Section 6 (Texas Traditional) Report Review

Attachment to letter dated MAY 02 2008

TPWD signature date on report March 27, 2008

Project Title: Protection on Private Lands and Research for Recovery of Large-fruited sand verbena

Final or Interim Report? Final

Grant #: E-58

Reviewer Station: Austin ESFO

Lead station was contacted and concurs with the following comments:
☐ Yes ☐ No ☒ Not applicable (reviewer is from lead station)

Interim Report (check one):	**Final Report** (check one):
☐ is acceptable as is	☐ is acceptable as is
☐ is acceptable as is, but comments below need to be addressed in the next report	☒ is acceptable, but needs minor revision (see comments below)
☐ needs revision (see comments below)	☐ needs major revision (see comments below)

Comments:

We appreciate the considerable effort that went in to this project. The comments below are mainly requested revisions for clarification.

- Under Location, the report lists the eight landowners with known populations and then discusses the property owners where access was granted for experimental augmentation. Please clarify that these later properties are within the historic range, but did not contain large-fruited sand verbenas naturally. It would also be helpful to discuss whether the properties had plants reintroduced on them as part of this study.

- Under Approach and Results, Population 9, the report states that the landowner was unable to be contacted for data. Was this data the landowner had, or were you unable to gain access to collect more data? If the later, does this mean Population 9 has not been revisited since the initial 2006 discovery?

- Please submit the GPS locations for all of the known and reintroduced populations, distinguishing between two.

- One area around Camp Creek Lake was thought to be suitable for reintroduction, but needed

further analysis. Were you able to conduct this analysis?

- Prior to any more reintroductions, we would like to meet to ensure the reintroductions are in accordance with our Controlled Propagation Policy (65 FR 56916). This policy provides guidance and establishes consistency for use of controlled propagation as a component of a listed species' recovery strategy. Please contact Alisa Shull at 512/490-0057 for further assistance.

FINAL REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. E - 58

Endangered and Threatened Species Conservation

Protection on Private Lands and Research for Recovery of Large-fruited Sand-verbena

Prepared by:

Paula S. Williamson

Carter Smith
Executive Director

Mike Berger
Division Director, Wildlife

27 March 2008

FINAL REPORT

STATE: Texas **GRANT NUMBER:** E - 58

GRANT TITLE: Endangered and Threatened Species Conservation

REPORTING PERIOD: 10/01/04 to 9/30/08

PROJECT TITLE: Protection on Private Lands and Research for Recovery of Large-fruited Sand-verbena

OBJECTIVE(S):

To achieve the established recovery criteria required to delist large-fruited sand-verbena (*Abronia macrocarpa*) and enable private landowners to participate in conservation of the species by managing their lands to protect populations from threats.

Significant Deviation:

None.

Summary Of Progress:

Please see Attachment A.

Preliminary Findings:

Please see Attachment A.

Location: Angelina, Newton, Sabine, and Tyler Counties, Texas.

Cost: Final financial report will be submitted later

Prepared by: Craig Farquhar **Date:** 27 March 2008

Approved by: C. Craig Farquhar **Date:** 27 Mar 08

ATTACHMENT A

FINAL REPORT

Protection on Private Lands and Research for Recovery of Large-fruited Sand-verbena

A three-year project
State fiscal year 2005 – fiscal year 2008

TPWD Contract Number 146696

Submitted by

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27 March 2008

INTRODUCTION:

The ultimate goal of endangered species research is the recovery of listed species. One listed species with a high probability of recovery, given the necessary research, is *Abronia macrocarpa*, the large-fruited sand-verbena. The plant was listed as a federally endangered species on September 28, 1988 (U. S. Fish and Wildlife Service, 1988) and received listing as an endangered species by the State of Texas on December 30, 1988 (U. S. Fish and Wildlife Service, 1992). The species recovery plan lists a recovery priority of 2 for *A. macrocarpa* indicating the species has a high degree of threat yet a high recovery potential. Recovery criteria set forth are to develop and implement management plans that insure continued protection of at least 20 viable populations, each at least 25 acres in size with a stable population of at least 600 plants (U. S. Fish and Wildlife Service, 1992).

Abronia macrocarpa is a member of the Nyctaginaceae or four o'clock family. The plant is an herbaceous perennial with a fleshy to semi-woody taproot, glandular-pubescent leaves, flowers grouped into a head, and large, thin-walled, papery anthocarps (Galloway, 1972, 1975). The species is a Texas endemic and apparently limited in distribution to three counties (Freestone, Leon and Robertson Counties). The plant grows in deep sandy soils in open areas of the Post Oak Savannah Woodlands (Galloway, 1972, 1975; Turner, 1983; Poole and Riskind, 1987; Bridges, 1988). At the time the species was listed only one population was known. The type locality is located in Hilltop Lakes Resort (Leon Co.) approximately nine miles northwest of Normangee, Texas (Galloway, 1972). At the time the species recovery plan was written, two additional populations had been identified (U. S. Fish and Wildlife Service, 1992), extending the species

distributional range into Freestone County and Robertson County. Recent surveys have identified additional populations (Williamson, 1996; Williamson and Janssen, 2002). Work in previous studies (Williamson and Werth, 1999; Williamson and Janssen, 2002) brought the number of known populations to eight (Figure 1) by the onset of this investigation. A ninth population was discovered in 2006. The known populations all occur on private land and are offered little protection by the Endangered Species Act, making the species especially vulnerable.

The Recovery Plan (U. S. Fish and Wildlife Service, 1992) indicates that the greatest existing threat to the species is habitat modification and destruction by man. Habitats have been modified by introduction of grasses and other non-native species for pasture improvement and soil stabilization. Clearing and fire repression have also contributed to habitat modification. Additional impacts by man include oil exploration and development, residential development, and recreational activities. Additionally, reproductive failure and low genetic variability have been suggested as potential limiting factors.

Little was known about the biology of the species when the recovery plan was written. Since implementation of species recovery plans requires an analysis of the taxon's ecology and biology, studies of the phenology, reproductive biology, and population genetics of *A. macrocarpa* were conducted (Corlies, 1991; Williamson, et al., 1994; Williamson and Bazeer, 1997; Williamson and Werth, 1999). These studies have improved our understanding of the species and provide critical information for developing land management plans to assist in alleviating threats to populations.

The need to protect *A. macrocarpa* from threats led to a three-year landowner technical assistance program conducted by Williamson and Janssen to assist landowners

in developing and implementing management plans to conserve the species. Each landowner was requested to voluntarily participate in our study. During the course of the study, landowners were contacted and made aware of the importance of the species and the critical need to preserve the existing populations. Literature was provided and laws governing endangered plant species and private landowner rights were discussed. Site visits were made to the populations to identify specific threats to each population. Studies of phenology, reproductive biology, and population genetics of *A. macrocarpa* (Williamson, et al., 1994; Williamson, 1996; Williamson and Bazeer, 1997; Williamson and Werth, 1999) were reviewed to glean data for use in compiling a list of compatible and noncompatible land management practices to assist landowners in developing workable land management plans. A Conservation Agreement, drafted for the particular needs and concerns of landowners, was presented to the landowners to provide a starting point for protection of the species.

To achieve recovery criteria set forth in the Recovery Plan (U. S. Fish and Wildlife Service, 1992), not only must presently known populations continue to be protected, but some of the known populations must be augmented to increase size and 12 additional populations must be discovered or created through a reintroduction program. The current study sought to contribute to discovery of suitable habitat and perhaps new populations through field surveys. The current study also sought to develop procedures to establish a successful population augmentation and reintroduction program to achieve recovery should insufficient numbers of wild populations exist. The Recovery Plan indicates that if continuous progress is made, delisting may be possible by 2015 (U. S. Fish and Wildlife Service, 1992). Five major actions are needed in order to achieve recovery of this endangered species (U. S. Fish and Wildlife Service, 1992) and three of these

(protect existing populations, search for new populations, develop plans for reintroduction into suitable habitat) were addressed in the current study.

OBJECTIVE:

The objective of this project was to address three of the five major actions needed to achieve the established recovery criteria required to delist large-fruited sand-verbena (*Abronia macrocarpa*) and enable private landowners to participate in conservation of the species by managing their lands to protect populations from threats.

LOCATION:

The study was conducted on private lands in Leon, Robertson and Freestone Counties, Texas. Permission for access was granted for eight known populations by the landowners (Womack, Mullenax, Winstead, Hilltop Lakes Resort, Rabe/Morris, Mancusso, Ingram/ Ruhland, Emmons/Woodard/Jones/Vernon). Permission was granted to conduct population augmentation studies on private lands with existing populations (Rabe/Morris, Ruhland). Landowner (Robertson) permission was granted in 2005 to establish experimental reintroduction field plots on two pieces of private property in Leon County. Landowner (Bourne) permission was granted in 2006 to establish experimental reintroduction field plots on private property in Leon County. Landowner permission was granted in 2007 to establish experimental reintroduction field plots on private property in Leon County (Karels, Thurston, Hardin, Slay, Allison) and Freestone County (Osborn). Seed germination experiments and propagation were conducted in the laboratory, in a walk-in growth chamber located at The University of Texas, Austin, and in a greenhouse located at the Lady Bird Johnson Wildflower Center, Austin.

APPROACH AND RESULTS:

The principal investigator is Dr. Paula S. Williamson, Professor of Biology at Texas State University-San Marcos. Gena K. Janssen was a project consultant. Anna Strong assisted as a research project specialist. Jacque Goodson and Carolyn Meredith, two Texas State Biology Graduate Students, assisted in the study. Specific recovery tasks addressed in this study are listed below. Each recovery task is numbered according to the numbers listed in the Recovery Outline section (pages 11-13) of the Large-fruited Sand-verbena (*A. macrocarpa*) Recovery Plan (U. S. Fish and Wildlife Service, 1992). The results shown below are from research conducted February 17, 2005 – March 17, 2008.

