollinator effectiveness was measured in spring of 2006 and 2007 to determine which floral visitors most successfully pollinate *A. asterias*. The study was conducted on Property 4. Treatment plants (n=73) were covered with a fine mesh bag prior to anthesis. On the day of flowering, bags were removed one at a time, and each flower was observed until it has been visited by an insect. Trained observers recorded the following variables for each observation: (1) species of the visitor, (2) date, (3) time of day, (4) duration of visit, (5) contact or no contact with the stigma, (6) landed on or did not land on the stigma when entering the flower. Once the insect had left the flower, the plant was re-covered with the mesh bag to prevent further pollination events. Additionally, the plants were covered with metal cages to prevent the herbivory of floral parts during fruit maturation. Once the fruits (n = 12) had matured, they were collected and the number of seeds for each fruit was recorded. Natural levels of fruit set and seed set were observed for open-pollinated control plants (n = 97) in order to determine the reproductive output of *A. asterias* under natural conditions and to provide a control with which to compare the effectiveness of various pollinators.

Pollinator effectiveness of each visiting insect species was calculated as the average seed set per visit (and percent seed set when possible) by each species. Mann-Whitney U tests were used to test the hypothesis that species differed from each other and from open-pollinated controls in their effectiveness at causing seed set. Additionally, tests of the difference between two proportions were used to determine whether or not visiting species differed from each other and from the open-pollinated controls in their ability to affect fruit set. Uncommon species and those whose visits did not result in fruit set were excluded from these analyses.

Visits from the bee *Diadasia rinconis* (n = 10) were found to be more effective in causing fruit set (95% C.I. of difference between proportions = 0.63 +/- 0.30) than those from the more abundant *Macrotera lobata* (n = 41) (Fig. 37). *Diadasia rinconis* was also more effective than *M. lobata* in terms of seed set per visit (U = 66, p < 0.001) (Fig. 38). Single visits to flowers by *Diadasia rinconis* did not differ significantly from open-pollinated controls in terms of fruit set (95% C.I. of difference between proportions = 0.09 +/- 0.30) (Fig. 37), seed set (U = 377.5, p = 0.450) (Fig. 38), or percentage seed set per fruit (U = 163, p = 0.483) (Fig. 39). Visits from *M. lobata* showed significantly lower rates of fruit set (95% C.I. of difference between proportions = 0.54 +/- 0.13) (Fig. 37) and seed set (U = 2762.5, p < 0.001) (Fig. 38) than controls.

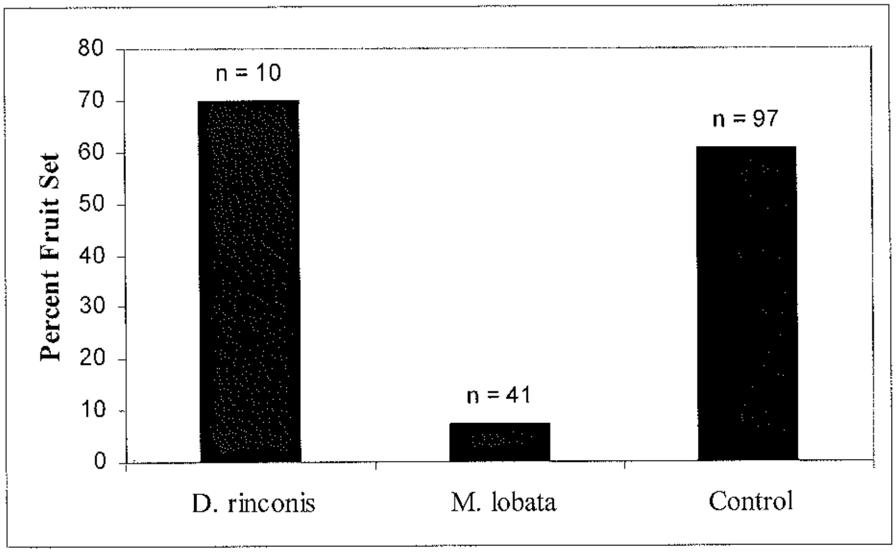


Figure 37. Percent fruit set by the two most abundant visitors to *Astrophytum asterias* compared to the open-pollinated controls.

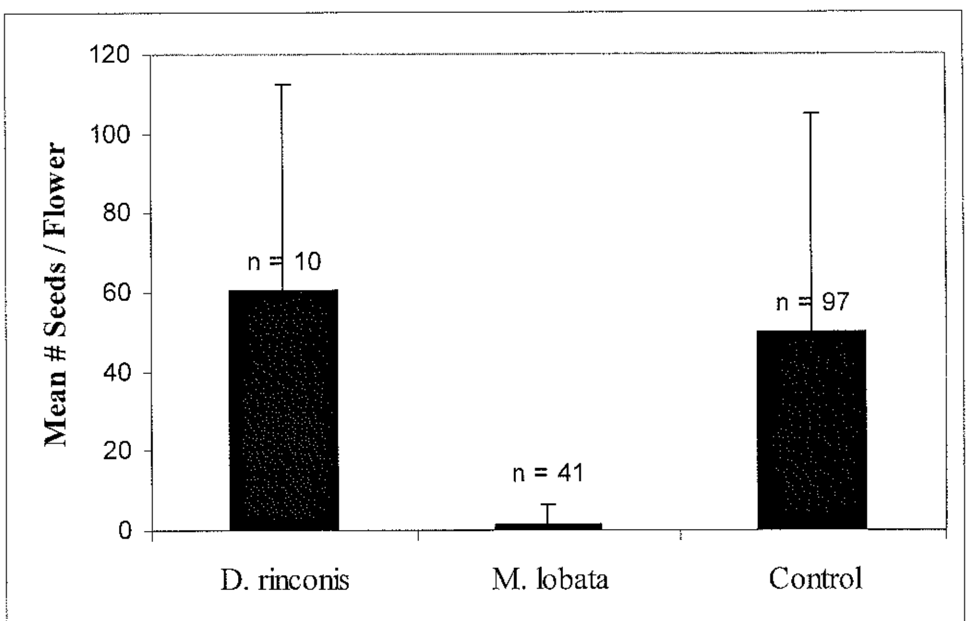


Figure 38. Mean number of seeds produced per flower visit by the two most abundant visitors compared to the open-pollinated controls. Error bars indicate 1 *SD*.

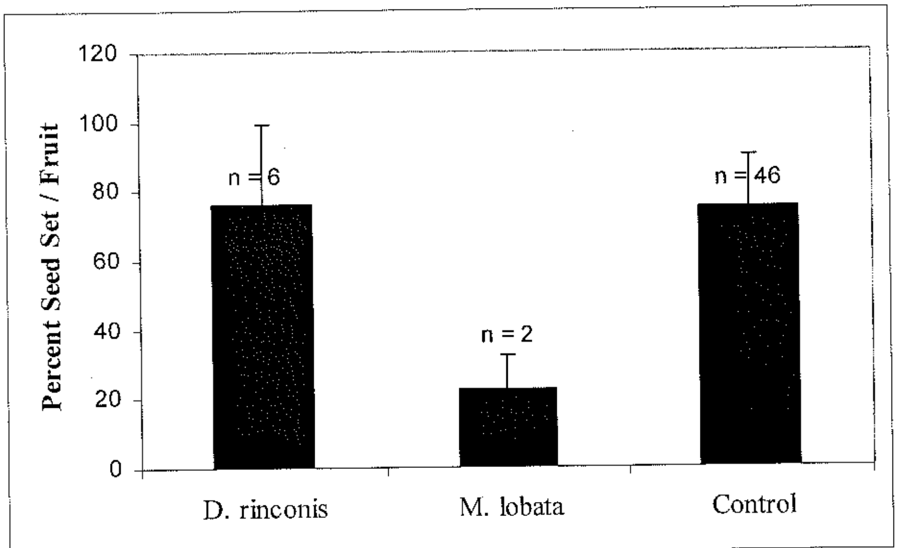


Figure 39. Mean percent seed set per fruit for the two most abundant visitors compared to open-pollinated controls. Error bars indicate 1 *SD*.

Visits from beetles (*Acmaeodera* sp. $n = 3$, and *Carpophilus* sp. $n = 2$) and ants (*Forelius mccooki* $n = 5$) did not result in fruit set and nothing about the behavior of these visitors suggests that they are effective pollinators of *A. asterias*. However, one visit from the bombyliid fly *Anthrax irroratus* ssp. *irroratus* ($n = 2$ visits) resulted in a fruit with 129 seeds. This is the first documented case of a fly successfully pollinating a cactus flower, and it highlights the necessity of empirically evaluating the effectiveness of all visiting species, even those that do not conform to the expectations of pollination syndromes.

Fruit set was not correlated with visit duration among insect species ($r = -0.43, p = 0.291, n = 8$). However, there was a strong positive correlation between fruit set and the proportion of visits in which a visitor landed on the stigma when entering the flower ($r = 0.94, p = 0.002, n = 7$). Thus, whether or not a visitor lands on the stigma is a more reliable predictor of effectiveness than visit duration.

Visitation frequencies of species were calculated as the total number of visits by each species divided by the total number of observation hours from the effectiveness study (20.78 hours). Pollinator importance was determined for visiting species by multiplying the effectiveness of a species (average seed set/visit) by its respective visitation frequency.

The small bee, *M. lobata,* had the highest visitation rate of any floral visitor (Table 19), but because of its low effectiveness, it is not considered the most important pollinator of *A. asterias*. Instead, the most important pollinator appears to be *D. rinconis*, despite having a lower visitation frequency (Table 19). Bees in the genus *Ashmeadiella* were relatively uncommon visitors in this study, but showed the potential to be effective pollinators and should thus be considered to have some limited importance to the pollination of *A. asterias* (Table 19). The bombyliid fly *A. irroratus* ssp. *irroratus*, was likewise a rare, but somewhat effective pollinator, and like

Ashmeadiella spp. should be considered to have some importance as a pollinator (Table 19). The full contribution of these last two visitors to the pollination services of *A. asterias* cannot be known from their limited number of visits in this study.

Table 19. Percent fruit set, seed set, visitation rates, relative visitation frequencies, and pollinator importance of visitors to *Astrophytum asterias*.

Visitor Species	Fruit Set (%)	Number of Seeds/Visit	Seed Set/Fruit (%)	Visits/ Hour	Relative Visitation Frequency	Pollinator Importance
Acmaeodera sp. (Coleoptera: Buprestidae)	0.00	0.00	0.00	0.14	0.04	0.00
Anthrax irroratus (Diptera: Bombyliidae)	50	64.50	*	0.10	0.03	6.19
Ashmeadiella spp. (Hymenoptera: Megachilidae)	17	8.17	89	0.29	0.08	2.36
Carpophilus sp. (Coleoptera: Nitidulidae)	0.00	0.00	0.00	0.10	0.03	0.00
Diadasia rinconis (Hymenoptera: Apidae)	70	60.70	76	0.48	0.14	29.20
Dialictus sp. (Hymenoptera: Halictidae)	0.00	0.00	0.00	0.19	0.05	0.00
Forelius mccookii (Hymenoptera: Formicidae)	0.00	0.00	0.00	0.24	0.07	0.00
Macrotera lobata (Hymenoptera: Andrenidae)	7	1.37	22	1.97	0.56	2.69
Control	61	50.07	75			

*percent seed set was undeterminable for some fruits that were damaged or deteriorated at the time of collection

The small bee, *Dialictus* sp. was both an infrequent and ineffective visitor, and should not be considered as an important pollinator of *A. asterias*. Similarly, *Acmaeodera* sp., *Carpophilus* sp., and *F. mccookii* were neither frequent visitors nor effective pollinators, and should not be considered important to the pollination of *A. asterias*.

Pollen dispersal

In the spring of 2007, a study was conducted using fluorescent dye as a pollen analogue to determine the extent of pollen dispersal within a population of *A. asterias*. This study was conducted on Property 4. During each bloom period, fluorescent powder dye was liberally applied to the anthers of 1-3 *A. asterias* flowers with a paintbrush using a different color of dye for each flower. Dye was applied shortly after anthesis. Between 24-48 hours after flowering, the stigmas of all *A. asterias* flowers (excluding the source flowers and the treatment and control flowers from the effectiveness study) in the study area were collected and stored in individual containers. Additionally, stigmas were collected from the flowers of all other species of cacti in the study area that were open during this experiment in order to determine whether or not individual pollinators visited multiple species. All stigmas were then observed in the laboratory under a microscope to determine which flowers received dye particles. The number of dye particles per stigma could not be reliably counted because dye particles tended to clump so stigmas were simply scored for the presence or absence of dye. The distance between donor flowers and all recipient flowers was then measured in the field, and these dye dispersal distances were used to calculate genetic neighborhood estimates (neighborhood size, area, and diameter) using the neighborhood model developed by Wright (1943, 1946, 1969).

The dispersal of fluorescent dye particles followed a leptokurtic distribution with a mean dispersal distance of 25.1m from source plants and an axially corrected variance of 153.2m. There were a total of 13 source plants and 69 recipient plants (*A. asterias*). Approximately 80% of all recipient plants were located within 30m of the source plant (Fig. 40). The longest dispersal event recorded was 142.2m. There were also dispersal events in which dye was transferred from the *A. asterias* source plant to the stigma of another cactus species (*Echinocereus reichenbachii* var. *fitchii* ($n = 2$), *Echinocereus enneacanthus* ($n = 2$), *Thelocactus bicolor* ($n = 1$) indicating that pollinators of *A. asterias* may visit multiple species of cacti in the same foraging bout.

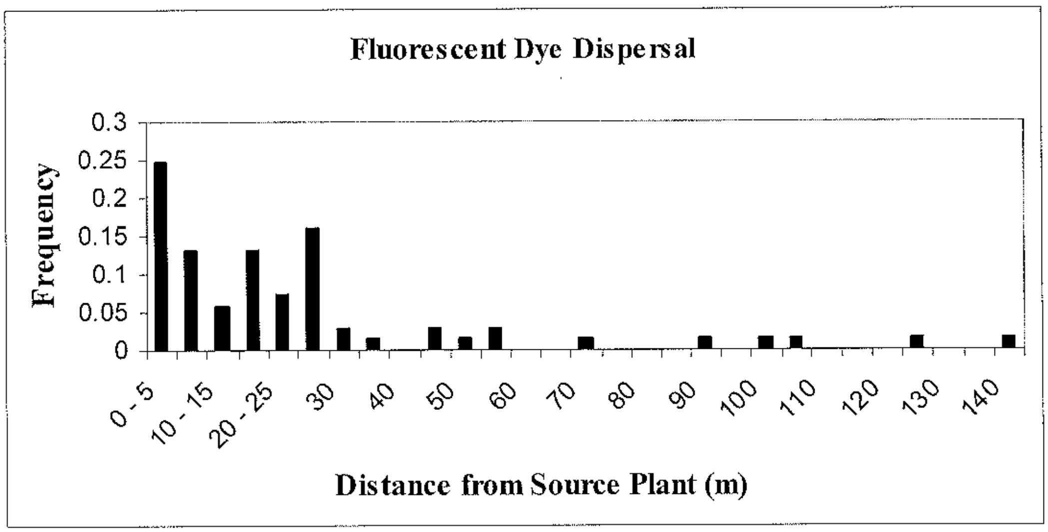


Figure 40. Frequency distribution of fluorescent dye dispersal distances.

Genetic neighborhood area adjusted for kurtosis was 1,876.5m^2 (~0.19 ha). Neighborhood size was 83.5 individuals with a 95% C.I. of 53.3 – 113.7 individuals. Neighborhood diameter calculated from the estimate of neighborhood area was 48.9m. These results suggest some level of population subdivision within the larger (1.9 ha, ~1146 individuals) patch due to restricted pollen dispersal.

Associate cacti insect visitors

In 2007, as a preliminary study, similar methods to the 2005 study were used to collect visitors at eight different cacti species that grow in association with star cactus. Cacti species were chosen purely depending upon flower availability at the time visitor data were collected. Table 20 shows the times spent collecting visitor data at each cactus species.

Table 20. Associated cacti species of *A. asterias* at Properties 2, 4, 9, 10, and 11 March to May 2007 and the hours spent collecting visitor data.

Associated Cactus Species of *A. asterias*	**Hours**
Ferocactus setispinus	5
Coryphantha macromeris var. *runyonii*	4.5
Echinocereus reichenbachii var. *fitchii*	4.5
Thelocactus bicolor	4
Echinocereus enneacanthus	3
Echinocereus pentalophus	1
Lophophora williamsii	1
Mammillaria sphaerica	1

Like the visitor studies conducted in 2005, there was a certain percentage from each cactus species that could not be caught or identified. These visitors were classified as unknown bees. As in *A. asterias*, *Macrotera lobata* was the most common visitor among all eight associate cacti species except for *Echinocereus reichenbachii* var. *fitchii*. *Diadasia rinconis* visited 75% of cacti species. Except for *Echinocereus reichenbachii* var. *fitchii* (where it was the most common visitor at 46%), *D. rinconis* was generally the second most common visitor (4% - 32%) at the six cacti species it visited. Bombyliids (bee flies) visited five of the eight cacti species and made up 1% - 14% of the visitors. Unknown bees accounted for 4%-22% of the associate cacti visitors that were monitored in 2007.

The difference between the 2005 *A. asterias* visitor data (Fig. 34) and the 2007 associate cactus species data (Appendix B) is that bee diversity was greatest at *A. asterias* (12 species) compared to 2-5 bee species at the other associate cacti. However, over 52 hours was spent at *A. asterias* and only 1-5 hours was spent at the associates. But in contrast, the visitation rate to *A. asterias* was higher than that of the eight associate cacti species. This could be because of environmental differences between the two years or differences between the different cacti species or because of the methodology.

Unlike 2005, bee flies were infrequent but general visitors since they visited several cacti species. Bee flies are hairy and therefore have the ability to more effectively collect pollen while visiting a flower. Pollination may be passive although bee flies are known to visit various species of plants for nectar or pollen or both (Deyrup 1988).

5) Propagation of Star Cactus Individuals for Founding Populations

Deviation from Proposed Research

One study objective was to utilize already propagated individuals of *A. asterias* [those housed at San Antonio Botanical Gardens (SABG)] in a pilot study to create an experimental population from which to learn transplanting and establishment techniques. This proved to not be feasible. At the onset of our study we met with Karl Hagenbuch, a botanist at SABG, to discuss using the plants as proposed. He informed us that only six *A. asterias* individuals remained at SABG. We are not aware of the fate of the other *A. asterias* plants that had previously been housed at SABG. Instead we propagated plants at the Lady Bird Johnson Wildflower Center (LBJWC) to use in the pilot reintroduction. Whereas the plants from SABG were of unknown origin, the plants propagated at LBJWC were from seed collected at Property 2.

Seed Collection and Propagation

Seed collection occurred at properties 1-9 (Table 21) in April-May 2004 and 2006. The 2004 seeds were deposited in 2005 with the Lady Bird Johnson Wildflower Center (LBJWC) in Austin, Texas. All seeds were transferred to the Desert Botanical Garden in Phoenix, Arizona in 2006 instead of the National Center for Genetic Resources Preservation in Ft. Collins, Colorado due to ease of transfer and ability to access seeds for future germinations. The repository of seeds are available, if necessary, for future population reintroductions or augmentations.

Table 21. Number of fruits and seeds collected at properties in 2004 and 2006.

Property	Collection Date	# Fruits	Avg No. Seed/Fruit
1	Apr-06	10	59
2	Apr, May-04	57	98
4	Apr, May-06	48	60
6	Apr, May-06	5	71
7	Apr, May-06	7	91
8	Apr, May-06	23	40
9	Apr, May-06	15	48

Seed collection for viability testing and propagations were done in accordance with the Center for Plant Conservation Guidelines (Falk and Holsinger 1991). Seed germination to test for viability is recommended in the literature (Dafni 1992) in addition to taking seed counts since variation between the two can occur. Therefore, seeds resulting from the breeding system and pollinator-limited experiments were germinated in January 2005 to determine viability. Three replicates of ten randomly chosen seeds from each fruit were weighed together, and an average weight was calculated for each replicate. A total of 1,563 seeds were placed on top of a 30:70 Sunshine general/universal potting mix: sand mixture and pots were arranged into blocks by treatment type within each tray. The trays were placed in a Sherer DualJet walk-in growth chamber set at 25-30° C at the University of Texas at Austin. The day the trays were placed in the chamber and initially watered was considered Day 0. Each day the trays were checked for germinated seeds and randomly rearranged within the chamber to reduce differences caused by chamber effects. Number of seeds germinated and percent germination per day was recorded. At the end of 18 days, all ungerminated seeds were tested for viability with tetrazolium to determine if lack of germination was due to non-viability or germination procedure (Baskin and Baskin 1998). Because of extreme variability in extent of staining with tetrazolium, all seeds that did not germinate were considered non-viable. The first seeds germinated on Day 3, between 72 and 96 hours after the first watering. The most seeds to germinate in one day were 284 (18.2%) by Day 5. By Day 18, 75.02% of the seeds had germinated (Fig. 41).

