Reintroduction of star cactus *Astrophytum asterias* by seed sowing and seedling transplanting, Las Estrellas Preserve, Texas, USA

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SUMMARY

Star cactus *Astrophytum asterias* is listed endangered in the USA. The few known extant populations are located in Starr County, Texas and adjacent north Mexican states. According to the United States Fish and Wildlife Service *A. asterias* recovery plan, reintroduction is an acceptable step in recovery of this species. This paper reports on a pilot *A. asterias* reintroduction program. Seeds and seedlings (2¼ and 2¾ years of age) were planted in the spring and autumn of 2007. Of the seeds sown (120 each in spring and autumn), less than 4% grew to produce seedlings (five from the spring planting and four from the autumn planting). After a monitoring period of 14 months, spring- and autumn-planted seedling survival was 55.0% and 72.5%, respectively. Mean diameter of the surviving spring-planted seedlings (n = 66) increased from 8.9 (± 1.6) mm at planting to 10.4 (± 2.0) mm. Mean diameter of surviving autumn-planted seedlings (n = 87) increased from 9.4 (± 2.0) mm to 11.3 (± 2.6) mm. Based upon these results, for *A. asterias* reintroduction purposes transplanting nursery reared seedlings appears a better strategy than sowing of seeds directly into the wild.

BACKGROUND

Astrophytum asterias is a rare cactus of northern Mexico and southern Texas, which is the northernmost extent of its range. Due to low population numbers and anthropogenic threats, this species was federally listed as endangered on 18 October 1993 and by the state of Texas on 30 January 1997 (United States Fish & Wildlife Service (USFWS) 1993, Texas Parks & Wildlife Dept. 1997). As of 22 October 1987, *A. asterias* was also listed in Appendix I by CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora). The 2003 USFWS *A. asterias* recovery plan assigns the species a priority ranking of 2, which indicates that it faces a high degree of threat, yet has high recovery potential. Recovery criteria include the maintenance or establishment of 10 fully protected populations in the USA or Mexico. The populations must be fully protected, with a minimum of 2,000 individuals each, and of an age class structure reflecting that the plants are reproducing and becoming naturally established (USFWS 2003). To ascertain this, surveys for new populations will continue. If sufficient populations are not found, reintroduction of *A. asterias* is considered an acceptable step in the recovery of this species.

There was only one known population in the USA, in Starr County (Texas) located on private property when *A. asterias* was federally listed in 1993. There were also reports of *A. asterias* from Cameron, Hidalgo and Zapata counties; however, none of those sites had been relocated (Damude & Poole 1990). At the initiation of the study described herein, there were nine known private properties in Starr County with extant populations of *A. asterias* (Janssen *et al*. 2005). Recent surveys in Mexico identified seven populations in Tamaulipas and two in Nuevo León (Martínez-Ávalos *et al*. 2004).

In Mexico recent research has documented mortality of *A. asterias* due to herbivory by Mexican ground squirrels *Spermophilus mexicanus*, a fungal plant pathogen *Phytophthora infestans*, and a cerambicid beetle (species unidentified) (Martínez-Ávalos *et al*. 2007). Mortality in Texas has been documented due to herbivory by desert cottontail *Sylvilagus audubonii* and possibly Mexican ground squirrel, fungal infection, and the cerambicid beetle *Moneilema armatum* (Janssen *et al*. 2010, Ferguson & Williamson 2009, Ferguson *et al*. in prep.). In Mexico and Texas mortality by the aforementioned causes occurs in all size classes. The presented research also documented mortality of *A. asterias* seedlings due to weevils, tentatively identified as *Gerstaeckeria* sp.

Habitat destruction/modification is the primary anthropogenic threat to the persistence of *A. asterias* in the wild. Natural rangeland in Texas is still being root-ploughed (i.e. slicing of shrub/brush roots) and seeded to non-native, forage grasses in particular buffelgrass *Pennisetum ciliare* (native to Africa) to enhance livestock grazing potential. Urban sprawl is also a threat in Texas as Rio Grande City is within 13 km of *A. asterias* populations and development (e.g.housing) is spreading towards these sites. A recently proposed highway bypass project would have bisected these *A. asterias* populations. Although, this project did not come to fruition, such projects remain a threat. Because *A. asterias* resembles peyote *Lophophora williamsii* it is accidentally collected and threatened by the peyote trade (Terry 2005). Over-collection of star cactus for the horticultural trade is also listed as a threat in the recovery plan (USFWS 2003). Collection for the horticultural and peyote trade is hard to quantify but still assumed to be of significance. Oil and gas exploration also presents a threat to star cactus populations. In autumn 2008-spring 2009, two 260 km^2 seismic survey projects (aimed at detecting gas reserves) crossed *A. asterias* populations. Despite coordination between the gas companies and a private consultant knowledgeable of star cactus populations, around 10% of the plants in the project area were destroyed (Janssen *et al*. 2010).

