TESTING REINTRODUCTION POTENTIAL OF *ABRONIA MACROCARPA* (NYCTAGINACEAE)

THESIS

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

for the Degree

Master of SCIENCE

by

Jacqueline Jean Goodson, B.S.

San Marcos, Texas
December 2007

ACKNOWLEDGMENTS

This has been an amazing educational journey over the last 2 years. There are so many people who have contributed to my success as a graduate student. Without them, my work would be incomplete. I am grateful to all who have encouraged me, and helped me stay focused. Below are a few very special thanks.

First and foremost I would like to acknowledge Dr. Paula Williamson for allowing me to further my education and knowledge in Botany and Conservation Biology. You have taught me many life skills and always provided encouragement. I would also like to thank my committee members, Dr. Tina Cade and Dr. David Lemke for all of their suggestions.

I would like to thank my mom, Dee Hilliard, for all of her love and support throughout my life. I am extremely grateful to my aunt and uncle, Thomas and Vicki Lumpkin, for all of their support and encouragement in pursuing my academic career.

I am thankful to Anna Strong, Carolyn Meredith and Gena Janssen for all of their assistance in the field. Thank you Flo Oxley and Dr. Butch Weckerley for help with statistics.

Special thanks to Courtney Brooks, who has been there to listen and provided countless hours counting seeds and assisting with the germination experiements.

This manuscript was submitted to my committee on October 31, 2007.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

TESTING REINTRODUCTION POTENTIAL OF

ABRONIA MACROCARPA (NYCTAGINACEAE)

by

Jacqueline Jean Goodson, B.S.

Texas State University-San Marcos

December 2007

SUPERVISING PROFESSOR: PAULA WILLIAMSON

This study tests reintroduction as a mechanism to assist in recovery of *Abronia macrocarpa,* a federally and state endangered plant endemic to Texas. A series of laboratory tests were conducted to determine the most effective method for seed germination. Germination ranged from 0% to 68.6% among the control and twelve treatments. Seed germination was highest when achenes were scarified and subjected to warm followed by cold stratification. Three parcels of private property with suitable habitat were used as experimental reintroduction properties. A split-plot design was used to test the effects of timing of planting seed in the field (spring planted seed vs. fall

planted seed). The mean percent germination of spring planted seed (4.2 to 16.67%) was higher than fall planted seed (0 to 0.83%) at all experimental properties. Germination of spring planted seed was significantly higher at experimental properties 1 and 2 when compared to experimental property 3 (p-value = 0.01303, F = 5.88, df = 2). The need for warm followed by cold stratification coupled with scarification may explain the higher percentage of germination of seed planted in the spring vs. the fall. Fall planted seed would not have been exposed to warm stratification. Experimental property 2 had a higher percentage of survivorship (87.5%) than experimental property 1 (19.4%). Plants at all stages of development (seedling, juvenile, anthesis) were observed at experimental property 2, but plants remained in the seedling stage at experimental properties 1 and 3. The levels of nitrate and potassium were higher at experimental property 2. This higher level of soil nutrients may be responsible for higher survivorship and plants reaching juvenile and anthesis stages in the first year of growth.

CHAPTER I

INTRODUCTION

Conservation biology is a science aimed at preserving biodiversity that has been impacted due to habitat degradation through the introduction of non-native species, construction, fragmentation and climatic changes (Pavlik, 1994). Reintroduction may be a necessary conservation tool for species with few remaining wild populations (Morse, 1996). Species reintroductions often involve introducing known genotypes into the species' historic range or a new suitable habitat (Falk et al., 1996).

Reintroduction is an emerging practice in conservation biology and is being used frequently in the United States by federal, state, and private conservation agencies. Reintroduction plays a role in implementation of the Endangered Species Act, as 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.*

Abronia macrocarpa was listed as a federally endangered plant species by the U.S. Fish and Wildlife Service on September 28, 1988 (U.S. Fish and Wildlife Service, 1988) and as a Texas state endangered plant species on December 30, 1988 (U. S. Fish and Wildlife Service, 1992). The nine known populations all occur on private property in Leon, Robertson, and Freestone Counties, Texas. *Abronia macrocarpa* populations occur

in the Oak Woods and Prairies region of Texas (Diamond et al., 1987). This area is characterized as having a warm climate with approximately 267 frost-free days annually and an average annual rainfall of 97.5 centimeters, with most precipitation occurring in April, May, and September. March, July, and August are the driest months (U.S. Fish and Wildlife Service, 1992). The plant is given a recovery priority of 2 by the U.S. Fish and Wildlife Service recovery plan, indicating a high degree of threat, but a high recovery potential (U.S. Fish and Wildlife Service, 1992). Threats to the plant include habitat modification and disturbances such as the introduction of non-native grasses for range improvement, fire suppression, oil and residential development, and recreational activities. Since the species is considered to have a high recovery potential, *A. macrocarpa* may benefit from a reintroduction plan.

