THE REPRODUCTIVE BIOLOGY OF STAR CACTUS

(*ASTROPHYTUM ASTERIAS*)

Presented to the Graduate Council
of Texas State University-San Marcos
in Partial Fulfillment
of the Requirements

for the Degree

Master of SCIENCE

by

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San Marcos, Texas
December 2005

Dedicated to
Maxine Trenck Wendler
and
Emily Jo Trenckmann Strong

ACKNOWLEDGEMENTS

I am extremely grateful to my mom, dad and brother for their continued support throughout all my endeavors.

Texas State (SWT!) would have been much less colorful without the support of my fellow graduate students who supported me emotionally and physically to complete that which is a graduate education.

My appreciation also goes out to the wonderfully helpful folks in the Valley, especially thank you to Minnie, our landlord and a property owner, and Eddie, also a property owner. I also thank Lisa Williams with the Nature Conservancy for enabling this project and allowing it to continue beyond my involvement.

I am grateful to Gena Janssen and Jackie Poole, without whom I would never have had the opportunity to get quite this caliber of an education.

Also, I am indebted to Dr. Chris Nice who has encouraged me to learn and has inspired my approach to teaching.

And finally, thank you Dr. Williamson. Your timing was perfect. You have allowed me to pursue my love of the plant world, to grow professionally and you have opened windows to the future.

This manuscript was submitted on October 28, 2005.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Conservation of rare plants involves identifying baseline biological data which can facilitate recovery through specialized management strategies applied to a target species. Schemske et al. (1994) recommends a three step process using demographic data to determine if a species is in decline and why: (1) Determine the stability of the population (2) If in decline, determine the life history stages which are most limiting to the species (3) Determine the biological causes of the limiting life history stages that impact demographics in the metapopulation. Answering these questions helps conservation managers alleviate the effect limiting life history stages may have on a target species and retain or promote population maintenance (Schemske et al. 1994).

The reproductive stage can be a limiting life history stage of rare plants. If vegetative reproduction does not occur, the reproductive capacity of adult plants can limit population growth. Understanding the importance of breeding systems and pollination biology is paramount to assessing whether reproductive capacity puts constraints on population size, which in turn is information critical to effectively manage rare plant species (Hamrick et al. 1991, De Mauro 1993). Late-stage reproduction, self-incompatibility, pollinator-specificity and short flowering seasons can all result in decreased seed set and, therefore, a population decline.

Self-incompatible, obligately outcrossing species depend solely on the availability of pollen from other plants to reproduce (Torres et al. 2002) as well as the presence of pollinators to transfer that pollen. In choosing sites for preserving rare plants, managers not only need to have a large enough population of target plants for outcrossing (Byers 1995) but also high plant densities to attract sufficient pollinators (Torres et al. 2002). Identification of pollinators, knowledge of the life history stages of pollinators and knowledge of potential negative impacts to pollinators is necessary. Larval food sites and foraging sites of pollinators often occur in different areas, therefore, incorporation of different habitat types within a conservation area may be an important consideration (Spira 2001). Use of adjacent lands and management practices of those lands can negatively impact plants and pollinators on lands targeted for conservation. Practices like the application of pesticides for crops can be detrimental to plants on adjacent lands by reducing pollinator numbers (Sipes and Tepedino 1995, Liu and Koptur 2003). In areas where pesticide drift is possible, buffer zones may be needed, especially during the flowering seasons (Tepedino et al. 1999). Land use on the targeted conservation area can also potentially affect plants and their pollinators. Disturbances created by cattle and other domesticated animals could negatively affect pollinator resources like ground nesting sites and forage plants (Tepedino et al. 1999).

Knowledge of plant pollination syndromes can also be helpful and make conservation plans more successful. Generalist plant species can be easier to maintain because they are more adaptable to changes in pollinator richness or abundance (Bond

1994), therefore, land managers might initially choose generalist plant species to manage. Deciding how to manage for long-term maintenance of conservation areas is becoming more obviously important in recent years due to global climate change. Knowing how the local climate is being influenced by global climate change could help with long-term management as conditions continue to change and influence plant phenology and, therefore, pollinator visitation (Wall et al. 2003). Being familiar with dominant plants occurring with the targeted species and their accompanying pollinators can help affect management decisions. Synchronously flowering species can increase overall pollination rates and this knowledge of phenology of associate species can lead to decisions to augment populations of associate species (Rathcke and Lacey 1985). Having knowledge of the phenologies of rare plants and their suite of pollinators as well as knowledge of land practices, climatic change and co-occurring plant species in the area can be used to determine appropriate management strategies to increase population growth rates and reduce probability of extinction.

Astrophytum asterias is a rare and endangered plant species occurring in south Texas. Historically, *A. asterias* was known from five localities in south Texas, however, only two of these localities remain. This range is becoming increasingly limited as Rio Grande Valley urbanization continues. While average population growth in Texas from 1990 to 2000 was 23%, growth in Starr County was far greater at 32% (U.S. Census Bureau 2005). No occurrences of *A. asterias* have been recorded in recent years in either Hidalgo or Cameron County. Additionally, extant Mexican populations of *A. asterias* are recorded to have lost 50% of individuals since 1998 (Martinez-Avalos 2004). Populations are in decline and reproductive biology studies may reveal if this life history stage is limiting to population growth and, if so, what specific biological causes are limiting to this life history stage.

The purpose of this study is to provide fundamental information about the reproductive biology of *A. asterias* to support recovery efforts that will be undertaken in the future. Specific aspects of reproductive biology to be investigated are: phenology, breeding systems and pollination biology. This study hopes to answer the following questions: (1) What is the phenology of *A. asterias*? (2) Are there any correlations between local climatology and phenology? (3) What is the breeding system of *A. asterias*? (4) Is reproductive capacity limited by pollinators? 5) Is seed weight correlated with viability? (6) Is there a difference in the germination capabilities of seeds when pollen is applied naturally versus by hand? (7) What are the potential pollinators of *A. asterias*? (8) How do the findings affect the conservation strategies for *A. asterias*?

CHAPTER II

METHODS AND MATERIALS

Astrophytum asterias Habitat and Habit

Astrophytum asterias (Zuccarini) Lemaire, commonly known as star cactus, is a United States federally listed cactus classified as endangered on November 17, 1993 by the United States Fish and Wildlife Service and by the state of Texas on January 30, 1997. Historically, *A. asterias* occurred in the United States in Hidalgo, Starr, and Cameron counties in Texas and the states of Nuevo Leon and Tamaulipas in Mexico (USFWS 2003). At present, *A. asterias* populations are only known from Starr County, Texas, and Tamaulipas and Nuevo Leon, Mexico.

Astrophytum asterias is a small, spineless cactus which occurs flush to the ground to no more than about 3 cm above the ground (USFWS 2003). The 2-15cm diameter cactus has flat ribs which are divided by grooves into eight sections. More mature cacti tend to have tufts of whitish wooly hairs that centrally line each rib. The plant can range in color from green or dark green to maroon to orange. Flowers are yellow with an orange to red center and open to 15cm in diameter (USFWS 2003). Fruit are oval to round, typically changing from a trichome-covered dark green to less pubescent maroonish-brown as they mature. The 1-2cm fruits contain glossy seeds with a flaring collar surrounding the hilum (Benson 1982).

Study Site

The study was conducted near Rio Grande City, Texas in the Tamaulipan thornscrub of Starr County. In 2004, the Nature Conservancy of Texas finalized purchase of a 168 hectare site called Las Estrellas. Phenology, breeding system, and pollinator-limitation data were collected at the population located at Las Estrellas. Phenological data was also collected at a small (< 8 hectares) private ranch (EE Site) 9-10 km from Las Estrellas. The pollinator survey experiment was conducted across the highway from Las Estrellas at a portion (1.9 hectares) of a large private ranch (MA Site). The Nature Conservancy and private landowners have requested that specific location data not be revealed, therefore, coordinates of study sites are not reported. All properties occur on the Catarina-Copita soil association of clayey saline and sandy loams and the Jimenez-Quemado soil association of gravelly loams (USFWS 2003).

Phenology

Astrophytum asterias phenology was investigated to determine length of the flowering season, length of bud, flower, and fruit development periods and peak flowering periods. Three transects were established in high density areas of the *A. asterias* population at Las Estrellas. Distribution was patchy and few areas of high density were present on the property, therefore, the number and placement of transects was limited. Orientation was chosen to incorporate as many *A. asterias* as possible in a transect line. Transect 1 was 25m long and Transects 2S and 2N were each 20m long. All plants located within a meter on one side of the transect line were tagged, measured and monitored. In the event that new plants were found, they were tagged and monitored.

Phenological data were collected every day during the flowering season from March 9-April 29, 2004. In 2005, phenological data were collected every third day from March 17-May 11. Following the peak flowering season, transects were monitored on a monthly basis. An additional 25m long transect was established through the small population at the EE Site. Again, orientation was chosen to incorporate as many *A. asterias* as possible in a straight line through the area. Phenological data were collected at this site every third day in both years (March 11-April 29, 2004 and March 17 – May 11, 2005). The number of buds, flowers and fruit per plant located in each transect was recorded. From these data, bud, flower and fruit development periods and percent fruit set were determined. Percent fruit set was calculated by averaging the number of fruit per flowers for each plant for both year of observation and then calculating yearly averages across all plants.

Precipitation Effects on Bud, Flower and Fruit Production

Precipitation data from 2004 and 2005 were obtained from the National Organization of Atmospheric Administration weather station located 12.2 km from Las Estrellas to determine whether number of buds, flowers and fruit produced by *A. asterias* is correlated with precipitation. The number of buds, flowers, and fruits produced by 50 plants within the Las Estrellas phenology transects was recorded in 2004 and 2005. The 50 plants were chosen because they were of reproductive age and alive both years phenological data were collected. Due to the non-normal distribution of this data set, a non-parametric Wilcoxon test was used to determine differences in bud, flower and fruit numbers between years.

Size Class Structure

Stem diameter of all plants located in the transects was measured with Mitutoyo Absolute Digimatic calipers to determine approximate size class structure. Flowering individuals were considered adults and non-flowering individuals were considered juveniles. Individuals that produced flowers were given a value of 1, while individuals that did not produce flowers were given a value of 0. The relationship between diameter and reproductive state (juvenile vs. adult) was examined with a Pearson's correlation test.

Breeding System

To determine extent of selfing versus crossing in *A. asterias*, different pollination treatments were applied to individual plants simulating a range of possible breeding systems. The treatments (Table 1) tested for non-facilitated and facilitated autogamy (self-fertilization), geitonogamy (self-fertilization by sibling flower), and xenogamy (outcrossing). Due to the distribution of a few high density areas of *A. asterias* plants at Las Estrellas, individuals for the breeding system treatments were selected within 20m of Transects 1, 2N and 2S in Las Estrellas. Due to low levels of flower production, plants with flowers were opportunistically designated to specific treatments. An attempt was made to conduct all five treatments in rounds each day flowers were open. Non-facilitated autogamy treatments were bagged prior to anthesis and no further action was taken. Facilitated autogamy treatments were bagged prior to anthesis and hand-pollinated with self pollen. Geitonogamy treatments were bagged prior to anthesis and flowers were hand-pollinated with pollen from flowers on the same plant. Both facilitated autogamy and geitonogamy treatments were hand-pollinated once a day until anthesis ended.