Determining the feasibility of reintroducing *A. asterias* is one of the tasks listed in the USFWS *A. asterias* recovery plan which could lead to the down-listing of this species. It is well known in the horticultural trade that *Astrophytum* species, including *A. asterias*, are easily grown in cultivation (Higgins 1960, Damude & Poole 1990, Anderson *et al*. 1994). However, whether seeds or transplanted seedlings would survive best in the wild was not known. Therefore, the authors conducted a pilot reintroduction of *A. asterias* using both seeds and seedlings planted in different seasons (spring and autumn).

ACTION

Vegetation cover estimates and soil analyses were conducted in 15 subpopulations (sites) of *A. asterias* in Texas prior to selection of the reintroduction site. Only three of the 15 sites had vegetation cover exceeding 50%. The lowest vegetative cover recorded was 21%. *Varilla texana* (a succulent shrub) was the most dominant species (11.6% cover). The other species with >5% cover included *Prosopis glandulosa* and *Acacia rigidula*. Sixty-eight plant species were documented as *A. asterias* associates at the 15 sites. Other species documented with ≥1% dominance included: *Opuntia leptocaulis*, *Castela erecta* subsp. *texana*, *Ziziphus obtusifolia* var. *obtusifolia*, *Suaeda conferta*, *Parkinsonia texana* var. *macra*, and *Monanthochloë littoralis*. The majority of the soils at the 15 sites have a clay component and are classified as clay or clay loam with varying levels of salinity. Many sites also have a gravel component. Soil analyses classified nine of the 15 sites as saline-sodic; two as saline; two as sodic; and two as non-saline, non-sodic.

The authors also conducted growth rate analyses of *A. asterias* in cultivation (n = 108). The plants demonstrated a linear growth pattern. Analysis has also shown that initial diameter of seedlings is not correlated with growth rate (confidence intervals: lower = -0.0993 and upper = 0.2526). Seventy-five percent of the estimated growth rates of the seedlings in cultivation were less than 2.92 mm/year.

Pilot reintroduction site: In 2007 the *A. asterias* reintroduction site was established at the 168 ha Las Estrellas Preserve, Starr County, owned by the Texas Chapter of The Nature Conservancy (TNC). The preserve has subpopulations of *A. asterias* scattered throughout and is the only protected site of this species in the USA. Soils comprised clay with a gravel component and soil analysis classified it as non-saline, non-sodic. Vegetation cover was 47.4%; most dominant species were *Castela erecta* subsp. *texana* (15.5%), *Acacia rigidula* (6.8%), and *Ziziphus obtusifolia* var. *obtusifolia* (5.5%). Other species observed (>2% cover) included: *Bouteloua trifida*, *Varilla texana*, *Sporobolus airoides* subsp. *airoides* and *Prosopis glandulosa*. Although the reintroduction site had greater shrub coverage than the 15 star cactus subpopulations, the overall vegetation cover was within the range of cover recorded for the 15 subpopulations. The majority of the species documented at the reintroduction site were also documented in the 15 subpopulations.

Experimental design: A split-plot experimental design was established. Two 1-m^2 quadrats were located along each of three 25-m transects established on a 30-m baseline in a stratified-random design for a total of six quadrats (Fig. 1). A randomly selected distance of 1-9 m was used to locate the first quadrat on each transect. The second quadrat was located 10 m north of the first quadrat. The quadrats were centered on the transect unless one of the following conditions was encountered: 1) 100% dense brush covered one or more of the subquadrats; 2) two or more of the planting rectangles were covered by shrub basal area; 3) a Mexican ground squirrel burrow was located within 1 m of the quadrat. In these instances the quadrat was rotated around the centre point until a feasible placement was obtained. Each of the six quadrats was subdivided into four 0.25 m^2 subquadrats. One of four treatments was randomly assigned to each subquadrat: a) 20 seeds planted in the spring (n = 120), b) 20 seedlings planted in the spring (n = 120), c) 20 seeds planted in the autumn (n = 120), and d) 20 seedlings planted in the autumn (n = 120) (Fig. 1). Three planting grids (each approximately 50 cm x 50 cm) comprising hardware cloth (wire mesh) with ¼ inch (0.6 cm) openings stretched within a wooden frame were constructed. Twenty planting rectangles approximately 3.2 cm x 4.5 cm in size were cut into the hardware cloth creating a grid of four columns and five rows (Fig. 2). The planting grid allowed for equal spacing of the seeds and seedlings and aided in monitoring.