1) Protect *A. macrocarpa* populations from existing and future threats and develop management plans (Recovery task 1):

This project task was to continue providing technical assistance to landowners that will assist landowners in managing their lands to protect existing populations of large-fruited sand-verbena from present and future threats. Landowners were contacted and site visits were made in 2005, 2006, and 2007 to survey and assess population status. We met with most landowners to provide technical assistance. There were a few landowners that we were not able to meet with face-to-face. However, we were able to contact them by phone to discuss the species. Landowners were presented with land management plans specific to their property in a previous Section 6 funded project (PIs Paula Williamson and Gena Janssen). The landowners have implemented the recommended land management practices such as delaying mowing in the spring until after seed are dispersed and applying herbicides during summer months when the plant is dormant. This project has not revealed new information that would necessitate modifying the land

management plans previously developed. Landowners have been provided with voluntary conservation agreements. Despite being very cooperative, to date, no landowner has been willing to sign such an agreement.

The status of each population is shown below.

Population 1 – stable, consists of about 28,000 individuals on just over 20 acres

Population 2 – stable, consists of approximately 6,200 individuals on 5.5 acres

We made contact with a new landowner that owns the adjacent property. The population extends on to his land. He was informed about the species and need for conservation. He is willing to participate in our project and we now have permission to work on his property as well.

Population 3 – stable, consists of about 12,000 plants on over 90 acres

Population 4 – stable, consists of about 8,000 individuals on 8.5 acres

Population 5 – consists of approximately 5,000 individuals on 2.7 acres

The population occurs along a property fence-line and extending to the neighboring property where the bulk of the plants now occur. In 2002, approximately 4,000 plants grew along the property fence line. The installation of a natural gas pipeline in November 2004 heavily impacted the population. The number of *A. macrocarpa* individuals located along the fence line in spring 2005 was 164. The population size increased slightly with 184 plants found along the fence line in 2006. The number of plants along the fence-line increased to 701 by spring 2007, indicating the plant has an ability to naturally reestablish following a severe habitat impact. The landowners are very proud to have this species on their property and assumed the pipeline company would take precautions to protect the plants, but that obviously didn't happen. This is a population now in

need of augmentation, which fortunately we can do because we had previously collected seed. We did not attempt population augmentation at this site in 2006 or 2007 because the spread of coastal Bermuda grass has left little available habitat. The landowners are attempting to restrict the growth of the grass and if they are successful we will attempt population augmentation in the future.

Population 6 – a 10.6 acre area, currently with an estimated 750 plants

This population had about 2,000 plants in 2002. But, when we surveyed the population in spring 2005 we found establishment of a food plot had impacted it. Only two plants were found in spring 2005. We were not able to contact the landowner to visit the property in spring 2006. We did survey the site in 2007. The food plot had been abandoned. Approximately 200 flowering plants were found in the area in spring 2007.

Population 7 – stable, consists of about 4,500 plants on 12 acres

We tested population augmentation at this site. Seed (n=240) collected from Population 7 were planted on May 2, 2006 in suitable habitat approximately 0.32 miles from the existing population. Six plots were set up along a 12 meter transect, 40 seeds were planted in each 1 m^2 quadrat. The plots were monitored and the number of seedlings was recorded in spring 2007. The mean germination percentage was 17.10% ($\bar{x}$ = 17.10, n = 6 plots).

Population 8 – stable, consists of over 30,000 plants on about 30 acres

Population 9 – population discovered April 30, 2006

Population structure and community composition data were not collected because it was so late in the growing season and plants had already gone dormant. We were not able to contact the landowner to collect data.

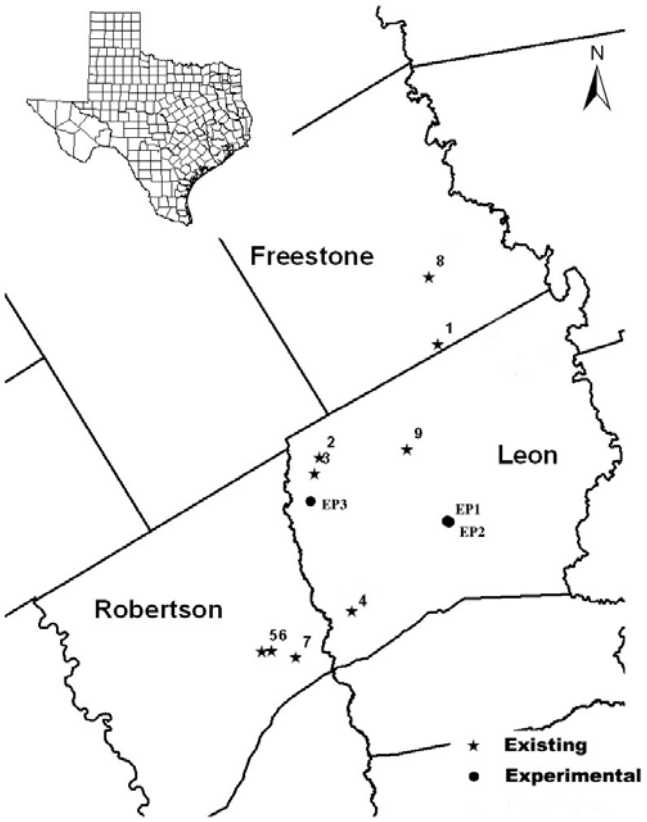

Figure 1. Map of Texas showing the nine known populations of *Abronia macrocarpa* and experimental reintroduction properties 1, 2 and 3.

An important step to generating support for the protection and conservation of endangered species is increasing the public's awareness. We made a public presentation on June 25, 2006, which was held at the Roberta Bourne Memorial Library in Marquez, Texas. The presentation was publicized in several local newspaper including the Jewett Messenger and Fairfield Recorder. The presentation was attended by some of the landowners that have *A. macrocarpa* populations on their property, as well as other interested landowners. The presentation was featured on a KBTX-TV Bryan/College Station newscast. This event is one example of our efforts to increase public awareness.

2) Search for new populations and for potential habitat **(Recovery task 4):**

Abronia macrocarpa may be delisted if at least 20 healthy, stable populations with a minimum of 600 plants in each can be located or established (U. S. Fish and Wildlife Service, 1992). Prior to the onset of this study we knew of eight populations. This project task was to survey potential suitable habitat to search for as yet unidentified existing populations as well as prime habitat and willing landowners that would enthusiastically participate in a population reintroduction program.

Abronia macrocarpa is known to occur in deep sandy soils of the Arenosa and Padina series (Williamson, et al., 1994). County soils maps were used to target sites to search for new populations. Field surveys to search for additional existing populations were conducted during the blooming season (March and April) in each year of the study.

The area around Camp Creek Lake was surveyed in spring 2005, but no new populations were discovered. One area was located that may be a suitable reintroduction site but, requires more analysis of the habitat. The area adjacent to the railroad track on Hwy 39 from Jewett to Normangee was extensively surveyed. This area was selected because it is thought that Dr. Walter Holmes, Baylor University, previously located a

population in this area between Robbins and Flynn, while on a class field trip. The most likely location is railroad property that is now extensively used by ATVs. If a population did occur there, it no longer exists.

As we surveyed for additional existing populations, we also searched to identify prime habitat and willing landowners that would enthusiastically participate in a population reintroduction program. This component of the study allowed us to identify areas within the historical range for future establishment of new populations.

In 2005 we did identify one landowner, with prime habitat on two pieces of property, willing to allow us to establish field plots to test reintroduction protocol. This particular landowner is very enthusiastic about conservation and our project, and not only welcomed our working on his land, but has also written several articles for local newspapers about our project. He is very well known and well respected in Leon County and his endorsement of our project gave us instant creditability with many people in the community. As a result, landowners contacted us asking us to survey their property to determine if they have populations of *A. macrocarpa* and if not, to assess their land to determine if it is suitable habitat for a reintroduction population.

As a result we were able to identify one additional population (Population 9, see Figure 1) in spring 2006. The population was discovered by a Jewett resident, who then contacted us. The population occurs approximately four miles southeast of Jewett just off of CR 317.

We conducted surveys of ten other private properties in Leon and Freestone Counties during the blooming season in spring 2006 and 2007. None of the properties surveyed supported populations, however seven properties appear to be suitable reintroduction

sites and the landowners enthusiastically endorse our using their lands as reintroduction sites.

3) Develop plans for reintroduction into suitable habitat (Recovery task 5):

This project task was to study techniques for successful population augmentation, use results of this study to establish a pilot reintroduction program and assess feasibility of the reintroduction program. This part of the project also provided important cultivation requirements concerning seed germination (a component of Recovery Task 3 – initiate studies to gather information necessary for protective management and restoration). Methodology and results are shown below for the project tasks:

• **Collect seed that represent known genomes.** (Recovery task 2):

Seed were collected in April and May, 2005 in accordance with the Center for Plant Conservation Guidelines (Faulk and Holsinger 1991) from seven of the eight populations. Seed was not collected from Population 6, which had been impacted by the establishment of a food plot. Seed has been deposited at the Lady Bird Johnson Wildflower Center in Austin, Texas.

• **Test seed germination requirements.** (Recovery task 3):

Abronia macrocarpa produces a fruit, termed an anthocarp, which consists of a dry papery portion formed by the lower calyx, which encases an achene. Achenes are dry, indehiscent, single-seeded, with the seed coat free from the pericarp. One aspect of seed biology that we were uncertain of was whether seed germinated in the spring immediately following anthesis, or if a period of dormancy occurred prior to germination. So we first performed a trial germination experiment.

Randomly selected whole fruits (anthocarps) collected in spring 2005 were planted 1/4” deep in a Schultz seed starter mixture. The trays were placed in a Sherer DualJet

growth chamber set at approximately 25º Celsius for a 3-4 week period. To mimic the natural day/night conditions of the environment the trays were exposed to 13/11 hr light periods. The trays were checked daily to record number of germinated seeds. We found that no seed germinated, indicating that a period of dormancy occurs.

Next, anthocarps were subjected to stratification to test whether a period of cold is required to break dormancy. Anthocarps planted in trays were placed in a refrigerator set at approximately 5º Celsius for 6-8 weeks. Following cold stratification, the trays were placed in a Sherer DualJet growth chamber set at approximately 25º Celsius for a 3-4 week period. Trays were subjected to the same light schedule used in the previous trial. The trays were checked daily to record number of germinated seeds. Seed (16%) did germinate in this trial, confirming that a cold period can break dormancy.

The percent germination in the previous experiment was relatively low indicating the seed of *A. macrocarpa* may require additional treatments to achieve greater germination. For example, exposure to summer and winter temperatures may be required to break dormancy (Baskin and Baskin, 2003). Therefore we conducted more extensive experiments to determine the most effective method to induce germination.