Xenogamy treatments were bagged prior to anthesis and hand-pollinated at anthesis using pollen from other plants. All treatments except controls were bagged prior to anthesis. After breeding treatment, all plants were caged to avoid fruit predation and were monitored until seeds matured. Extent of self-incompatibility was determined by comparing average fruit and seed set between all treatments. Fruit were totaled for each individual of each treatment and an average fruit number was calculated for all five treatments. Seeds were counted for each fruit and in the case where multiple fruit resulted for an individual plant, an average seed set was calculated. An average seed set was then calculated for all five treatments. Due to the non-normal distribution of this data set, a non-parametric Kruskal-Wallis test was used to determine if differences in fruit and seed set existed between treatment means.

Table 1. Breeding system treatments.

	Treatment	Bagged	Hand-Pollinated	Emasculated
1	Control	N	N	N
2	Non-facilitated Autogamy	Y	N	N
3	Facilitated Autogamy	Y	Self	N
4	Geitonogamy	Y	Sibling Pollen	N
5	Xenogamy	Y	Outcross	Y

Pollen Limitation

To determine extent of pollen-limitation in *A. asterias*, seed set in two flowers on an individual plant (n=20 plants) were compared at Las Estrellas. As in the breeding system experiment, individuals for the pollen limitation experiment were selected from within 20m of Transects 1, 2N and 2S at Las Estrellas due to the patchy distribution of a few high density areas of *A. asterias* plants. 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). Both treatments were conducted on one plant to limit environmental variation between plants separated spatially. Plants with flowers were opportunistically chosen and used as maternal plants in this experiment. Pollen removed from emasculated, outcrossed treatments in the breeding system experiment was used to pollinate maternal plants. Neither flower was bagged, so both were available for pollinator visitation. Type of treatment and date of anthesis for each flower were recorded. A substantial amount of outcross pollen was applied to hand-pollinated flowers by loading the paintbrush liberally with pollen and brushing stigmas multiple times. A pollen amount larger than what would have been deposited naturally was sought, however, no quantitative measure was taken to ensure a "larger than naturally deposited" amount. As with breeding treatments, pollen limitation treatments were caged.

Total number of fruit was counted for each treatment. Seeds were counted for each fruit and an average seed set was calculated for both treatments. Due to the non-normal distribution of this data set, a non-parametric Wilcoxon Signed Rank test was used to determine whether fruit number between control and open xenogamy flowers was significantly different. Additionally, to investigate whether differences in seed set exist between hand-pollinated flowers and control flowers, a Single Factor Repeated Measures ANOVA was performed. This test was used due to the dependent, unbalanced nature of the data sets. Treatments were not considered independent because manipulated and control flowers occurred on the same plant. Additionally, because the control treatment resulted in far fewer fruits than the hand-pollinated treatment, comparison of the number of seeds was unbalanced and so a paired t test could not be used.

Seed set has been used to determine if close in proximity individuals influences are genetically more similar than individuals located further apart (Irwin 2001, Robertson and Ulappa 2004). Seed set is expected to decrease as outcrossing distance decreases (Sobrevila 1988) due to reduced genetic variability between closely related/spaced plants. The relationship between proximity of individuals and seed set was examined with a Pearson's Correlation. This relationship was analyzed using the data from the pollen limitation experiment. Therefore, although not specifically designed for an outcrossing distance/offspring fitness question, results give preliminary indications for managers concerning *A. asterias* fitness. Distance between outcrossed flowers from the pollen limitation experiment was measured and seed set from resulting fruit were counted. Distances classes (and sample sizes) were: 1-5m (4), 5-10m (7), 10-15m (3), 20-30m (3) and 200+m (2).

Seed Germination

To ensure that seeds used to analyze the breeding system and pollen-limitation experiment results were viable, seeds from each fruit were germinated. Seeds capable of germination were assumed to be viable. Seeds of fruit from the breeding system and pollen limitation experiments were collected. The germination procedure followed the methods of Maiti et al. (2002b), although the resulting design also incorporated ideas from local horticulturists and others experienced in germinating cacti. Three replicates of ten seeds from each fruit were placed on top of a 30:70 Sunshine general/universal potting mix: sand mixture. The sand was sterilized at 70°C. Since seeds were too small to weigh individually, ten seeds were weighed together and an average weight was calculated for each replicate. Fruit from each treatment were grouped into randomly arranged blocks within each tray. Trays were covered with a plastic cover to reduce evaporation and retain moisture. The trays were placed in a Sherer DualJet walk-in growth chamber set at 25-30°C for 18 days. The temperature range selected was based on a study by Maiti et al. (2002a), which found that a 25-30°C temperature range was needed for inducing germination in more than forty cacti species. A photoperiod of 13L/11D was used to mimic average south Texas spring day length. Each day the trays were checked for germinated seeds and rearranged randomly with a random number generator within the chamber to reduce differences caused by chamber effects. Germinations were terminated on day 18, because few germinations had occurred since day 14. Number of seeds germinated and percent germination per day were recorded.

Percent germination of seeds from breeding system and pollen limitation experiments was compared to determine if there was a difference in seed viability in control treatments versus treatments pollinated by hand. A Kruskal-Wallis test was used to analyze viability in seed resulting from the breeding system experiment and a Single Factor Repeated Measures ANOVA was used to analyze seed viability from the pollen limitation experiment. The relationship between seed weight and seed germinability was also tested.

Insect Visitors

Floral visitor species diversity was assessed throughout the flowering season to identify potential pollinators and potential pollinator visitation rates. These data will be useful in future pollinator studies to determine which visitors are effective pollinators. In 2004, a preliminary survey was conducted, whereas, in 2005, a more in-depth investigation of pollination biology was conducted at the MA Site. Insect activities were recorded, but emphasis was on collection and identification of species.

On each day that flowers were open, insect visitation was observed for ten minutes at each flower. An attempt was made to capture all visitors. Date, time of day, a description of the visitor and floral organs the insect contacted were recorded. All visitors not captured were recorded, a description given and noted as not captured. This initial survey estimated *A. asterias* visitor abundance and richness. Percentage of total individuals of each insect taxa visiting *A. asterias* was determined.

To examine temporal variation in insect visitation rate, time, day, visitor activity and visitor type were recorded throughout the flowering season. Individual plants were examined for ten minute intervals. Mean hourly visitation rates for the day and mean visitation rate/hour for the whole season for potential pollinators were determined.

CHAPTER III

RESULTS

Phenology

In 2004, anthesis began in mid-March. Results of the phenological investigation show a flowering peak (38 flowers out of a total 98 flowers produced in 2004) in the first week of April, 2004 at Las Estrellas population (Figs.1, 2, 3, 4). The greatest number of flowers (9 out of a total of 21 flowers) observed in the EE Site population occurred the last week of April 2004 (Fig. 5). Several smaller flowering peaks occurred in March to May (Figs. 1, 2, 3, 4, 5). Occasional buds were observed in both populations during June-September, 2004.

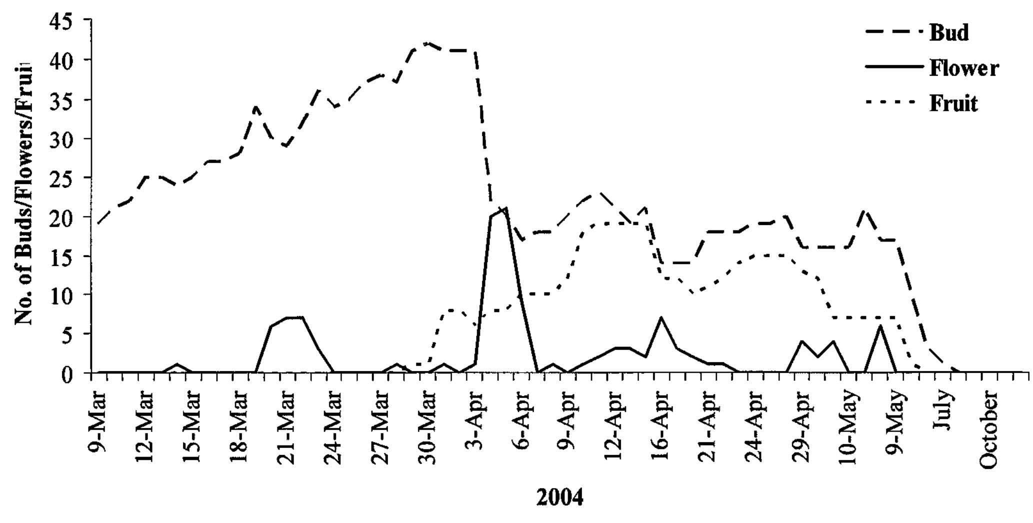

Figure 1. Number of *Astrophytum asterias* buds, flowers, fruit recorded within Transect 1 in Las Estrellas population, 2004.

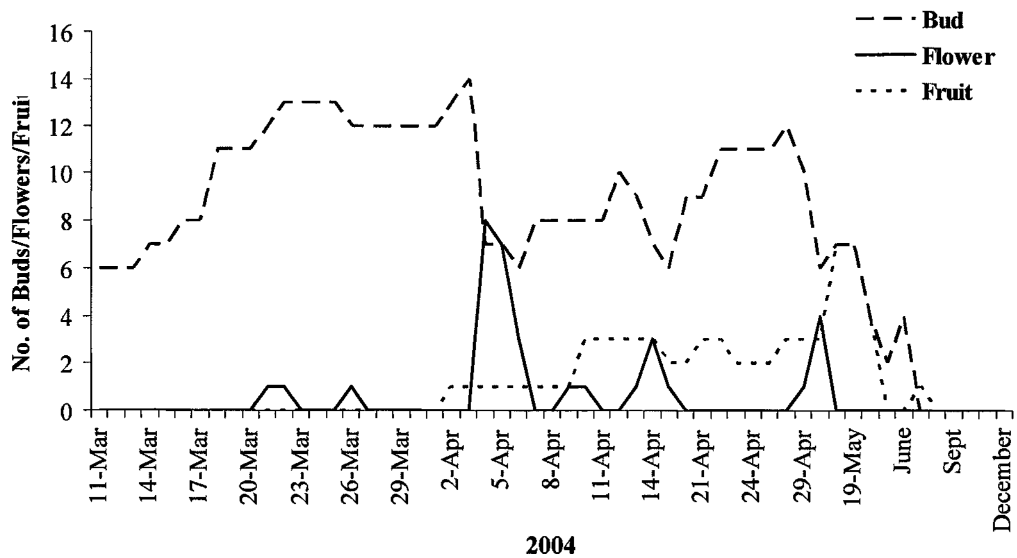

Figure 2. Number of *Astrophytum asterias* buds, flowers, fruit recorded within Transect 2S in Las Estrellas population, 2004.