Propagules and planting methodology: Twenty seeds were planted in six quadrats on 14 March (spring; n = 120) and 22 September 2007 (autumn; n = 120). No site preparation was conducted prior to planting. A 60d 6 inch (15.4 cm) nail was inserted approximately 1 cm into the ground to create a divot in which one seed was planted per planting rectangle. The seeds used in this intervention were derived from a breeding system and pollen-limitation experiment (Strong & Williamson 2007) conducted at the Preserve. The seeds for the plantings were haphazardly chosen from all the seed collected (i.e. seeds scattered on table and undamaged ones selected until the desired number for planting had been reached).

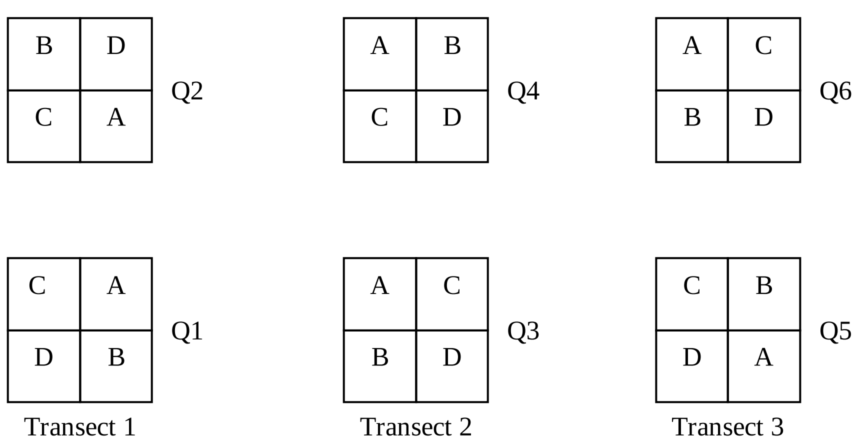

Figure 1. Randomly assigned treatments for each 0.25 m^2 subquadrat: A = seeds planted in spring; B = seedlings planted in spring; C = seeds planted in autumn; and D = seedlings planted in autumn.

Figure 2. A planting grid (50 cm x 50 cm) comprising hardware cloth (wire mesh) within a wooden frame, with 20 planting rectangles (3.2 cm x 4.5 cm) cut into the mesh.

Twenty seedlings were planted in six quadrats 19-20 April (spring; n = 120). The seedlings were approximately 2 ¼ years of age with diameters averaging 8.78 ± 1.7 mm (±SD; range 4.96-13.50 mm). Twenty seedlings were also planted in six quadrats 20-21 October (autumn; n = 120) 2007. These were approximately 2 ¾ years of age. The diameters of the seedlings averaged 9.30 ± 2.1 mm (range 5.10-15.17 mm). As with the seed sowing experiment, no site preparation was conducted prior to planting. A set methodology for planting of seedlings was developed. Approximately 6-10 days prior to planting, seedlings were removed from the greenhouse and housed out-of-doors in Starr County to acclimatize them. The seedlings were each watered with approximately 3 mL of water immediately after planting. Any uprooted seedlings found subsequent to transplanting were replanted and given a further 3 mL of water.

The seedlings used in this intervention were grown from the seeds of the aforementioned Strong and Williamson (2007) experiment. The seedlings were maintained in a greenhouse at the Lady Bird Johnson Wildflower Center (Austin, Texas) and a randomly chosen subset was used in this study.

Monitoring: Each planting treatment was monitored two weeks after planting to record any losses. Thereafter, data were collected every four weeks. Data collection concluded on 1 June 2008 and 15 November 2008 for the spring and autumn treatments of seeds and seedlings, respectively. If the seeds germinated, the month in which the seedling was first observed was documented. At the conclusion of each 15-month monitoring period of the seed treatments, the diameter of each seedling was measured with calipers. At the end of each 14-month monitoring period of the seedling treatments, the diameter of each seedling was recorded. *A. asterias* will shrink and retract into the soil. Therefore, diameter measurements of the planted seedlings were not taken more often as seedlings were often flush with the soil surface or buried. It was feared that exposing the seedlings regularly could make them more vulnerable to desiccation and possibly herbivory and thereby jeopardize their survival. When a seedling died, if cause of death could be determined, this was documented.