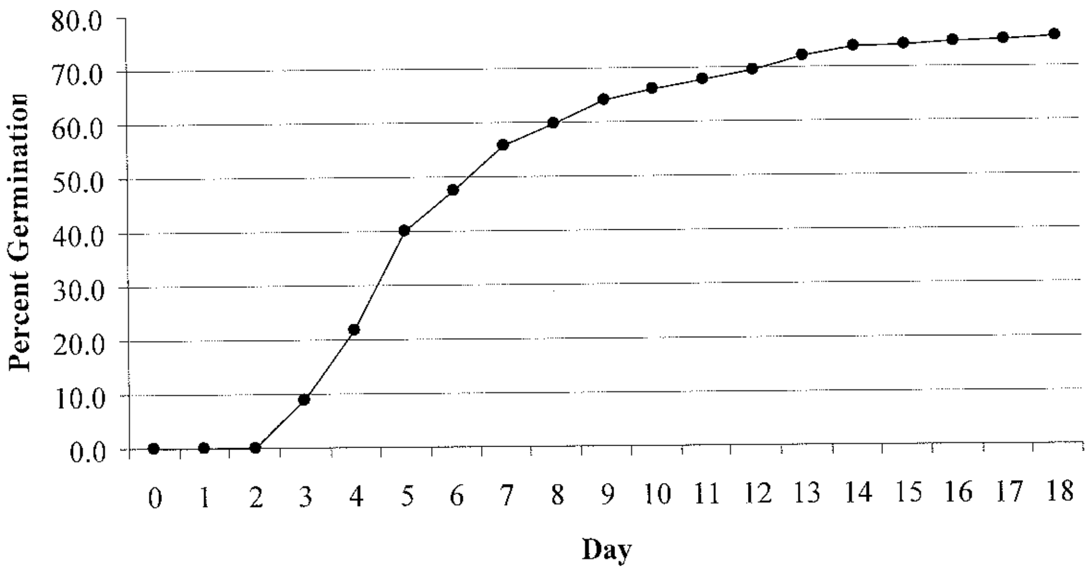


Figure 41. Percent germination of *Astrophytum asterias* seeds resulting from breeding system and pollinator-limited experiments (n=1,563 seeds).

After germinations were conducted, percent germination of seed from breeding system and pollinator-limited experiments was compared to determine any difference between seed viability in control treatments versus hand-pollinated treatments. In both tests (Kruskal-Wallis for the breeding system and Single Factor Repeated Measures ANOVA for the pollinator-limited experiment), a p-value above 0.05 resulted in no significant difference between viability of seeds pollinated naturally or by hand. P-values were 0.1779 and 0.1473, respectively. This indicates that viability among seeds is similar and seed counts are an appropriate measure to detect differences between treatments.

A direct correlation could not be made between each individual seed and its viability because *A. asterias* seeds weigh approximately 1.3mg and available scales did not allow for accurate results. To see if there was a correlation between seed weight and viability, the percent germination of each group of ten seeds was compared to the average seed weight per group of ten seeds. A Pearson's Correlation resulted in a p-value of 0.0264 and r^2 value of 0.31 (Fig. 42). These results indicate that there is a significantly weak relationship between seed weight and seed viability. The low association in *A. asterias* may be due to methodology. If a more exact scale was available for weighing individual seeds, a direct relationship could be analyzed instead of an average of the weight and viability of a group of ten seeds.

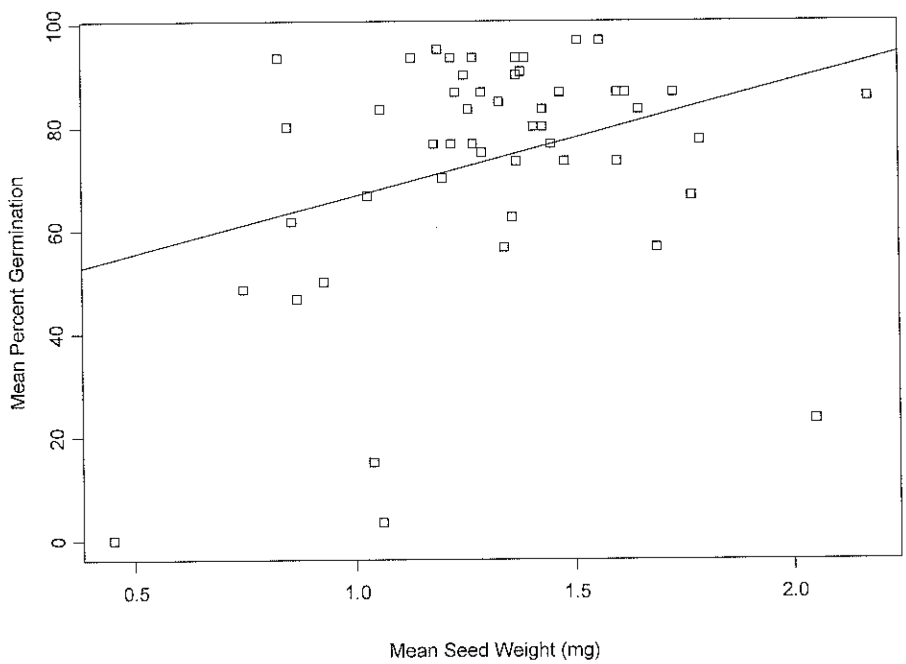


Figure 42. Relationship between the mean seed weight (mg) and mean percent germination for groups of ten seeds from breeding system and pollinator-limited experiments. (Pearson's Correlation; r^2=0.314; p-value=0.0264).

Seedlings from germinations are housed at the LBJWC until they can be reintroduced. In January 2006, there were 682 seedlings in cultivation. As of 8 September 2006 and 17 August 2007 there were 651 and 528 seedlings, respectively. A total of 240 seedlings were outplanted as part of the pilot reintroduction in 2007. Subsequent counts of the seedlings in cultivation yielded 406 seedlings as of 2 February 2008 and 382 seedlings as of 23 September 2009. The majority of the 382 seedlings in cultivation will be used in the restoration of 13 subpopulations impacted by seismic surveys earlier this year (2009). The restoration project is being lead by The Nature Conservancy (TNC). The remaining plants in cultivation will be divided between and housed at the LBJWC and TNC's Southmost Preserve nursery.

In January 2006, the seedlings (n = 682) in cultivation were individually numbered and diagrams created 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. The cacti were counted every two weeks beginning in January 2006 to document mortality. After 10 months, mortality leveled-off, so counting was done once a month. During the 25-month study period (January 2006-February 2008) 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.

Over the 25-month study period, the diameters of a randomly selected subset, representing ~25% of the seedlings (n = 170), were measured to analyze growth rate of *A. asterias* in cultivation. Individuals were measured monthly initially. In October 2006, measuring was switched to every two months as little change in the diameter was noted on a monthly basis. Therefore, a total of 18 measurements for each seedling over the 25-month study period were used in the analysis. 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. When initially measured in January 2006, 87% of the seedlings (n = 108) were in the 4.01-7.00 mm size class (Fig. 43). 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. 43).

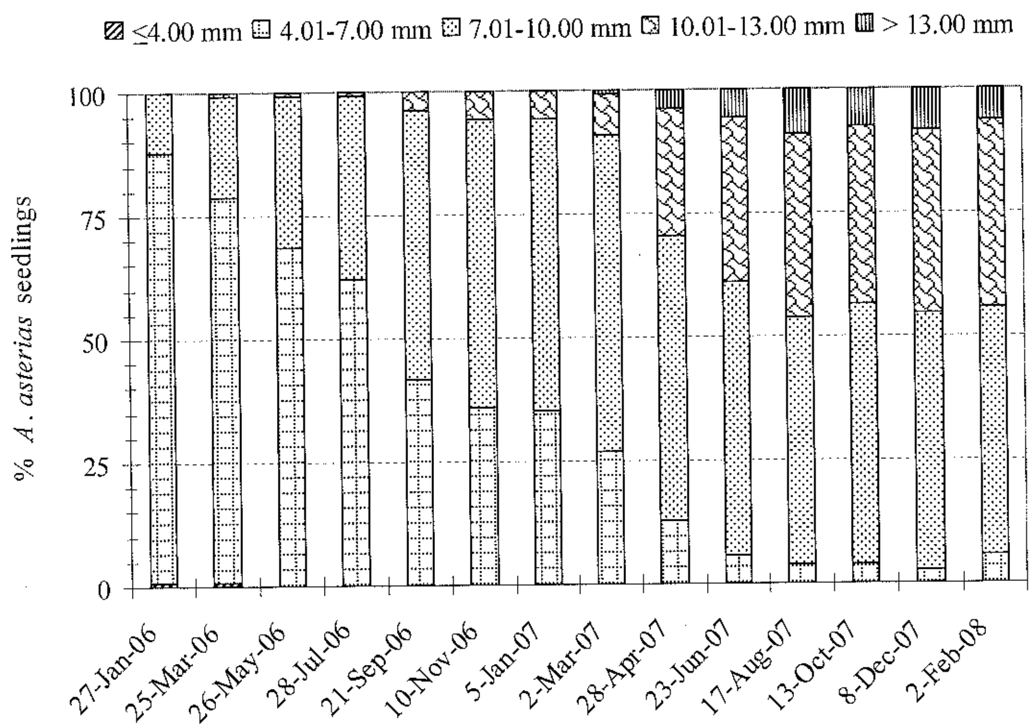


Figure 43. Size classes of 108 seedlings in cultivation across the 25-month study period.

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. Simple linear regression was used to determine whether the raw diameter data or the natural log transformed diameter data best represented the data over time. 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 ($r^2 = 0.45$, $P < 0.0001$; $n = 108$). A likelihood ratio test was then used 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). The following linear mixed models were compared: 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 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 and was 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. 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. 44). 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. 44).

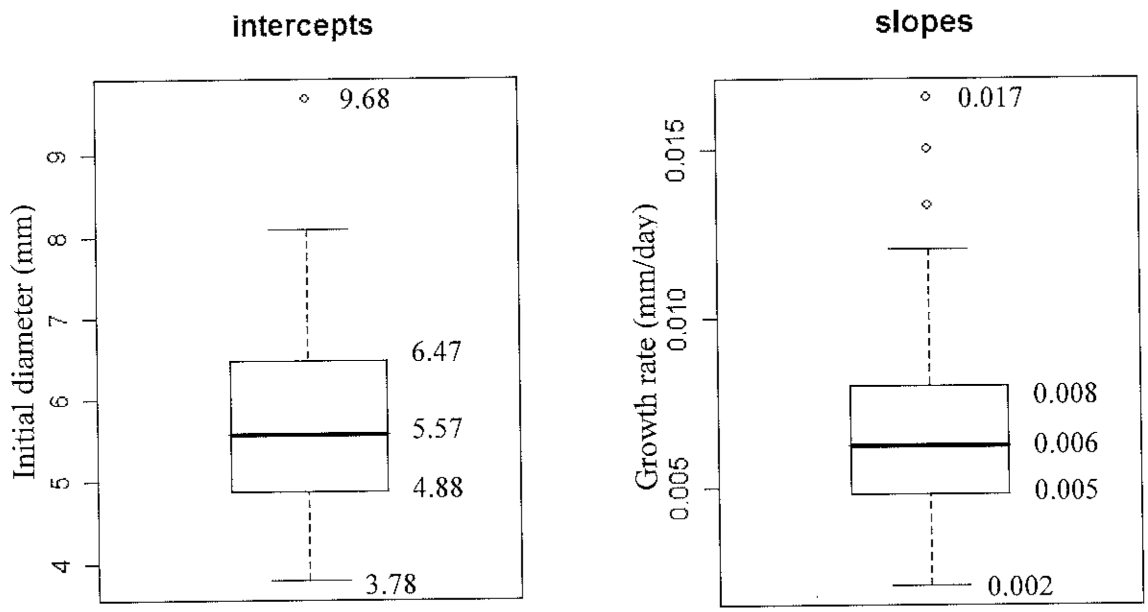


Figure 44. Box plots of the 108 coefficients estimating initial diameters (intercepts) and growth rates (slopes) of the cultivated seedlings.

6) Establishment of a pilot reintroduction site

The objective of this study is establish a pilot reintroduction site to determine which propagule type (seeds or seedlings) has greatest success dependent upon season of planting (spring versus fall).

A pilot reintroduction site was established in March 2007 at Property 2. 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*) (see Herbivory section). Property 2 is also a logical choice as a reintroduction site as it is owned by a conservation organization and is highly likely to be maintained as such. Lastly, according to the USDA-NRCS Starr County Soil Survey (NRCS 2005) the soils are mapped as Catarina soils which is a soil type that supports subpopulations of *A. asterias*.

Vegetation transects were conducted and a soil sample collected at the pilot site following the same methodology as outlined in the Habitat Analysis section. The data for the reintroduction site are provided in the Habitat Analysis section.

The pilot reintroduction site is a split-plot design. Two 1-m^2 quadrats are located along each 25-m transect of the vegetation transect for a total of 6 quadrats (Fig. 45). Location of the quadrats along the 25-m transect followed a set protocol. The first quadrat was randomly located using a random numbers table between 1 and 9 meters with the second quadrat 10 m north of the first one. The initial placement of the quadrat was centered on the transect. Quadrats were repositioned if at least one of the subquadrats contained 100% dense brush cover, at least two of the planting rectangles had dense basal coverage, or a Mexican ground squirrel (*Spermophilus mexicana*) burrow was located within the quadrat. The quadrats were repositioned in a predetermined order always keeping one edge or corner in contact with its initial starting position. The quadrats were rotated to the north, east, south, west, northwest, northeast, southeast, and southwest, respectively. If none of these positions were feasible, the quadrat was moved 0.5 m north from its starting point and the placement process started again. A planting grid, following the example of Pavlik (1994), was used to plant the seeds and seedlings. The wooden grid was 50 cm x 50 cm with a total of 20 planting rectangles (4 columns and 5 rows) which allowed for exact placement of the seeds and seedlings and aids in relocation during monitoring. The locations of the quadrats and placement of the planting grid were permanently located using nails.

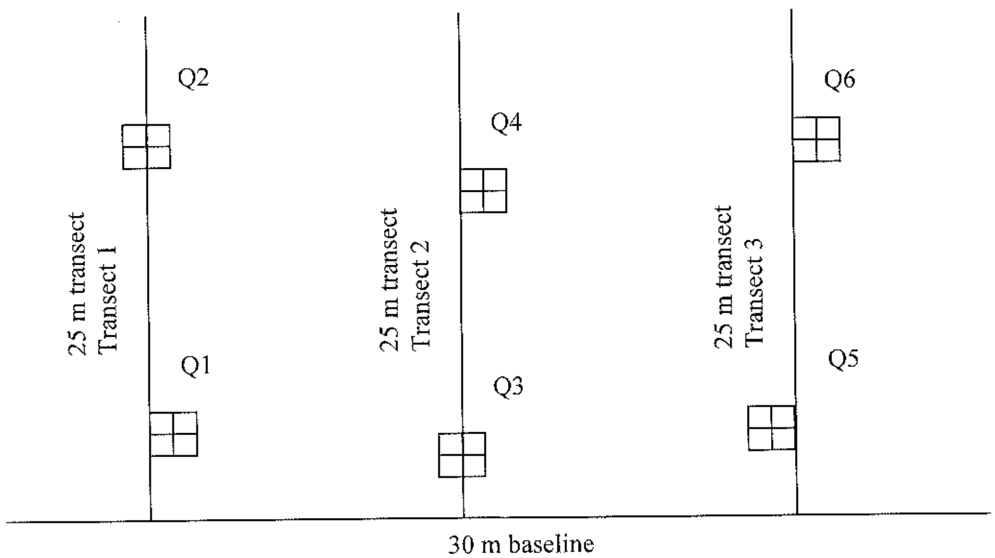


Figure 45. Stratified-random design of the 6 quadrats of the pilot reintroduction site.

Each of the 6 quadrats was sub-divided into 0.25 m^2 subquadrats. Each subquadrat was randomly assigned one of four treatments: 20 seeds planted in the spring (n = 120), 20 seedlings planted in the spring (n = 120), 20 seeds planted in the fall (n = 120), 20 seedlings planted in the fall (n = 120; Fig. 46). The propagule material used for the pilot reintroduction originated from Property 2. Seeds were collected prior to 2005 and stored at the Desert Botanical Garden in Phoenix, Arizona which is the Center for Plant Conservation designated repository for this species (see Propagation of Star Cactus Individuals for Founding Populations section). Seeds for the reintroduction were haphazardly chosen from these. The seedlings used for the pilot reintroduction are from the germination tests of 2005 (see Propagation of Star Cactus Individuals for Founding Populations section). The 240 seedlings used in the reintroduction were randomly chosen from the seedlings in cultivation.

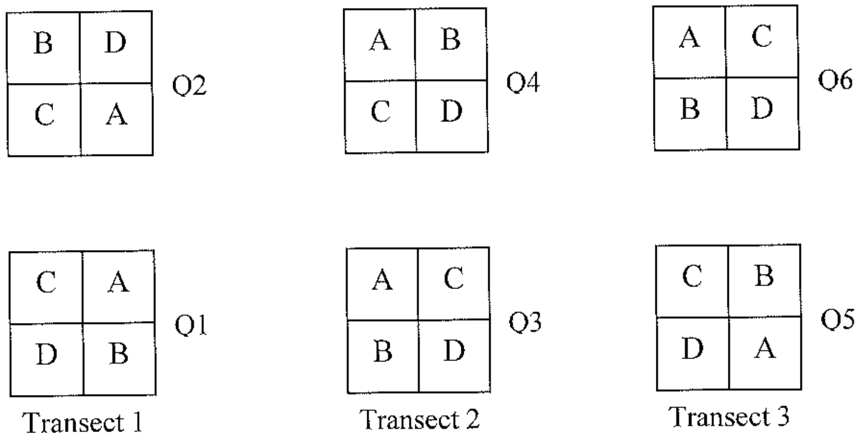


Figure 46. Randomly assigned treatments for each 0.25 m^2 subquadrat. A = seeds planted in the spring, B = seedlings planted in the spring, C = seeds planted in the fall, and D = seedlings planted in the fall.

Standardized planting procedures for the seeds and seedlings were established at the time of the spring planting treatment. This methodology was also used for the fall planting treatment. For the seeds a large nail was used to make an ~1 cm deep divot in the planting rectangle. The seed was placed in the divot and left uncovered. If there were many small rocks and a divot could not be created, the seed was dropped among the rocks. Craft pins were placed approximately 1 cm north of the seed location to aid monitoring efforts. 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. The subquadrats receiving spring planted seeds had greater percentages of bare ground than the subquadrats in the fall (Fig. 47). 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.