A series of tests were conducted in the laboratory using seed collected in spring 2006 to determine the most effective method for seed germination. Germination of seeds was compared among twelve treatments and a control (Table 1). The anthocarps not subjected to stratification, scarification, or a chemical treatment served as the control.

Each treatment was replicated three times with 36 seed per replicate for a total of 108 seed per treatment. In the warm stratification treatment, seeds were placed in a sand mixture at a 20-30° C for 2 weeks. Seed were cold stratified by placing seed in a refrigerator set at approximately 5º C for 8 weeks. Seed subjected to the scarification

treatment were mechanically rubbed with fine sand paper to abrade the seed coat. Randomly chosen seed were used in the treatments and control. The achene/anthocarp was either planted 0.6 cm deep in 75:25 sand: sphagnum moss mixture in rows in trays or placed on filter paper in a petri dish. The trays and petri dishes were placed in a Sherer DualJet growth chamber for 4 weeks. To mimic the natural day/night conditions of the environment seed were exposed to alternating temperatures and photoperiods (28ºC, 13 hr light/20ºC, 11 hr dark). Each day the trays and petri dishes were randomly rearranged to minimize differences within the growth chamber. The number of germinated seed was recorded daily. Percentage germination was calculated for each treatment and control. A single-factor analysis of variance was conducted using S-Plus ($p<0.05$) for Windows to determine if differences exist among treatment means. The Tukey's multiple comparison procedure was performed using S-Plus for Windows to compare treatment means.

Table 1. Laboratory Germination Treatments. The + symbol indicates the type of material planted in each treatment.

	Treatment	Substrate	Achene	Anthocarp
1	Cold stratified	Sand mixture		+
2	Cold stratified	Sand mixture	+	
3	Warm stratified, cold stratified	Sand mixture		+
4	Warm stratified, cold stratified	Sand mixture	+	
5	0.2% KNO3	Filter paper	+	
6	0.2% KNO3	Sand mixture	+	
7	Gibberellic acid	Sand mixture	+	
8	Gibberellic acid	Filter paper	+	
9	Scarified	Sand mixture	+	
10	Warm stratified, cold stratified, scarified	Sand mixture	+	
11	Warm stratified	Sand mixture	+	
12	No treatment	Sand mixture	+	
13	No treatment (control)	Sand mixture		+

Results indicate that a significant difference exists between the germination treatments (p-value = <0.000001, F = 16.37, df = 12). Germination ranged from 0% to 68.6% among the control and treatments (Figure 2). Seed germination was highest when achenes were scarified and subjected to warm followed by cold stratification. In the

control and treatments without stratification or scarification, there was a 0% germination rate. Subjecting the seed to a period of cold stratification positively influenced germination. Removing the achene from the papery part of the anthocarp increased germination in the cold stratification treatment from 0.93% when anthocarp was left intact to 6.5% when the achene was removed. Removing the achene from the anthocarp increased germination in the warm followed by cold stratification from 2.8% when anthocarp was left intact to 5.6% when the achene was removed (Figure 2). The Tukey's multiple comparison showed the scarified, warm-cold stratified treatment was significantly different from all other treatments (p value = <0.000001) (Figure 2).

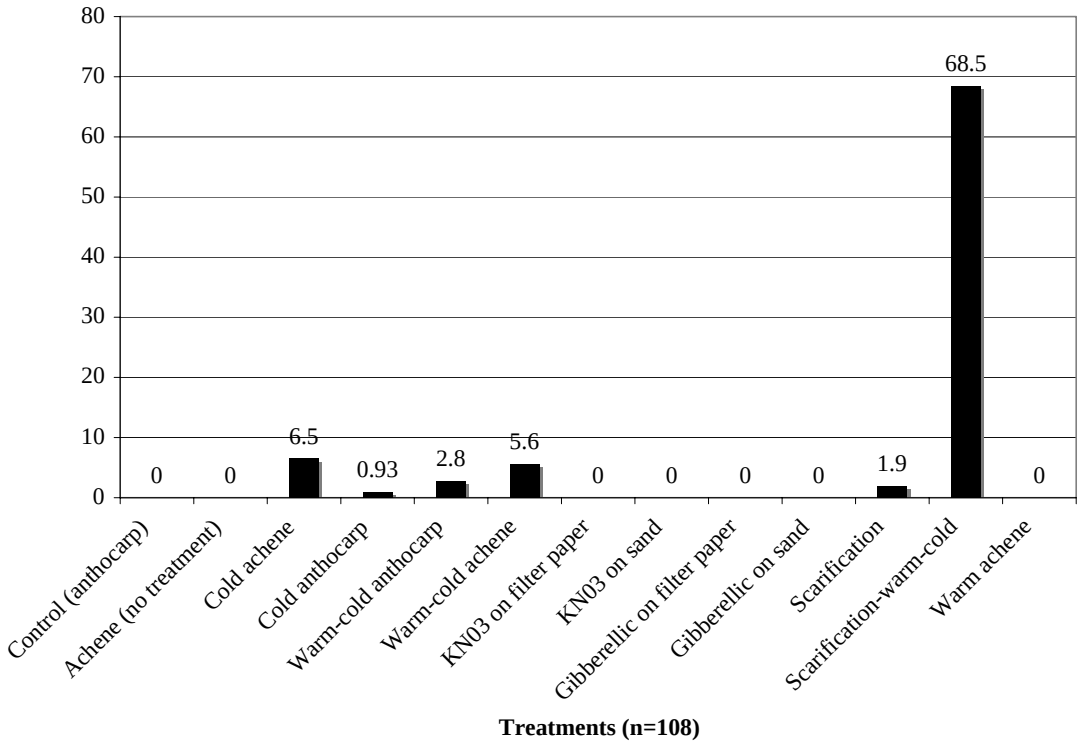

Figure 2. Percentage germination of control and achenes subjected to the twelve treatments, p-value = <0.000001; F value = 16.37; df = 12.

• **Characterize habitat of existing populations.** (Recovery task 3):

This task allowed us to identify appropriate habitat, within the historical range, suitable for future establishment of new populations should a reintroduction program be deemed necessary. We first characterized community structure, community similarity and edaphic features of sites supporting existing populations of *A. macrocarpa*. These data were compared to data collected from potential reintroduction sites to determine habitat suitability for reintroduction.

Community Structure of Existing Populations:

To examine community structure, twenty $1m^2$ quadrats were placed randomly throughout seven existing *A. macrocarpa* populations. We divided each quadrat into four sections, to ease counting and estimation, and recorded the number and type of associated plant species. Species identification followed the *Manual of Vascular Plants of Texas* (Correll and Johnston, 1979). The percent bare ground, vegetative cover, and litter were also estimated. We calculated the relative density of plant species occurring within the quadrats to determine community composition using the following formula:

$$\text{Relative Density} = \frac{\text{Number of plants of a given species}}{\text{Total number of plants}} \times 100$$

We used principal component analysis (PCA) to determine if there were major trends in the species composition found among the sites sampled. The PCA identifies linear patterns of correlated change among several variables and arranges each sample unit along the trend represented by the principal component (PC) (Anderson, et al., 1983). Data were analyzed using S-Plus 7.0 Student Statistics package.

The analysis of community structure (Figure 3) indicates that the majority of plants occurring along with *A. macrocarpa* are small annuals, such as Indian Blanket (*Gaillardia pulchella*), Chickweed (*Cerastium glomeratum*), and Scale Seed (*Spermolepis echinata*). Small annuals had combined relative densities ranging between 47.5 per m^2 to 92.3 per m^2. Grasses make up a smaller portion of the community, between 9.7 per m^2 to 20.7 per m^2. Grasses include Rescuegrass (*Bromus unioloides*), Sixweeks Grass (*Vulpia octoflora*), and Little Bluestem (*Schizachyrium scoparium*).

Several annual and perennial species occurred at high enough densities in each community to be reported individually. One of the most common associated species was *Rhododon ciliatus* (Sand Mint). Relative density of *R. ciliatus* ranged from 0.02 per m^2 at the site 3 location to 25.7 per m^2 at site 2. Various species of *Plantago* occurred at densities ranging from 3.2 per m^2 to 15.7 per m^2. *Tradescantia occidentalis* density ranged from 0.9 per m^2 to 7.5 per m^2 within the communities.

Sixty-one percent of the variance in species composition is explained by Principal Component 1 (PC 1) (Table 2). Relatively strong correlations exist between *Rhododon ciliatus* and PC 2, *Plantago* sp. and PC 3, *Croton argyranthemus* and PC 4, and *Opuntia compressa* and PC 5 (Table 2). Table 3 includes a list of associated species found commonly at sites supporting populations of *A. macrocarpa.*

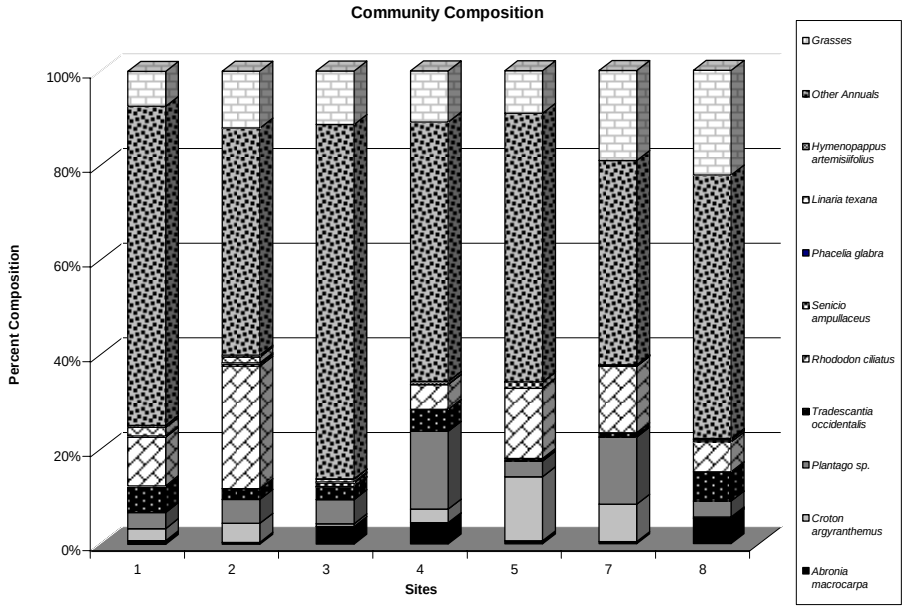

Figure 3. Relative density of associated species within communities supporting *Abronia macrocarpa*.