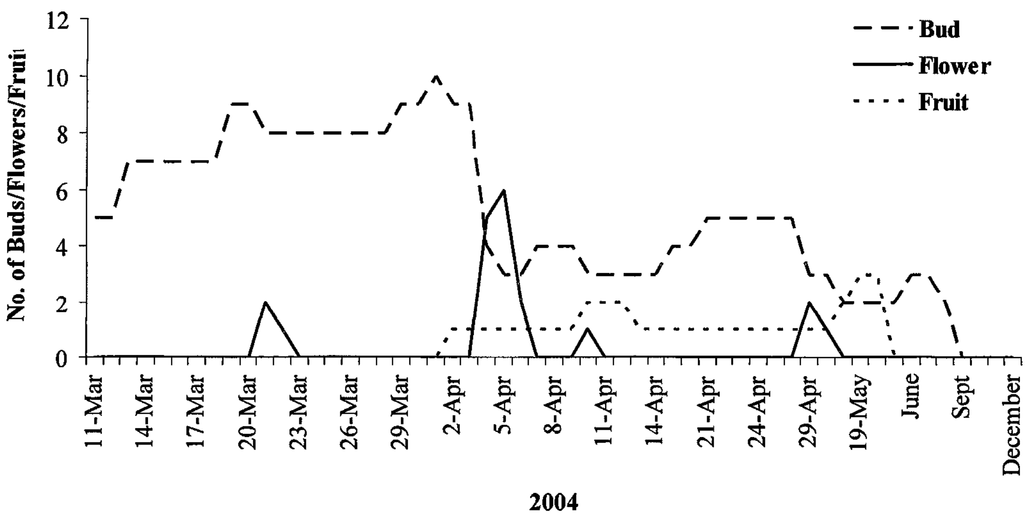

Figure 3. Number of *Astrophytum asterias* buds, flowers, fruit recorded within Transect 2N at Las Estrellas population, 2004.

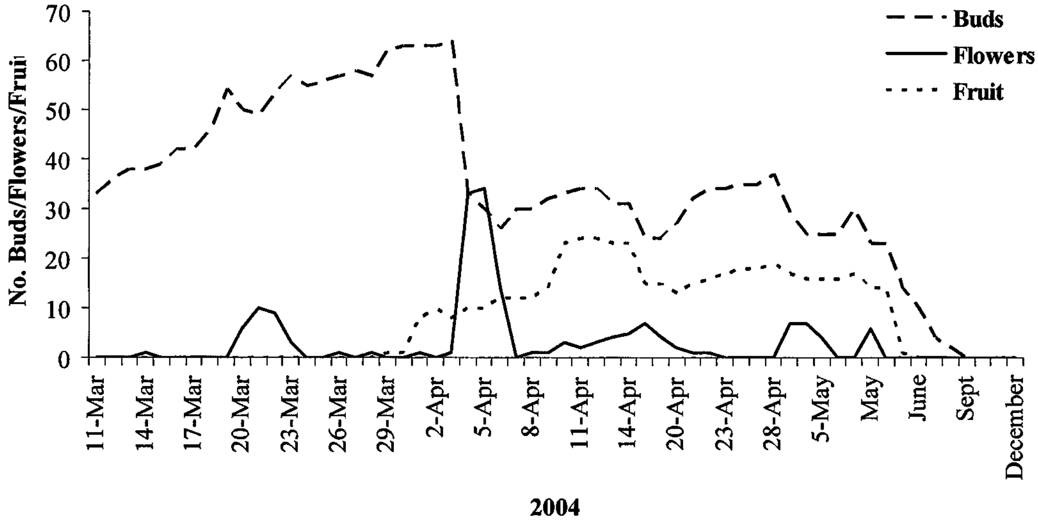

Figure 4. Number of *Astrophytum asterias* buds, flowers, fruit recorded within the three transects at Las Estrellas population, 2004.

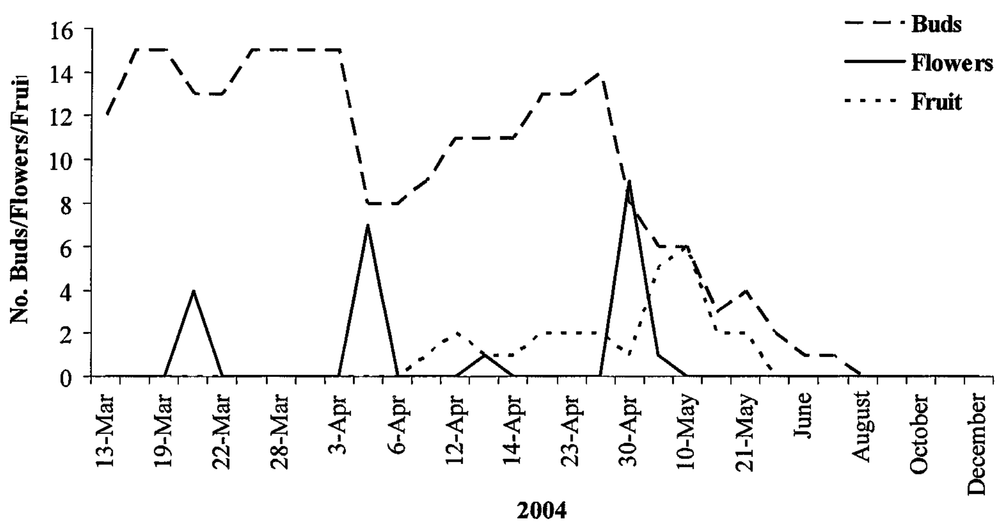

Figure 5. Number of *Astrophytum asterias* buds, flowers, fruit recorded within the EE Site transect, 2004.

Anthesis began mid-March, 2005. Results of phenological data show a flowering peak (33 flowers out of a total 54 flowers) the first week of April, 2005 in Las Estrellas population (Figs. 6, 7, 8, 9). The greatest number of flowers (4 out of a total of 6 flowers) observed in the EE Site population occurred the first week of April 2005 (Fig. 10). Several smaller flowering peaks occurred in March through May and again in July (Figs. 6, 7, 8, 9, 10). Buds were observed in both populations during June-October, 2005. However, the number of buds observed from June-October, 2005 was much lower compared to the spring flowering season.

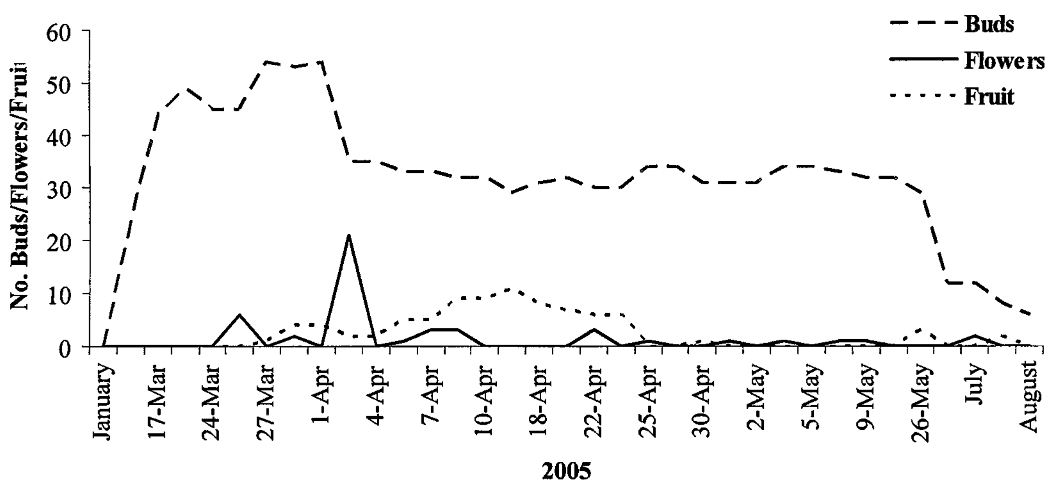

Figure 6. Number of *Astrophytum asterias* buds, flowers, fruit recorded within Transect 1 in Las Estrellas population, 2005.

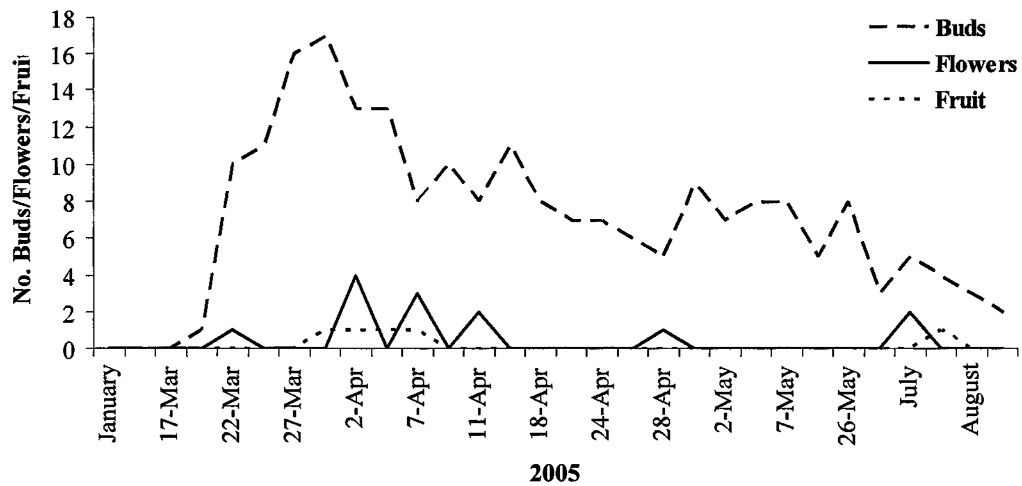

Figure 7. Number of *Astrophytum asterias* buds, flowers, fruit recorded within Transect 2S in Las Estrellas population, 2005.

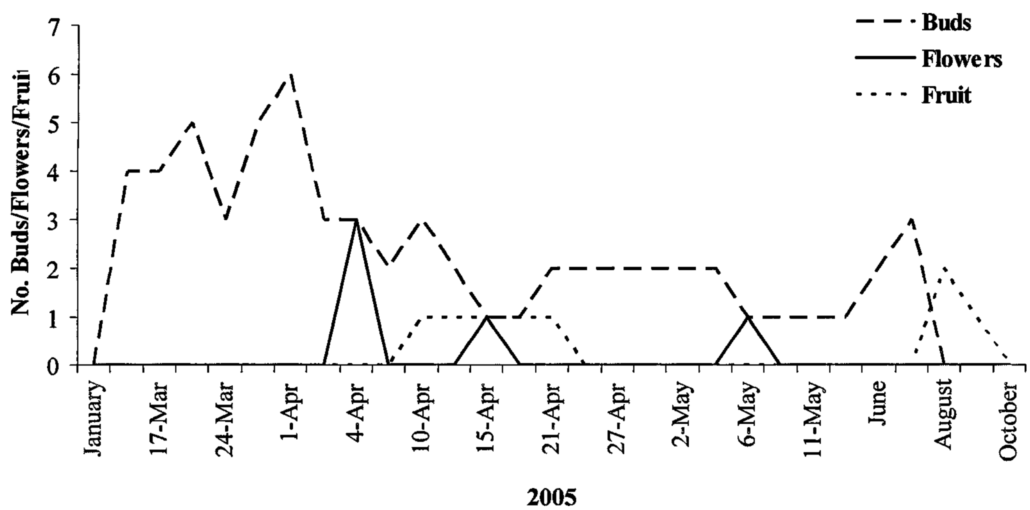

Figure 8. Number of *Astrophytum asterias* buds, flowers, fruit recorded within Transect 2N at Las Estrellas population, 2005.