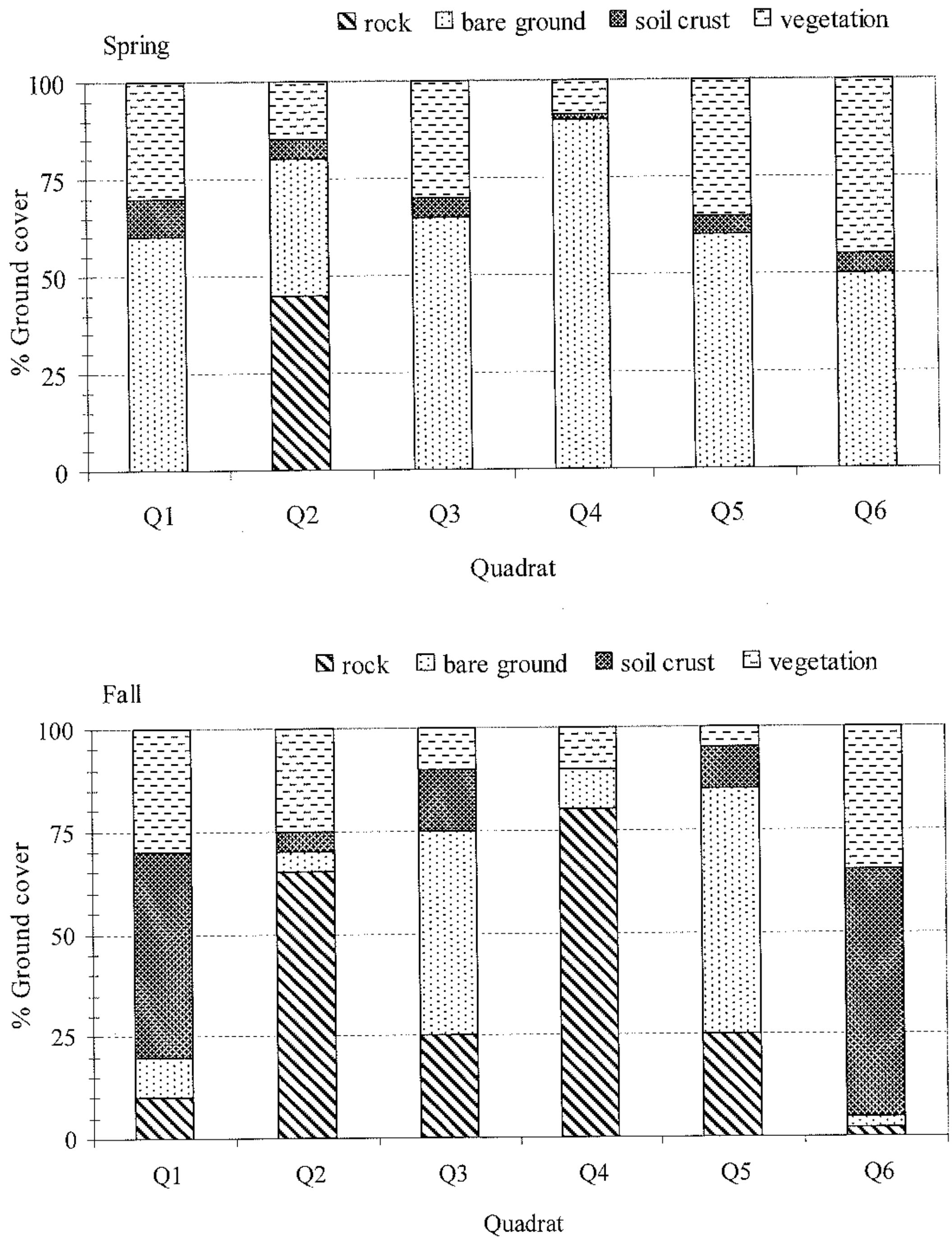
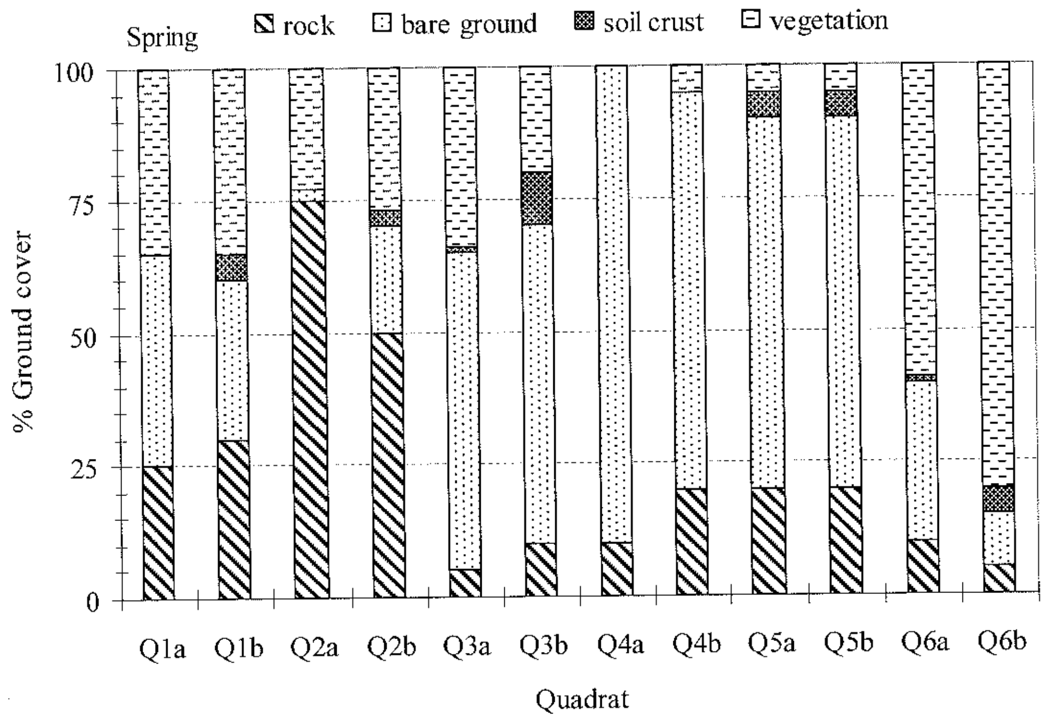


Figure 47. Percent rock, bare ground, soil crust, and vegetation of each subquadrat when seeds were planted, March and September 2007.

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. Initially a trowel was used to plant ~40 seedlings. However, it disturbed too large of an area, so a 3/8" x 12" slotted screwdriver was used to plant the remaining seedlings. The screwdriver minimized disturbance and created a deeper, well-defined hole which allowed the roots to be kept straight while planting. Each planted seedling was also watered with ~ 3 mL of water. There were minimal differences between measurements of ground cover at the beginning and ending of the study periods for each treatment (Fig. 48). The amount of soil crust documented for spring planted seedlings was higher at the conclusion of the study period (Fig. 48). The opposite was true for fall planted seedlings (Fig. 48). The spring planting of seedlings occurred on 19-20 April 2007 and the fall planting of seedlings occurred on 20-21 October 2007. At the time of planting, two diameter measurements perpendicular to one another were taken and averaged to obtain the diameter of each seedling at planting (initial diameter). 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).



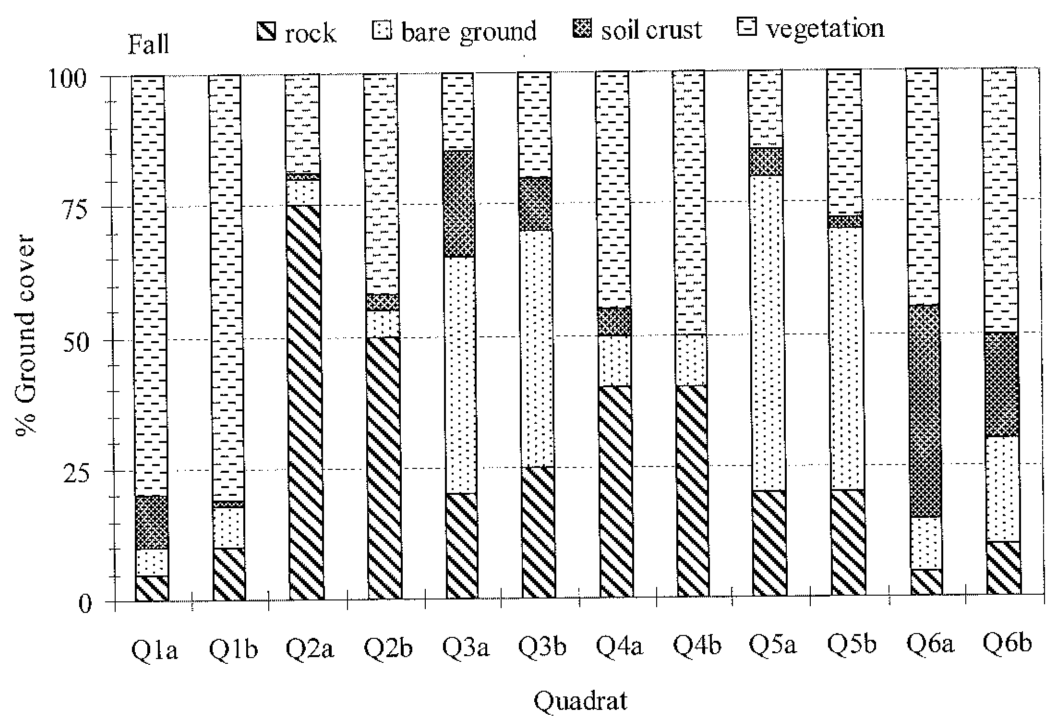


Figure 48. Percent rock, bare ground, soil crust, and vegetation of each subquadrat when seedlings were planted April and October 2007 (Q1a, Q2a, etc.) and at the end of the respective study periods, June and November 2008 (Q1b, Q2b, etc.).

7) Pilot Reintroduction Monitoring

The seeds and seedlings were checked two weeks after the initial planting to document if a catastrophic loss had occurred. Thereafter, presence/absence data for the seeds and seedlings was collected every four weeks. At the two-week check-up, it was noted if the seeds and/or divot were still visible. At the two week check-up for the spring and fall planting of seeds, it was possible to see the seeds and/or divot. However, by the first monthly monitoring of both the seed plantings it had rained which caused the divots to fill in. The monthly monitoring of the seeds consisted of checking to see if the seeds germinated and documenting the date 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. Of the 120 seeds planted in the spring, five produced seedlings (Table 22). 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 22). Monitoring of fall planted seeds concluded November 2008 and all four seedlings of this treatment were alive. Monitoring of the reintroduction plot has continued beyond the initial study period. As of August 2009 only 2 seedlings that germinated from seeds were still alive.

Table 22. 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

For the seedlings, only presence/absence data were collected. The state of each seedling was documented: 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. If causes of death could be determined, this was also documented.

A total of 66 *A. asterias* seedlings (55.0%) of the spring planted treatment survived the 14-month study period (Fig. 49). 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. 49). A total of 87 (72.5%) survived from the fall treatment (Fig. 49). The number of seedlings surviving per quadrat for spring planted seedlings ranged from 1-16 (Fig. 50). For the fall planted seedlings, the number of seedlings surviving per subquadrat ranged from 12-19 (Fig. 50). Monitoring of the pilot reintroduction has continued beyond the initial 14-month study period. As of August 2009, a total of 44 (36.7%) and 43 (35.8%) of the spring and fall planted seedlings, respectively, were alive.

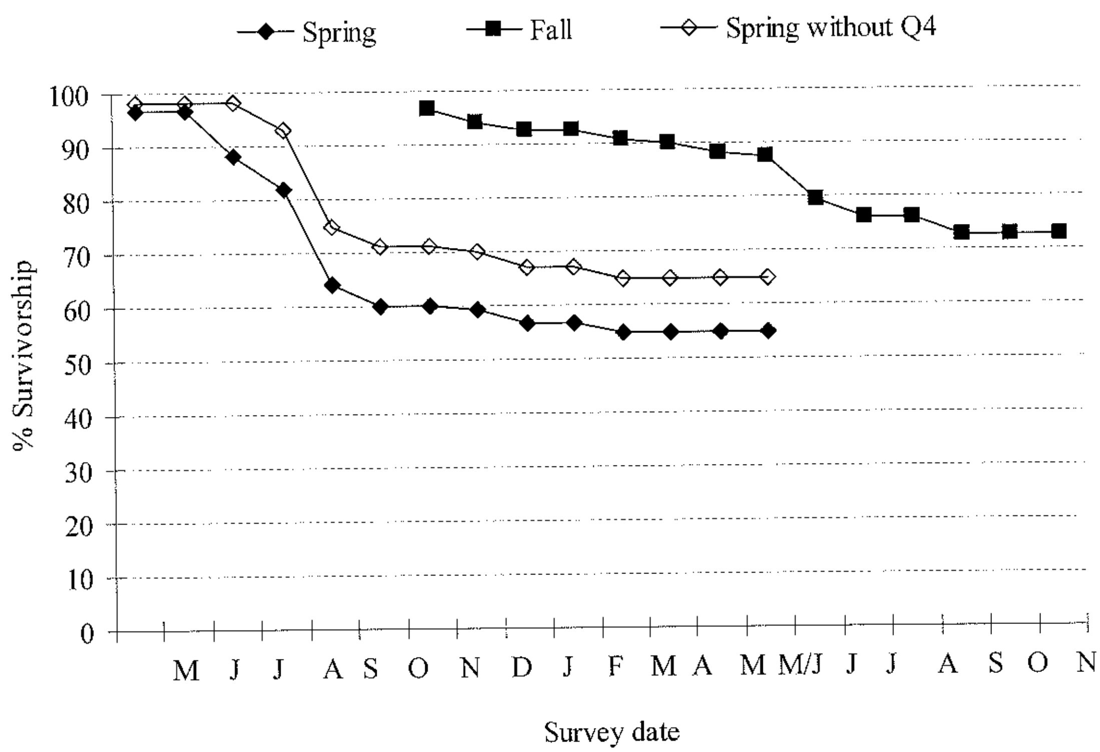


Figure 49. Percent survivorship per month of the seedlings (n = 120) planted April and October 2007. The "spring without Q4" line is the survivorship of seedlings planted in the spring without the 20 seedlings lost in Q4.

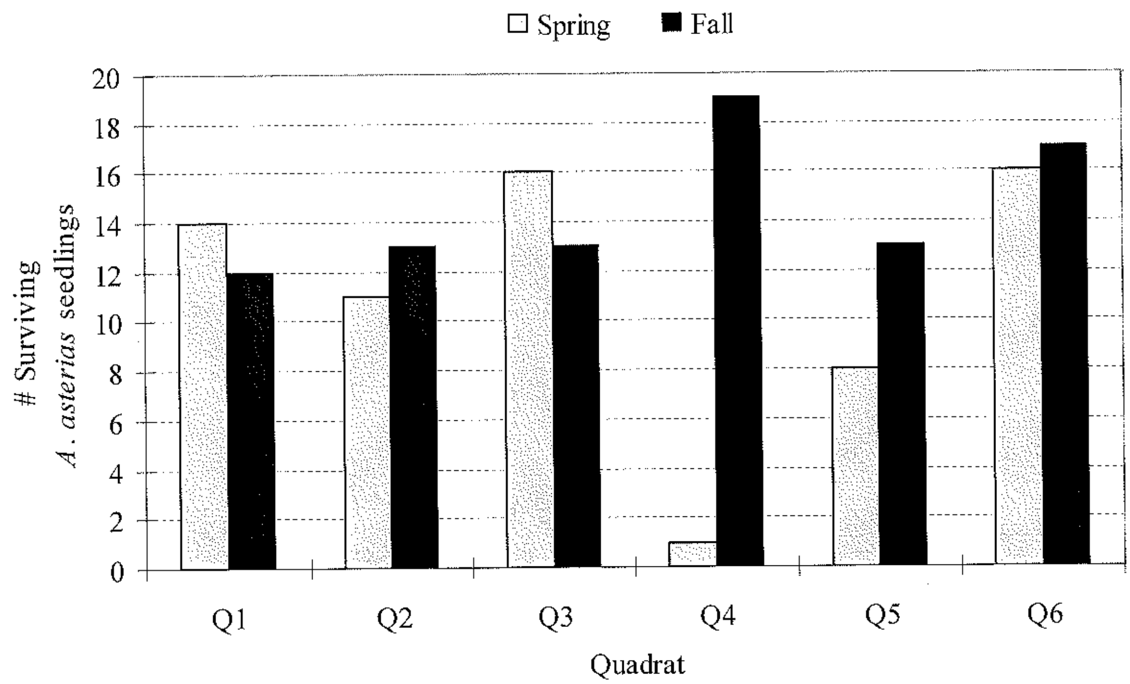


Figure 50. Number of *A. asterias* seedlings per quadrat out of 240 planted in the spring and fall that were alive at the end of the 14-month study periods.

Causes of mortality included burrowing activity by Mexican ground squirrel, desiccation, herbivory, infestation by weevils, and other causes (Fig. 51). 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. 51).

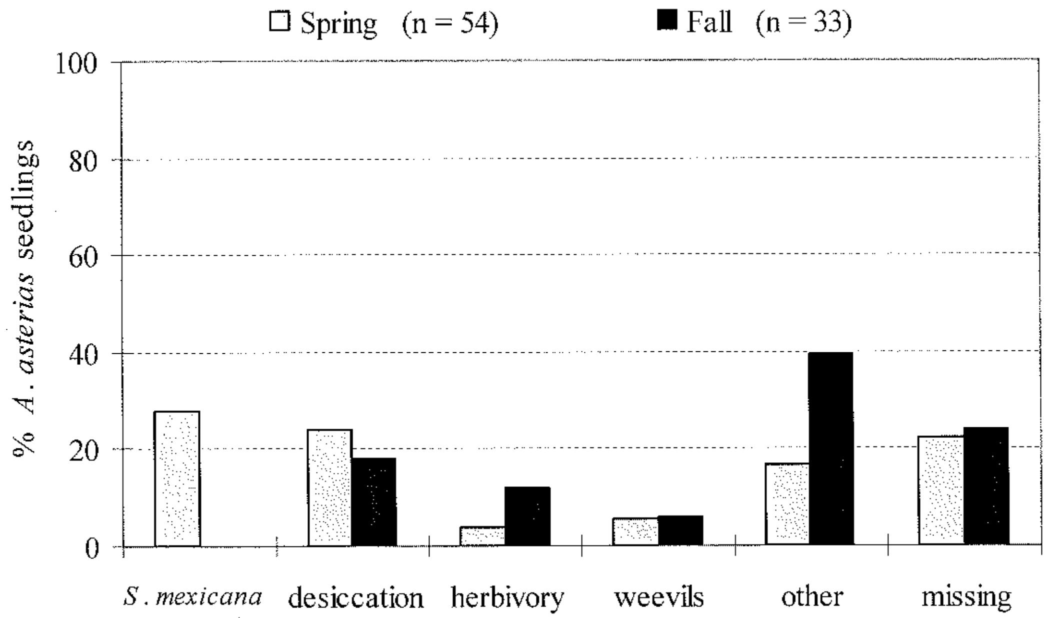


Figure 51. Causes of mortality for reintroduced *A. asterias* seedlings.

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. 51). 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, they were replanted and given ~3 mL of water each.

Weevil infestation accounted for 6% of the total deaths (Fig. 51). In January 2008, two seedlings (one each planted in the spring and fall) containing larvae were collected; these died before identification could be made. In March 2008, three more seedlings (two planted in the spring and one in the fall) which contained larvae were collected. After approximately one month two adult weevils emerged. The specimens are preserved but have not been positively identified or deposited with a natural history museum. Preliminary identification is to the genus *Gerstaerckeria*. 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. One seedling was observed with 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. 52.