CONSEQUENCES

Survivorship of seedlings derived from seeds: Of the 120 spring-planted seeds, five produced seedlings; these were first observed six to nine months after sowing (Table 1). Four of the 120 autumn-planted seeds produced seedlings, first observed 11 to 12 months after sowing. Although more seeds may have germinated, the overall percentage of seedlings observed from seed was only 4%.

Four of the five seedlings (80% survival rate) derived from spring-planted seeds were alive and healthy at the end of the spring planting monitoring period (June 2008) whilst 100% of the seedlings from autumn-planted seeds were alive and healthy at the end of the respective monitoring period (November 2008). On-going monitoring of the intervention indicated overall survivorship of the seedlings derived from seed at 67% as of 29 May 2009. However by 27 August 2009 only two of nine of the seedlings produced from planted seeds were alive. As of 31 March 2011 only one of the seedlings was still alive (Table 1).

Table 1. *A. asterias* seedlings derived from spring-sown (March) and autumn-sown (September) seed, date seedling first observed, final diameter at the end of the respective monitoring periods (June and November 2008), and status as of 31 March 2011. The date a seedling went missing and therefore considered dead is noted.

Planted	Date seedling first observed	Diameter (mm) at end of monitoring period	Status on 31 March 2011
14 March 2007	22 September 2007	3.47	dead 26 March 2010
14 March 2007	22 September 2007	3.56	alive
14 March 2007	22 September 2007	dead	dead 15 December 2007
14 March 2007	15 December 2007	4.23	dead 29 May 2009
14 March 2007	15 December 2007	3.51	dead 27 August 2009
22 September 2007	2 August 2008	3.82	dead 27 August 2009
22 September 2007	2 August 2008	3.38	dead 27 August 2009
22 September 2007	23 August 2008	4.24	dead 27 February 2009
22 September 2007	20 September 2008	3.98	dead 27 August 2009

Survivorship of transplanted seedlings: A total of 66 *A. asterias* seedlings (55.0%) of the spring-planted seedlings were alive at the end of the 14-month monitoring period (June 2008; Fig. 3). Almost all (19 of 20) of the spring-planted seedlings in a single subquadrat (Q4) were lost due to digging activities of a Mexican ground squirrel. Excluding Q4 from the survivorship calculations, spring survivorship was 65.0%. Of autumn-planted seedlings, 87 (72.5%) survived to the end of the monitoring period (November 2008; Fig. 3). Monitoring of the surviving *A. asterias* plants is on-going. As of 29 May 2009, 52 and 83 of the spring- and autumn-planted seedlings, respectively, were still alive. However, by 27 August 2009 surviving seedlings only numbered 42 and 43. As of 31 March 2011, 35 and 39 of the spring- and autumn-planted seedlings, respectively, were still alive, representing an overall survivorship of 31%.