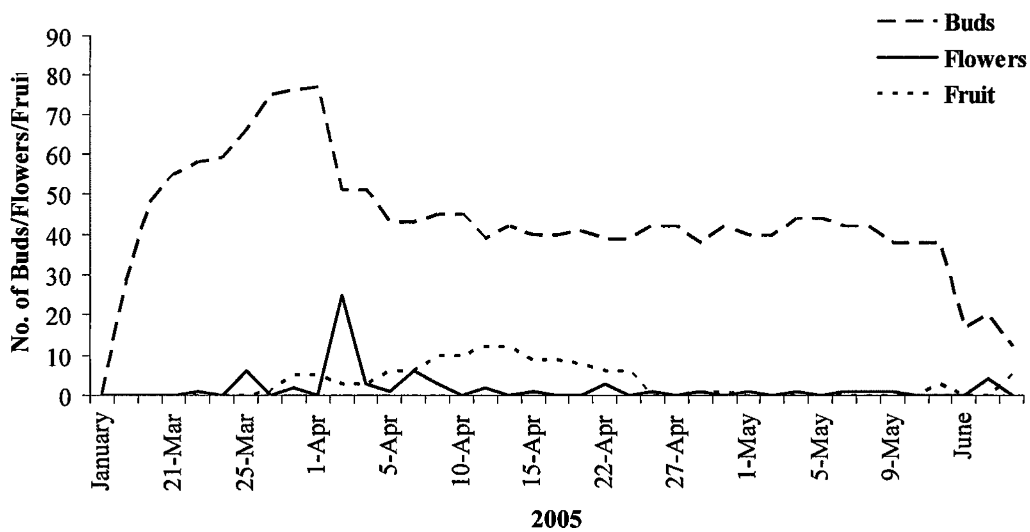

Figure 9. Number of *Astrophytum asterias* buds, flowers, fruit recorded within the three transects at Las Estrellas population, 2005.

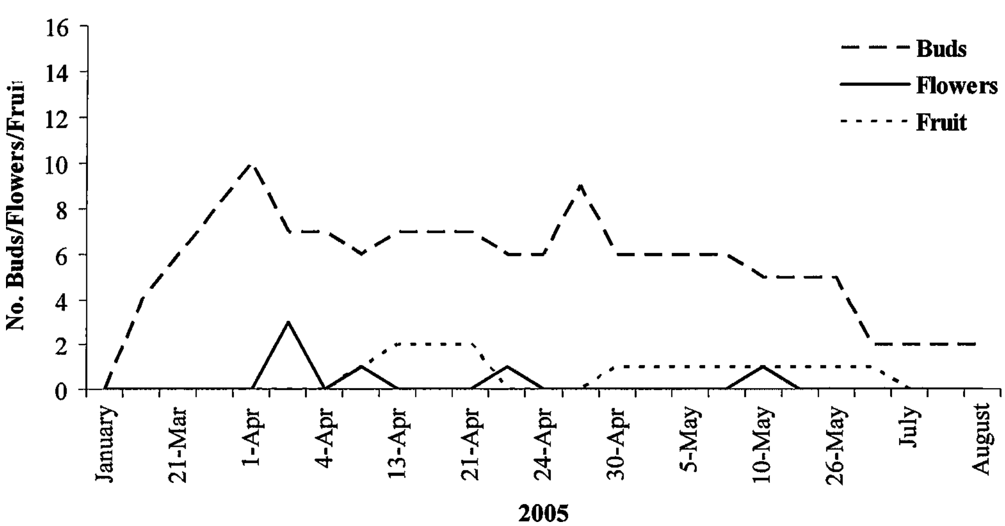

Figure 10. Number of *Astrophytum asterias* buds, flowers, fruit recorded within the EE Site transect, 2005.

Most flowers in 2004 opened two consecutive days or for a single day, however some flowers opened for a three-day period (Fig. 11). This information could not be assessed for 2005 due to the collection of data every third day. When 50 reproductive plants from within the Las Estrellas transects were compared, the mean number of flowers/plant decreased from 1.76 flowers/plant in 2004 to 1.0 flowers/plant in 2005 (Fig. 12). Mean number of fruit/plant decreased from 0.76 fruit/plant in 2004 to 0.36 fruit/plant in 2005 (Fig. 12). Fruit set was compared in 29 (of the original 50) plants that were of reproductive age and which had flowers for both years. Fruit set for these 29 plants was 45.9% in 2004 and 37.4% in 2005. Fruit set was not significantly different between years (Paired t-Test: p=0.1982; T=0.6813; df=28).

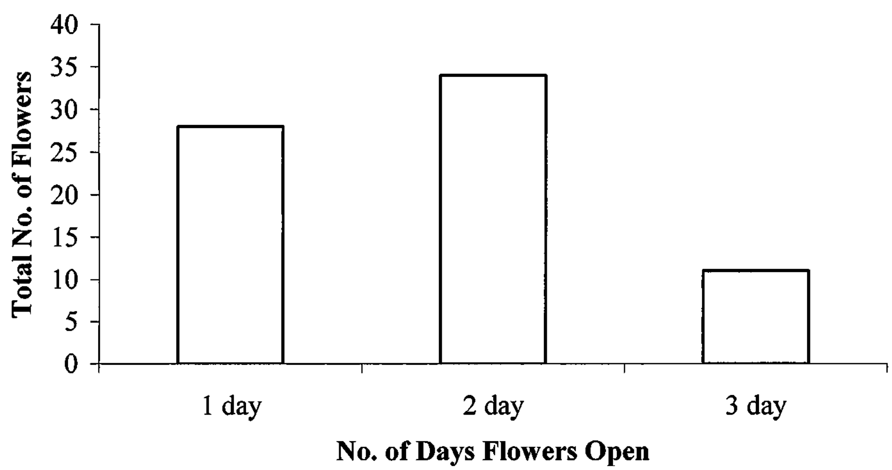

Figure 11. Period of time a flower opens during anthesis. Data collected from *Astrophytum asterias* flowers (n=98) within the three transects in Las Estrellas population, spring 2004.

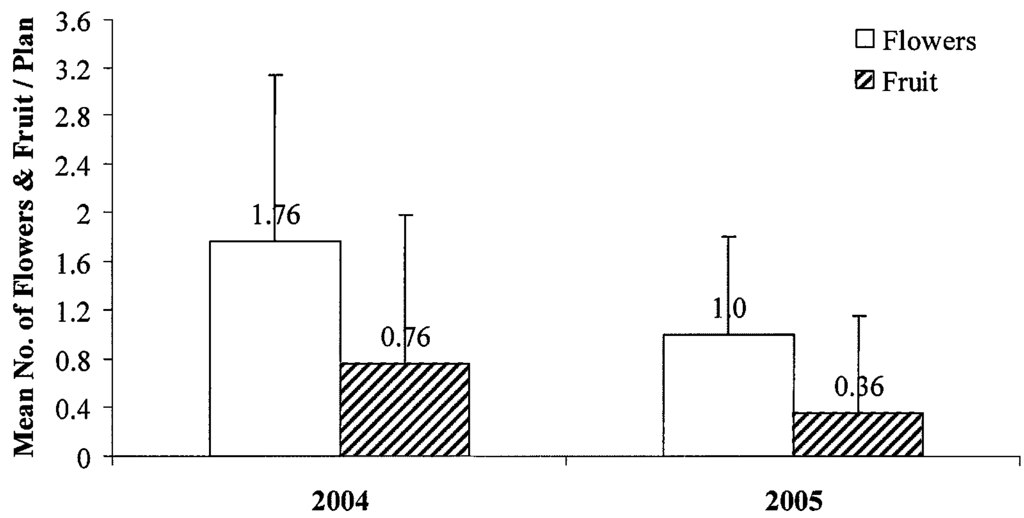

Figure 12. Mean number of flowers and fruit per plant (n=50) in Las Estrellas transects for 2004 and 2005; SD=1.

Precipitation Effects on Bud, Flower, and Fruit Production

When 50 reproductive plants within the Las Estrellas transects were compared, no significant difference was detected between total number of buds produced between 2004 and 2005 (Fig. 13). However, a significantly greater number of flowers and fruit developed in 2004 (Figs. 14, 15). The greater number of flowers and fruit produced in 2004 may be due to the greater amount of precipitation (9.7 inches January to May, 2004 and 7.1 inches January to May, 2005) during the flowering season, which was over three inches more than occurs in an average year during this time period.

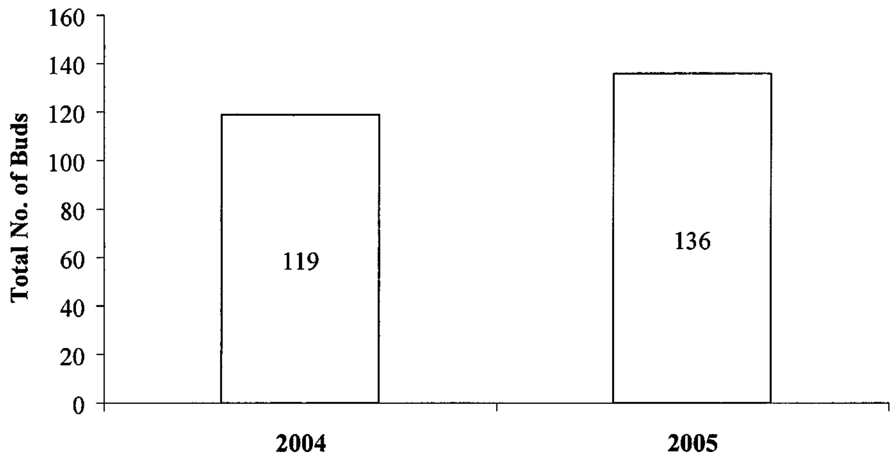

Figure 13. Total number of buds produced by plants (n=50) within the Las Estrellas transects between years (Wilcoxon; p=0.42; Z = 0.2019; df=49).

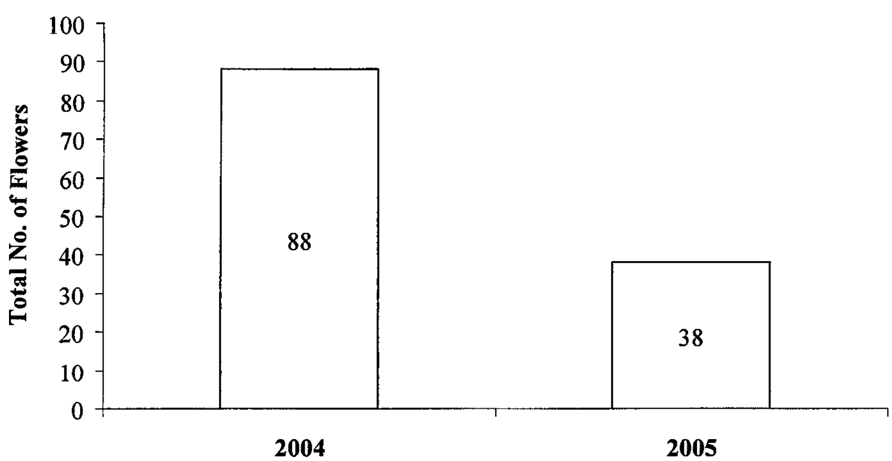

Figure 14. Total number of flowers produced by plants (n=50) within the Las Estrellas transects between years (Wilcoxon; p=0.0025; Z = 2.8033; df=49).