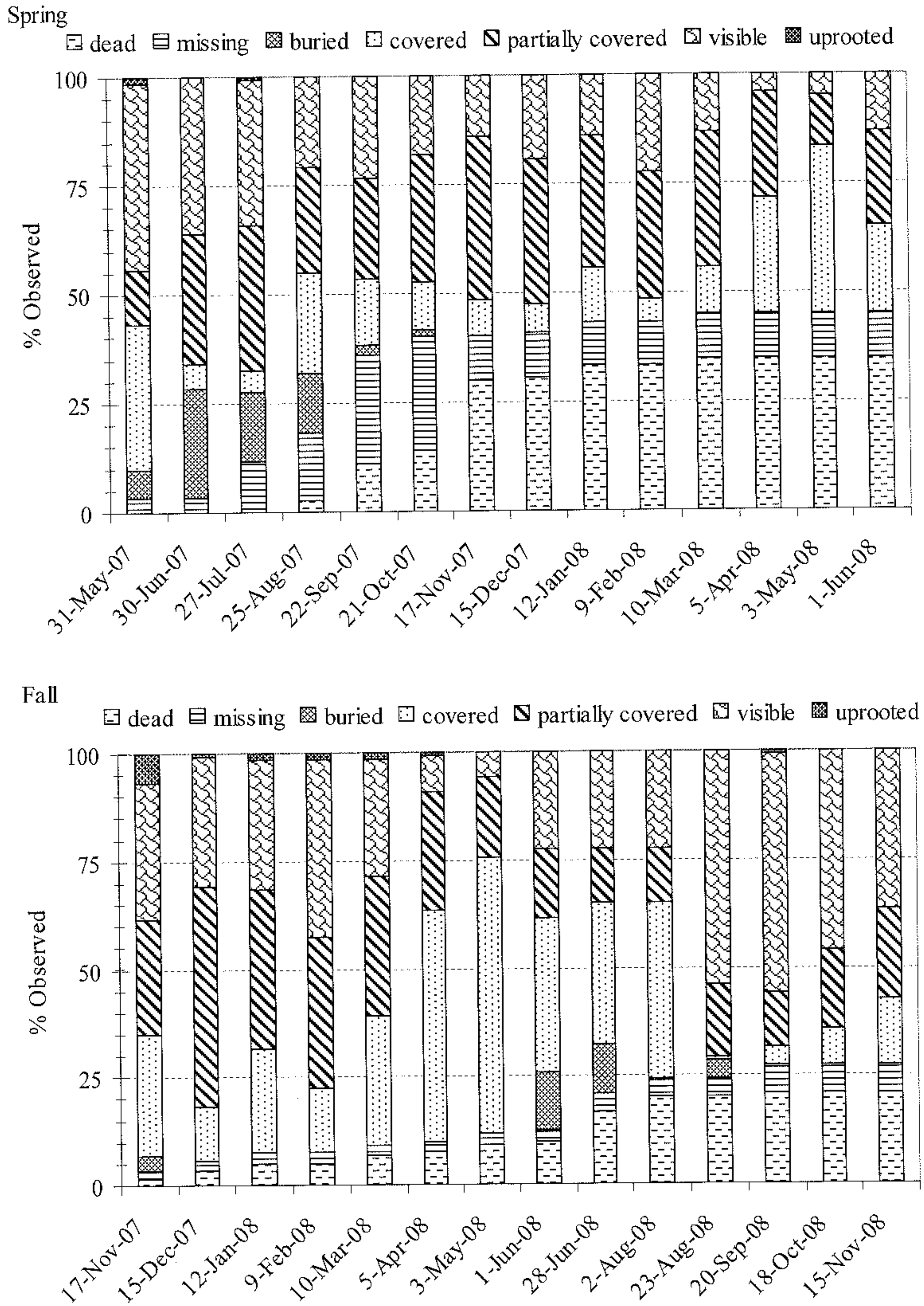


Figure 52. Percent of spring and fall planted seedlings observed in the various states per month of monitoring.

Diameter measurements were taken when the seedlings were planted and at the conclusion of the 14-month study period. The seedlings were often flush with the ground or buried and it was feared that exposing the seedlings on a monthly basis could jeopardize their survival. The diameters of the seedlings at the end of each 14-month study period were used to group the seedling in 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.

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. 53). 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. 54). Over the 14-month study period, the diameters of reintroduced seedlings increased by 0.8358 mm (Fig. 54). This equates to an estimated growth rate of 0.73 mm/year.

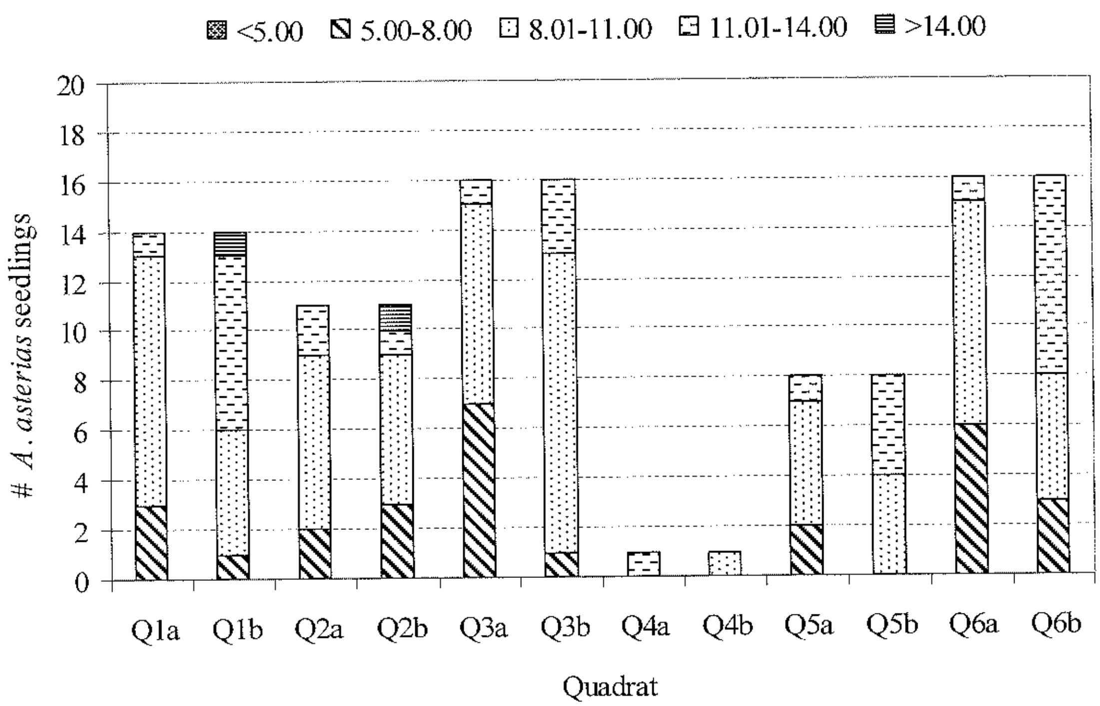


Figure 53. Size classes per quadrat of the 66 seedlings when planted in April 2007 (Q1a, Q2a, etc.) and at the end of the study period, June 2008 (Q1b, Q2b, etc.).

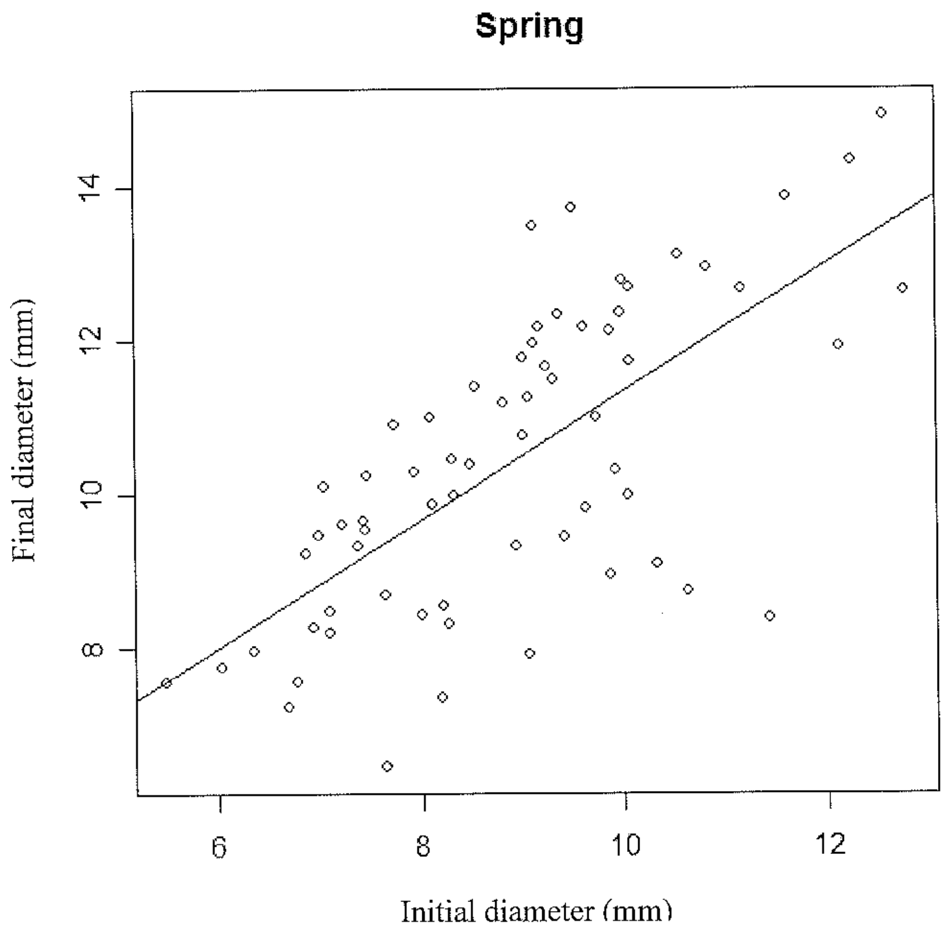


Figure 54. Linear regression of final diameter of spring planted seedlings on initial diameter ($r^2 = 0.47$, $P < 0.0001$, $n = 66$; $y = 2.9676 + 0.8358x$).

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. 55). 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. 56). Over the 14-month study period, the diameters of reintroduced seedlings increased by 1.0752 mm (Fig. 56). 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 ($t = -2.41$, $P = 0.0173$, $df = 151$).

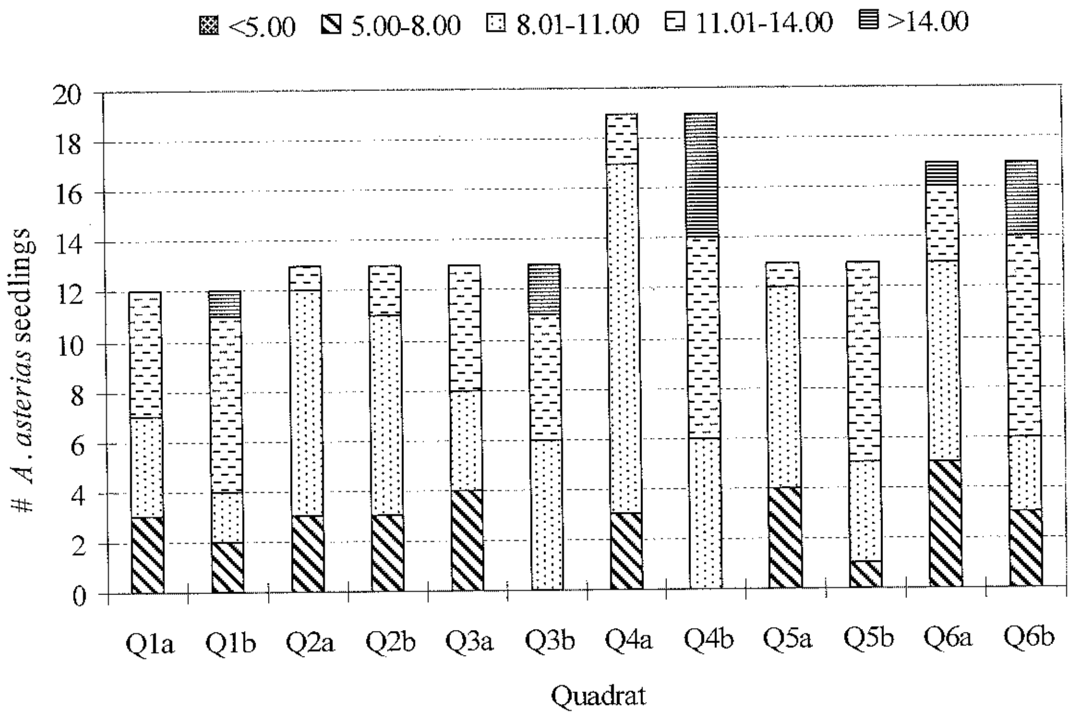


Figure 55. Size classes per quadrat of the 87 seedlings when planted in October 2007 (Q1a, Q2a, etc.) and at the end of the study period, November 2008 (Q1b, Q2b, etc.).

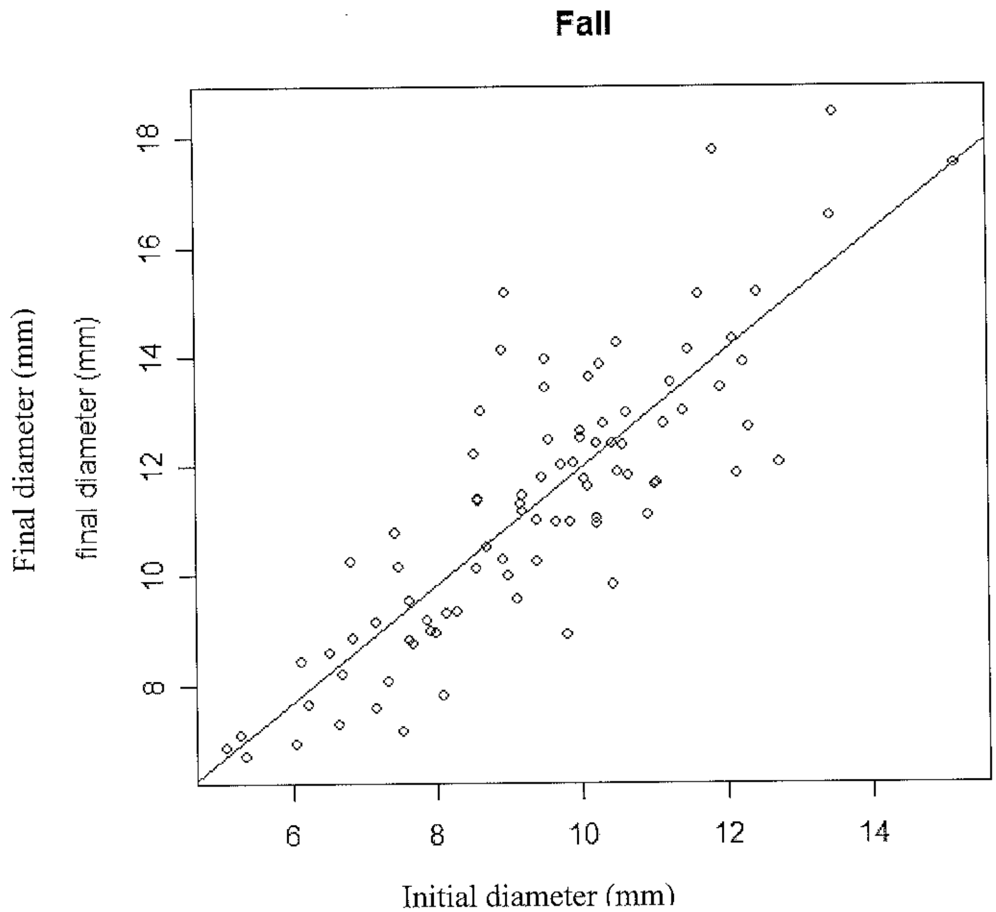


Figure 56. Linear regression of final diameter of fall planted seedlings on initial diameter ($r^2 = 0.71$, $P < 0.0001$, $n = 87$; $y = 1.1987 + 1.0752x$).

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. Three 12-bit temperature smart sensors were installed to record soil temperature. Three soil moisture smart sensors were also installed 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 the weather data was downloaded 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. Weather data collected at the reintroduction site is summarized in Appendix C.

8) Surveys for Additional Extant Populations in Texas

Surveys to identify additional populations of *A. asterias* in Texas were accomplished using two different strategies. The first was to simply identify all landowners surrounding Property 2 and begin getting access permission, doing surveys, and methodically working out from there. The second was to follow up on leads or rumors that have surrounded this species for years. There is a kind of lore associated with *A. asterias*, and stories abound. The survey facet of this study has tracked down each story and rumor and established whether it is fact or fiction.

All private ranches were surveyed for *A. asterias* with landowner permission. Survey effort on each ranch varied. Some ranches were surveyed with many biologists invited to help with the search for *A. asterias*, while other ranches were surveyed with one to four members of the *A. asterias* research team.

When many biologists helped with a survey, there were many individual volunteer data collections, mostly collected on Garmin GPS units (Garmins), but also other hand-held devices. This is especially true for the data collected on Properties 1, 2 and 8. Over time, survey data collected for this project became more controlled and streamlined. When *A. asterias* or other endangered or rare plant species were identified on private ranches, a Trimble GeoXH Handheld GPS unit was used to collect the geographic location. Locality data was collected by GPS in the form of points, lines and polygons. Point coordinates were collected for individual plants as well as for tightly clustered individuals, while lines and polygons usually represent multiple individuals over a larger area. Epochs were collected every second (or 7/8ths of a second) for a minimum of 100 seconds and averaged for each point. Along with location, species, number of individuals, and notes were collected with the GPS. During the project, the GeoXH Handheld GPS was allowed a clear view of the sky, maintaining a lock on the carrier frequency which is critical for high precision positions.

In the office, Trimble PathFinder Office 4.10 was used for data transfer, differential correction, and export to the GIS (Geographic Information System). After the rover files are transferred from the GPS to the GIS workstation, they are differentially corrected against TxDOT base stations located in Pharr and Laredo, as well as CORS (Continuously Operating Reference Stations) sites located in Kingsville, Texas, and Monterrey, Mexico. The accuracy of the positions after carrier-based differential correction ranges from 10 to 30 cm in precision. The corrected output is then exported into ESRI shapefiles based on the Universal Transverse Mercator (UTM) North American Datum 83 (NAD 83) projection and datum, which is the standard used for the 2008/2009 Texas Orthoimagery (TOP). All spatial analysis and map products are created using ESRI ArcGIS 9.3 software and extensions.

Individual ranch maps depicting rare species occurrences were created for each landowner and will be forwarded as an addendum to this report, along with Excel files by ranch, and the shapefiles.

When the proposal for the research project was written, there were only two properties known to have *A. asterias* in Texas (Properties 1 and 2). To date there are 25 verified properties with *A. asterias*. All 25 private properties cover an approximate 56 square mile area of south-central

Starr County, Texas. Approximately 5,124 *A. asterias* individuals have been recorded on these properties. New localities for the endangered *Frankenia johnstonii* were recorded on seven properties, and the endangered *Physaria thamnophila* was recorded on one property. Other rare plant localities were recorded for *Asclepias prostrata* (two properties), *Cardiospermum dissectum* (one property), *Coryphantha macromeris* var. *runyonii* (14 properties), and *Manfreda longiflora* (13 properties).

Descriptions of Properties with Star Cactus

Property 1 - For years this was the only extant site of *A. asterias* in Texas known to conservation. In the past, relationships and access permission for this property were shaky at best. Recently new relationships were established with the landowner by members of the Nature Conservancy of Texas and Texas Parks and Wildlife Dept. (TPWD). The landowner and her family are interested in their *A. asterias* sites and allowed access for surveys. A two-day survey was conducted on this 741 acre ranch on March 11th and 12th, 2004, with over 15 volunteers from various conservation organizations. Most of the data was collected as point data by volunteers with individual Garmins, but there were a few lines and polygons collected with Trimble GPS units. A total of 277 individual *A. asterias* plants were found in five areas of the ranch according to field notes. This ranch has not been resurveyed since 2004. This ranch was revisited in 2008 to flag *A. asterias* occurrence areas before seismic activity, and then again in 2009 after seismic activity. While always polite, this family remains quite private, and has been slow to warm up to further conservation initiatives.

***A. asterias* total individuals: 277**
Other endangered plants recorded: none
Other rare plants recorded: *Asclepias prostrata, Coryphantha macromeris* var. *runyonii,* and *Manfreda longiflora*
TPWD Permission: Yes
Lower Rio Grande Valley (LRGV) Candidate Conservation Agreement: No
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: No

Property 2 - Approximately a mile north of the property mentioned above, a new locality for *A. asterias* was verified in the early 2000s. Through the diligent efforts of a local South Texas plant conservation enthusiast, Texas Parks and Wildlife, U. S. Fish and Wildlife Service and the Nature Conservancy of Texas, the Nature Conservancy now owns this 419 acre property and has appropriately named the preserve Las Estrellas. Various surveys have taken place on the property annually (2001-2009) and the total number of star cacti data points continues to increase. *A. asterias* has been verified on many areas of this preserve with the exception of the northeast quadrant. The first organized survey of this entire property using multiple volunteers was January 2001. Additional site specific surveys have occurred over the years as various aspects of this research project were initiated. The last survey effort capturing new data (again site specific) was in July and August 2009 before the seismic was allowed access. Early on much of the data was taken on individual Garmins or other hand-held GPS devices by many volunteer surveyors, and later also with Trimble units (by Poole and Janssen). Although interpretation of all these data collections is difficult, currently as the data stands today, an approximate total of 328 individual *A. asterias* have been recorded on this preserve. This number is approximate at best since some of the data points recorded for this property are missing total number of individuals, while other points collected may have been an effort to encircle a group of individuals. A more organized survey effort should be conducted on this preserve in the future to create a more comprehensive and accurate data set and map of the preserve.