Knowledge of plant demography, environmental factors, and genetics is essential in the development of a reintroduction program (Friar et al., 2001). It is important when reintroducing a new population to create populations that closely mimic the characteristics of the naturally occurring population (Pavlik, 1996). Rarity of plants is often the result of the species' extremely specific habitat requirements (Falk et al., 1996). In order to successfully select a reintroduction site, habitat conditions must be taken into consideration. Meredith (2006) found that when bare ground increases, the density of *A. macrocarpa* increases. Populations that had over 50% bare ground had the highest *A. macrocarpa* density (Meredith, 2006). 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 macrocapa* 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 moderately to slightly acidic, 4.8 to 6.6 (Meredith, 2006). Nitrate levels are low varying between 2 and 11 ppm (Meredith, 2006).

Knowledge of a plant's associated species is also helpful in delineating suitable habitat for the reintroduced plant population. Meredith (2006) found that communities supporting populations of *A. macrocarpa* are very similar with a subset of species in common. Plant species commonly associated with *A. macrocarpa* populations include *Rhododon ciliatus, Croton argyranthemus, Tradescantia occidentalis*, *Ilex vomitoria,* and *Quercus stellata.*

The *A. macrocarpa* recovery plan states that before the species can be delisted 20 viable populations, each at least 10.11 hectares (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.*

Successful reintroduction of a species relies upon knowledge of seed germination and cultivation requirements (Falk et al., 1996). Techniques for propagating rare plant species are seldom well developed. Unless there have been previous studies on the taxon under investigation or a close relative, it is difficult to determine the best method for germinating seeds that undergo a period of dormancy (Baskin and Baskin, 2003). It is important to conduct seed germination trials to determine a method for creating and maintaining a cultivated reintroduction population to prevent total loss of the species in a catastrophic event.

Developing a reintroduction plan requires testing experimental reintroduction techniques. If a reintroduction plan proves to be successful, delisting of *A. macrocarpa* may be possible by 2015 (U. S. Fish and Wildlife Service, 1992). The primary objective of this research is to test reintroduction potential as a mechanism to assist in recovery of *A. macrocarpa.* This study will investigate habitat requirements as well as germination and cultivation techniques both in the laboratory and under field conditions.

Objectives of this study are to:

1. Conduct laboratory germination experiments to determine the most effective method for germinating seeds.
2. Compare edaphic features and community composition/similarity data of potential reintroduction properties to data previously collected on existing *A. macrocarpa* populations to select experimental reintroduction properties.
3. Test experimental reintroduction using seed planted in the field.