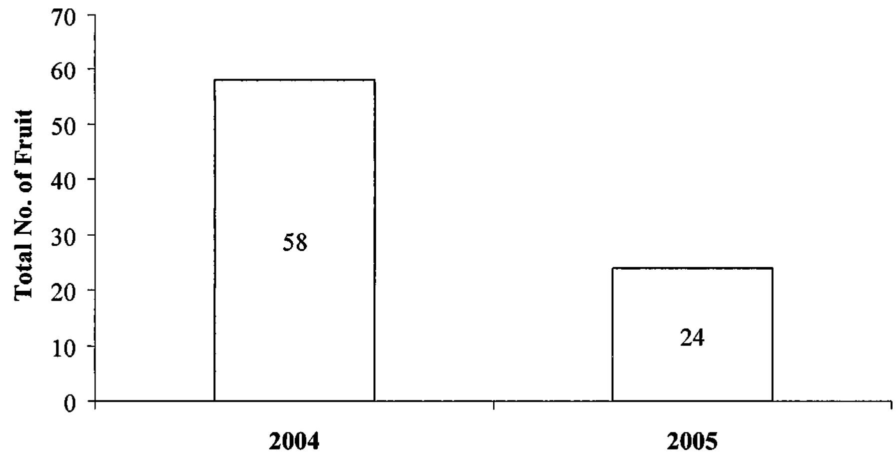

Figure 15. Total number of fruit produced by plants (n=50) within the Las Estrellas transects between years (Wilcoxon; p=0.0028; Z = 2.7647; df=49).

Size Class Structure

Diameter of plants located within the transects was recorded to determine approximate age class distribution. The size distribution is fairly even (Figs. 16, 17), although in 2004 there were fewer individuals reaching diameters in the range of 70.01-80mm and 80.01-90mm (Fig. 16). No individuals over 97mm were observed within the transects.

The relationship between plant diameter and flower production was examined in individuals in the three transects at the Las Estrellas population. Individual plants monitored daily from March – December, 2004 and from January – October, 2005 were scored based on production of flowers. In 2004, 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 (r^2=0.78; p<0.000001;

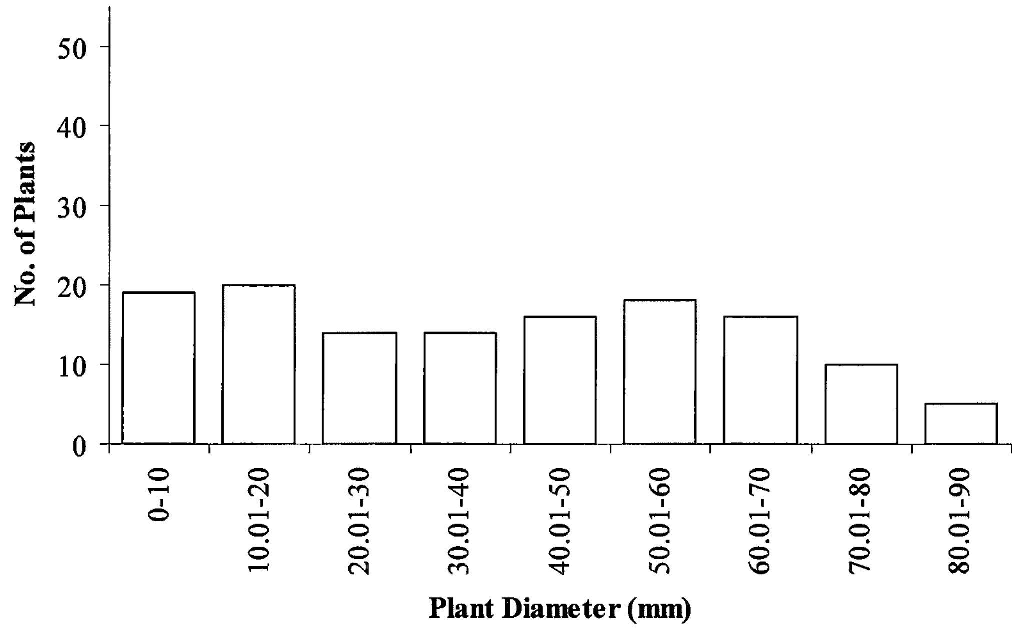

Figure 16. Size class distribution of *Astrophytum asterias* within transects at Las Estrellas and EE Site populations (n=109 plants), fall 2004.

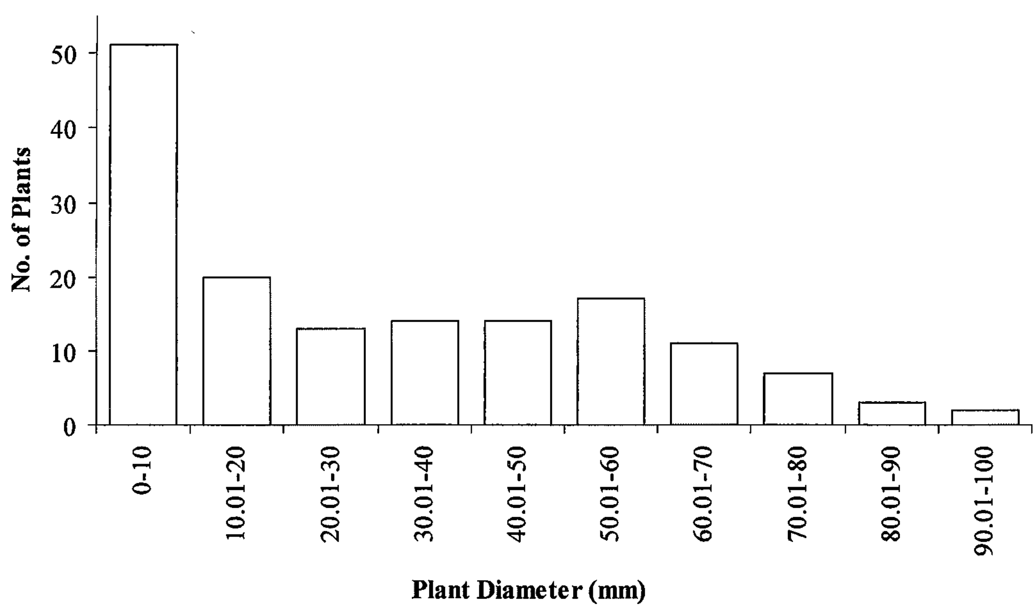

Figure 17. Size class distribution of *Astrophytum asterias* within transects at Las Estrellas and EE Site populations (n=150 plants), fall 2005.

n=109). In 2005, the mean diameter of non-reproductive individuals (n=117) was 29.94mm, with a range of 3.59-73.67mm. The mean diameter of reproductive individuals (n=33) was 62.47mm, with a range of 39.38-101.93mm. Results of a Spearman's Correlation revealed a moderately strong positive relationship between plant size class (based on diameter) and maturation to a reproductive stage (r^2=0.55; $p<0.000001$; n=150). 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. Many cactus species establish under nurse plants to decrease effects caused by the arid environment in which they reside (Callaway 1995). It was observed that cacti deep within a nurse plant and receiving minimal direct light would not produce buds even though they were well over the diameter of average reproductive size.

Breeding System

Fruit and seed resulted only from controls and the xenogamy treatment (Fig. 18). These results show that the species is an obligate outcrosser. Fruit set results show that only facilitated emasculated xenogamous crosses are significantly different from all other treatments (Figs. 19, 20). The median mean fruit and seed set was 0 for all treatments except the xenogamy treatment. The median mean fruit set for the xenogamy treatment was 1 (Fig. 19) and the median mean seed set for the xenogamy treatment was 92.7 (Fig. 20).

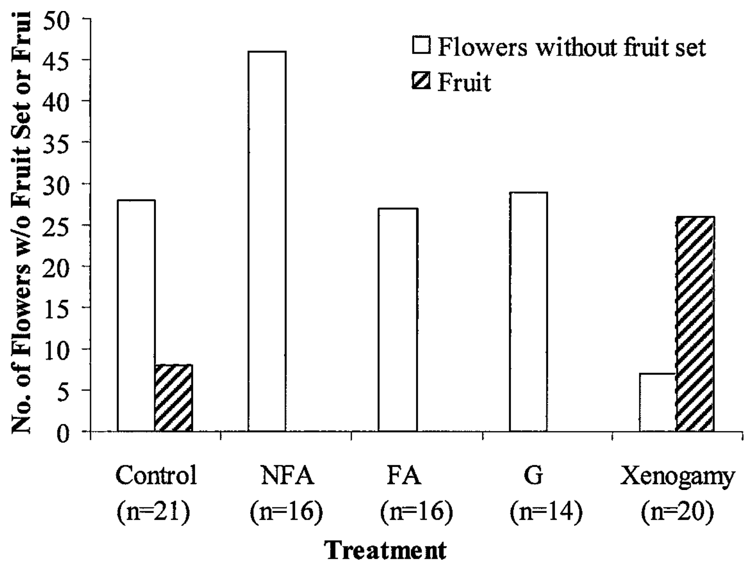

Figure 18. Number of flowers without fruit set and fruit resulting from controls, non-facilitated autogamy (NFA), facilitated autogamy (FA), and geitonogamous (G) treatments and xenogamy crosses of *Astrophytum asterias* plants within Las Estrellas population, spring 2004 (No. of plants in parentheses).