***A. asterias* total individuals: Roughly 328**
Other endangered plants recorded: none
Other rare plants recorded: *Coryphantha macromeris* var. *runyonii,* and *Manfreda longiflora*
TPWD Permission: Yes
LRGV Candidate Conservation Agreement: Yes
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: Yes

Property 3 - This 15,200 acre high-fenced ranch is a long narrow rectangle running north to south to the east of Property 2. This ranch is owned by a large Trust that also owns property in the Hill Country with endangered plant species, so they were already familiar with what it meant to have endangered plants on their property. After conducting surveys on other nearby ranches and seeing all the various species of cacti on those ranches, the lack of cacti of any kind on this very large ranch was disconcerting. The ranch manager confirmed that there has been considerable poaching on the ranch not only by trespassers, but also he believes, by a former ranch manager who was bringing groups out to the ranch to dig up cacti. Despite the tremendous amount of available suitable habitat on the ranch and after two weeks of surveys in 2004 by Janssen and Poole, only 196 *A. asterias* individuals were verified in six different areas. No additional surveys have been conducted on this property, but all the *A. asterias* and *Frankenia johnstonii* sites were visited before and after the seismic activity in 2008 and 2009.

This property is worthy of a large-scale multi-biologist survey effort in the future. This may also be a good property to begin a population augmentation study if the ranch managers and other neighbors can keep a more watchful eye on the property. The Trust members have been asked and are agreeable to future augmentation efforts on this ranch and are also interested in ongoing conservation initiatives.

***A. asterias* total individuals: 196**
Other endangered plants recorded: *Frankenia johnstonii*
Other rare plants recorded: *Coryphantha macromeris* var. *runyonii,* and *Manfreda longiflora*
TPWD Permission: Yes
LRGV Candidate Conservation Agreement: Yes
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: No, but interested

Property 4 - This is an 80 acre ranch to the east of Property 2. Initially regarded as part of Property 10, this site is owned by a separate landowner. In 2004, this ranch was surveyed by Poole, Janssen, Williamson and Strong, and 968 *A. asterias* were verified in two different areas. Although a portion of this property was again intensely surveyed during herbivory studies from 2007 to 2009, the entire property has not been resurveyed for *A. asterias* since 2004. In September 2009, there was an explosion on the Enterprise TX-150 pipeline (Zapata-Penitas 16") that traverses this property and separates the two clusters of *A. asterias*. The fire associated with this explosion burned for at least a day. The fire impacted 13 acres and burned into the area containing *A. asterias*. The fire, although very hot at the explosion site, moved beyond this point only along the tops of the brush. At this time, the *A. asterias* individuals do not appear to have been affected because of the lack of fuel load along the rocky ground, but a portion of the overall habitat has been impacted. Although the cause of this explosion is still undetermined, according to the Railroad Commission report, Enterprise was fined $5,000 during the investigation for exceeding pressure control limits along the La Victoria southern segment of this pipeline. This landowner has a store on Alvarez Road, and has a wonderful attitude about his endangered species and helping with conservation and further conservation initiatives.

***A. asterias* total individuals: 968**

Other endangered plants recorded: none

Other rare plants recorded: *Coryphantha macromeris* var. *runyonii,* and *Manfreda longiflora*

TPWD Permission: Yes

LRGV Candidate Conservation Agreement: Yes

Las Estrellas Conservation Cooperative Gate Signs & Management Plan: Yes

Property 5 - This 750 acre ranch was accessed briefly in the 1990s, and one *Frankenia johnstonii* site was recorded. In October 2004 a one day survey of this ranch revealed a total of 92 *A. asterias* individuals on five areas of the ranch. Additional surveys were conducted by Janssen in 2006, 2008 and 2009 and additional *A. asterias* and *F. johnstonii* sites were recorded. The last survey was conducted in December 2009, and all sites were revisited in April 2010. The landowner is a retired school teacher and one of the nicest people you would ever want to meet. The owner is always open to ranch visits, surveys, and conservation initiatives.

***A. asterias* total individuals: 143**

Other endangered plants recorded: *Frankenia johnstonii*

Other rare plants recorded: *Coryphantha macromeris* var. *runyonii,* and *Manfreda longiflora*

TPWD Permission: Yes

LRGV Candidate Conservation Agreement: Yes

Las Estrellas Conservation Cooperative Gate Signs & Management Plan: Yes

Property 6 – Even a small property can contain *A. asterias.* Despite only about a three hour survey in October 2004 by Poole and Janssen, a total of 70 *A. asterias* individuals were discovered in three areas of this small 30 acre ranch. This ranch has not been resurveyed, but the *A. asterias* sites were visited before and after the seismic activity in 2008 and 2009. The husband and wife that own this property are wonderful, but speak very little English. Their son lives and works locally, and is very helpful.

***A. asterias* total individuals: 70**

Other endangered plants recorded: none

Other rare plants recorded: *Coryphantha macromeris* var. *runyonii,* and *Manfreda longiflora*

TPWD Permission: Yes

LRGV Candidate Conservation Agreement: Yes

Las Estrellas Conservation Cooperative Gate Signs & Management Plan: No

Property 7 - This very small ranch (ca. 20 acres) was first visited by members of the Nature Conservancy of Texas in 2002 at the invitation of the landowner. In 2004 he agreed to let his ranch be part of the research project. This site is important because it occurs approximately five air miles to the west of most of the properties. It is a very interesting little remnant of plants surrounded by root plowing and cross-fencing. The several person survey included the landowner and his sons. A total of 303 *A. asterias* individuals were located on this property in 2004. This property was resurveyed in 2007 in preparation for herbivory studies, and 487 individuals were recorded. According to Dr. Martin Terry (2005), it is his opinion based on preliminary genetic diversity results of three *A. asterias* sites, that this site would benefit the most from population augmentation. This landowner, a local school teacher, and his son have always had a wonderful attitude about conservation and allowing studies on the ranch.

***A. asterias* total individuals: 487**
Other endangered plants recorded: none
Other rare plants recorded: *Coryphantha macromeris* var. *runyonii,* and *Manfreda longiflora*
TPWD Permission: Yes
LRGV Candidate Conservation Agreement: Yes
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: Yes

Property 8 - This 400 acre site was first discovered by Texas Parks and Wildlife during a visit for a wildlife management plan consultation. The landowners knew that they had *A. asterias* on a large saline area on the ranch. On May 12 and 13, 2005, approximately 10 to 15 biologists (depending upon the day) helped to survey this 400 acre property. Some volunteers used individual Garmins to record data, while some data was being recorded with a Trimble. The wife of the property owner was by far the greatest *A. asterias* finder! 587 *A. asterias* were verified in one large saline area of the ranch. Although this entire ranch has not been resurveyed since 2005, a portion of the *A. asterias* population on this ranch was intensely surveyed during herbivory studies from 2007 to 2009, and these sites were also visited before and after the seismic activity in 2008 and 2009. The owners live in McAllen, but are currently building a house on the ranch and are there almost every weekend.

***A. asterias* total individuals: 587**
Other endangered plants recorded: *Frankenia johnstonii*
Other rare plants recorded: *Coryphantha macromeris* var. *runyonii,* and *Manfreda longiflora*
TPWD Permission: Yes
LRGV Candidate Conservation Agreement: Yes
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: No

Property 9 - Initially thought to be part of Property 10 because it is not fenced, this site is owned by a separate landowner who is very nice. The property, which is approximately 20 acres, was surveyed in 2004 and 142 plants were flagged. This site has not been resurveyed since, but has been utilized intensively by many aspects of this study. The final visit to this site was April 2010. The cows from the adjacent property walk through this site often, leaving deep holes in the clay. This site would benefit from a small amount of fencing in the future.

***A. asterias* total individuals: 142**
Other endangered plants recorded: none

Other rare plants recorded: ***Coryphantha macromeris*** **var.** ***runyonii,*** **and** ***Manfreda longiflora***
TPWD Permission: Yes
LRGV Candidate Conservation Agreement: Yes
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: No

<u>Property 10</u> - The owner of this property recently passed away, but the same woman has been leasing this ranch for over 25 years. She knew of only one place on the ranch where *A. asterias* grew. Over 25 years ago she collected two *A. asterias* near the front gate and took them home. She said that they had multiplied into many more in her garden. In 2004 there were 181 individuals in her driveway garden area, 4 in pots (1 with 15 seedlings around it), and another 3 in her yard. From the 2 star cacti that she collected over 25 years ago, she now has her own conservation garden of 203 *A. asterias* in her yard! The last visit to this garden was April 2010, and it was still going strong.

This ranch has been surveyed multiple times with one (usually) to four team members. The first survey was May 2004, and the final survey was December 2009. A total of 175 *A. asterias* have been verified in seven areas of this 200 acre ranch. Now that the owner has passed away, the woman who leases the property wants to buy it, but for now the estate is up in the air. She is interested in further conservation initiatives; however, she would feel more comfortable as the "owner" of the property, not the leaser.
A. asterias **total individuals: 175**
Other endangered plants recorded: none
Other rare plants recorded: ***Coryphantha macromeris*** **var.** ***runyonii,*** **and** ***Manfreda longiflora***
TPWD Permission: Yes
LRGV Candidate Conservation Agreement: No
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: No

<u>Property 11</u> – As with Property 7, this 416 acre ranch is also to the west and disjunct from the majority of *A. asterias* occurrences. This ranch not only has the endangered *A. asterias*, but also the endangered *Physaria thamnophila* and *Frankenia johnstonii*. A thorough two-day census for *A. asterias* was conducted in May 2007 by 10 biologists of many disciplines. The final count was 655 *A. asterias*. A subset of the *A. asterias* on this ranch was intensely surveyed from 2007 to 2009 as part of the herbivory studies. Visits to this ranch have continued over the years, with the last visit to all *A. asterias* sites in April 2010. This ranch is truly a treasure among treasures with three endangered plants and three additional rare plant occurrences. The landowner is very kind and so interested in everything (plant and animal) on the ranch.
A. asterias **total individuals: 655**
Other endangered plants recorded: ***Frankenia johnstonii*** **and** ***Physaria thamnophila***
Other rare plants recorded: ***Asclepias prostrata, Coryphantha macromeris*** **var.** ***runyonii,*** **and** ***Manfreda longiflora***
TPWD Permission: Yes
LRGV Candidate Conservation Agreement: No
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: Yes

Property 12 - A 107 acre ranch, or possibly a parcel of a larger ranch. A half-day survey by one botanist in June 2007 revealed 41 *A. asterias*. There is most certainly more *A. asterias* out there to be verified. The two sisters that own and live on this property are somewhat peculiar and extremely reclusive.
***A. asterias* total individuals: 41**
Other endangered plants recorded: *Frankenia johnstonii*
Other rare plants recorded: none
TPWD Permission: Yes
LRGV Candidate Conservation Agreement: No
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: No

Property 13 - This 630 acre ranch has been owned by the same family for many generations, and the siblings are now spread out all over Texas. However, they still keep the home in Rio Grande City, and visit frequently. This property has been surveyed on and off for the past three years by Janssen as time and visits with the landowner allowed. The first survey was in July of 2007, and the final survey was December 2009. All the *A. asterias* sites were revisited in April 2010. To date, over 235 *A. asterias* (occurring in seven areas) have been flagged on this ranch. Although this ranch belongs to three siblings, contact has been primarily with the only brother. He is a geologist in Houston, and has always been very receptive to conservation issues on the ranch. It is interesting that although his family has owned that ranch for generations (and he grew up and went to high school in Rio Grande City), he does not know his neighbors, and he has expressed a keen interest in the developing Las Estrellas Conservation Cooperative for that very reason.
***A. asterias* total individuals: 235**
Other endangered plants recorded: *Frankenia johnstonii*
Other rare plants recorded: *Cardiospermum dissectum, Coryphantha macromeris* var. *runyonii,* and *Manfreda longiflora*
TPWD Permission: Yes
LRGV Candidate Conservation Agreement: No
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: Yes

Property 14a and 14b – I am still not sure if these two brothers own this property jointly or separately. It just depends on the day and which brother you talk to. Both brothers have *A. asterias* on their "portion". Much of this total 850 acres has been root-plowed. One botanist spent one long day on this ranch in September 2007 and located 90 *A. asterias*. More surveys are definitely needed on the areas with good habitat. *A. asterias* sites were revisited in 2008 and 2009 (before and after seismic activity).
***A. asterias* total individuals: 90**
Other endangered plants recorded: none
Other rare plants recorded: none
TPWD Permission: Yes
LRGV Candidate Conservation Agreement: No
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: No

Property 15 – This 250 acre ranch is just west of Property 5. The first survey was in June 2008 by one surveyor. This ranch consists of mostly salt-flat country, with one very large *Frankenia*

johnstonii population at the back of the ranch. While GPSing the perimeter of the *F. johnstonii*, a cluster of *A. asterias* was discovered. The individuals were huge and protruding out of the ground tripping the surveyor. Approximately 32 were flagged, although there are probably more. This property has been visited multiple times since June 2008, with the last visit taking place in April 2010. This landowner is very nice; he and his family are very excited and proud to know that they have and are conserving endangered plants.
***A. asterias* total individuals: 32**
Other endangered plants recorded: *Frankenia johnstonii*
Other rare plants recorded: none
TPWD Permission: Yes
LRGV Candidate Conservation Agreement: No
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: Yes

Property 16 – This 200 acre ranch is just south of the property above and was also first accessed in June 2008. Properties 15 and 16 are brothers. *A. asterias* was located on a rocky saladillo hill near the southern fence line and the pipeline easement. Approximately 100 individuals were flagged over a couple of short field days by one surveyor in August 2008. This property has been visited multiple times since June 2008, with the last visit taking place in April 2010.
***A. asterias* total individuals: 100**
Other endangered plants recorded: none
Other rare plants recorded: none
TPWD Permission: Yes
LRGV Candidate Conservation Agreement: No
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: Yes

Property 17 – This 111 acre tract is just south of the above property and was first accessed in June 2008. Although thoroughly root-plowed, one *A. asterias* individual was discovered just inside the fence adjacent to the Property 16 population. The contact person for this property is the ex-wife of the owner who now is apparently in prison. A return visit in the fall of 2009 revealed that the one *A. asterias* just inside the fence was completely gone.
***A. asterias* total individuals: 0**
Other endangered plants recorded: none
Other rare plants recorded: none
TPWD Permission: Verbal by phone
LRGV Candidate Conservation Agreement: No
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: No

Property 18 – This 100 acre tract is just south of Property 17 and was accessed one time in March 2009. There are multiple sibling owners, and a few live in Rio Grande City. There seems to be quite a bit of habitat on this tract (little to no clearing or root-plowing), but only 8 individuals total were flagged from two different areas in one day by one surveyor. The field conditions for locating *A. asterias* in the 2009 field season were not the best. It was very hot and dry and many *A. asterias* had pulled under or were yellow. More could probably be found on this tract.
***A. asterias* total individuals: 8**
Other endangered plants recorded: none

Other rare plants recorded: none
TPWD Permission: Yes
LRGV Candidate Conservation Agreement: No
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: No

Property 19 – North of Property 10 and west of Property 5, sits this 213 acre tract. This ranch was first accessed in March 2009. The owner lives in San Antonio, but his father (who is very reclusive) lives west of Rio Grande City and takes care of the cattle. After three days of one-person surveying, three areas of *A. asterias* were located on the ranch, and a total of 61 were flagged.
***A. asterias* total individuals: 61**
Other endangered plants recorded: none
Other rare plants recorded: none
TPWD Permission: Verbal by phone
LRGV Candidate Conservation Agreement: No
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: No

Property 20 – North of Property 2 is a 545 acre ranch filled with prime *A. asterias* habitat. While traversing this ranch to get to another in January 2009, the surveyor actually saw *A. asterias* from her truck window. Sixteen individuals were counted in this one area along the road along the power line easement. The landowner has been slow to warm up to the idea of allowing further surveys or a big thorough survey with multiple people, but communication continues. He wanted to read the Endangered Species Act before he allowed further access, and we haven't spoken since.
***A. asterias* total individuals: 16**
Other endangered plants recorded: none
Other rare plants recorded: none
TPWD Permission: Yes
LRGV Candidate Conservation Agreement: No
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: No

Property 21 – Just south of Property 2, this 518 acre ranch was first accessed in June 2009 and surveyed for three days by one surveyor. This ranch was also surveyed one day in July by one surveyor, and then again in October 2009 by four biologists. *A. asterias* was found on twelve areas of the ranch, and over 294 were flagged. There are multiple siblings involved in the property, and at least one seems very interested in the *A. asterias* and future conservation initiatives.
***A. asterias* total individuals: 294**
Other endangered plants recorded: none
Other rare plants recorded: *Coryphantha macromeris* var. *runyonii*, and *Manfreda longiflora*
TPWD Permission: Yes
LRGV Candidate Conservation Agreement: No
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: No

Property 22 - South of Property 21, this 55 acre tract was visited at the start of this study and *A. asterias* was verified on the property. The landowner (one of the two local peyote dealers) was leery, and eventually refused to communicate. Although communication was started again in 2009 and things seemed more positive, he again shut down, and efforts to include his ranch in this study failed.
***A. asterias* total individuals: none**
Other endangered plants recorded: none
Other rare plants recorded: none
TPWD Permission: No
LRGV Candidate Conservation Agreement: No
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: No

Property 23 – North of Property 8 and now the ranch with our northern-most *A. asterias* site, this 100 acre ranch was accessed in March 2009. There are only two *A. asterias* verified on this ranch, and the landowner took me right to them. She said that is all she has ever had and always tries to take good care of them. Two more days were spent surveying in very good habitat, but no other *A. asterias* were found. This ranch is worthy of more surveys, and possible future augmentation. This landowner is very interested in further conservation initiatives and also possible augmentation of her *A. asterias*.
***A. asterias* total individuals: 2**
Other endangered plants recorded: *Frankenia johnstonii*
Other rare plants recorded: *Coryphantha macromeris* var. *runyonii*
TPWD Permission: Yes
LRGV Candidate Conservation Agreement: No
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: No

Property 24 – This 36 acre triangular lot is actually the northeastern corner of Property 2 that the family decided to keep. A survey was conducted in November 2008 with many volunteer surveyors. Although many surveyed and flagged *A. asterias* and other rare plants, data was only collected by Poole and Janssen. Although a late lesson to learn, this method of using volunteers and then having only one or two people actually collect the data once the survey is complete works fabulously. A total of 149 individual *A. asterias* were recorded.
***A. asterias* total individuals: 149**
Other endangered plants recorded: none
Other rare plants recorded: *Coryphantha macromeris* var. *runyonii*, and *Manfreda longiflora*
TPWD Permission: Yes
LRGV Candidate Conservation Agreement: No
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: Yes

Property 25 – Located in-between Properties 4 and 5 sits this smallish 63 acre tract that has not been touched in years. A survey was conducted in March 2010 with two family members and two friends, and 68 star cacti were verified. Many were very large! The family members stated that they had never found it anywhere else on the ranch, but further surveys should be done.
***A. asterias* total individuals: 68**
Other endangered plants recorded: none

Other rare plants recorded: none
TPWD Permission: Yes
LRGV Candidate Conservation Agreement: No
Las Estrellas Conservation Cooperative Gate Signs & Management Plan: No

Negative Survey Results

Numerous ranches were surveyed within and outside of this 56 square mile area of south-central Starr County that *A. asterias* is now known to occur with negative results.

Ambiguous Occurrences

More so than with any other endangered plant species it seems that there have always been rumors and stories about localities of *A. asterias* that have never been verified by conservation biologists. To find an answer to some of these mysteries or to dispel some of the myths, each story was investigated.