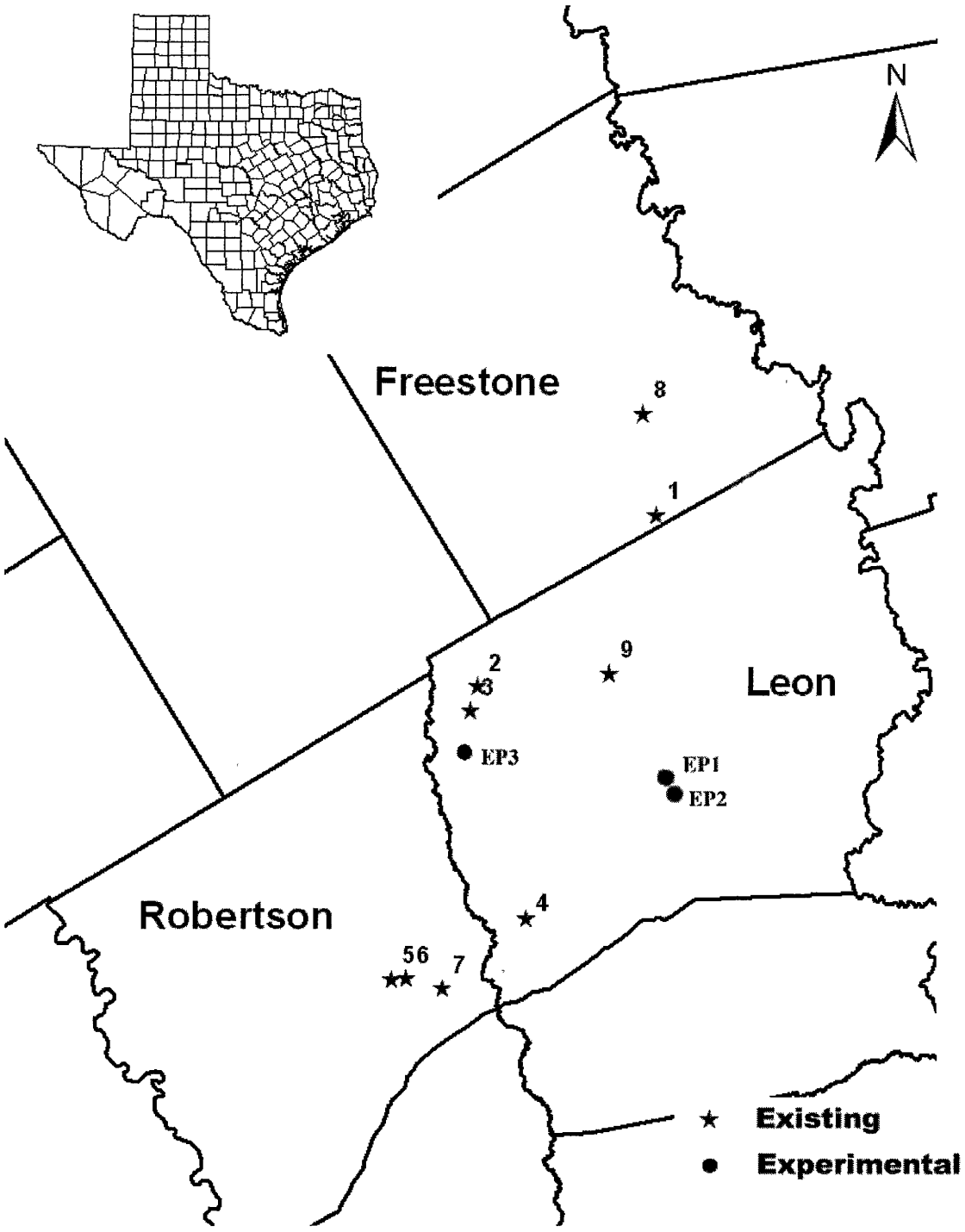

Figure 1. Map of existing *A. macrocarpa* populations (1-9) and experimental reintroduction properties 1, 2, and 3.

CHAPTER II

MATERIALS AND METHODS

Study Species

Abronia macrocarpa, commonly known as Large-Fruited Sand Verbena is a member of the Nyctaginaceae or four o' clock family. *Abronia macrocarpa* was first described and named by Galloway (1972). The species is a tap-rooted, herbaceous perennial that grows up to 20 centimeters tall. The leaves are opposite, oval shaped and are covered with glandular hairs. The plant flowers from February to June producing capitula composed of approximately 27 to 40 flowers each (Williamson et al., 1994). The flowers are tubular in shape and are up to 3.2 centimeters long. Flower color varies from light pink to fuchsia.

Abronia macrocarpa produces a fruit, termed an anthocarp, consisting of a dry papery portion formed by the lower calyx, which encases an achene. Achenes are dry, indehiscent, single-seeded fruits, with the seed coat free from the pericarp. The dry papery portion will develop even in the absence of an achene developing. The fruit is larger and more papery than that of other species of *Abronia* (Galloway, 1972). This species is self-incompatible and relies on effective pollinators for fruit set; observed pollinators were primarily hawk moths (Sphingidae) and noctuid moths (Noctuidae) (Williamson et al., 1994, Williamson and Bazeer, 1997).

Laboratory Germination Experiment

One aspect of seed biology that was unknown was whether seed germinated in the spring immediately following anthesis, or if a period of dormancy occurred prior to germination. So I performed a series of trial germination experiments.

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 seeds per replicate for a total of 108 seed per treatment. Randomly chosen seed were used in the treatments and control. 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 placement in a refrigerator set at approximately 5° C for 8 weeks. Seed subjected to the scarification treatment were mechanically rubbed with fine sandpaper to abrade the seed coat. This treatment was used to mimic the sandy soils, which may be responsible for scarification thereby, weakening the seed coat. The seeds subjected to the gibberellic acid (GA3) and 0.2% KNO3 solution was soaked for eight hours. Gibberellic acid (GA3) is known to promote germination in seeds, which experience physiological dormancy (Baskin and Baskin, 2004). A 0.2% KNO3 solution was recommended as a treatment to increase germination in *Abronia umbellata* ssp. *breviflora* (Kaye, 1999). The achene/anthocarp was either planted 0.6 cm deep in a 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 (28°C, 13 hr light/20°C, 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 Kruskal-Wallis one-way analysis of variance was conducted using S-Plus ($p<0.05$) for Windows to determine if differences exist among 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		+

Selection of Experimental Reintroduction Sites

Edaphic Features

Potential experimental properties were identified using county soil maps and through landowner outreach. Landowners with potential *A. macrocarpa* habitat were contacted for permission to assess their land for use as an experimental reintroduction property. Composite soil samples were collected at each property. Composite soil samples were collected using a trowel to clear the surface vegetation and then a hole was dug and soil was removed. The sample was taken at 10.2 cm of depth. This technique was repeated in 8–10 random areas throughout the property in order to minimize differences that may exist in an area of the study site. The samples were 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.

Community Composition and Similarity

Community composition of potential experimental reintroduction properties was examined and compared to that of existing *A. macrocarpa* populations to determine similarity and suitability of the habitat. Fifteen 1 m^2 quadrats were randomly placed throughout each property. The number and type of associated plant species were recorded. All species were identified according to the *Manual of Vascular Plants of Texas* (Correll and Johnston, 1979). The percentages of litter, bare ground, and vegetative cover were estimated. The relative density and relative frequency of plant species occurring within the quadrats were calculated to determine community composition using the following formula:

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

$$\text{Relative Frequency} = \frac{\text{Frequency of a given species}}{\text{Total Frequency of all Plants}} \times 100$$

The presence and absence of plant species along with their density and frequency were used to determine community composition and compared with data collected from existing populations (Meredith, 2006). A Coefficient of Community Index (Cheetham and Hazel, 1969) was used to compare potential experimental reintroduction property communities to communities with populations of *A. macrocarpa* for similarity. Communities that have no species in common are represented using a 0 and communities that have all species in common are represented using a 1.

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

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

Field Reintroduction Design and Treatments

I identified and received permission to work on three parcels of private property with suitable habitat in the species' historical range (Leon County) to test reintroduction methods (Figure 1). The criteria used to determine that habitat was suitable were soil pH within the range of soils supporting existing populations and a coefficient of community of 0.50. This value was chosen because all of the existing *A. macrocarpa* populations share at least 50% of the same species (Meredith, 2006).