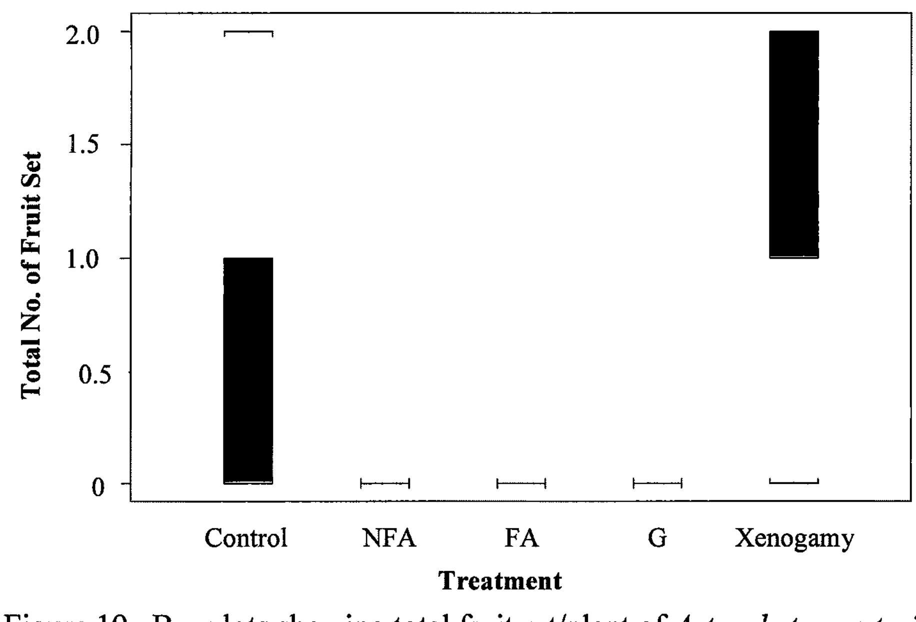

Figure 19. Boxplots showing total fruit set/plant of *Astrophytum asterias* plants in controls, non-facilitated autogamy (NFA), facilitated autogamy (FA), and geitonogamous (G) treatments and xenogamy crosses within Las Estrellas population, spring 2004. White line is median fruit set, black box is middle 50% of fruit set values, dotted lines are outer 25% of fruit set values, and brackets are minimum or maximum values. Statistical difference only exists for xenogamy treatment compared to all other treatments (Kruskal-Wallis; $p<0.000001$; $x^2=55.3829$; df=4).

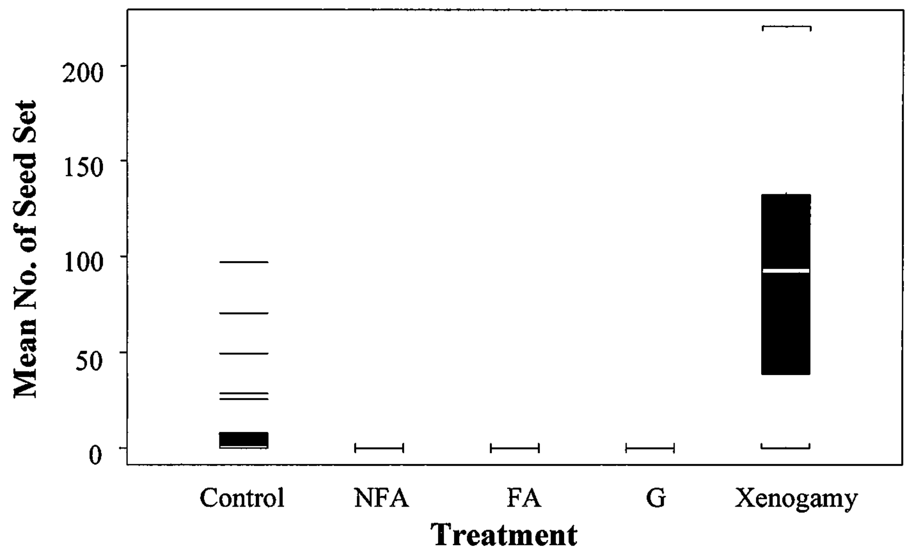

Figure 20. Boxplots showing mean seed set/plant of *Astrophytum asterias* plants in controls, non-facilitated autogamy (NFA), facilitated autogamy (FA), and geitonogamous (G) treatments, and xenogamy crosses within Las Estrellas population, spring 2004. White line is median fruit set, black box is middle 50% of fruit set values, dotted lines are outer 25% of fruit set values, brackets are minimum or maximum values, and black lines are outliers. Statistical difference only exists for xenogamy treatment compared to all other treatments (Kruskal-Wallis; $p<0.000001$; $x^2=62.8261$; df=4).

Pollen Limitation

Not only did the quantity of fruit from hand-pollinated crosses significantly increase compared to controls (Fig. 21), but the quality of those crosses was significantly different with fruit from hand-pollinated flowers setting more seeds than controls (Fig. 22).

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). However, Oostermeijer et al. (1995) found

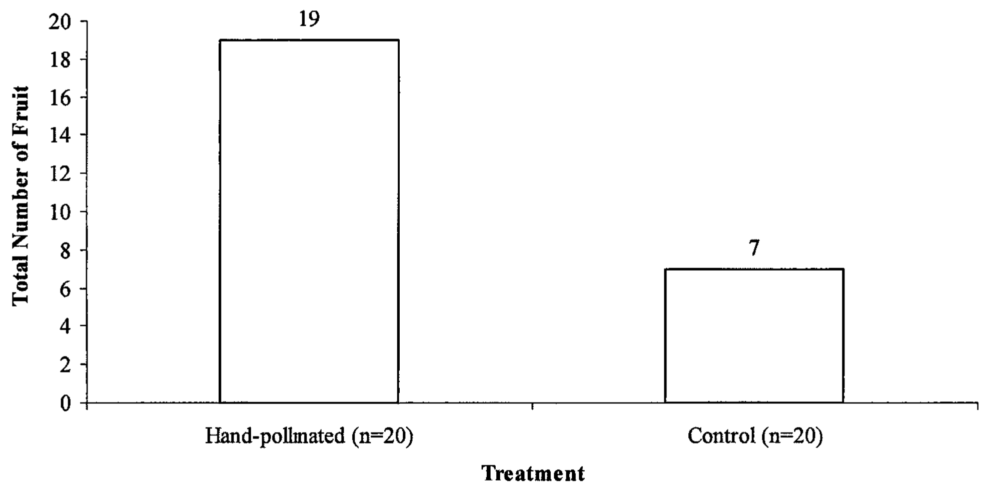

Figure 21. Comparison of fruit set by individual plants (n=20) in which one flower was the control and one flower was experimentally outcrossed (hand-pollinated) (Wilcoxon Signed Rank Test; p<0.000001; Z-value= -5.8443).

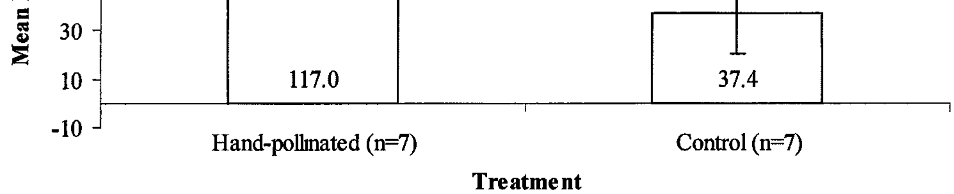

Figure 22. Comparison of seed set by individual plants (n=7) in which one flower was the control and one flower was experimentally outcrossed (hand-pollinated). Graph shows only plants that resulted in fruit for both the control and hand-pollination, SD=1 (Single Factor Repeated Measures ANOVA; p=0.0499; F-value= 5.99184; df=6).

that outcrossing distance was not correlated to fitness of progeny. The distance between the plants serving as pollen donor and pollen recipient in the xenogamous crosses was compared to determine if there is a relationship between distance and seed set. Results show that distance between parental plants had no significant effect on seed set (p-value=0.1427; r^2=0.122).

Seed Germination

The first seeds germinated on day 3, between 72 and 96 hours after the day they were planted and first watered. The largest number of seeds to germinate in one day was 284 (18.2%) on day 5. By day 18, 75.02% of the seeds had germinated (Fig. 23).

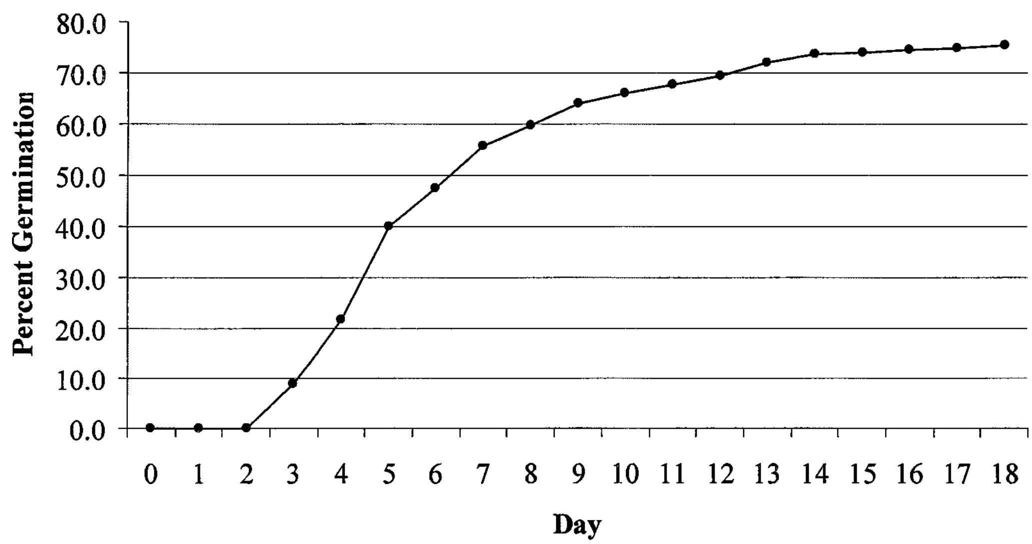

Figure 23. Percent germination of *Astrophytum asterias* seeds resulting from breeding system and pollen limitation experiments (n=1,563 seeds).

Results indicate that no significant difference exists between germination of seed resulting from hand-pollinated flowers compared to controls in either the breeding system experiment (p-value=0.1779; x^2=1.8153; df=1) or the pollen limitation experiment (p-value=0.1473; F-value=2.7666; df=6). This indicates that viability among seeds is similar and seed counts are an appropriate measure to detect differences among 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 detect any 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 indicated a significantly positive relationship between seed weight and seed viability (p-value=0.0264, r^2=0.31) (Fig. 24).

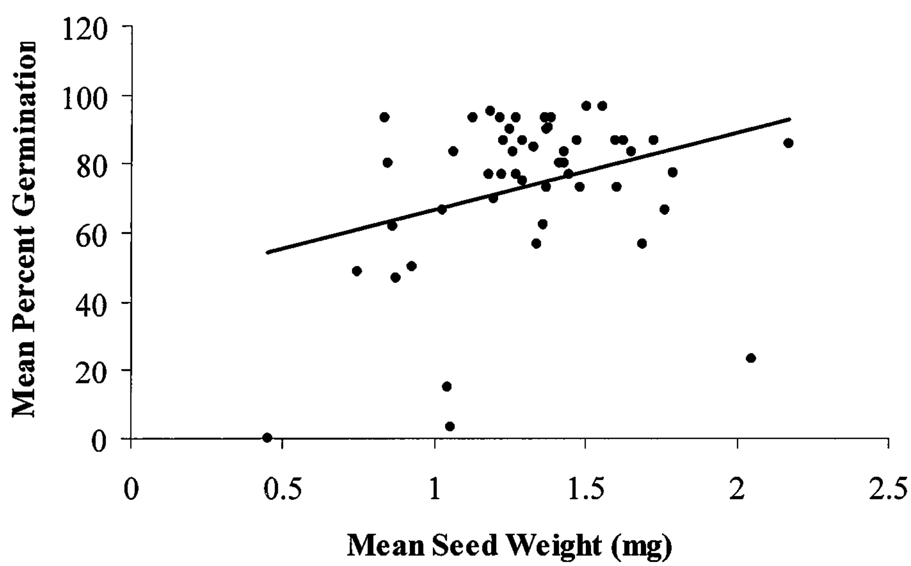

Figure 24. Relationship between the mean seed weight (mg) and mean percent germination for groups of ten seeds from breeding system and pollen-limited experiments. (Pearson's Correlation; r^2=0.314; p-value=0.0264).