<u>Dr. Martin Terry (Chief Redhawk claims to pray on a ranch with star cactus near Roma)</u> – In 2007 Dr. Martin Terry put me in touch with Redhawk, Chief of the Choctaw Nation. He stated that when he is in Rio Grande City visiting the local peyote dealer, Mauro Morales, that one of his peyoteros, Leo Trevino, will take him to his mother's ranch to pray and give thanks to the peyote. Chief Redhawk stated that there is also the star medicine in the area that he prays and that the star medicine is very abundant. Chief Redhawk and his wife visited my home several times. He promised to take me to this ranch the next time he was in Rio Grande City, but that time never came. I tried to catch Leo at Mauro's several times, but I would always miss him. Finally Mauro told me where his house was and what his truck looked like and for me to just go over there, and if the truck is there, he'll be there. So I found Leo's house and the truck was there. I knocked on the door, and a man fitting Leo's description answered. I introduced myself and asked if he was Leo Trevino, and he said, "No!" and slammed the door in my face. When I went back to Mauro's and told him what had happened, he said, "Oh well, he is always in trouble with the law and was probably scared of you." Repeatedly I would ask Mauro to help me visit this ranch, but he was reluctant to help—reluctant unless I was willing to pay him to take me there was the impression I got. He asked me often, "How much is it worth to you?" I would just laugh it off and pretend like I thought he was joking, but I know darn well he was serious. I am sorry that I could never gain the trust of these people or the access to this ranch. I believe it would have meant a lot to this study, and hopefully one day I will finally gain access. It is worth mentioning that Mauro Morales' prayer garden at his house contains a number of star cacti.

<u>The Glen Spraker letter of 1997</u> - In a phone conversation in 2003, Dana Price (TPWD botanist) followed up with Mr. Spraker. Although in his letter he stated that he had found *A. asterias* in northern Jim Hogg County, he said that was a mistake, and he was actually in southwestern Jim Hogg County. He said he turned west from highway 649 onto an unpaved road southwest of Guerra (road easy to get lost on). Today there is a sign designating the road as "Javalina County Road". Less than a half mile down the road it forks. To the right, it goes into Zapata County; to the left it eventually goes to Starr County and joins Loma Blanca Road. However, there are no

signs of any sort designating where the Zapata or Starr County lines are. According to the Jim Hogg Soil Survey (Sanders et al. 1974) (aerials 28 & 33), the road to the right goes into Zapata County within approximately four miles. The soils of Jim Hogg County along that road before the Zapata County line are classified as Copita and Tela soils, which are very sandy. In Zapata County, the soils eventually become more rocky and saline. The fork to the left hits the Starr County line within approximately six miles. The soils of Jim Hogg County along that road before the Starr County line are classified primarily Copita, and Tela, with one little segment of Zapata soils and one little segment of Brennan Soils. It is only further into Starr County where the "little rocky knolls" that Mr. Spraker refers to start to show up.

Since there are no signs along this road designating where the county lines are, and since the soils in Jim Hogg County are too sandy in the southwest corner, Mr. Spraker probably made his way into Starr County, or possibly even Zapata County, without realizing it. After surveying the area in 2006 and going back and forth on those roads, it is evident that there is no potential habitat in the Jim Hogg County portion of the Javelina County Road.

<u>Jim Everitt (vicinity of the Rio Grande City airport and his book photo)</u> - In an e-mail from 2006, Jim stated that, "I only found this species in one location back in the late seventies near the Starr County airport west of Rio Grande City. It was growing in association with *Varilla* (saladillo). There were only a hand full of plants at that site." The owners of the airport property are actually the same owners of the *A. asterias* site referred to as Property 3 in this report. Permission was granted to access this property, however the key is different for the airport portion than it is for the rest of the ranch. The key for the airport section of the ranch was secured, but a couple of days of surveys of this area only revealed one really large *Frankenia johnstonii* population.

When questioned about the picture of *A. asterias* that appears in *Trees, Shrubs & Cacti of South Texas* (Everitt et al. 2002), Everitt stated that the photo was taken in his yard; however, he did not elaborate as to where he actually got the plant.

<u>Zach Labus (hills northwest of the Villa Real Apartments)</u> - Back in the early 1990s, Zach Labus, a local school teacher, notified Angie Brooks (then botanist with the USFWS Corpus Christi office) that he had found an *A. asterias* in a colonia and dug it up. He described this colonia as in the Los Saenz area about a quarter mile northwest of the Villa Real Apartments. When called at the onset of this research project, Zach Labus confessed that his memory was foggy regarding where he was exactly. He was not interested in driving to the area. He said that it was all changed and developed now, and it wouldn't be the same. When asked what happened to the one *A. asterias* that he dug up, he stated that he had it in a pot on his porch for a while but it eventually, as he put it, "croaked".

Lisa Williams of the Nature Conservancy of Texas said that Zach had taken her to that place. Although she did not know or remember that Zach had found *A. asterias* there, her memory was of all the petrified wood that was being destroyed by all the construction and excavations.

The Villa Real Apartments are at the intersection of Highway 83 and the San Julian Road. This area, technically, is in Escobares. (Los Saenz morphs into Escobares, so it is easy to see how

Zach thought he was around Los Saenz.) There is now a new housing development/trailer park behind the Villa Real apartments, and then right after that, "the hills" start. There are a lot "holes" that look like small quarries, and a lot of buffelgrass invasion. Several days were spent driving around the hills, and an area with petrified wood was searched thoroughly, but no *A. asterias* were located. Many areas around there had great potential. However, one population of *Frankenia johnstonii*, up in the hills north of Escobares was found.

There is probably still *A. asterias* in the hills just north of Escobares, but it may take a long time to find it.

<u>Loretta Pressly (Bass Ranch)</u> – Loretta Pressly, former botanist for USFWS in south Texas, provided this lead. The owner of the Bass Ranch was contacted in 2006, and was very interested in the project. Through e-mails and photographs, the owner decided that his ranch in Hidalgo County probably had the most potential, but he'd check out the others in Brooks and Kenedy counties too.

In regard to this lead, Loretta said that Mr. Kratz of Kratz's Nursery told her that he had dug up *A. asterias* on the Bass Ranch. (For years Kratz's nursery has been selling *A. asterias*.) Mr. Kratz (who is now in poor health and has closed the nursery, but not his bait shop) when asked him if he knew of any *A. asterias* localities, only said "Just follow the peyote, and wherever you find peyote, you'll find *A. asterias*." When asked where that would be, he said, "Oh you know, over around Mirando City and places like that." When the owner of the Bass Ranch was e-mailed that Mr. Kratz was the source of the information of *A. asterias* on the Bass Ranch, the owner never replied despite several attempts.

<u>Angie Brooks (Kratz Ranch)</u> – See Loretta Pressly and the Bass Ranch above. These two stories may be somehow confused or connected.

<u>Jose Guadalupe Martinez-Avalos (west of the town of Zapata)</u> – In March 1998, Martinez-Avalos, along with a group of students, reported they had located eight individual *A. asterias* west or southwest of the town of Zapata in Zapata County. They had recorded the locality using a GPS unit, and he provided the coordinates. In October 2002, using the GPS coordinates, the coordinates lead deep into the inundation zone of Falcon Reservoir. In 2003, Martinez-Avalos came to Texas to visit with Dana Price and Christ Best (USFWS botanist), and they went to visit the site. According to Chris Best, Martinez-Avalos seemed a little confused, as if things had changed. They surveyed the property on the south side of the road just before the inundation zone, but did not cross the fence onto the adjacent private property.

In January 2005 the landowner of this tract was contacted, and permission to survey was obtained. An entire day was spent hiking the tract although it did not look like any of the sites in Starr County. Additionally, approximately two more days were spent surveying nearby tracts that had more suitable habitat. No *A. asterias* was found to the west of the town of Zapata.

<u>Dana Price (La Gloria café specimen)</u> – In April 2003, Dana Price and Chris Best were dining at the La Gloria Café in La Gloria in Starr County. After noticing that the pair was interested in plants, the café owner invited them out back to see her "cactus garden" (whether it was an actual

garden or if the cacti were all in pots is unknown). The café owner had both peyote and *A. asterias* that she said her son had dug up from a ranch that he works on in Starr County.

After visiting with the café owner and her sister, it was learned that the son worked a ranch belonging to the owners of Property 3. A ranch manager stated that they use all the same cowboys and workers on the two Starr County ranches owned by the Trust. Although the son was never located, it is most likely that he dug up both the peyote and the *A. asterias* off of Property 3, not the other ranch. The other ranch has been surveyed by several botanists (Janssen, Poole, Carr, Williamson, and others), and no *A. asterias* has been located (the soils are not right, and there were many improved pastures).

<u>Chris Best (Noel Benevides and *A. asterias* in petrified wood at his home)</u>
Noel Benevides was interviewed in April 2005. Noel owns a subdivision called the Pablo A. Ramirez Subdivision #1. His house is in this subdivision. When they were building the homes for this subdivision, they were bringing in fill material for the foundations from north of Escobares (see Zach Labus story above). This material was packed with petrified wood. Noel said that he would walk around the construction sites and pick out some of the best pieces. He placed these pieces of petrified wood in various places around his home. One day he noticed an *A. asterias* growing from a crack in one of the pieces of petrified wood. Additionally, there is another source of information to corroborate that there used to be an abundance of *A. asterias* just north of Escobares. The wife of the owner of Property 8 is from Escobares, and she and her husband still own a home just north of Escobares. After they moved to McAllen, they rented the house, and the renters root plowed the site and planted buffelgrass. The owner said that the property used to have *A. asterias* all over it.

<u>Chris Best (Noel Benevides and the Starr County Judge Eloy Vera know of *A. asterias* sites off Loma Blanca Road)</u> - After interviewing both Noel Benevides and Judge Eloy Vera, it was clear that these two men had never seen *A. asterias* on ranches along the Loma Blanca Road. They both had gotten their information secondhand from a local peyotero named Chuy Vera. Noel and the Judge both own ranches along the Loma Blanca Road. Noel and the Judge know Chuy fairly well since apparently they run into him often on their ranches. Chuy has told both Noel and the Judge that he has seen *A. asterias* on their ranches and on other ranches along the Loma Blanca Road. Chuy has never said exactly where he has seen it. Two botanists spent a day surveying Noel's ranch, but found no *A. asterias*. The Judge also allowed access, but his ranch has not been surveyed.

It is also clear that Noel and the Judge each have a different relationship with Chuy. For example, Noel told Chuy that if he ever collected *A. asterias* accidentally, to bring it to him, and he would give it to Chris Best. Over the years, Noel estimates that he has given Chris Best about 10 star cacti from Chuy. On the other hand, the Judge had Chuy arrested for trespassing on his ranch.

It was obvious to me that the man that I needed to talk to was Chuy Vera. I figured out where he lived, and decided to drop by one day. His humble home was surrounded by a very high chain link fence that was gated. The gate was not locked—the chain and the lock were just dangling there. So, I opened the gate and walked up the driveway to the front door. Big Mistake. While

I'm standing there in my shorts and hiking boots, clip board in hand, knocking on the door, suddenly I hear this vicious, snarling, growl behind me. I turn around and there is a dog whose face is all exposed gums and teeth, ears pulled back, ready to attack. My first response was to say to the very big dog in a very sweet voice, "Oh, it's okay baby. I just want to talk to your daddy." Big Mistake #2. Apparently, mean dogs don't like baby talk. The dog took a lunge at me stopping herself (I know it was a "her" because her teats were almost dragging the ground) just at my kneecaps. She slid to sliding halt, and I could hear her claws trying to grasp the concrete to stop. So, I thought at this point I would pretend that I wasn't scared of the evil devil dog, and turn around and walk away. Big Mistake #3. The dog then took after me, again stopping herself by making that awful claw sliding sound on the concrete, and this time letting her wet nose hit the back of my knee and leaving it there, just growling and snarling. At this point, I am really scared to death but trying not to let it show, and thinking that this has got to be one, if not THE stupidest, things I have ever done. I had about 12 more feet until I could reach the gate. I then turned around slowly to face the dog, and started walking backwards towards the gate. Every two steps I took, the dog would lunge again, slide, and let her nose hit my knees. Finally, I reached the gate, got through and lock it. And as I am locking it, I look up and to my right there is a sign, a very sun and heat faded, nearly unreadable sign that said "*Beware of Dog*".

Education/Outreach

In May 2004, the Associated Press prepared an article about the Las Estrellas preserve and the beginning of this research project. They interviewed both Lisa Williams and Gena Janssen, and took many photographs. It ran in newspapers all over Texas (and perhaps elsewhere). No leads or contacts ever came from that article.

In April 2006, three south Texas newspapers carried an article entitled *"In Search of the Very Rare and Endangered Star Cactus"*. This article ran in the Starr County Town Crier (with one very poor black and white photo) on April 26, 2006; in The Zapata County News (with color and black and white pictures) on April 27, 2006; and, The South Texas Report (with no accompanying picture) on April 27, 2006. Each newspaper ran the article just as it was written by the *A. asterias* research team. Although this article was also submitted to the Rio Grande Herald and the McAllen Monitor, they chose not to run the article. Representatives at the McAllen Monitoring stated that they were trying to focus their outdoor articles on the drought and its effect on local wildlife at the time. At The Rio Grande Herald the article apparently slipped through the cracks, but they promised to run the article in the future. Unfortunately, these newspaper articles did not result in any new leads or contacts for *A. asterias*.

In 2008 and 2009, a vast majority of the education and outreach was constant communication with the two active seismic/gas companies in the area. There was always more data to share, someone new to meet with, and someone new to take to see *A. asterias* in the field. Training sessions were provided for crew members, but their crews changed tremendously every couple of months, and then education, outreach and training had to start all over again.

The most successful education and outreach in Starr County was one on one visits. Whether it's a private landowner, a local restaurant owner, or members of the Starr County NRCS office and Soil and Water Board members, meeting people face to face and talking and sharing pictures and descriptions of *A. asterias* was really the only outreach that worked. Color laser pictures on 8.5 x 11 paper were always a big hit and were readily shared with interested parties.

The people of Starr County are very fragmented, and there is no great sense of community that brings people together. There is not one major newspaper that everyone reads to keep up with county politics and happenings as in surrounding counties where the local paper is the life-blood of the community. People are very private and secretive in Starr County, and keep to themselves more often than not. Even landowners whose families have owned property for decades in Starr County do not know their neighbors. Some landowners are not even sure where their property is exactly. Landowners often asked ***the surveyor*** who their neighbors were, and for a copy of ***her*** map!

One of the greatest ways to identify landowners and property boundaries is through gas companies. Legally they have to know proper ownership and boundaries, and have teams of people constantly researching this. The two gas companies that recently performed the seismic surveys in Starr County both had at least five to 10 property researchers that worked for over five years identifying landowners before the project ever started. One of the gas companies, Edge Petroleum, has since filed for bankruptcy and one of the primary reasons given was the amount of time and money they spent on the landowner research.

Other Relevant Conservation Initiatives

Voluntary Conservation Agreements and Las Estrellas Conservation Cooperative

Although inviting private landowners to sign Voluntary Conservation Agreements to protect *A. asterias* on their property is not technically part of the original proposal, a brief mention of this information is included in this report because of its significant conservation implications.

Voluntary Conservation Agreements have been signed by six of the 25 verified private land sites for *A. asterias* as part of a separate federally funded conservation project (Section 6—Lower Rio Grande Valley Candidate Conservation Agreement). Voluntary Conservation Agreements are now in place for: Property 2 (415 acres); Property 3 (15,200 acres); Property 5 (750 acres); Property 6 (30 acres); Property 7 (ca. 10 acres); and Property 8 (400 acres). The area voluntarily protected by private landowners for the preservation and conservation of *A. asterias* into the future totals almost 16,815 acres.

In yet another separate project funded by Texas Parks and Wildlife Dept., The Nature Conservancy of Texas, and the U. S. Fish and Wildlife Service Partners for Wildlife, nine of the 25 *A. asterias* properties (Properties 2, 4, 5, 7, 11, 13, 15, 16 and 24) have signed management plans for rare species conservation and are now displaying "Las Estrellas Conservation Cooperative" gate signs (Photo 1). These gate signs were created specifically for the landowners surrounding The Nature Conservancy's Las Estrellas Preserve (Preserve; Property 2) that have the endangered *A. asterias*. Developing this "Cooperative" of landowners with *A. asterias* will foster a sense of community along with conservation. It will open lines of communication by helping neighbors get to know each better, and encourage landowners to keep a watchful eye on each other's properties. By working together with the Preserve, this Cooperative of ranches can more effectively conserve the *A. asterias* populations in Texas. It is the goal of this fledgling cooperative to eventually have all 25 properties become cooperative members. Road signs keeping ranch visitors on the roads and off of the sensitive habitats have also been posted throughout these properties.

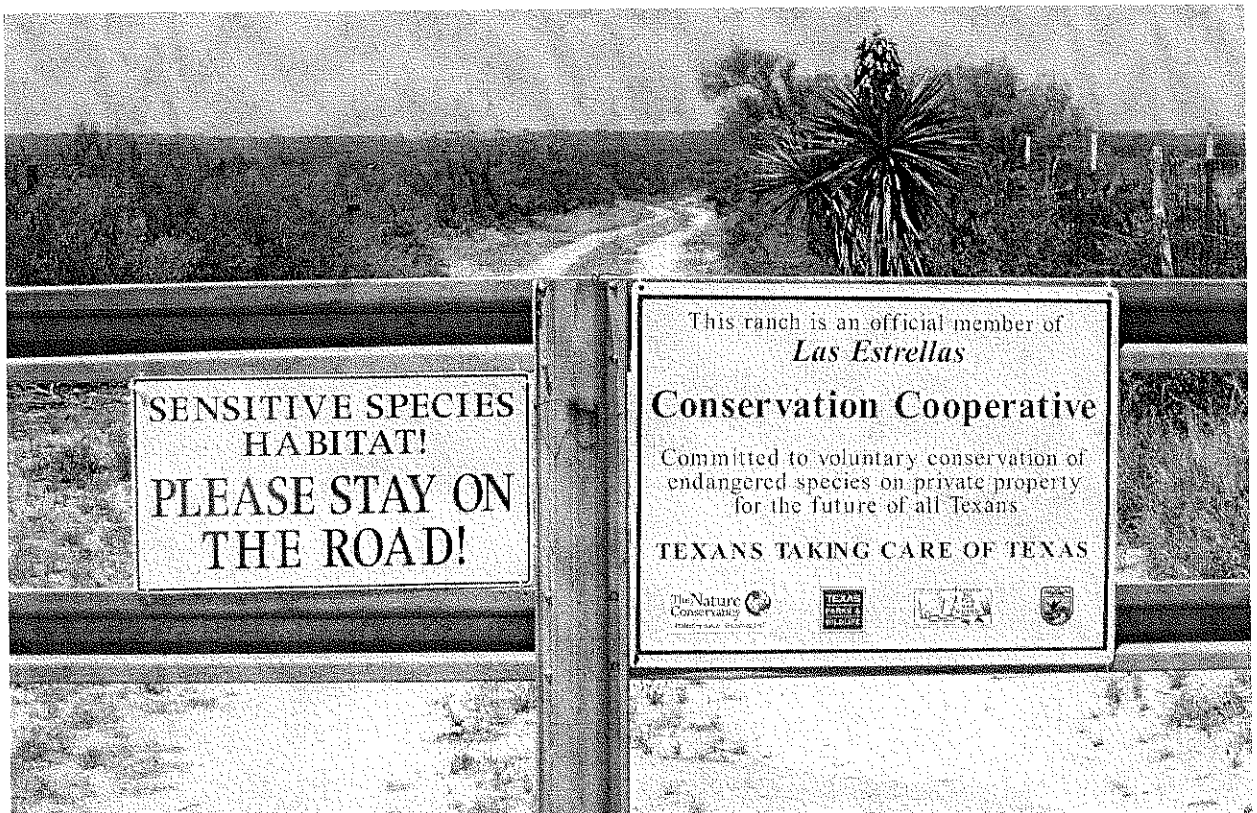


Photo 1. Las Estrellas Conservation Cooperative gate sign displayed together with a road sign on the front gate of an *A. asterias* property.