Table 2. Results of Principle Components Analysis.

	Principal Components					
Plant	**1**	**2**	**3**	**4**	**5**	**6**
Abronia	0.046	0.210	0.062	-0.080	0.500	
Allium	0.013			0.030	-0.028	
Annuals	0.839	-0.292	-0.033		0.296	0.202
Croton	-0.158	-0.263	-0.428	-0.738	-0.204	0.187
Grass	-0.147	0.406	0.486	-0.402	0.553	0.186
Hymenopappus	-0.012	0.051	-0.030	-0.013	0.013	
Linaria	0.012	-0.051	0.041	0.110	0.021	
Mimosa	0.221	0.174	0.034	-0.462	0.518	
Cnidosulus			-0.048			
Cheamacrista	0.011			0.043	0.058	
Phacelia		0.013	0.018	0.019	0.014	
Phlox	0.010	-0.023	-0.016	0.029	-0.062	
Plantago	-0.141	0.398	-0.692	0.333	0.339	0.207
Q. stellata		-0.012	-0.015	0.053	-0.105	
Rhododon	-0.472	-0.645	0.172	0.299	0.262	0.191
Tradescantia	0.060	0.100	0.183	0.262	-0.389	0.260
Vicia		0.016	-0.027	-0.147	0.036	
Ilex		0.025	-0.040	0.013		
Sececio	-0.024	-0.087	0.203	-0.123		
Yucca			0.027			
Opuntia	0.021		-0.013	0.012	0.719	

Table 3. Plants commonly associated with communities supporting *Abronia macrocarpa.*

Family	Scientific Name	Common Name
Agavaceae	*Yucca arkansana*	Arkansas Yucca
Apocynaceae	*Apocynum cannabinum*	Indian Hemp
Apiaceae	*Spermolepsis echinata*	Scale seed
Asteraceae	*Aphanostephus ramosissimus*	Dozy Daisy
	Coreposis tinctoria	Golden Tickseed
	Helenium amarum	Sneezeweed
	Heterotheca subaxillaris	Camphorweed
	Hymenopappus artemesiifolius	Old Plainsman
	Senecio ampullaceus	Texas Groundsel
	Rudbeckia hirta	Black-Eyed Susan
Aquifoliaceae	*Ilex vomitoria*	Yaupon
Brassicaceae	*Lepidium virginicum*	Peppergrass
Cactaceae	*Opuntia compressa*	Eastern Prickly Pear
Capperaceae	*Polanisia erosa*	Clammy-weed
Caryophyllaceae	*Cerastium glomeratum*	Chick-weed
Commelinaceae	*Tradescantia occidentalis*	Spiderwort
Convulvulaceae	*Stylisma pickeringii*	Pickering's Dawnflower
Cuppressaceae	*Juniperus virginiana*	Eastern Red Cedar
Euphorbiaceae	*Croton argyranthemus*	Silver Croton
Fabaceae	*Chamaecrista fasciculata*	Partridge Pea
	Coreopsis tinctoria	Tick-seed
	Gaillardia amblyodon	Maroon Blanketflower
	Gaillardia pulchella	Indian Blanket
	Mimosa pudica	Sensitive Plant
	Vicia ludoviciana	Vetch
Fagaceae	*Quercus stellata*	Post Oak
	Quercus incana	Sandjack Oak

Table 3, continued.

Family	Scientific Name	Common Name
Hydrophyllaceae	*Phacelia glabra*	Phacelia
Lamiaceae	*Monarda citriodora*	Lemon Beebalm
	Rhododon ciliatus	Sand Mint
Liliaceae	*Allium drummondii*	Wild Onion
	Nothoscordum bivalve	Crow-poison
	Smilax bona-nox	Greenbrier
Onagraceae	*Oenothera laciniata*	Cut Leaf Evening Primrose
Papaveraceae	*Argemone albifora*	White Prickly Poppy
Plantanaceae	*Plantago aristata*	Bracted Plantain
	Plantago major	Common Plantain
	Plantago virginica	Dwarf Plantain
Poaceae	*Bromus catharticus*	Rescue Grass
	Dicanthelium oligosanthes	Rosette Grass
	Schizachryrium scoparium	Little Bluestem
	Vulpia octoflora	Sixweeks Grass
Polemoniaceae	*Phlox drummondii*	Phlox
Primulaceae	*Anagallis arvensis*	Scarlet pimpernel
Scrophulariaceae	*Penstemon murrayanus*	Beard-Tongue
	Linaria texana	Toad-Flax
Rosaceae	*Rubus trivialis*	Southern dewberry
Vitaceae	*Vitis mustangensis*	Mustang Grape

The communities where *A. macrocarpa* grows demonstrate some interesting trends concerning cover categories. The percent bare ground ranges from 25% (Site 1) to 66.75% (Site 4). The percent litter ranges from 9.4 % (Site 3) to 29.25% (Site 1). The percent vegetative

cover ranges from 16.25% (Site 4) to 40% (Site 2) (Figure 4). The four sites with the highest density of *A. macrocarpa* plants all have greater than 50% bare ground (Figure 5). This demonstrates a trend that as bare ground reaches 50%, the density of *A. macrocarpa* increases.

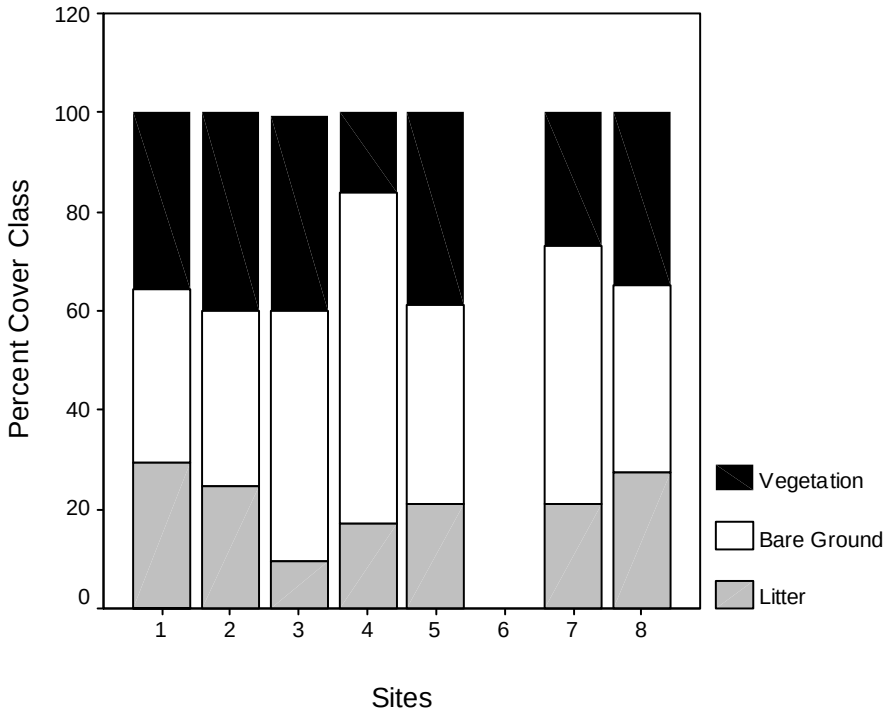

Figure 4. Mean percent cover class for each community containing *Abronia macrocarpa.*

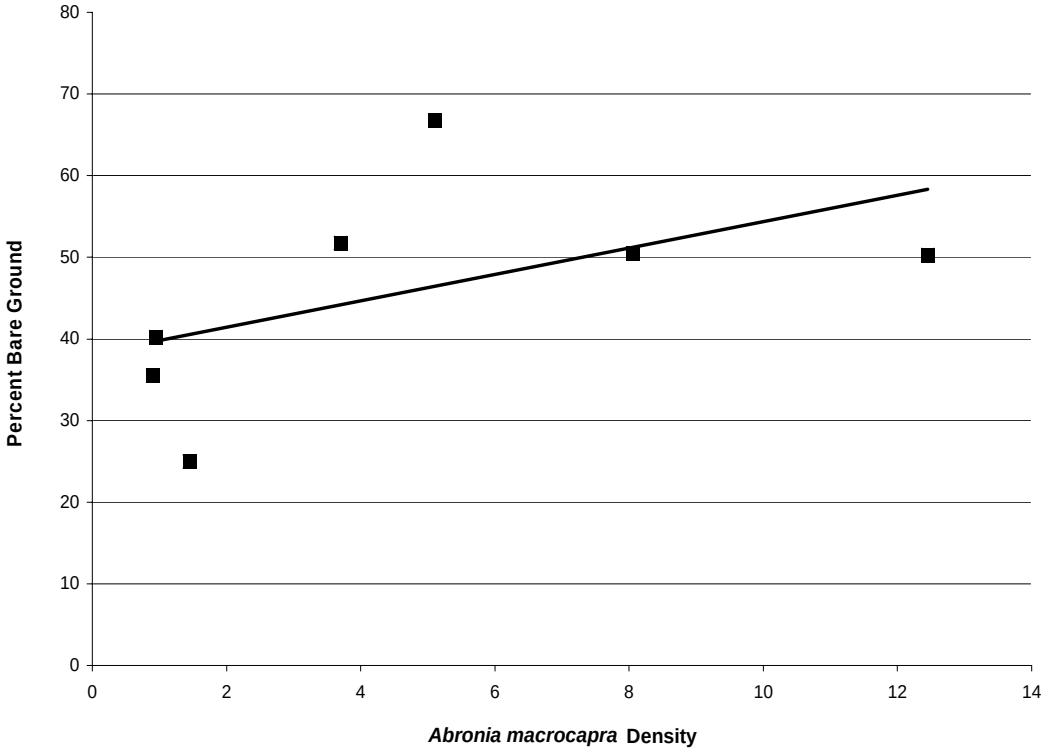

Figure 5. *Abronia macrocarpa* density as compared to the percentage of bare ground at population sites.