Insect Visitors

The preliminary investigation of pollination biology conducted in 2004 resulted in collection of two orders of insects: Coleoptera and Hymenoptera. Insects belonging to the Coleoptera that have been identified to genus or species include: *Carpophilus* sp./spp. (n=1), *Euphoria kerni* (Haldeman) (n=3) and *Acmaeodera* sp. (n=4). Insects belonging to the Hymenoptera that have been identified to genus or species include: *Macrotera lobata*, (n=5), *Lassioglossum/Dialictus* sp. (n=2), and *Osmia subfasciata* (n=1).

Observations made in 2005 revealed that flowers remain open between 10:00am and 7:00pm. Twenty species of insects, four orders and 281 individuals were documented visiting *A. asterias* within a 3,130-minute observation period. Twelve Apoidea (n=84), six coleopteran (n=120), one formicid (n=75) and one syrphid (n=2) species were documented during 11 days of observation (Fig. 25 and Table 2).

Two species, *M. lobata* (Timberlake) and *Ashmeadiella maxima* (Michener), made up 40.0% of all bees (Apoidea) visiting *A. asterias* (Fig. 26). Another 27.1% of visits were by the other ten bee species (Fig. 26). The remaining visits (32.9%) were by unidentified bees that were recorded but not collected.

Beetles were almost never seen flying from flower to flower but rather had to be prodded out from the base of the flower and between all the filaments to be counted and collected. Bees, on the other hand, visited flowers briefly and would then continue foraging in the area. Out of the 120 visits by beetles, only 18

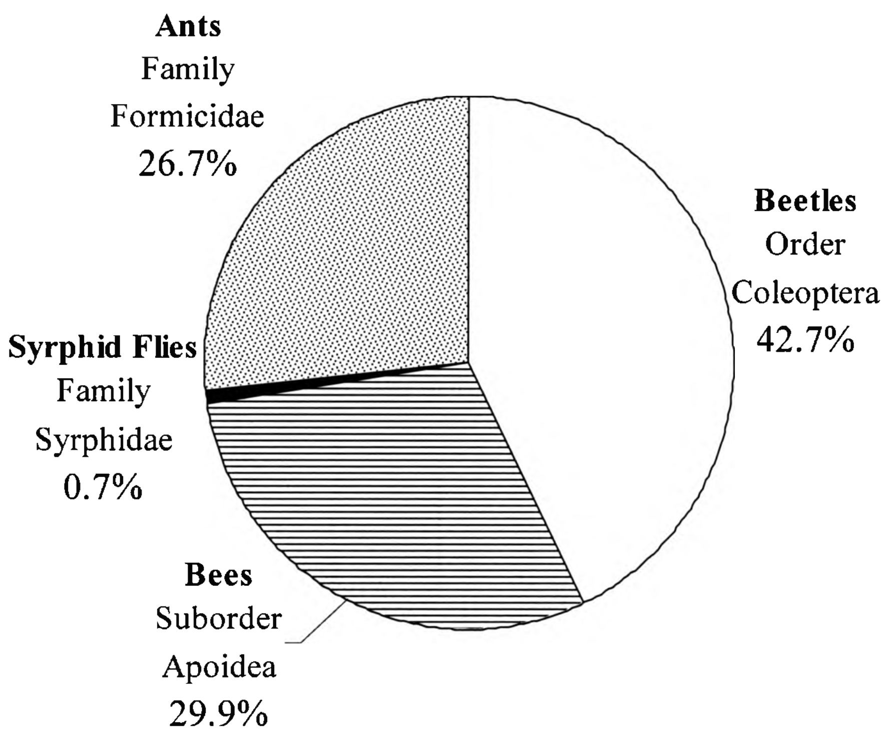

Figure 25. Percentage of total individuals of insect taxa visiting *Astrophytum asterias* March 17 – May 7, 2005 at MA Site.

(15.0%) of those visits had beetles touching either the anther or stigma. This was in contrast to the 22 out of 48 visits (45.8%) of bees that involved touching of the stigma or anthers. An additional 36 visits did not have recorded data for visitation actions due to emphasis placed on bee collection. Bee visitations were interrupted to collect the bee, therefore, a higher percentage of bees may have touched the stigma or anthers if visitations were uninterrupted. Bees were commonly seen landing on the stigma and then crawling down into the anther by way of the style.

Table 2. Identified insects observed visiting flowers of *Astrophytum asterias*, between March 17 – May 7, 2005 at MA Site.

Genus	Family	No. of Individuals	% Total	Order
Macrotera lobata (Timberlake)	Andrenidae	27	9 61	Hymenoptera
Anthophorula compactula (Cockerell)	Apidae	3	1.07	Hymenoptera
Diadasia rinconis (Cockerell)	Apidae	4	1.42	Hymenoptera
Agapostemon angelicus/texanus	Halictidae	1	0.36	Hymenoptera
Agapostemon tyleri (Cockerell)	Halictidae	1	0.36	Hymenoptera
Augochlorella bracteata (Ordway)	Halictidae	1	0.36	Hymenoptera
Lassioglossum/Dialictus sp.	Halictidae	4	1.42	Hymenoptera
Ashmeadiella cactorum (Cockerell)	Megachilidae	1	0.36	Hymenoptera
Ashmeadiella maxima (Michener)	Megachilidae	7	2.49	Hymenoptera
Ashmeadiella meliloti (Cockerell)	Megachilidae	4	1.42	Hymenoptera
Dianthidium discors (Timberlake)	Megachilidae	2	0.71	Hymenoptera
Osmia subfasciata (Cresson)	Megachilidae	1	0 36	Hymenoptera
Unidentified Hymenoptera		28	9.96	Hymenoptera
Apoidea subtotal		**84**	**29.89**	Hymenoptera
Forelius mccooki (Forel)	Formicidae	75	26.69	Hymenoptera
Hymenoptera subtotal		**159**	**56.58**	Hymenoptera
Acmaeodera sp.	Buprestidae	66	23.49	Coleoptera
Acanthoscelides *sp.*	Chrysomelidae	1	0.36	Coleoptera
Selvadius sp.	Coccinelidae	1	0.36	Coleoptera
Carpophilus sp.	Nitidulidae	40	14.23	Coleoptera
Dasytinae	Melyridae	10	3.56	Coleoptera
Euphoria kerni (Haldeman)	Scarabaeidae	2	0.71	Coleoptera
Coleoptera subtotal		**120**	**42.70**	Coleoptera
Syrphidae	Syrphidae	2	0.71	Diptera

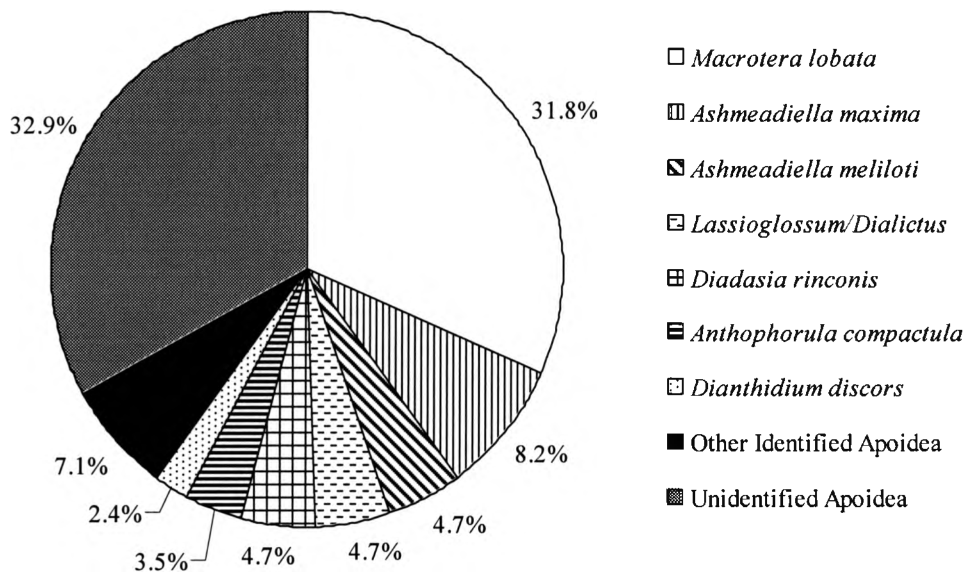

Figure 26. Percentage of total individuals of Apoidea visiting *Astrophytum asterias*, between March 17 – May 7, 2005 at MA Site.

The Apoidea had an overall visitation rate of 3.08 bees/hour. Rate of peak visitation for bees was 4.2 bees/hour between 12:00 - 2:00pm with a secondary peak of 4.0 bees/hour between 5:00 - 6:00pm. Rate of peak visitation was 3.5 bees/hour for *M. lobata* between 12:00 - 2:00pm (Fig. 27). *Ashmeadiella meliloti, A. maxima* and *Diadasia rinconis* account for identified bee visits during the hours between 4:00 - 7:00pm (Fig. 27). During the entire blooming season, *M. lobata* had the highest mean visitation rate of 1.1 bees/hour (Fig. 28). Visits by all other bee species ranged between 0.05 bees/hour and 0.47 bees/hour (Fig. 28).

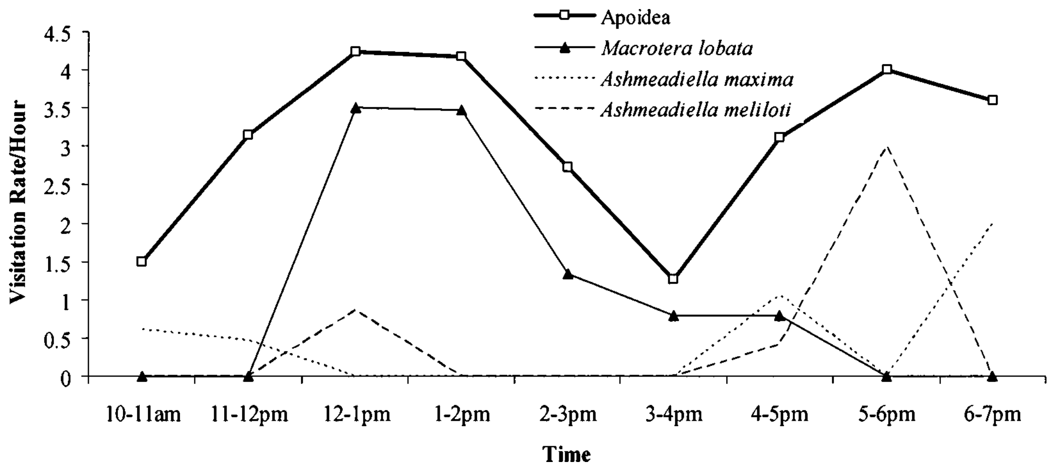

Figure 27. Mean hourly visitation rates of Apoidea, between March 17 – May 7, 2005 at MA Site.