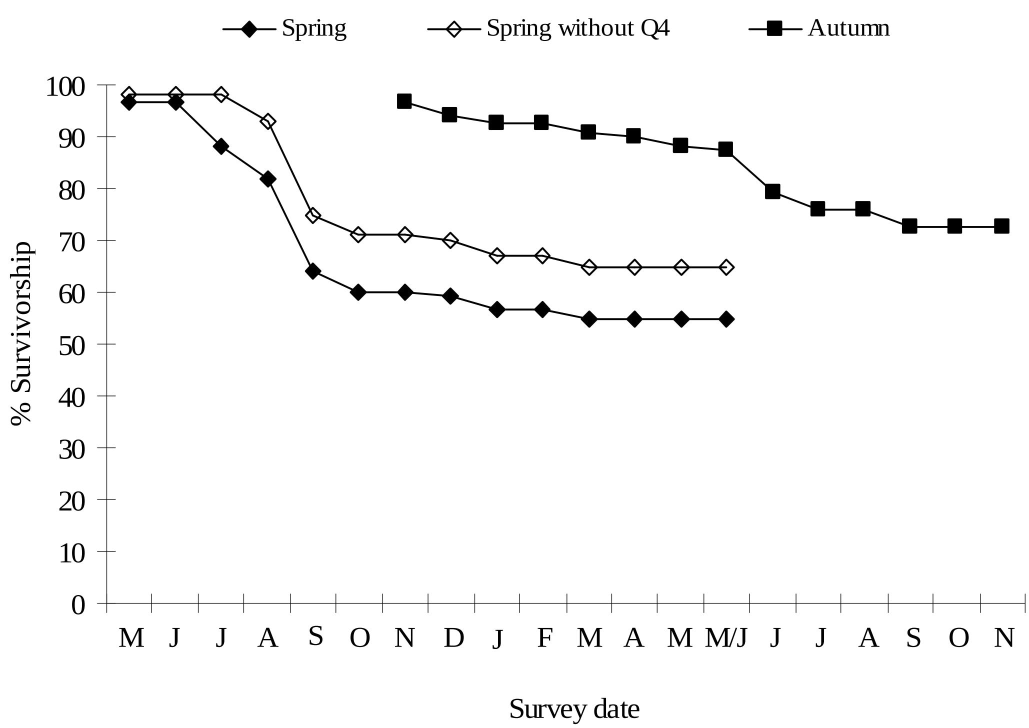

Figure 3. Percent survivorship per month of seedlings (n = 120) planted April and October 2007. The ‘spring without Q4’ line is the survivorship of spring-planted seedlings without the Q4 subquadrat which sustained a catastrophic loss of 19 seedlings.

The difference in the final diameters of the spring- and autumn-planted seedlings was significant (t = -2.41, P = 0.0173, df = 151). At the end of the 14-month monitoring period, average diameter of the surviving spring-planted seedlings (n = 66) had increased from 8.89 ± 1.6 mm (range 5.48-12.76 mm) at planting to 10.40 ± 2.0 mm (range 6.43-14.92 mm; Fig. 4). Nearly 85% of the 66 seedlings alive at the end of the spring monitoring period had diameters ranging from 8.01-14.00 mm (Fig. 5). At the end of the 14-month monitoring period, average diameter of the surviving autumn-planted seedlings (n = 87) had increased from 9.40 ± 2.0 mm (range 5.10-15.17 mm) at planting to 11.31 ± 2.6 mm (range 6.67-18.45 mm; Fig. 4). Seventy-seven percent of the 87 seedlings alive at the end of the autumn monitoring period had diameters ranging from 8.01-14.00 mm (Fig. 5).

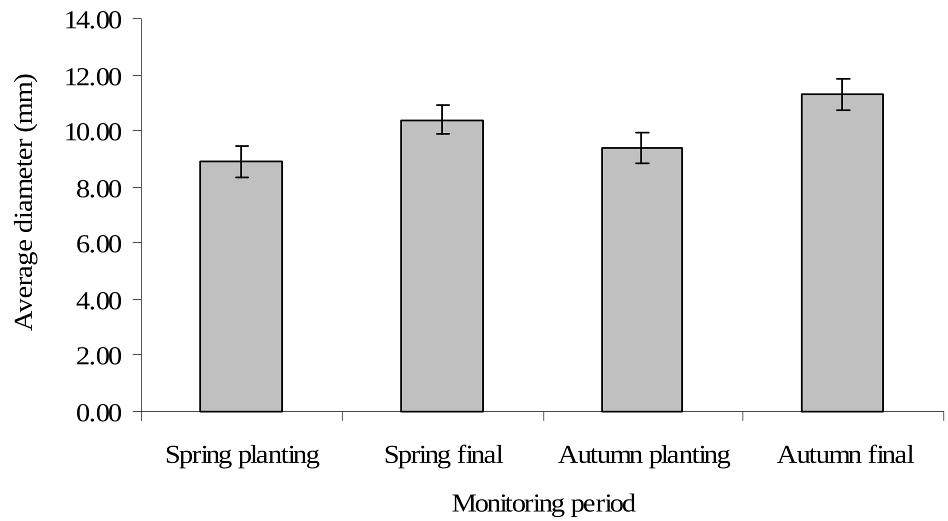

Figure 4. Average diameter with standard error bars of the spring- (n = 66) and autumn-planted (n = 87) *A. asterias* seedlings at the time of planting and at the end (final) of each respective 14-month monitoring period.