Community Similarity of Existing Populations:

We used the presence and absence of individual species and their density to determine community similarity. A Coefficient of Community Index (Cheetham and Hazel, 1969) was used to compare communities with populations of *A. macrocarpa* for similarity. This index uses 0 to represent those communities that have no species in common to 1 for communities that have all species in common.

The following equation was used:

$$\text{Coefficient of Community} = \frac{2C}{N_1+N_2}$$

C = Sum of lower of the two values for shared species

N_1 = Sum of values for community 1

N_2 = Sum of Values for community 2

The Coefficient of Community Index indicates that the communities are very similar. The coefficient ranges from 0.67 between sites 2 and 4 and 0.99 between sites 1 and 7 (Table 4). The data indicate that the communities usually have more than half of the species in common between various sites.

Table 4. Coefficient of Community Index. Coefficient ranges from 0 to1. Communities with all species in common have an index value of 1 whereas communities that share no species in common have a value of 0.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 7	Site 8
Site 1	x	0.89	0.95	0.71	0.85	0.99	0.87
Site 2	0.89	x	0.95	0.67	0.75	0.9	0.96
Site 3	0.95	0.95	x	0.7	0.78	0.94	0.91
Site 4	0.71	0.67	0.7	x	0.85	0.72	0.71
Site 5	0.85	0.75	0.78	0.85	x	0.86	0.72
Site 7	0.99	0.9	0.94	0.72	0.86	x	0.87
Site 8	0.87	0.96	0.91	0.71	0.72	0.87	x

Soil Analysis of Existing Populations:

Soil samples were collected at each population site. Composite samples were collected by taking soil from 8 to 10 random sites throughout the population. The samples were then sent to the Texas Cooperative Extension Soil, Water, and Forage Testing Laboratory to determine pH, levels of nitrates, phosphorus, potassium, calcium, magnesium, sulfur, sodium, iron, zinc, manganese, copper, salinity and conductivity. Data for seven chemical parameters were collected for each of eight sites. Four of these parameters, (nitrogen, phosphorous, potassium, and pH) were analyzed to determine if there was a correlation between chemistry and relative density of *A. macrocarpa* observed at seven of the eight sites. Data were analyzed by testing for multicoliniarity and using multiple regression analysis. Data were analyzed using S-Plus 7.0 Student Statistics package.

Soil analysis indicates that the majority of populations have a pH that is slightly to moderately acidic, with a range from 5.3 to 6.6. The exception to this is population site 6 which had a pH of 4.8 and is strongly acidic (Figure 6).

Nitrates were considered to be low to very low, ranging from 2 to 11 ppm (Figure 7). Those sites with the highest levels of nitrates both occurred in Freestone County. Phosphorus was present at low to high levels ranging from 13 to 29 ppm (Figure 7). Potassium was detected at low levels ranging from 24 to 39 ppm (Figure 7). Calcium levels were moderate to high ranging from 87 to 398 ppm. The lowest level of calcium was detected at site 6. Magnesium levels were low to moderate ranging from 11 to 26 ppm. Moderate to high levels of sulfur were detected ranging from 8 to 10 ppm.

Of the micronutrients, sodium was present in the soil of all sites at moderate levels ranging from 164 to 197 ppm. Iron was detected at very high levels at all sites, ranging from 6.03 to 33.20 ppm. Zinc was present in moderate to very high levels ranging from 0.24 to

3.78 ppm. The highest level of zinc was detected at site 6. Manganese was considered to be at very high levels at all sites ranging from 1.16 to 12.19. Copper was present at moderate to very high levels ranging from 0.05 to 0.38 ppm.

No multicoliniarity was detected in the data analyzed. Residual standard error was 20.35 with 5 degrees of freedom. Multiple R^2 value was 0.06436. F-statistic was 0.3439, df = 1,5, and p value was 0.5830. There is no correlation between the chemical parameters and the relative density of *A. macrocarpa* (F = 0.1598, df = 4,2, p= 0.9413). Residual standard error was 2.896 and multiple R^2 value was 0.2422.

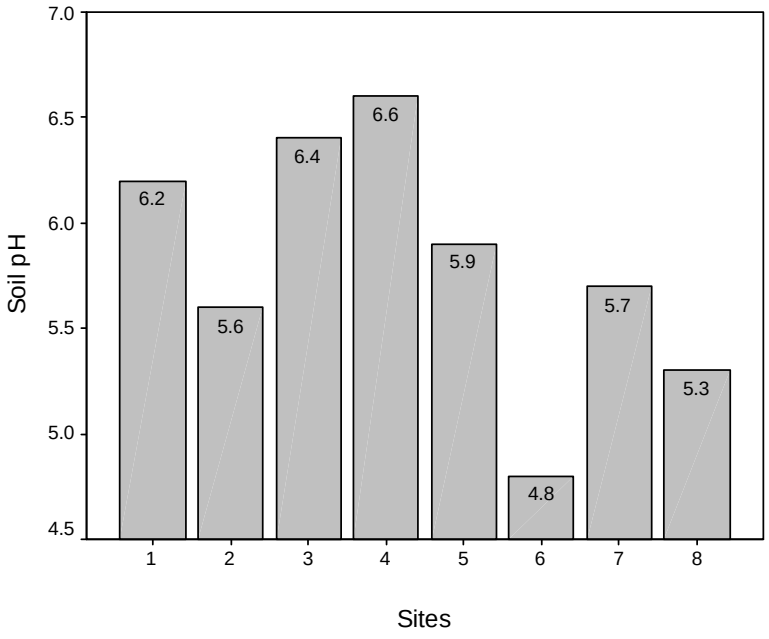

Figure 6. Soil pH of sites supporting populations of *Abronia macrocarpa*.

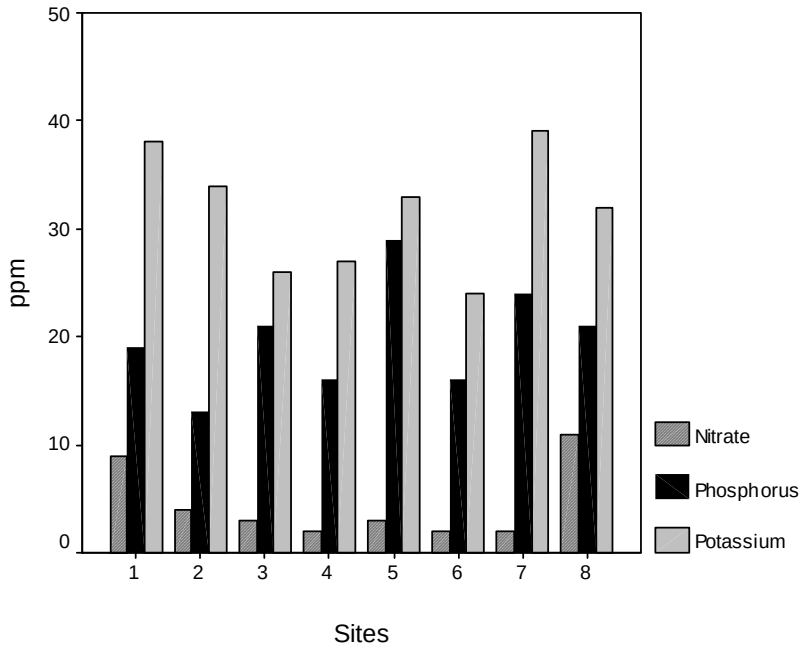

Figure 7. Nitrate, phosphorus, and potassium soil concentrations (ppm) of sites supporting *Abronia macrocarpa*.

• **Establish a pilot experimental population of varying age classes.** (Recovery task 5):

Assess population density and structure of existing populations:

This task required that we first analyze *A. macrocarpa* population density and structure in existing populations. This enabled us to use existing wild populations as a reference to compare demographics of experimental reintroduction populations. Seven populations of *A. macrocarpa* were included in this section of the study. We placed 20 1 m^2 quadrats (Krebs, 1999) randomly throughout each of seven population sites of *A. macrocarpa*. Population 6 was not included due to recent clearing of the land which greatly reduced population size. We recorded the number of *A. macrocarpa* seedlings (only cotyledons present), vegetative plants, and flowering plants for each quadrat. Seedlings were characterized as those plants with one or two leaves, the other plants were classified as vegetative or at anthesis (flowering). These data were compiled and analyzed to determine the number of individual

plants per structure class. *Abronia macrocarpa* density was calculated using the following formula:

$$\text{Density} = \frac{\text{Number of plants}}{\text{Number of 1 m}^2 \text{ quadrats sampled}}$$

Population density was compared among the populations using a one way analysis of variance ($p<0.05$) using SPSS 9.0 for Windows.

Three age groups were identified: seedlings, vegetative, and adults. Percent composition for each age group was calculated at each site. Percent composition was analyzed using a single-factor ANOVA ($p< 0.05$). Tukey's Multiple Comparison was performed to determine where differences occur. Normality and homoscedasticity were checked before analysis. Data were analyzed using S-Plus 7.0 Student Statistics package.

Population density of *A. macrocarpa* varied significantly ($p<0.005$, $F=8.387$, $df=6$) among the seven sites supporting populations of the taxon. There was more variation among the sites than within each individual site. Site 8 was significantly different from other populations, with the exception of site 3. Density ranged from approximately 1.0 individual per m^2 at sites 2 and 5 to 12.45 individuals per m^2 at site 8 (Figure 8).

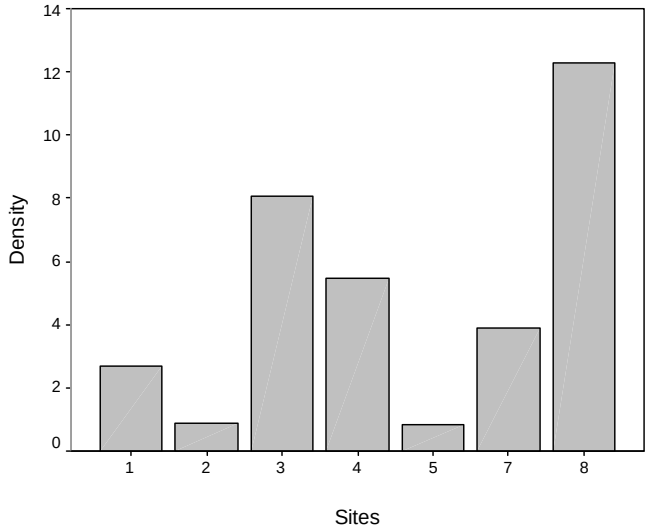

Figure 8. Density of *Abronia macrocarpa* at seven sites. Density ranges from 0.75 to 12.45 per m^2. Site 8 is significantly different from other populations with the exception of site 3 ($p<0.005$, $F=8.387$, $df=6$).