Impacts to *A. asterias* Properties

Two overlapping 100 square mile seismic projects that included all the private ranches with *A. asterias* but two (Properties 7 & 11 which are the furthest to the west) was completed by August 2009. The two gas companies, Edge Petroleum and EOG, have now left Starr County. Both Edge and EOG were aware of the *A. asterias* sites and the Voluntary Conservation Agreements with Texas Parks and Wildlife Dept.. Both companies were amiable to crew field training for *A. asterias* and allowed for all the sites to be flagged to insure there would be no accidental impacts on the ground. Both companies used existing *A. asterias* and *Frankenia johnstonii* shapefiles and overlaid them with their seismic line map. Assurance was given that lines were moved via the computer before any heavy equipment hit the ground. The companies did not, however, want a biological monitor. All sites were first flagged in the late summer and early fall of 2008, and then reflagged with yellow and black "Caution" flagging in January 2009. Despite these efforts and relatively constant communication, 13 sites were impacted by the roller chopper that cleared the seismic lines. Approximately 160 individual star cacti were lost.

Edge Petroleum has since filed for bankruptcy, but EOG is still in business. The two seismic companies that were subcontracted by the above mentioned gas companies have now expressed an interest in possibly financing restoration efforts of these sites. The preserve manager of Las Estrellas Preserve is taking the lead on this and is planning restoration efforts within the next year if funding from the responsible parties is forthcoming.

CONCLUSIONS:

The results of our breeding system study show that *A. asterias* is an obligate outcrosser. Self-incompatible species are more likely to produce new genetic recombinations that may allow for adaptation to a changing environment compared to self-compatible or inbreeding species. However, reproductive success in an outcrossing species is dependent on a vector to transfer pollen for seed set. As a self-incompatible species, *A. asterias* might be experiencing reproductive constraints in terms of pollinator availability or pollinator effectiveness.

Our pollinator effectiveness and pollinator importance study of *A. asterias* indicate that the most common visitor, *Macrotera lobata*, is a relatively ineffective pollinator, while the less common *Diadasia rinconis* is the most effective and important pollinator. Effective pollination is important in maintaining genetic diversity in this species. Therefore efforts should be made to protect habitat for the effective pollinator, *D. rinconis*, as well as *A. asterias*. The bee species identified as floral visitors in this study are solitary species (excluding the semi-social *Dialictus* sp.) that either nest in patches of bare ground (*D. rinconis, M. lobata*) or in hollow twigs (*Ashmeadiella* spp.). Areas set aside for *A. asterias* will need to provide adequate nesting sites for these pollinators. *Diadasia rinconis* often nests in large aggregations; sometimes with hundreds of nests clustered in a patch of suitable nesting habitat (Ordway, 1987). Thus, it may be possible to maintain a sizeable population of *D. rinconis* in a relatively small area of appropriate nesting habitat.

Bees are particularly sensitive to many insecticides used to control crop pests (Peach et al., 1993; Cane and Tepedino, 2001), so pesticide treatments might need to be adjusted (i.e. use treatments that are less toxic to bees, spray at times when pollinators are not active) to minimize non-target effects on pollinators. Overall, the complex network of interactions between plants, pollinators, and the surrounding landscape makes it necessary to adopt conservation measures that are ecosystem-oriented, rather than those that are simply species-oriented.

Astrophytum asterias is dependent on its pollinators in order to maintain viable populations and these pollinators are in turn dependent on their pollen sources in order to reproduce and maintain viable populations. Cactus-specialist bees such as *D. rinconis*, *M. lobata*, and *Ashmeadiella* spp. likely depend on the presence of multiple species of cacti blooming throughout their foraging season to provide a continuous source of pollen. Moeller (2004) provides evidence that plants can benefit reproductively from the presence of other plant species sharing the same pollinators. This result in conjunction with the obligate need for pollen for bee offspring production suggests that diversity and abundance of other cacti at a given site might be needed to maintain adequate pollinator populations for *A. asterias*. The common south Texas range management practices of root-plowing vegetation and planting pastures with non-native grasses are not only detrimental to *A. asterias* directly, but they also likely decrease the abundance and diversity of other species of cacti which might reduce habitat quality for pollinators of *A. asterias*. These practices should thus be discouraged on lands managed for *A. asterias*.

In plants, gene flow occurs via seeds and pollen; therefore pollen dispersal can have a substantial influence on the genetic makeup of plant populations. We used fluorescent dye as a pollen analogue to track pollen dispersal within a 1.9 hectare patch of *A. asterias*. The longest dispersal event recorded was 142.2m, with a mean dispersal distance of ~17m. Eighty percent of recipient plants were located within 30m of the source plant. Close proximity of populations enables pollen dispersal and thus gene flow among populations. To maintain pollinator movement between populations, future urban expansion in Starr County should be planned so as to not further fragment existing locations of *A. asterias*.

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 in order to determine biological success of our pilot reintroduction. Project success results from contributions that increase the knowledge regarding the specific taxon, even if the biological aspect of a reintroduction fails (Pavlik, 1996). The pilot reintroduction of *A. asterias* has achieved project success. For plants, knowledge of the type of propagule to use in reintroduction, season of planting, site preparation, post-planting care, etc. are essential (Guerrant and Kaye, 2007). Our pilot reintroduction study demonstrated that seedlings are a better choice for propagule type and that seedlings planted in the fall had a higher growth rate than spring planted seedlings. The study also showed that seedlings should be planted at least 3 m from active *Spermophilus mexicanus* burrows, since this species constitutes a threat of herbivory to *Astrophytum asterias*.

Our research into the impacts of mammalian and insect herbivory on populations of *A. asterias* clearly indicate that these two groups pose a serious threat to established populations of *A. asterias* in Texas. Our research identified a number of species that may potentially feed upon and ultimately kill *A. asterias* including: desert cottontail (*Sylvilagus audubonii*), Mexican ground squirrel (*Spermophilus mexicanus*), southern plains woodrat (*Neotoma micropus*), cotton rat (*Sigmodon hispidus*), and larva of the cerambycid beetle *Moneilema armatum*. Combined with other mortality factors (desiccation, fungus/rot, and unknown) the average percent mortality among 5 populations of *A. asterias* (totaling 1606 individual plants) was 46.4% (range: 25.6% - 78.8%). One monitored population was reduced from 146 plants to only 11 individuals from September 2007 – September 2009. The long-term impacts on these populations is unknown and continued monitoring of at least a subset of these populations should continue to examine if successful reestablishment of *A. asterias* can occur in areas where populations have been extirpated due to herbivore-induced mortality. This type of research may help in understanding the metapopulation dynamics of this species, which could prove critical to its successful recovery (Schemske et al. 1994). Future research into mammalian and insect herbivory of *A. asterias* should include assessments of the influence of environmental parameters on rates of herbivory, methods to prevent excessive amounts of herbivory and or protect individual plants or threatened populations, and the long-term impacts of herbivory on population dynamics such as growth rates, demographic structure, and recruitment rates.

Our research on the biology and ecology of *A. asterias* culminated in development of a reintroduction plan as required by Federal Register 65(183):56916-22 (USFWS, 2000) (see Reintroduction Plan section). We initially envisioned reintroduction as a likely necessary step in recovery of *A. asterias*. However, our surveys resulted in significant finds of previously unknown wild populations. Therefore, we do not find a current need to use reintroduction as a recovery method. We do believe reintroduction is a viable means of augmenting wild populations that suffer impacts.

REINTRODUCTION PLAN:

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. In Mexico, several populations were known from Tamaulipas and Nuevo León.

Using the soil types as defined in the Soil Survey of Starr County, Texas, 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. 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.

Astrophytum asterias is an obligate outcrosser with a slow growth rate, has low flower production, and low fruit and seed set compared to other cacti 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. Anthropogenic threats to *A. asterias* included habitat destruction/modification and over-collection by cactus enthusiasts. 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 occurs infrequently, but even an incidental harvest rate of 0.1% has profound implications since peyote harvest in Texas has fluctuated around 2,0000,000 buttons. Other threats include gas exploration (seismic surveys) and urbanization/sprawl.

Astrophytum asterias is assigned a priority ranking of 2 by the USFWS, which indicates it faces a high degree of threat, yet has high recovery potential. The recovery criteria as outlined by the recovery plan includes 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." 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 24 properties in a 56 square mile area of Starr County with subpopulations of *A. asterias*. Recent research in Mexico recognizes seven populations in Tamaulipas and two in Nuevo León with population numbers ranging from 10-704. A pilot reintroduction of *A. asterias* was established 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. 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. A complete list of associated species in order of dominance as documented in 15 vegetation transects are provided in the appendix. 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%.

Table 1. Averages (*Avg*), standard deviations (*SD*), and ranges of soil parameters from routine soil analyses of soil samples collected within 15 vegetation transects 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 = parts per million.

	pH	Cnd	NO_3	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
Avg	8.3	2256	10	16	300	19099	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

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.

Pollinators

Effective pollination is important in maintaining genetic diversity in this species. Therefore efforts should be made to protect habitat for the effective pollinator, *D. rinconis*, as well as *A. asterias*. Areas set aside for *A. asterias* will need to provide adequate nesting sites for these pollinators. Close proximity of populations enables pollen dispersal and thus gene flow among populations. To maintain pollinator movement between populations, future urban expansion in Starr County should be planned so as to not further fragment existing locations of *A. asterias*. Bees are particularly sensitive to many insecticides used to control crop pests (Peach et al., 1993; Cane and Tepedino, 2001), so pesticide treatments might need to be adjusted (i.e. use treatments that are less toxic to bees, spray at times when pollinators are not active) to minimize non-target effects on pollinators

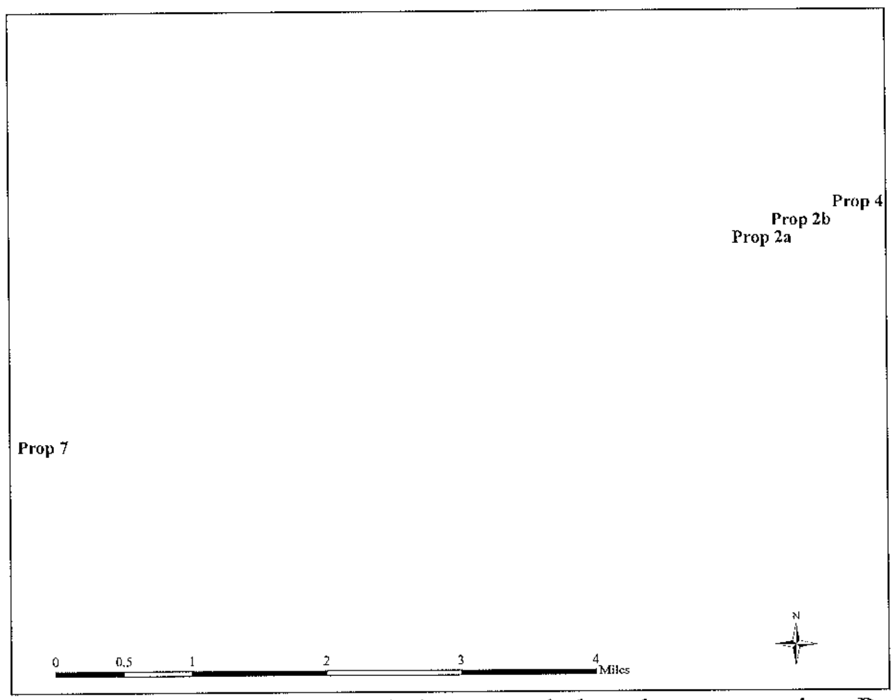


Figure 1. Location of the four subpopulations sampled on three properties. Property numbers correspond to those used in the Section 6 reports by Janssen, et al.

Propagation

Seedlings are the preferred propagule for reintroduction as <4% of the seeds planted in the pilot reintroduction germinated. 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). 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. 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. 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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United States Fish and Wildlife Service. 2003. Recovery plan for star cactus (*Astrophytum asterias*). United States Fish and Wildlife Service, Albuquerque, New Mexico.

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 A

Table A.1. 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 A.2. Species intercepted and dominance values (%) of each by study site. Sites AM1, AM2, AM3 = Property 1; CA = Property 9; EE = Property 7; JB1, JB2 = Property 5; KR = Property 3; LA = Property 4; LM = Property 6; NC1, NC2 = Property 2; PP1, PP2 = Property 8; RE = Property 2

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

Table A.2-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																
Guajacum angustifolium						0.01	0.04		0.92	0.13		0.24				1.11
Gutierrezia texana		4.07												1.08	0.56	
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		

Table A.2-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																
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
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						

Table A.3. 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 A.4. Routine soil analysis results of soil samples collected within the 15 vegetation transects, pilot reintroduction site (RE; Property 2), and one sample (Out; Property 5) collected adjacent to site JB1. Samples collected March, May 2006 and March 2007. Sites AM1, AM2, AM3 = Property 1; CA = Property 9; EE = Property 7; JB1, JB2 = Property 5; KR = Property 3; LA = Property 4; LM = Property 6; NC1, NC2 = Property 2; PP1, PP2 = Property 8. 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 A.5. 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. Sites AM1, AM2, AM3 = Property 1; CA = Property 9; EE = Property 7; JB1, JB2 = Property 5; KR = Property 3; LA = Property 4; LM = Property 6; NC1, NC2 = Property 2; PP1, PP2 = Property 8. 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

Table A.6. 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 A.6-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 B

Table B.1. Visitors to *Astrophytum asterias* from March 17 – May 7, 2005 at Property 4. N = number of individuals observed within 3,130 minutes.

Apoidea

Genus	*Family*	*No. of Individuals*
Agapostemon angelicus/texanus	Halictidae	1
Agapostemon tyleri (Cockerell)	Halictidae	1
Anthophorula compactula (Cockerell)	Apidae	3
Ashmeadiella cactorum (Cockerell)	Megachilidae	1
Ashmeadiella maxima (Michener)	Megachilidae	7
Ashmeadiella meliloti (Cockerell)	Megachilidae	4
Augochlorella bracteata (Ordway)	Halictidae	1
Diadasia rinconis (Cockerell)	Apidae	4
Dianthidium discors (Timberlake)	Megachilidae	2
Lassioglossum/Dialictus sp.	Halictidae	4
Macrotera lobata (Timberlake)	Andrenidae	27
Osmia subfasciata (Cresson)	Megachilidae	1

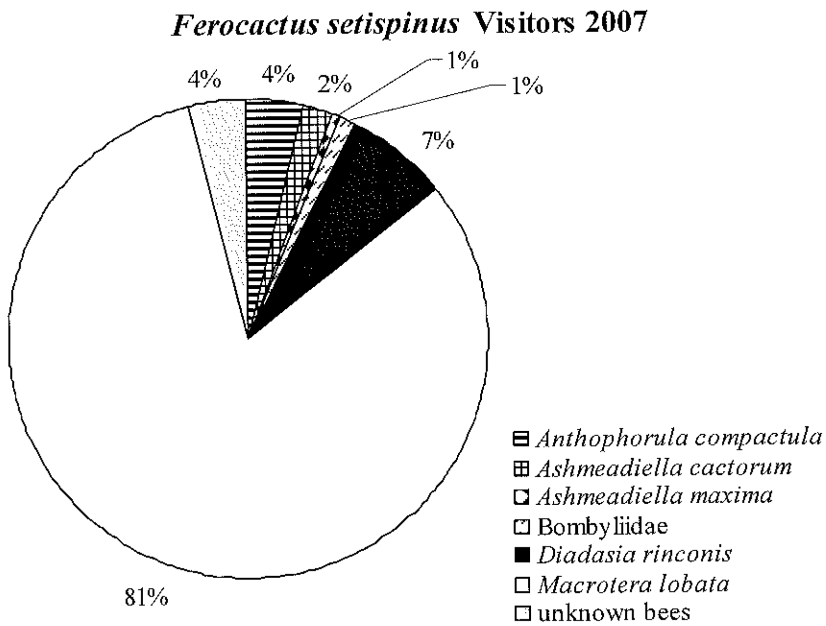
Coleoptera

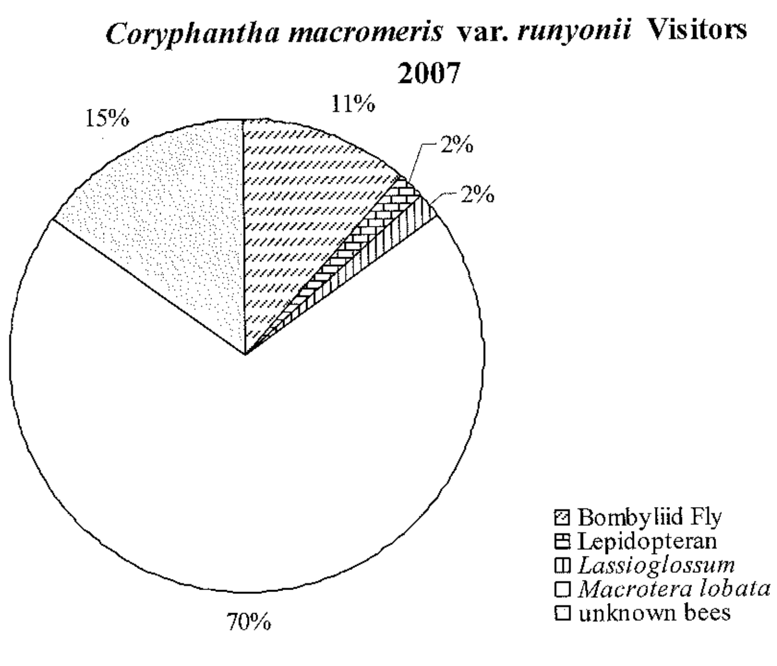
Taxa	*Family*	*No. of Individuals*
Acanthoscelides sp.	Chrysomelidae	1
Acmaeodera sp.	Buprestidae	66
Carpophilus sp.	Nitidulidae	40
Dasytinae	Melyridae	10
Euphoria kerni (Haldeman)	Scarabaeidae	2
Selvadius sp.	Coccinelidae	1

Other

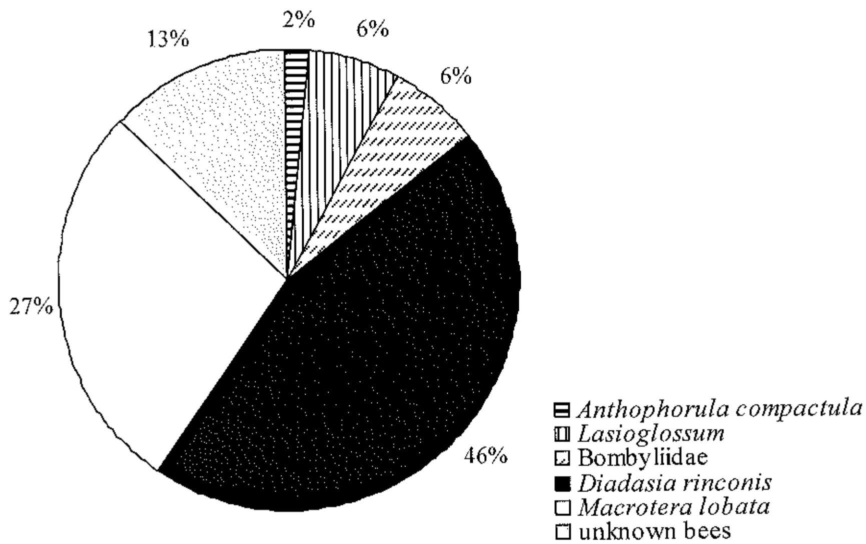
Taxa	*No. of Individuals*
Syrphidae	2
Forelius mccooki (Forel)	75

Figure B.1. Pie charts showing floral visitors.