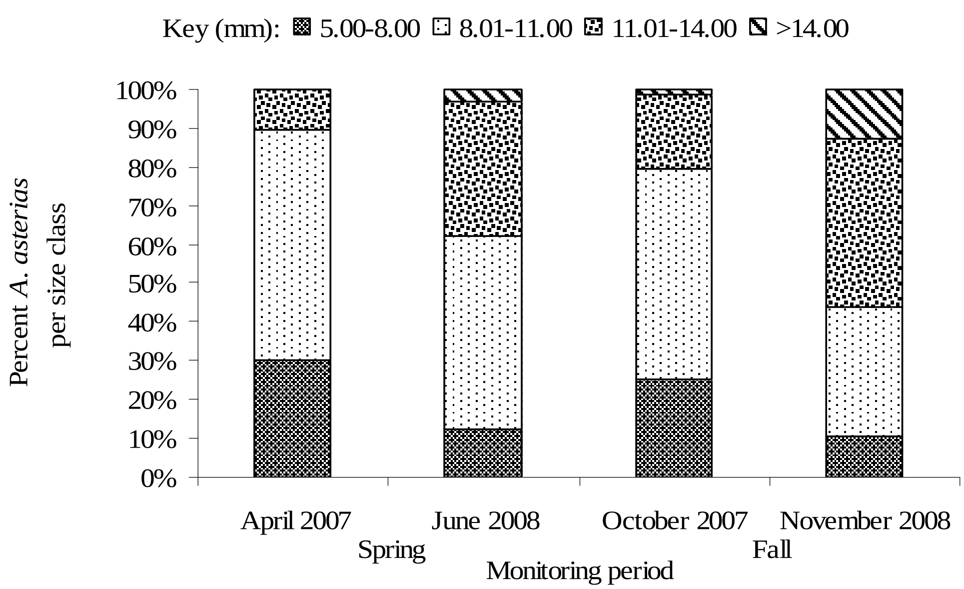

Figure 5. Percentage of *A. asterias* seedlings per size class when planted (April and October 2007) and at the conclusion of the respective monitoring periods (June and November 2008).

The simple linear regression of final diameter of spring planted seedlings on planting (initial) diameter was significant ($r^2 = 0.47$, $P < 0.0001$, $n = 66$; Fig. 6). Over the 14-month study period, the diameters of reintroduced seedlings increased by 0.84 mm (Fig. 6). This equates to an estimated growth rate of 0.73 mm/year. The simple linear regression of final diameter of fall planted seedlings on planting (initial) diameter was significant ($r^2 = 0.71$, $P < 0.0001$, $n = 87$; Fig. 7). Over the 14-month study period, the diameters of reintroduced seedlings increased by 1.08 mm (Fig. 7). This equates to an estimated growth rate of 0.99 mm/year.

The average diameter of the 54 spring-planted seedlings that died was 8.64 ± 1.9 mm (range 4.96-13.50 mm) whilst the average diameter of the 33 autumn-planted seedlings was 9.04 ± 2.3 mm (range 5.15-12.81 mm). Causes of mortality included burrowing activity of Mexican ground squirrels that uprooted/buried the plants, desiccation, herbivory, infestation by weevils, and other causes (Fig. 8). Seedlings were classified as dead when a dead plant or part(s) of it were located. The category "other" includes seedlings which were soft, uprooted, or otherwise damaged and eventually died. The "missing" category represents seedlings not relocated at the end of the monitoring periods and for purposes of data analysis, missing seedlings were assumed dead (Fig. 8).

A total of six seedlings died from herbivory as evidenced by rodent teeth marks. Another impact noted, that could possibly be attributed to rodents, was uprooting of the seedlings. Twenty of the 120 autumn-planted seedlings were uprooted at least once. Of these only nine were alive at the end of the monitoring period. Two died as a direct result of uprooting (included in the "other" category; Fig. 8). 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 monitoring period despite being uprooted (and replanted) in November 2007, and February, March and April 2008.