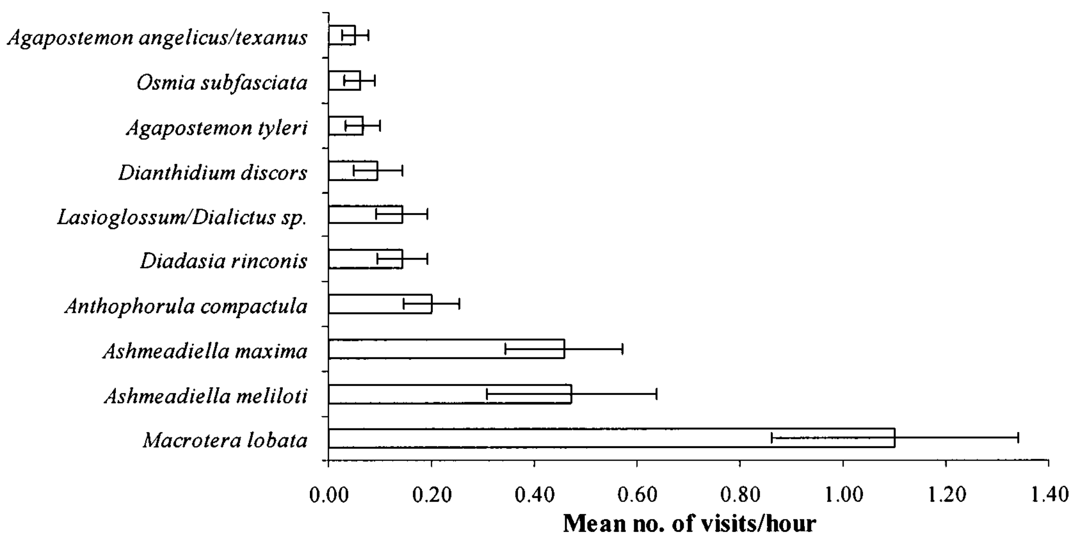

Figure 28. Mean visitation rate/hour of Apoidea, between March 17 – May 7, 2005 at MA Site; SD=1.

CHAPTER IV

DISCUSSION

This study provides fundamental information about the reproductive biology of *A. asterias* that will support recovery efforts in the future. Results of this study concerning phenology, the breeding system, pollen limitation, and potential pollinators provide information important for conservation of the species. *Astrophytum asterias* is limited by its need for a vector to transfer pollen and set seed. Additionally, the reproductive capacity of *A. asterias* is pollen-limited and even when pollinators are present, seed set is decreased compared to hand-pollinated flowers. Peak flowering days (4-6 days) result in 39%-61% of the year's total number of flowers. These data suggest that sufficient pollinator abundance is very important on these few days. Overall, enhancing pollinator abundance to maintain populations of *A. asterias* is critical.

Previous observations reported *A. asterias* blooming from March through May and fruiting from April through June with possible blooming occurring after rain in summer months (USFWS 2003). The 2004/2005 phenological results of this study show a similar pattern with flowering from March to May and fruiting from April to June with a few flowers opening in late July and producing fruit by early August. However, it was also observed between 39% (2004) and 61% (2005) of the total number of flowers for the year opened in the first week of April. Flowers open for one to three days and on average plants have 1-2 flowers and less than one fruit per year. For a short-columnar hermaphroditic cacti, *A. asterias* has low flower production. *Echinocereus chisoensis*, another rare Texas cactus with similar morphology and floral production, produces on average 3.6 flowers/plant (Amos and Vassiliou 2001), which is twice the number of flowers/plant produced by *A. asterias*. Many other columnar cacti have multiple stems and meristems and can produce 2-20 flowers a week (Fleming et al. 2001). Without vegetative reproduction, only one meristem and no branching, *A. asterias* is extremely restricted simply in numbers of meristems available to produce reproductive structures.

A relationship between precipitation and number of flowers and fruit produced has been found in studies of other cacti. Petit (2001) found that *Pilosocereus lanuginosus* was significantly and positively effected by precipitation on the week immediately prior to sampling dates. In contrast, Petit (2001) also found that *Stenocereus griseus* was significantly and negatively affected by precipitation due to bud loss from hard rains. A direct correlation between precipitation and number of flowers and fruit produced was not found to be significant in this study. However, the significantly greater number of flowers and fruit produced in 2004 may indeed be influenced by the amount of precipitation. From January to May 2004, 2.6 inches more rain fell compared to 2005, which received an amount closer to average annual precipitation for the area. If more years of phenological data were collected, a total number of flowers, fruit, and buds could be compared to a total yearly precipitation for a direct correlation. Comparison of fruit set between years indicates that although precipitation may influence the total number of resulting flowers and therefore fruit for each year, precipitation does not influence percent fruit set between years. If a relationship between precipitation and flower and fruit production exists and if precipitation increases fruit set, one would expect to see an increase in fruit in years with more rain. With the present data set this is not the case. Fruit set data (45% in 2004 and 37% in 2005) indicate that a similar proportion of flowers and fruit are set between years.

In terms of the flowering plants, *A. asterias* has a comparatively high fruit set. Sutherland and Delph (1984) found that of 316 plants from at least 43 angiosperm families analyzed, self-incompatible hermaphroditic plants on average resulted in 22.1% flowers producing fruit. However, in terms of the Cactaceae, *A. asterias* has a low fruit set compared to an average of 70.4% (range of 21-99%) found among five other hermaphroditic, self-incompatible columnar cactus species with published fruit set data (Fleming et al. 2001, Casas et al. 1999, Amos and Vassiliou 2001, Ibarra-Cerdeña et al. 2005) (See APPENDIX). Seed set in *A. asterias* is also far below the average seed set of other cacti species (Fleming et al. 2001, Casas et al. 1999, Amos and Vassiliou 2001, Ibarra-Cerdeña et al. 2005).

Astrophytum asterias is an obligate outcrosser and, by definition, self-incompatible. Many other species of cacti have been found self-incompatible including *Stenocereus stellatus, Carnegia gigantean, Stenocereus thurberi, Echinocereus chisoensis, Stenocereus eruca,* and *Stenocereus queretaroensis* (Casas et al. 1999, Fleming et al. 2001, Amos and Vassiliou 2001, Clark-Tapia and Molina-Freaner 2004, Ibarra-Cerdeña et al. 2005). As a self-incompatible species, *A. asterias* might be experiencing reproductive constraints in terms of pollinator availability/effectiveness. The significantly greater fruit and seed set resulting in the experimental outcrossing performed by hand compared to the controls left open and available for pollinator visitation is presumably the result of limited pollen deposition and may be correlated to pollinator limitation. Pollinator limitation is widespread in natural populations (Bierzychudek 1981) and can be caused by visitation variability and low pollinator abundance (Burd 1994, Johnson and Bond 1997). Pollinator limitation can also be caused by a variety of environmental factors affecting pollinator behavior.

Macrotera lobata and *D. rinconis* are cactus specialists and all other bees observed in this study are generalists (J. Neff personal communication 2005). Most of the bees observed visiting flowers of *A. asterias* are common in south Texas except for *M. lobata* and *D. discors* which are rare in collections (J. Neff personal communication 2005). However, the number of *M. lobata* specimens collected in this study is large, indicating that the taxon can be locally abundant (J. Neff personal communication 2005). *Macrotera lobata* has been documented on only cactus species (Danforth 1996, Michener 2000) and may have a morphology that increases its effectiveness as a pollinator compared to other generalist bees visiting *A. asterias*. *Macrotera lobata*, as the most abundant visitor to *A. asterias,* may be a more significant pollinator quantitatively. A plant population saturated by a large number of less effective pollinators may set more seed than a population visited by less abundant, but more effective pollinators (Jennersten and Morse 1991). However, Thomson and Thomson (1992) caution that qualitatively, plants occupying an area saturated in common, yet ineffective pollinators might never equal the seed set of plants occupying an area with fewer, yet more effective pollinators. Mayfield et al. (2001) found this to be true with *Bombus appositus* visiting *Ipomopsis aggregata*.

Otero-Arnaiz et al. (2003) and Cruz and Casas (2002) found Apoidea to be the most frequent visitor of two central Mexican cactus species. Apoidea were not the most frequent visitors to *A. asterias*, but were probably the most frequent effective pollinators. Other visiting insects, including beetles, ants and syrphid flies, do not appear to be effective pollinators. Due to minimal movement between flowers and lack of contact with the stigma in the flower, beetles appear to be ineffective as *A. asterias* pollinators. Similar observations have been made in other cactus species by several researchers (Grant and Grant 1979, Parfitt and Pickett 1980, McFarland et al. 1989, Escaravage and Wagner 2004). Ants secrete antibacterial and antifungal substances, which have been shown to interupt pollen germination and pollen-tube growth (Beattie et al. 1984, 1985). These pollen-inhibiting substances are purportedly the reason few ants are considered to play a role as plant pollinators (Peakall et al. 1991). Since syrphids accounted for only 0.7% of all visits to *A. asterias*, they were not considered significant pollinators. In addition, syrphids have been observed to carry small pollen loads and have short foraging times (Escaravage and Wagner 2004). In terms of *A. asterias*, further pollinator effectiveness studies should be conducted to determine which species are most effective.

Astrophytum asterias may be experiencing limitations on population growth due to its reproductive biology. *Astrophytum asterias* does not reproduce vegetatively and only produces 1-2 flowers a year, on average. Because *A. asterias* requires pollinators to reproduce, sufficient numbers of effective pollinators are very important. However, this study also shows that *A. asterias* may be fairly resilient to changes in its habitat or to human manipulation. Habitat changes that cause reduction in visitor numbers might not affect the reproductive output of *A. asterias,* if effective pollinators are not reduced.

Managers interested in reducing the impact of the limiting reproductive stage of *A. asterias* should continue to find ways to increase fruit and seed set. Hand-pollinations to augment populations may be required and therefore, further outcrossing studies should be conducted to find if an optimal outcrossing distance exists to optimize seed set. Due to the reliance of *A. asterias* on pollinators, further studies are needed to explore effective pollinator biology. Finally, identifying ranching and farming practices potentially detrimental to pollinators will help to determine agricultural practices that can enhance *A. asterias* populations on private lands in south Texas.

APPENDIX

Self-incompatible, hermaphroditic cacti species with published fruit and seed set data compared with *Astrophytum asterias* data from this study.

Cactus species	**Fruit set**	**Mean Seed set**	**Author**
Carnegia gigantea	64-74%	1358	Fleming et al. 2001
Stenocereus thurberi	21-30%	536	Fleming et al. 2001
Stenocereus stellatus	65-75%	1111	Casas et al. 1999
Echinocereus chisoensis	90-99%	401	Amos and Vassiliou 2001
Stenocereus queretaroensis	93%	923	Ibarra-Cerdeña et al 2005
Astrophytum asterias	**41-43%**	**13.3 +/- 27.1**	**Current study**

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