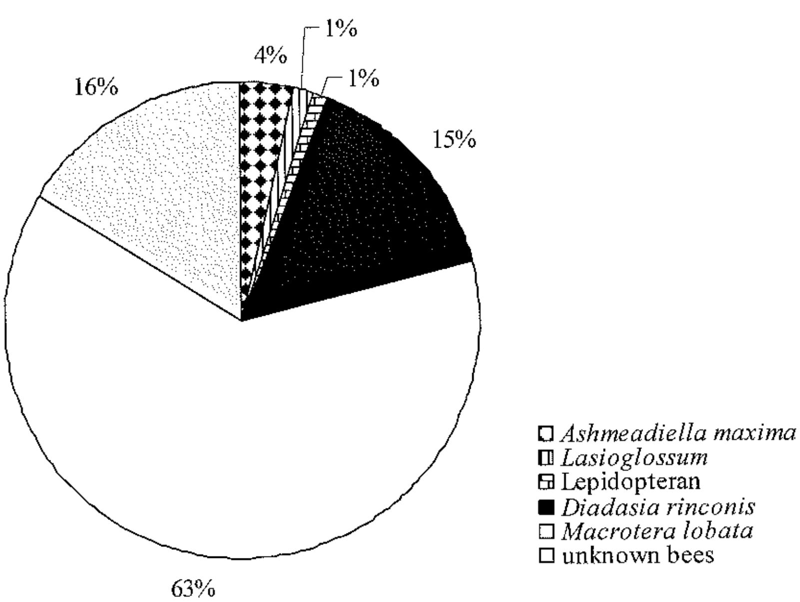




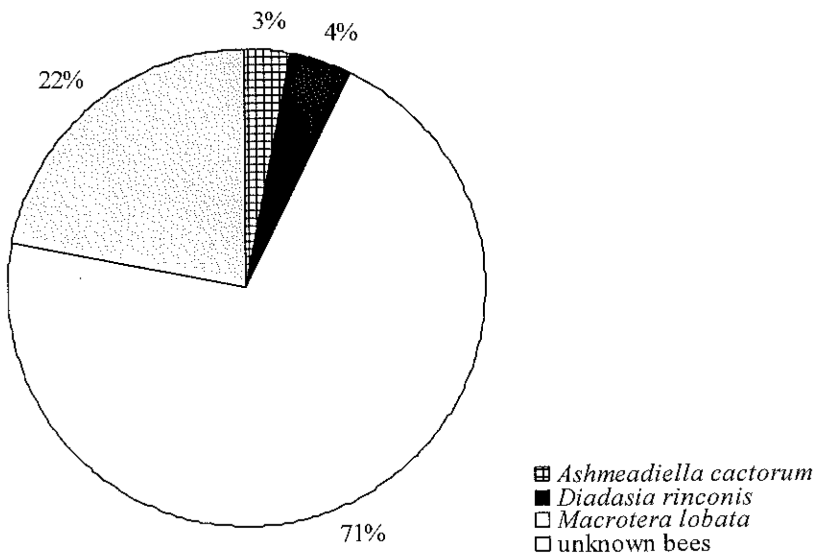
***Echinocereus reichenbachii* var. *fitchii* Visitors 2007**



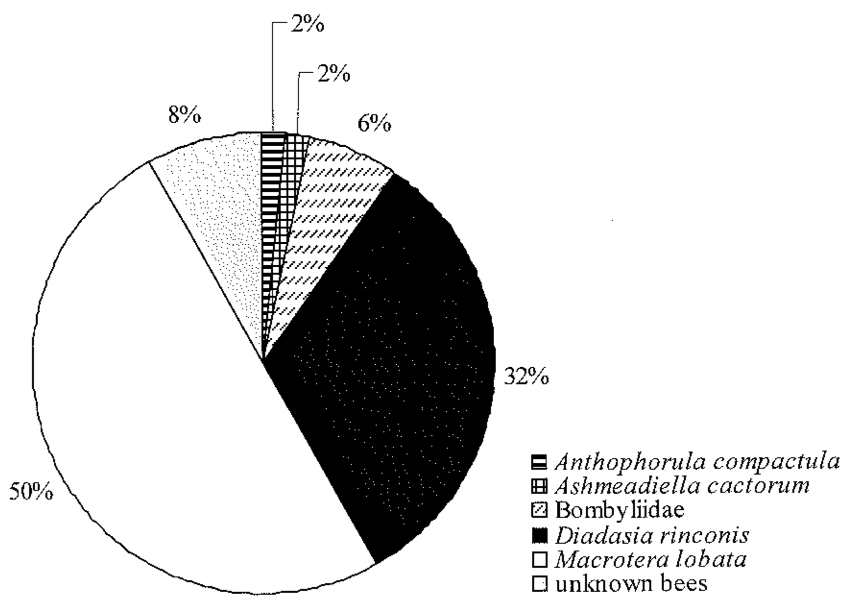
***Thelocactus bicolor* Visitors 2007**



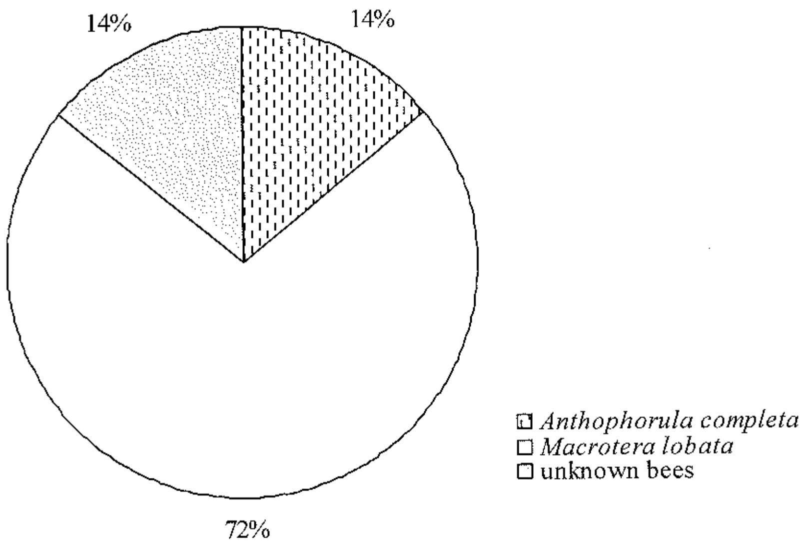
***Echinocereus enneacanthus* Visitors 2007**



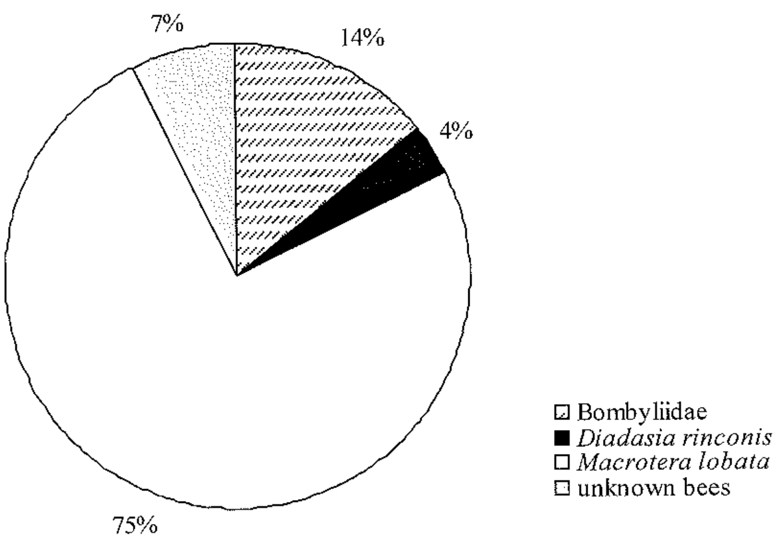
***Echinocereus pentalophus* Visitors 2007**



Lophophora williamsii Visitors 2007



Mammillaria sphaerica Visitors 2007



APPENDIX C

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. C.1). 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. C.1). 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. C.1). 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.

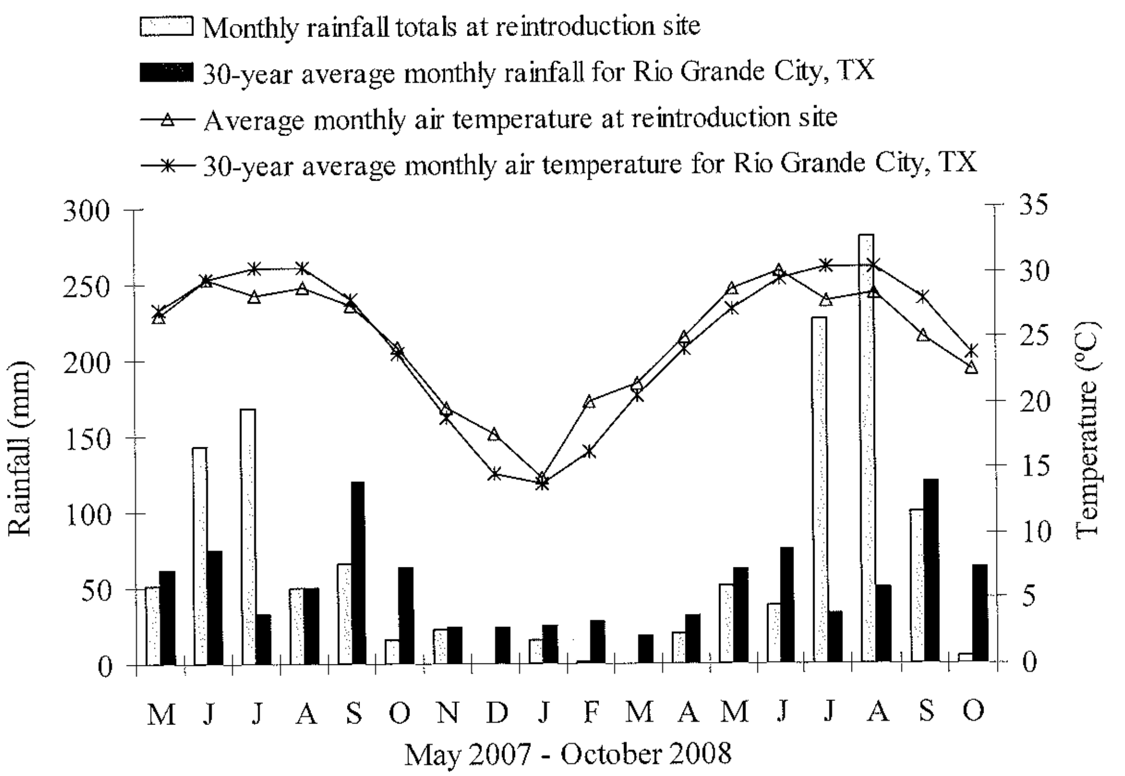


Figure C.1. Monthly rainfall and average monthly air temperature at the reintroduction site and the average monthly rainfall and air temperature for Rio Grande City, Starr County, Texas, 1971-2000 (National Climatic Data Center, 2002).

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. C.2). 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. C.2). 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.

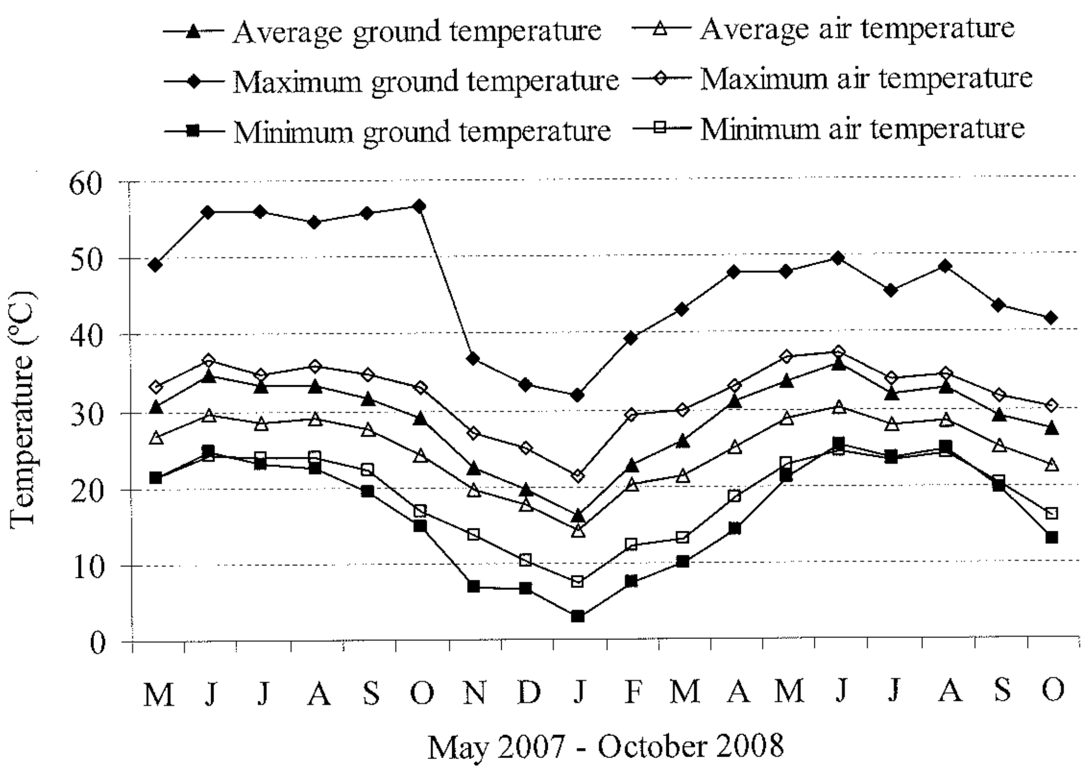
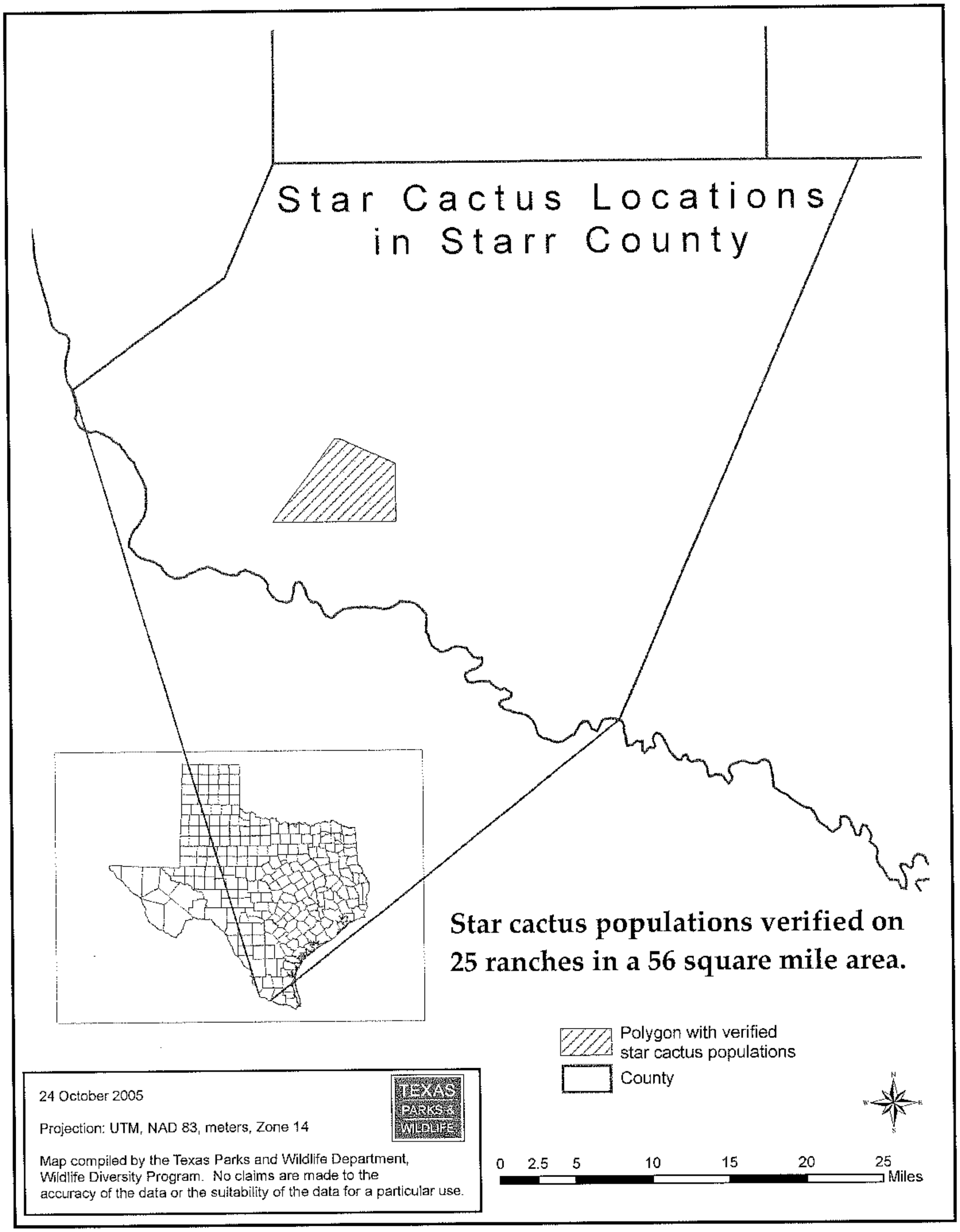


Figure C.2. Average, maximum, and minimum monthly air and ground temperatures at the reintroduction site. .

The highest monthly average ground temperature of 35.9°C was recorded for June 2008 (Fig. C.2). 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 >50°C with 7 of these days ≥55°C. The highest maximum ground temperature of 56.7°C was recorded on 1 October 2007. The lowest monthly average ground temperature of 16.3°C was recorded for January 2008 (Fig. C.2). For only a total of 2 days, the daily average ground temperature dropped below 10°C. The lowest daily average ground temperature of 7.9°C was recorded 18 January 2008. The minimum daily ground temperature was <10°C for 21 days; only 2 of which it was <5°C. The minimum ground temperature of 2.7°C 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 D



Star Cactus Locations
in Starr County
Star cactus populations verified on
25 ranches in a 56 square mile area.
Polygon with verified
star cactus populations
County
24 October 2005
Projection: UTM, NAD 83, meters, Zone 14
Map compiled by the Texas Parks and Wildlife Department, Wildlife Diversity Program. No claims are made to the accuracy of the data or the suitability of the data for a particular use.
TEXAS PARKS & WILDLIFE
0 2.5 5 10 15 20 25 Miles

APPENDIX E

In Search of the Very Rare and Endangered Star Cactus

Texas Parks and Wildlife (TPW) and Texas State University (TSU) are cooperatively working on a research and recovery project for the elusive star cactus (*Astrophytum asterias*). Federally and State listed as endangered, the only place in the United States that star cactus can be found is within a 20 square mile area in south-central Starr County, Texas. Although a Mexican Botanist reported finding star cactus in Zapata County in the 90's, no one has been able to verify this occurrence.

Currently known from only a handful of private ranches in Starr County, this spineless cactus that grows flush with ground can often be mistaken for peyote. But look closely! Star cactus is always divided into 8 triangular sections that resemble pieces of pie. Each one of the triangular "pies" will have small white circular tufts of hair and even smaller white scales. Peyote is not divided into 8 distinct sections; rather it can have varying numbers of "nipples" around the outside. And while peyote is always a grayish, blue-green color, star cactus is usually a much darker army green, but it can also change to yellow, red, or brown depending on rainfall and other factors. Star cactus can usually be found on the more open, rocky, saline areas of the monte where there is usually quite a bit of saladillo (*Varilla texana*), goat bush or amargosa (*Castela texana*), and many other species of cacti. Star cactus can be found out in the open gravelly areas, hiding within the saladillo, around the base of amargosa, or frequently finding safety under and around the tasajillo (*Opuntia leptocaulis*). Star cactus has been found primarily on soils mapped as Catarina, but has also been located within Jimenez-Quemado, Montell Clay, Eroded Maverick, Garceño Clay, and Ramadero Loam.

TPW and TSU researchers are trying to verify additional localities for the first phase of their research project. Can you help? Have you seen this cactus? Do you have it on your ranch? If so, please contact us! We would like to visit with you and verify your star cactus locality. We are currently trying to verify all the existing star cactus sites in Starr County to collect data including a count of the star cactus, soil analysis, vegetation analysis, and other important information such as pollinators, predation (something seems to be eating them!), and collection pressures. Once this phase of the research has been completed, the second phase will be to start trying to actually "recover" this species in Texas. This will be accomplished by reintroducing (or planting) star cactus onto ranches with the proper habitat and willing landowners who want to help protect, preserve, conserve and recover this vanishing Texas treasure.

Please contact Gena K. Janssen at Cingular 512.461.4684, Home Office 512.282.7222, or e-mail: gena@janssenbiological.com. Gena Janssen is a TPW subcontractor and the lead investigator for this project.

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