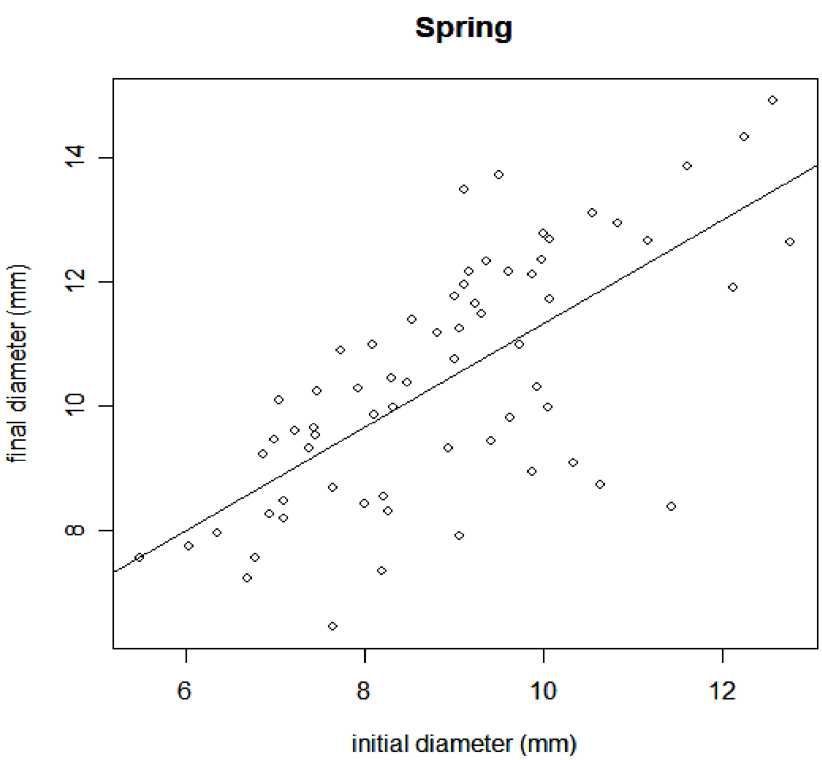

Figure 6. Linear regression of final diameter of spring planted seedlings on initial (planting) diameter ($r^2 = 0.47$, $P < 0.0001$, $n = 66$; $y = 2.9676 + 0.8358x$).

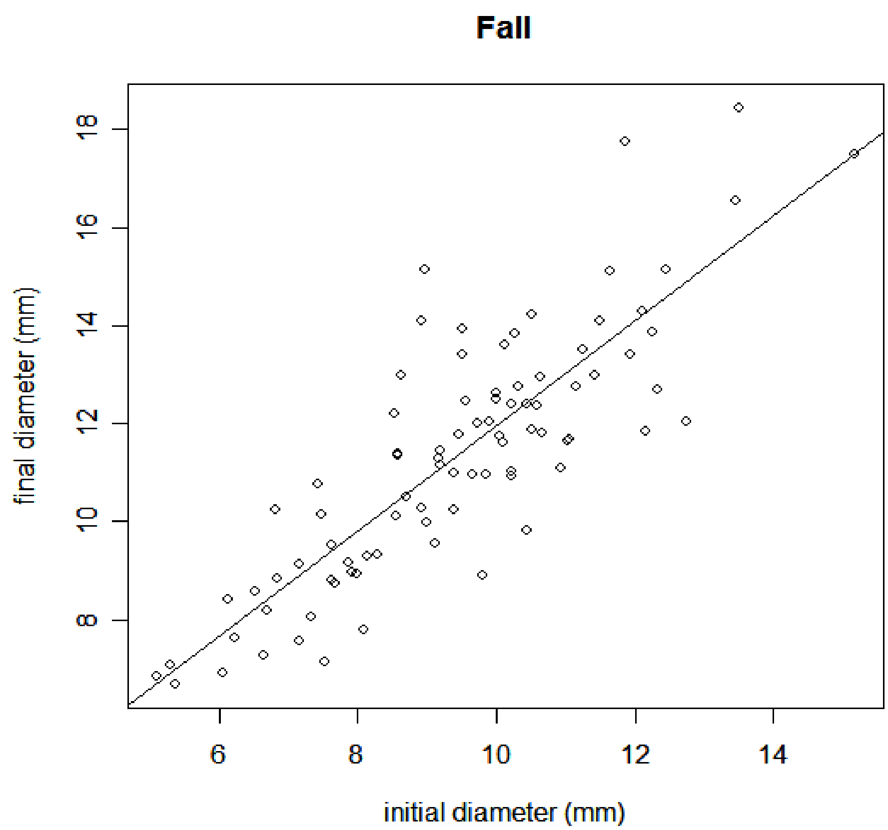

Figure 7. Linear regression of final diameter of fall planted seedlings on initial (planting) diameter (r^2 = 0.71, *P* <0.0001, *n* = 87; *y* = 1.1987 + 1.0752*x*).