A population genetic study conducted by Williamson and Werth (1999) showed that *A. macrocarpa* has a high degree of genetic variability within and among populations. The study showed that populations in close proximity were genetically more similar to one another than to more distant populations. Based on the results of the genetics study, it was deemed important to use seed from the nearest existing population to plant at the reintroduction properties. I measured straight-line distance in air miles between the experimental properties and the existing *A. macrocarpa* populations and found that *A. macrocarpa* population 3 (Figure 1) was closest in proximity to the experimental properties. Therefore, anthocarps collected from this population were used to plant the experimental field plots.

Reintroduction Experiment 2005

A split-plot design was used to test the effects of timing of planting seed in the field at two properties. In spring 2005, I ran transects at experimental property 1 and experimental property 2. Six plots were established at each experimental property. Each plot was divided into two 1 m^2 quadrats and separated from the next plot by 2 meters. The 1 m^2 quadrats within each plot were randomly assigned one of two treatments (seed planted in spring vs. seed planted in fall). Forty anthocarps (seed) were planted in each plot in April 2005 (spring treatment, n= 240). Twenty anthocarps (seed) were planted in each plot in November 2005 (fall treatment, n= 120). Anthocarps were planted in the field at a depth of 0.6 centimeters. Germination data were collected in spring 2006 and survivorship data were collected in 2007 at experimental properties 1 and 2. Plants were monitored monthly during the spring to determine the number of seedlings, juveniles, and plants at anthesis.

Reintroduction Experiment 2006

In 2006, new transects were set up at experimental property 1 and experimental property 2 using the split-plot design. Transects were run and six plots were established at experimental property 1 and experimental property 2. A transect was run and six plots were also set up at a new reintroduction property, experimental property 3. The plots followed the same design as in 2005. The 1 m^2 quadrats within the plots were randomly assigned one of two treatments (seed planted in spring vs. seed planted in fall). Seed collected from *A. macrocarpa* population 3 in spring 2006 was planted at all experimental reintroduction properties. Forty anthocarps (seed) were planted in each plot in April 2006 (spring treatment) (n= 240). Anthocarps (n=240) were planted in the experimental plots in November 2006 (fall treatment), with 40 seed in each plot. Anthocarps were planted in the field at a depth of 0.6 centimeters. The plots established in 2006 were monitored and number of germinated seed recorded in spring 2007. Plants were monitored monthly during the spring to determine the number of seedlings, juveniles, and plants at anthesis.

Field Reintroduction Data Analysis

I calculated the percentage of germinated seed and analyzed these data to determine the most effective time to plant seed into the field. A paired t-test was conducted using R software to determine if a difference existed between the spring and fall planted seed. The paired t-test was conducted separately for all experimental properties on the six plots established in 2005 and on the six plots established in 2006. At experimental property 2 (plots established in 2005) the spring and fall planted treatments in the split plot design could not be distinguished as separate treatments. The

seed was planted on a slope and with rainfall the seed washed down the slope and the spring and fall treatments intermixed, therefore the 2005 germination results at experimental property 2 include combined spring and fall planted seed. A single factor ANOVA was conducted using R software to determine if a difference existed in spring planted seed (plots established in 2006) among experimental properties 1, 2, and 3.

CHAPTER III

RESULTS

Laboratory Germination Experiment

Results indicated that a significant difference exists between the germination treatments (p-value = 0.022418, H = 23.68, 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 warmth followed by cold stratification. In the control and treatments without stratification or scarification, there was a 0% germination rate. The 0.2% KNO3 and gibberellic acid (GA3) treatments both resulted in 0% germination. 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).