Population structure of the seven *A. macrocarpa* populations varied significantly in developmental stages (Figure 9). The percent of seedlings ranged from 3.7 to 42.2%. The number of vegetative individuals ranged from 20 to 88.9%. The number of individuals at anthesis ranged from 5.5 to 40%. Sites 3, 4, and 8 had the highest number of seedlings. Sites 1, 2, and 4 had a higher percentage of vegetative individuals. Sites 5, 7, and 8 had a higher proportion of individuals at anthesis. Data met assumptions of normality and homoscedasticity. There was six times more variation between sites than within sites ($F = 6.472435$). Significant differences in age groups exist between the sites ($p = 0.007$, $df = 2$). Tukey's Multiple Comparison results indicate that there are significant differences between seedlings (A) and vegetative plants (B) (Figure 10). There are no significant differences between seedlings (A) and adults (C) or vegetative plants (B) and adults (C).

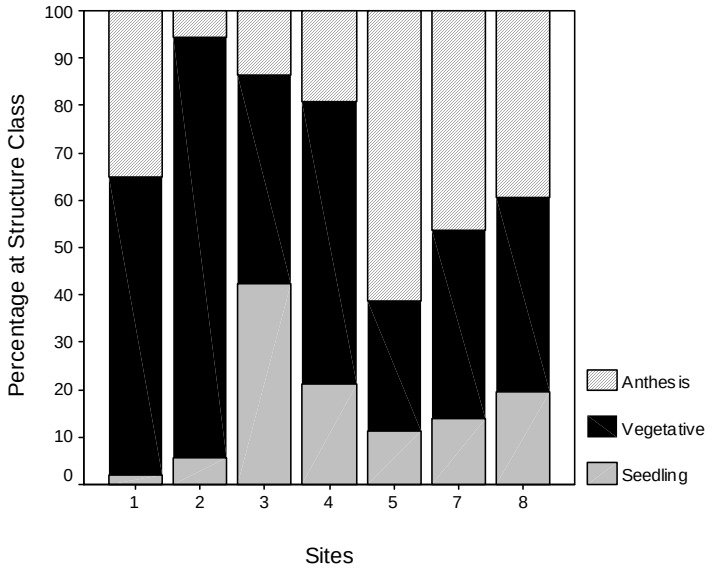

Figure 9. Percentage of *Abronia macrocarpa* plants at three structure classes. Sites varied significantly ($p = 0.007$, $df = 2$) in the number of seedlings compared to the number of vegetative plants.

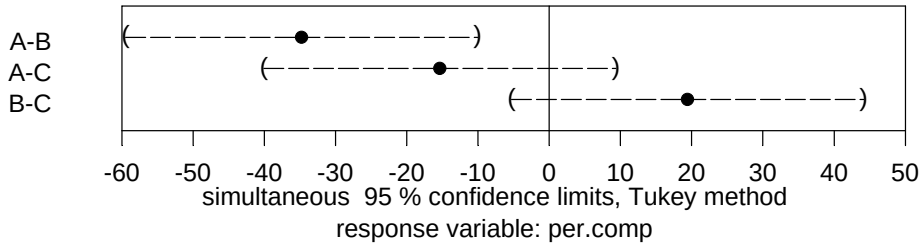

Figure 10. Ninety-five percent confidence intervals for specified linear methods.

Use analyses of community structure and similarity and edaphic features to select appropriate habitat and establish experimental reintroduction plots:

We identified and received permission to work on three pieces of property in Leon County (Figure 1) to test reintroduction methods. Community composition data and edaphic data were collected from the potential reintroduction properties following the same protocols used to collect these data from existing *A. macrocarpa* populations. We compared

community composition and edaphic features of potential reintroduction properties with the data from existing populations to verify the habitat was similar and suitable. The soil pH of the experimental reintroduction properties (5.5 and 5.6) is similar to soil pH of existing populations (Figure 11). Comparing community similarity index of the existing populations and the three experimental reintroduction properties revealed that the experimental properties share at least 50% of the same species with known populations (Table 5).

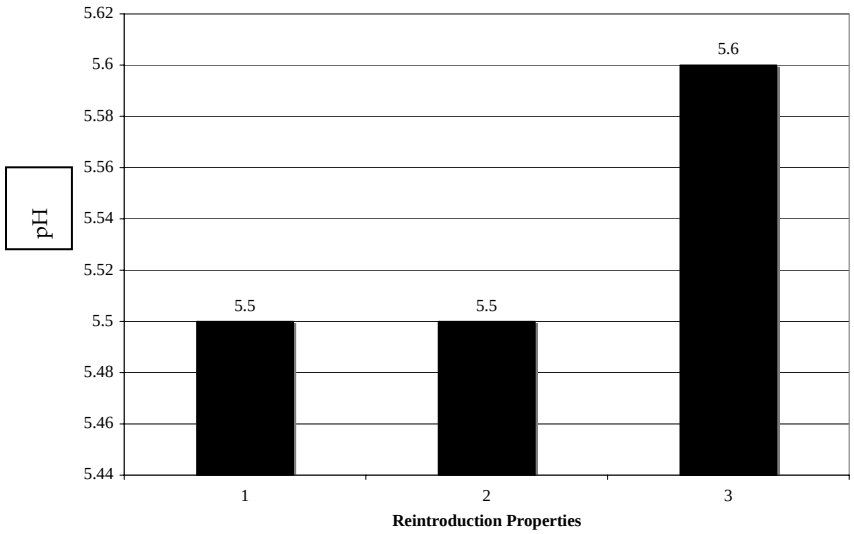

Figure 11. Soil pH of reintroduction experimental properties 1, 2, and 3.

Table 5. Coefficient of Community Index comparing existing *A. macrocarpa* populations to experimental properties 1, 2, and 3. Coefficient ranges from 0 to 1.

Coefficient of Community			
	Experimental Property 1	Experimental Property 2	Experimental Property 3
Population 1	0.85	0.71	0.46
Population 2	0.75	0.43	0.54
Population 3	0.80	0.67	0.50
Population 4	0.53	0.63	0.64
Population 5	0.69	0.76	0.61
Population 7	0.69	0.73	0.69

2005 Reintroduction Experiments:

Experimental plots were set up at experimental property 1 and experimental property 2 (Figure 1) using a split-plot design to test effects of timing of planting seed in the field. Six plots were set up at each site for a total of 12 plots. The $1m^2$ quadrats within each plot were randomly assigned one of two treatments (seed planted in spring vs. planted in fall). *Abronia macrocarpa* produces a fruit, termed an anthocarp, which consists of a dry papery portion formed by the lower calyx, which encases an achene. Achenes are dry, indehiscent, single-seeded, with the seed coat free from the pericarp. In nature, the seed germinates while still encased in the dry, papery portion of the anthocarp (pers. obs.). Although, this structure aids in dispersal to some degree, we believe a primary function is to prevent the achene from becoming buried too deeply in the sandy soil. If buried too deeply, stored food reserves would not be sufficient to support enough growth of the seedling for it to break the soil surface. To duplicate natural events as closely as possible, anthocarps were planted in the field. The dry papery portion will develop even in the absence of an achene developing. Therefore all anthocarps were checked to ascertain an achene was present prior to planting.

The population genetics study conducted by Williamson and Werth (1999) showed that this species has a high degree of genetic variability within and among populations. The study showed that populations in closer proximity were genetically more similar than they were to populations of other regions. We measured distance in air miles between the two reintroduction sites and the known populations and found that Population 3 is closest in proximity to the reintroduction sites. So, we used anthocarps collected from this population to plant in the experimental field plots.

A total of 240 anthocarps (n=40 per plot) were planted at experimental properties 1 and 2 in April 2005. A total of 120 anthocarps (n=20 per plot) were also planted in the November 2005. Plots were monitored in spring 2006 and percent seed germinated determined.

By March 11, 2006 seedlings from seed planted in 2005 had emerged in plots at both experimental properties. At experimental property 1, of the seed planted in spring 2005, the percent germination was 28% and germination of fall planted seed was 4% (Figure 12). At experimental property 1 all plants remained in the seedling stage throughout the growing season.

The seed at experimental property 2 was planted on a slope and with rainfall the seeds planted in spring and fall were washed down the slope. Therefore, the spring and fall planted treatments in the split plot design could no longer be distinguished from one another as separate treatments. All seed germinated at experimental property 2 includes the combined spring and fall planted seed. Combined percent germination was 9% (Figure 13). In previous studies it was thought that in the first year seedlings put all of their increase in biomass into root production. In this study we observed seedlings, vegetative plants, and plants in flower at experimental property 2. On April 12th, 2006 we counted fourteen seedlings, eleven vegetative, and six plants at anthesis. One of the plants produced anthocarps (n=3), which were collected and planted in a 1m^2 quadrat to contribute to establishing varying age classes.

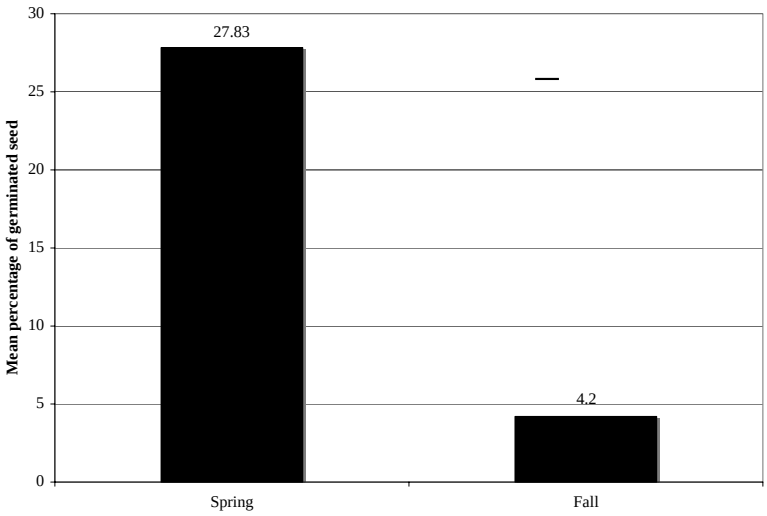

Figure 12. Mean germination percentage of seed planted in spring and fall at experimental property 1 (seed planted in 2005, data collected in spring 2006), p-value = <0.0006722; t value = 7.49; df = 5.