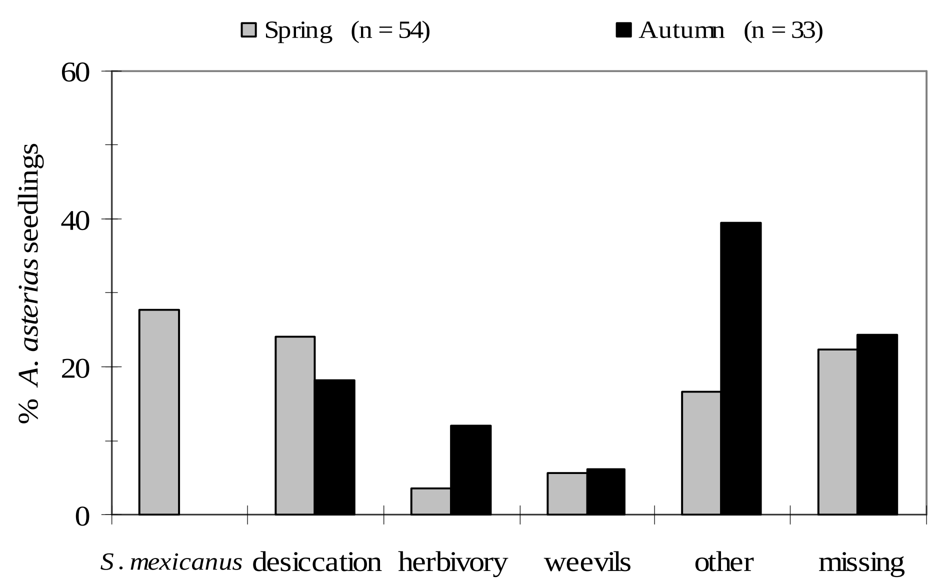

Figure 8. Causes of mortality for planted *A. asterias* seedlings. Note: Mexican ground squirrels *S.mexicanus* caused death by uprooting/burying seedlings.

Weevil infestation accounted for 6% of the total deaths (Fig. 8). In January 2008, two seedlings (one each planted in the spring and autumn) containing larvae were collected, but the larvae died before they matured and thus no identification could be made. In March 2008, three more seedlings (two planted in spring and one in autumn) which contained larvae were collected. After approximately one month, two adult weevils emerged. Specimens were tentatively identified to the genus *Gerstaeckeria*. All confirmed seedling deaths due to weevils were located in a single quadrat.

Conclusions: The *A. asterias* reintroduction trial suggests that transplanting nursery reared seedlings is a better choice of propagule than planting of seeds directly into the wild. Survival of autumn transplants (72.5%) was greater than spring transplants (55.0%) as was growth rate, but further studies are required to assess year to year variations. Further analyses of growth rate and survivorship of reintroduced seedlings is needed to determine if there is an optimum seedling size or age which ensures survivorship.

Future reintroductions should not employ a completely random design as this study documented spatially-related mortality from Mexican ground squirrels and weevils. A stratified random design can incorporate adequate spacing to spread the risks spatially and avoid areas where threats are high (e.g. burrows). A distance of about 3 m is suggested based on disturbance around burrows. Although the authors found an association between *A. asterias* and nearby plants in wild populations, nurse plants were not investigated in the intervention. Future interventions should further examine the benefits of ‘nurse plants’ for reintroduced seedlings as the quadrats adjacent to existing shrubs, thereby receiving shade during a portion of the day, had the highest percentage (35-85%) of surviving seedlings as of 31 March 2011. Based upon this pilot intervention, a draft reintroduction plan for *A. asterias* was developed (Janssen *et al*. 2010) as required by the U.S. Fish and Wildlife Service (USFWS 2000).

Due to the intrinsic biological characteristics (slow growth rate, low fecundity and recruitment) of *A. asterias*, as well as natural and anthropogenic threats, the outlook for star cactus is uncertain. Although this study demonstrates that fairly successful reintroduction of *A. asterias* is possible (as demonstrated by high seedling transplant survival), successful conservation of this species can only be ensured through protection of extant wild populations and preservation of its habitat and associated ecological processes. Hobbs (2007) discusses the importance of propagating plants, but stresses that little benefit will be achieved if the plants are simply reintroduced back into the same degrading environment. Currently the number of *A. asterias* in wild populations is not adequate to fulfill the recovery criteria as outlined by the USFWS recovery plan. Thus unless good quality habitat can be protected or degraded habitat restored, it is highly likely that more reintroductions of *A. asterias* will be necessary to maintain its presence in the Tamaulipan Thornscrub ecoregion of Texas within which the few extant populations persist. If the reintroduced plants continue to survive, it will take several more years before they become reproductively mature. This present intervention can only be deemed truly successful if these seedlings survive to maturity and their offspring become established.

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