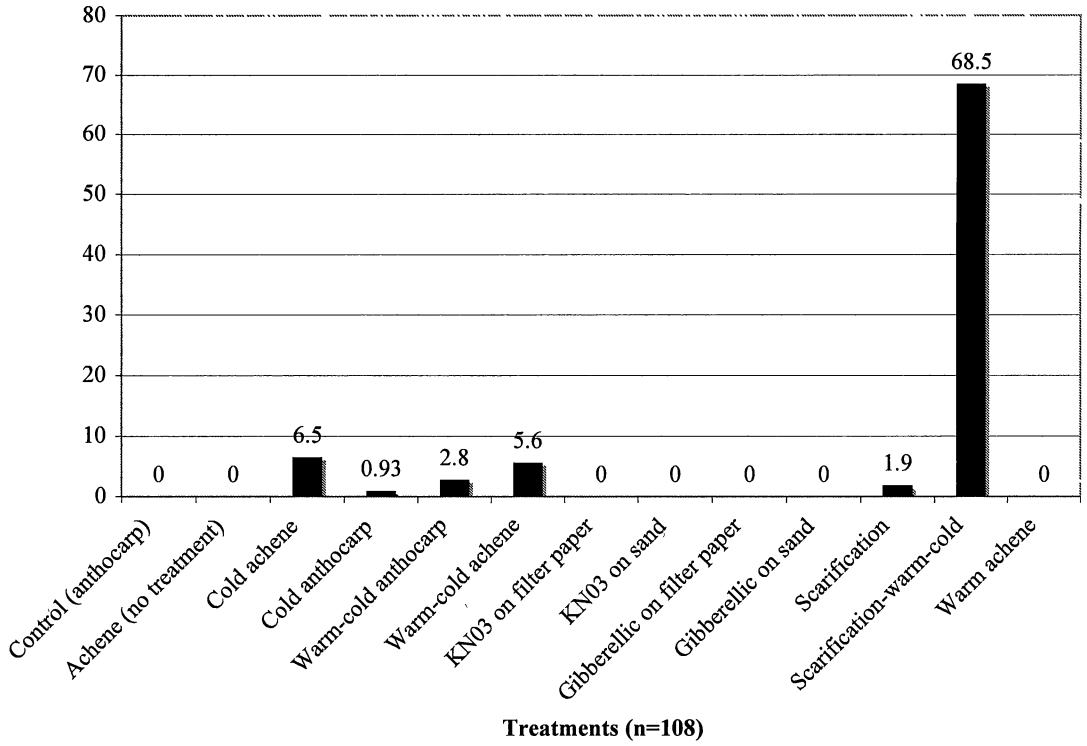

Figure 2. Percentage germination of control and achenes subjected to the twelve treatments, p-value = 0.022418; H value = 23.68; df = 12.

Selection of Experimental Reintroduction Properties

During spring 2005 and 2006 three parcels of private property were deemed suitable habitat to be used as experimental reintroduction properties (Figure 1) based on community composition/similarity and edaphic features.

Edaphic Features

Soil pH ranged from 5.5 to 5.6 at the three experimental properties (Figure 3). Nitrate levels ranged from 6 to 15 ppm (Figure 4). Phosphorus was detected at low levels ranging from 11 to 29 ppm (Figure 4). Potassium levels were low (36 to 45 ppm) at experimental properties 1 and 3 and high (81 ppm) at experimental property 2 (Figure 4).

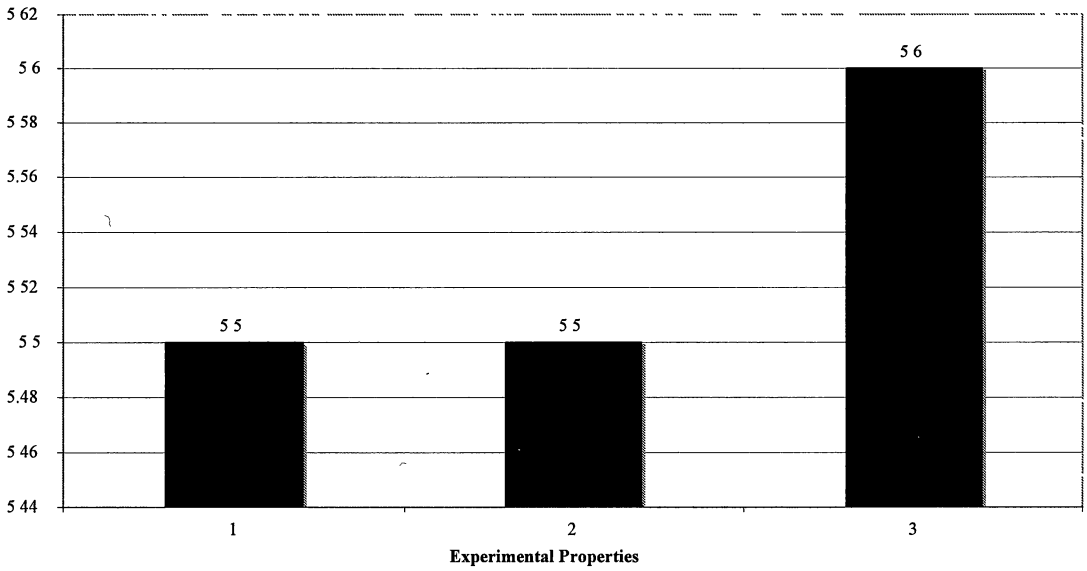

Figure 3. Soil pH of experimental reintroduction properties 1, 2, and 3.