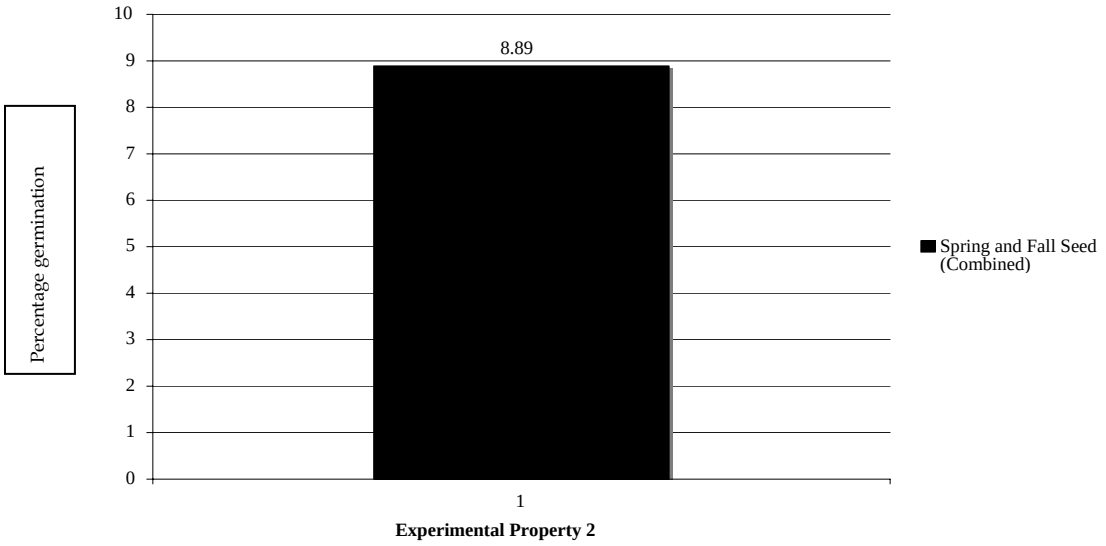

Figure 13. Mean germination percentage of combined seed planted in spring and fall at experimental property 2 (seed planted in 2005, data collected in spring 2006).

Assess survivorship over multiple years:

The survival of seedlings from 2006 to the following spring 2007 at experimental property 1 was 19.4% and the mean percentage of seedling survivorship at experimental property 2 was 87.5%. Experimental property 2 had a higher percentage of survivorship. Although experimental property 1 had the highest germination rate initially, by 2008 no plants remained (Table 6). A large colony of cutter ants was observed at this site, which may have been the cause of the loss of plants at this site. These results show the importance of monitoring over multiple years.

We monitored the pilot populations in 2006, 2007 and 2008 to measure success and/or failure of reintroduction and record evidence that the pilot populations are developing a complex demographic structure over time (Guerrant, 1996; Pavlik, 1996). While reintroduction at experimental property 1 was not successful, experimental property 2 is developing age structure classes that fall within the range of natural populations. Natural populations range in age classes with seedlings representing 8-42%, vegetative plants 20-89%, and plants at anthesis 6-40% of the population (Figure 9). By 2008, experimental property 2 had a demographic structure with 10% in the seedling stage, 59% vegetative plants and 31% plants at anthesis.

Table 6. Number of individuals in age classes at experimental properties 1 and 2 in 2006-2008.

Reintro. Prop.	Age Class	No. in 2006	No. in 2007	No. in 2008
Exp. Prop. 1				
	seedling	115	22	0
	vegetative	0	0	0
	anthesis	0	0	0
Exp. Prop. 2				
	seedling	14	8	3
	vegetative	11	16	17
	anthesis	6	4	9

2006 Reintroduction Experiments:

In spring of 2006, additional new plots were set up at experimental properties 1 and 2. We also selected a third property (experimental property 3), of suitable habitat in Leon County, to test reintroduction techniques using the same experimental design used at experimental properties 1 and 2. Six plots were established at each of the three properties. The $1m^2$ quadrats within the plots were randomly assigned one of two treatments (seed planted in spring vs. planted in fall). Anthocarps collected from Population 3 in spring 2006 were planted in the plots at experimental property 3 on April 30, 2006 and at experimental properties 1 and 2 on May 1, 2006. At each experimental property anthocarps (n=240) were

planted in the six plots (n=40 per plot). The plots were monitored in spring 2007 and number of germinated seed and varying structure classes were recorded.

At experimental property 1, the mean percentage of germinated spring seed was 16.33% ($\bar{x}$ = 16.33, n = 6 plots) (Figure 14). The mean percentage of germinated fall planted seed was 0.83% (Figure 14). Spring and fall planted treatments at experimental property 1 (t = 11.36, df = 5, p-value = < 0.0001) significantly differ. At experimental property 2 the mean percentage of germinated spring seed at this property was 16.67% ($\bar{x}$ = 16.67, n = 6 plots) (Figure 15). The mean percentage of germinated fall planted seed was 0.83% (Figure 14). A significant difference was detected between the spring and fall planted seed at experimental property 2 (t = 3.99, df = 5, p-value = 0.01036). The mean percentage of germinated spring seed at experimental property 3 was 4.2% ($\bar{x}$ = 4.2, n = 6 plots) (Figure 16). No seed planted in the fall germinated (Figure 16). Seed germination was not significantly different between spring and fall planted seed at experimental property 3 (t = 2.29, df = 5, p-value = 0.07057). All plants resulting from seed planted in 2006 were found to be at the seedling stage in 2007. None were categorized as vegetative or at anthesis.

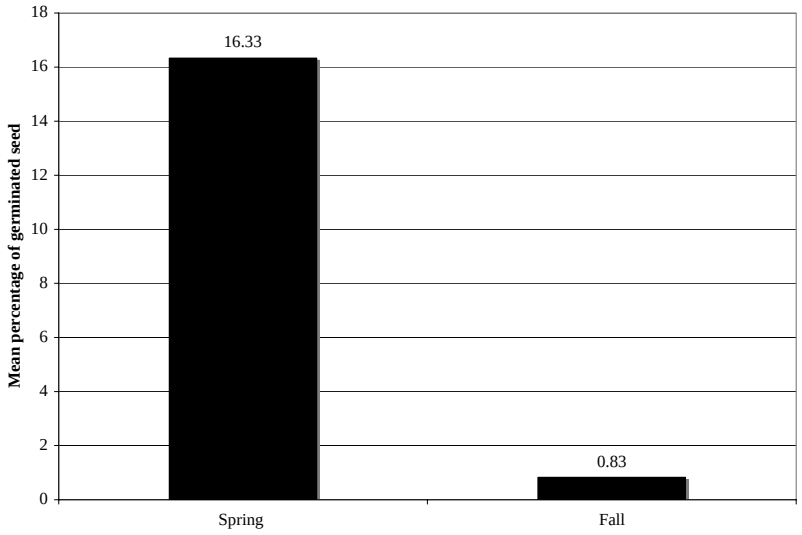

Figure 14. Mean germination percentage of seed planted in spring and fall at experimental property 1 (seed planted in 2006, data collected in spring 2007) p-value = <0.000001; t = 11.36; df = 5.

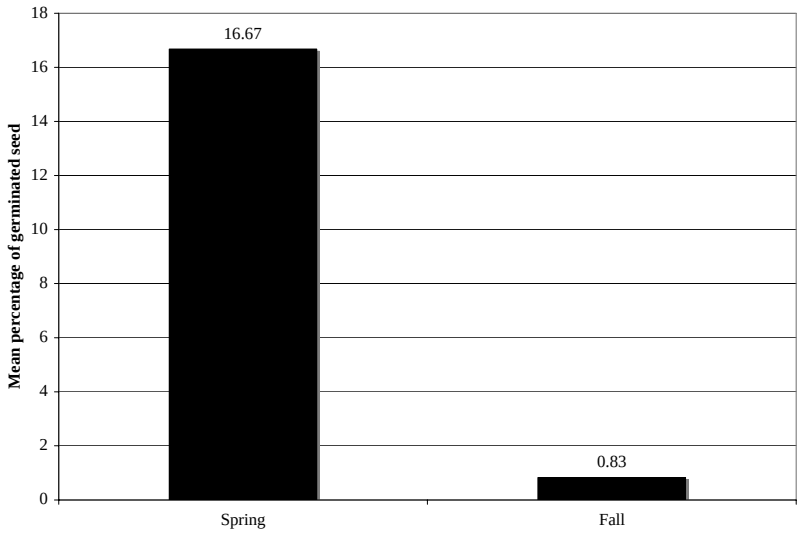

Figure 15. Mean germination percentage of seed planted in spring and fall at experimental property 2 (seed planted in 2006, data collected in spring 2007), p-value = <0.01036; t value = 3.99; df = 5.

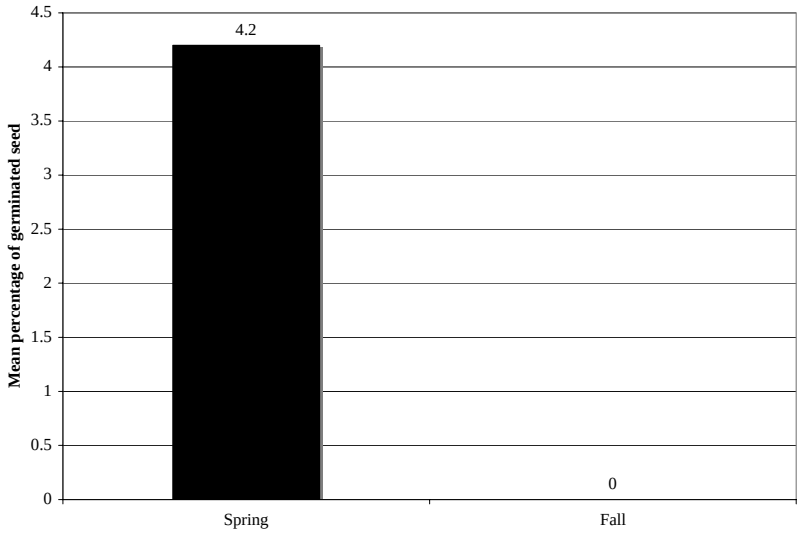

Figure 16. Mean germination percentage of seed planted in spring and fall at experimental property 3 (seed planted in 2006, data collected in spring 2007), p-value = 0.3632; t value = 1; df = 5.

2007 Reintroduction Experiments:

In 2007 we received permission to test reintroduction at additional sites. We collected community composition data and soil data as previously described and selected the sites deemed those with the highest potential. We established experimental field plots following the methodology described above at five properties in Leon County and one property in Freestone County (Table 7). Germination data were collected in March, 2008. Results are shown in Table 7. Seedlings and vegetative plants were observed at experimental properties 4, 7, 8 and 9. In addition to seedlings and vegetative plants, one plant at anthesis was observed at experimental property 9. Only seedlings were observed at experimental properties 5 and 6. Additional years of monitoring would be required to determine whether reintroduction properties of varying age classes establish at these reintroduction properties.

Table 7. Description of experimental reintroduction properties and percentage of germination of seed planted in spring 2007. Germination data collected spring 2008.

Reintroduction Property No.	Location	Soil pH	Seed Source & Coefficient of Community Index	% Germination (n = no. seed)
Exp. Prop. 4	Freestone Co. 10 miles E of Fairfield	6.5	Population 8 0.87	6% n = 240
Exp. Prop. 5	Leon Co. 18 miles SE of Buffalo	7.2	Population 1 0.66	2% n = 240
Exp. Prop. 6	Leon Co. 16 miles SE of Buffalo	6.6	Population 1 Not determined	2% n = 120
Exp. Prop. 7	Leon Co. 12 miles E of Buffalo	5.2	Population 1 0.46	13% n = 240
Exp. Prop. 8	Leon Co. 10 miles E of Buffalo	4.7	Population 1 0.67	*4% n = 240 *% germination may have been higher, but plots were impacted by an accidental burn prior to data collection
Exp. Prop. 9	Leon Co. 10 miles SW of Normangee	6.7	Population 4 0.53	4% n = 120

4) Utilize all the data and information garnered to develop a reintroduction plan for the purpose of establishing viable, self-sustaining populations.

All data collected in this study from existing populations, augmented populations, and experimental reintroduction plots were used to develop a reintroduction plan. The plan is based on guidelines for developing a rare plant reintroduction plan from Falk, Millar and Olwell (1996).

Abronia macrocarpa Reintroduction Plan

Appropriateness of Reintroduction:

Reintroduction is an emerging practice in conservation biology and is being used more frequently in the United States by federal, state, and private conservation agencies. Reintroduction plays a role in implementation of the Endangered Species Act, nearly one-fourth of all U.S. plants listed under the act include reintroduction in their recovery plan (Falk and Olwell, 1992). One listed species that may be a prime candidate for reintroduction is *Abronia macrocarpa*.

The *A. macrocarpa* recovery plan states that before the species can be delisted 20 viable populations, each at least 25 acres in size with a population of at least 600 individuals, must exist and be well established (U. S. Fish and Wildlife Service, 1992). These criteria are considered sufficient to protect the species from extinction in the case of a catastrophic event. Currently nine populations are known (four in Leon Co., three in Robertson Co. and two in Freestone Co.) (Figure 1). All known populations occur on privately owned land; therefore, the plant is offered very little protection by the Endangered Species Act. To achieve the recovery plan criteria the known populations must be protected and new populations must be located or created. If eleven additional populations are not discovered,

establishing new populations through reintroduction will be crucial for the recovery of *A. macrocarpa*.

Measuring Success:

A reintroduction effort should be considered a success if, overtime, it results in establishment of a viable, sustainable population with a population structure, reproductive capacity and genetic variability similar to existing naturally occurring populations.

Selection of Location of Reintroduction Sites:

Reintroduction should occur within the historical range of the species. *Abronia macrocarpa* is known to occur in only three counties in Texas (Freestone, Leon, Robertson Counties). Little state or federal lands occur in this region. Therefore, reintroductions will likely occur on private lands. Reintroduction sites on private lands should be limited to those with landowners that enthusiastically support conservation of the taxon and are committed to permanent protection of the site. If possible, a signed conservation agreement with the landowner should be obtained to solidify the voluntary commitment.

Ecological Criteria for Selection of Suitable Reintroduction Sites:

Knowledge of plant demography, environmental factors, and genetics is essential in the development of a reintroduction program (Friar et al., 2001). Rarity of plants is often the result of the species' extremely specific habitat requirements (Falk et al., 1996). Pavlik (1994) points out the need to identify suitable habitat for a reintroduction program. In order to successfully select a reintroduction site for *A. macrocarpa* the following habitat characteristics must be taken into consideration:

Edaphic Features:

Soil type including the soil pH, texture, and mineral nutrients are crucial in choosing a successful location. According to a review of mitigation-related introductions of rare plant species in California the majority of introductions failed due to unsuitable soil characteristics at the receptor site (Fielder, 1991). *Abronia macrocarpa* is known to occur in openings of deep sandy soils (Galloway, 1972), characterized as Arenosa Fine Soils in Leon County (Neitsch et al., 1998), Pinkton Loamy Fine Soils in Freestone County (Janeck and Griffin, 2002), and Silsted-Padina Soil in Robertson County (U.S. Fish and Wildlife Service, 1992). Soils supporting *A. macrocarpa* populations are in a pH range that is slightly to moderately acidic, 4.8 to 6.6. Nitrate levels are low varying between 2 to 11 ppm. Sites selected for reintroduction of *A. macrocarpa* should fall within the edaphic parameters listed above.

Cover:

Sites that support known populations of *A. macrocarpa* have bare ground ranging from 25% to 66.75%. When bare ground increases the density of *A. macrocarpa* increases. Populations that have over 50% bare ground have the highest *A. macrocarpa* density. Only sites with at least 25% bare ground should be selected as reintroduction sites. When possible, efforts should be made to select sites with over 50% bare ground.

Associated Species:

Knowledge of a plant's associated species is also helpful in delineating suitable habitat for the reintroduced plant population. Communities supporting populations of *A. macrocarpa* are very similar with a subset of species in common. Since data suggest that *Rhododon ciliatus*, *Plantago* sp., and *Croton argyranthemus* may be particularly strong indicators of suitable habitat, these species should be components of the community of sites selected for reintroduction.

Mycorrhizal Associations:

Abronia macrocarpa exhibits a mycorrhizal association (pers. obs.). While some reintroduction projects bring soil from an existing population to inoculate the soil of the reintroduction site, there is risk of introducing disease organisms (Falk, Millar and Olwell 1996). Instead of transporting soil to the site, a reintroduction site with similar community composition that will likely have similar soil microorganisms should be selected.

Source Material:

This pilot reintroduction study used seed collected from the closest population, which is recommended as the conservative practice (Falk, Millar and Olwell 1996). However, higher rates of germination were found in sites that had the highest community similarity to the source population. Selecting reintroduction sites that are ecologically similar to the source populations will aid in the recovery success of restored populations (Montalvo and Ellstrand, 2000). Therefore, seed selected as source material should come from the closest population with the highest coefficient of community index.

A population genetics study conducted by Williamson and Werth (1999) showed that this species has a high degree of genetic variability within and among populations. Since populations have high rates of genetic variability, seed source collected from one population should be sufficient to establish a genetically diverse reintroduction population.

Founder Population Size and Stage Structure:

Given the percentage germination and plant survivorship found in this pilot study, approximately 3600 seed would be required to establish a population of 600. Collecting this number of seed from an existing population in a single year is not a viable option because it

would violate the Center for Plant Conservation guidelines (Faulk and Holsinger, 1991). Therefore, creating demographically stable populations will require planting seed in spring for subsequent years. This method will also ensure establishment of a reintroduction population with varying age-class structure.

Plant community development largely depends on the availability of soil nutrients (Deyn et al., 2004). The mineral nutrient, nitrogen, is needed in greatest abundance for vegetative growth and development (Crawford, 1995). Egilla et al. (2001) found that potassium improves drought resistance and promotes root survival in drought stressed plants. The nitrate content of the soils at the experimental reintroduction properties was relatively low with the exception of experimental property 2. The level of potassium was also significantly higher at experimental property 2. This higher level of soil nutrients at experimental property 2 may be responsible for plants reaching vegetative and anthesis stages in the first year of growth. Experimental property 2 also had the greatest plant survivorship, which could be attributed to higher potassium levels promoting root longevity through the dry summer months. Given the apparent importance of nitrogen and potassium in seedling establishment of *A. macrocarpa*, the addition of soil amendments or liquid fertilizers may facilitate establishment and survivorship of a reintroduced population.

Pollination and Dispersal:

The reproductive biology of *A. macrocarpa* has been well documented (Williamson, et al., 1994; Williamson and Bazeer, 1997). Since the species is an obligate outcrosser, it is essential that a reintroduction site community contain effective pollinators. Dispersal of seed is less understood, but appears to occur primarily by wind (pers. obs.). Therefore, specific

biotic seed dispersal agents do not appear to be required components in selection of a reintroduction site.

Monitoring:

Monitoring of a reintroduction population will require a multiple year approach to assess recruitment of new individuals and determine whether the reintroduction population has reached a population structure similar to existing populations. A population genetics study should also be conducted to assess levels and patterns of genetic variability of the reintroduction population.

SIGNIFICANT DEVIATIONS FROM PROPOSED STUDY:

We originally proposed to test reintroduction by seed vs. seedlings. The two possibilities for obtaining seedlings to test as agents for reintroduction would be 1) germinate seeds and grow them in the greenhouse or 2) collect seedlings from existing populations. Early in the investigation we determined that using seedlings was not feasible due to the following factors: 1) the space, time, effort and expense required to grow seedlings in the greenhouse; 2) the high potential of skewing population structure of existing populations by collecting seedlings; 3) the high risk of unsuccessful transplanting in reintroduction plots due to the fragile tap root. Therefore, only seeds were used in establishing experimental reintroduction field plots.

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