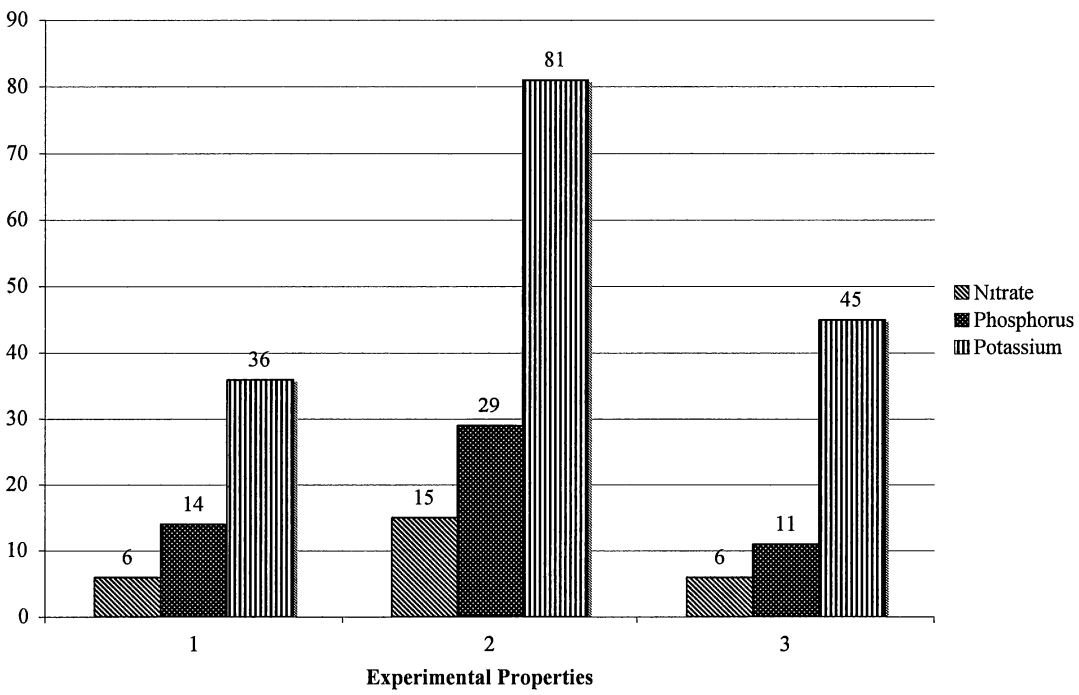

Figure 4. Nitrate, phosphorus, and potassium soil concentrations (ppm) of soils at experimental reintroduction properties 1, 2, and 3.

Community Composition

The majority of plants observed at the experimental properties were small annuals such as Prickly Poppy (*Argemone* sp.), Chickweed (*Cerastium glomeratum*), Indian Blanket (*Gaillardia pulchella*), and Plantago (*Plantago* sp.). The relative density of annuals ranged from 64.92 per m^2 at experimental property 1 to 37.37 per m^2 at experimental property 3. Grasses made up a significant portion of the community composition at the experimental properties, with relative densities ranging from 37.31 per m^2 at property 3 to 12.62 per m^2 at property 1. Grasses at these properties include Rescuegrass (*Bromus unioloides*), Sixweeks Grass (*Vulpia octoflora*), and Little Bluestem (*Schizachyrium scoparium*). The relative density of Silver Croton (*Croton argythamnia*) ranged from 14.70 per m^2 to 2.28 per m^2. Sand Mint (*Rhododon ciliatus*) occurred at experimental property 1 and 2 (8.45 per m^2 to 1.53 per m^2), but did not occur at experimental property 3. Phlox (*Phlox drummondii*) (relative density= 6.28) occurred at experimental reintroduction property 3.

The percent bare ground ranged from 26% to 37.33% among the experimental reintroduction properties (Figure 5). The percent litter ranged from 13% to 29% and the percent vegetative cover ranged from 20% to 61% among the properties (Figure 5).

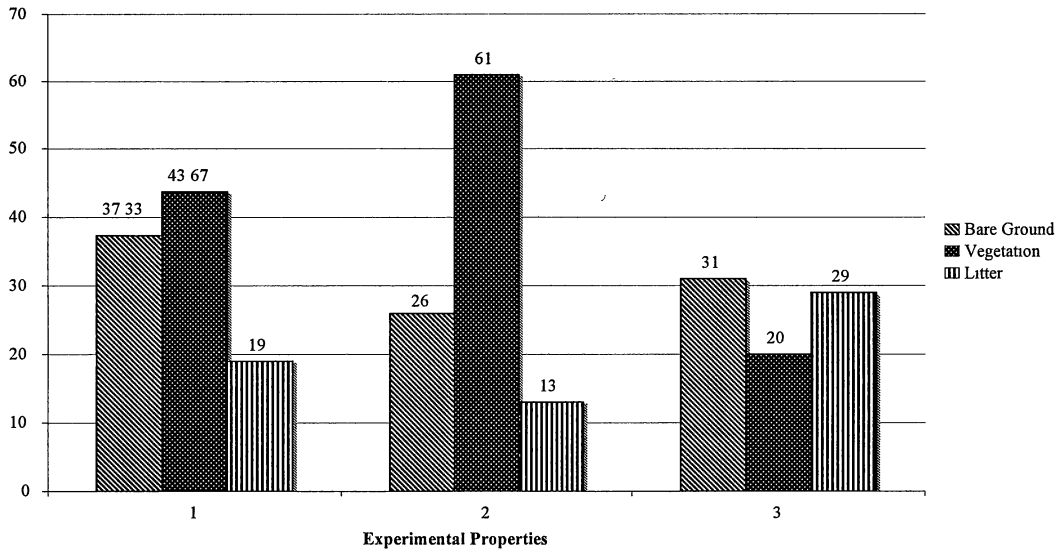

Figure 5. Mean percent cover class at experimental reintroduction properties 1, 2, and 3.

Community Similarity

Results from the Coefficient of Community Index are presented in Table 2. These results showed a range of index values when comparing the experimental properties to the existing *A. macrocarpa* populations. The values ranged from 0.43 for experimental property 2 and population 2 to 0.85 for experimental property 1 and population 1 (Table 2). Experimental property 1 had index values in the range of 0.53 to 0.85. Experimental property 2 shared over 50% of the same species with almost all of the existing populations (0.63 to 0.74), the exception being population 2 (0.43). The index values at experimental property 3 ranged from 0.46 to 0.69.

Experimental property 1 and population 3 were very similar with a coefficient index of 0.80, experimental property 2 and population 3 had a coefficient index of 0.67, and population 3 and experimental property 3 had an index value of 0.50 (Table 2).

Table 2. Coefficient of Community Index comparing existing *A. macrocarpa* populations to experimental reintroduction 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

Field Reintroduction Experiment

Reintroduction Experiment 2005

By March 11, 2006 seedlings had emerged at experimental property 1 and at experimental property 2. At experimental property 1, germination of seed planted in spring 2005 ranged from 18 to 40% among the six plots (Figure 6). The mean percentage of germinated spring seed at property 1 was 27.83% (Figure 6). Germination of fall planted seed ranged from 0 to 5% among the six plots at experimental property 1. The mean percentage of germinated fall seed at property 1 in spring 2006 was 0.83% (Figure 6). A significant difference exists between spring and fall planted treatments at property 1 (p-value = 0.0006722, t = 7.49, df = 5). At experimental property 1, all seed germinated remained in the seedling stage throughout the growing season.

Seed germinated at experimental property 2 included combined spring and fall planted seed. The percentage of germinated seed planted in spring 2005 at experimental

property 2 ranged from 0% to 20% among the six plots. The mean percentage of germinated seed at this property in spring 2006 was 8.89%. Plants at all stages of development were observed at experimental property 2 (Figure 7). Fourteen seedlings, eleven juveniles, and six plants at anthesis were recorded. One of the plants at anthesis produced anthocarps.

Seedling survivorship data of seed germinated in 2006 experimental properties 1 and 2 were collected in spring 2007. 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 property 2 was 87.5% (Figure 8). Experimental property 2 had a higher percentage of survivorship. In spring 2007 eight seedlings, sixteen juveniles, and four plants at anthesis were recorded at property 2 (Figure 7). All seed germinated remained in the seedling stage at property 1.

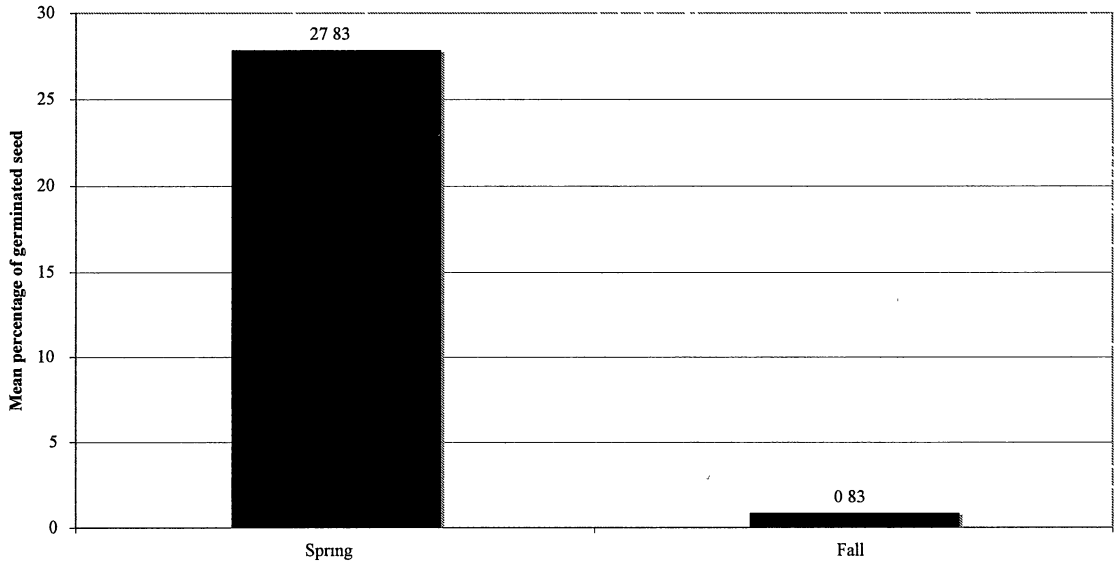

Figure 6. Mean germination percentage of seed planted in spring and fall at experimental property 1 (seed planted in 2005, data collected in spring 2006), p-value = <0.0006722; t = 7.49; df = 5.

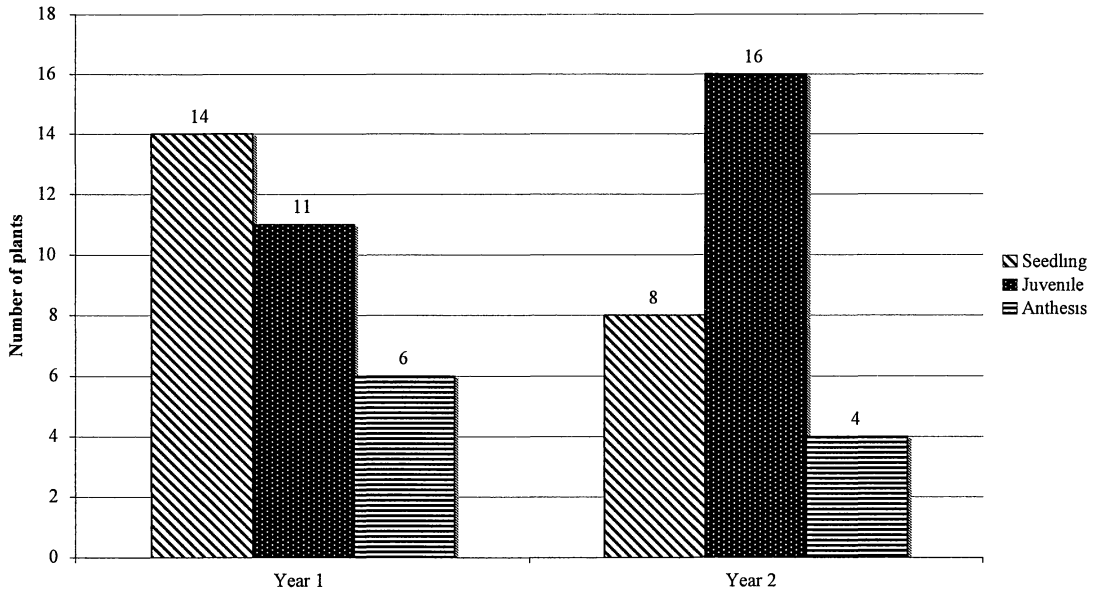

Figure 7. Number of plants at experimental property 2 in seedling, juvenile, and anthesis stages (seed planted in 2005, data collected in spring 2006 and 2007).

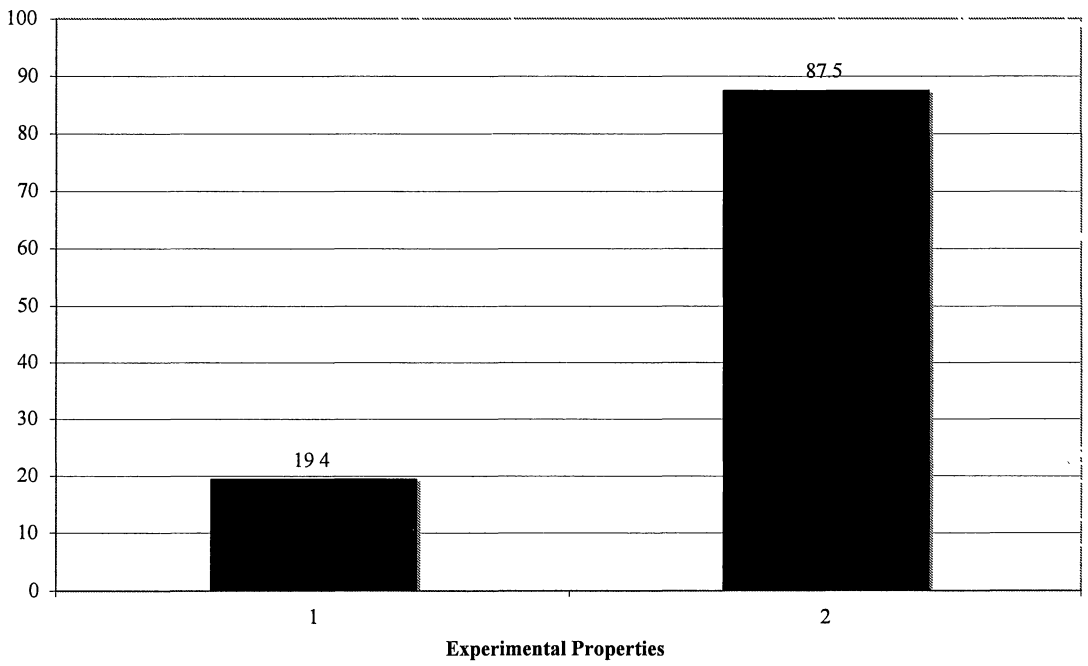

Figure 8. Mean survivorship of spring and fall seed at experimental property 1 and 2.

Reintroduction Experiment 2006

At experimental property 1, germination of seed planted in spring 2006 ranged from 10 to 20% among the six plots. The mean percentage of germinated spring seed at this property was 16.33% (Figure 9). Germination of fall planted seed ranged from 0 to 5% among the six plots. The mean percentage of germinated fall planted seed was 0.83% (Figure 9). Spring and fall planted treatments at property 1 (p-value = < 0.0001, t = 11.36, df = 5) significantly differed. At experimental property 2 germination of seed planted in spring 2006 ranged from 2.5 to 27.5% among the six plots. The mean percentage of germinated spring seed at this property was 16.67% (Figure 10). Germination of fall planted seed ranged from 0 to 2.5% among the six plots. The mean percentage of germinated fall planted seed was 0.83% (Figure 10). A significant difference was detected between the spring and fall planted seed at experimental property 2 (p-value = 0.01036, t = 3.99, df = 5). At experimental property 3, the maximum germination of spring planted seed was 10% within the six plots. The mean percentage of germinated spring seed at this property was 4.2% (Figure 11). No seed planted in the fall germinated (Figure 11). Seed germination was not significantly different between spring and fall planted seed at property 3 (p-value = 0.3632, t = 1, df = 5). Germination of spring planted seed was significantly higher at experimental properties 1 and 2 when compared to experimental property 3 (p-value = 0.01303, F = 5.88, df = 2) (Figure 12).

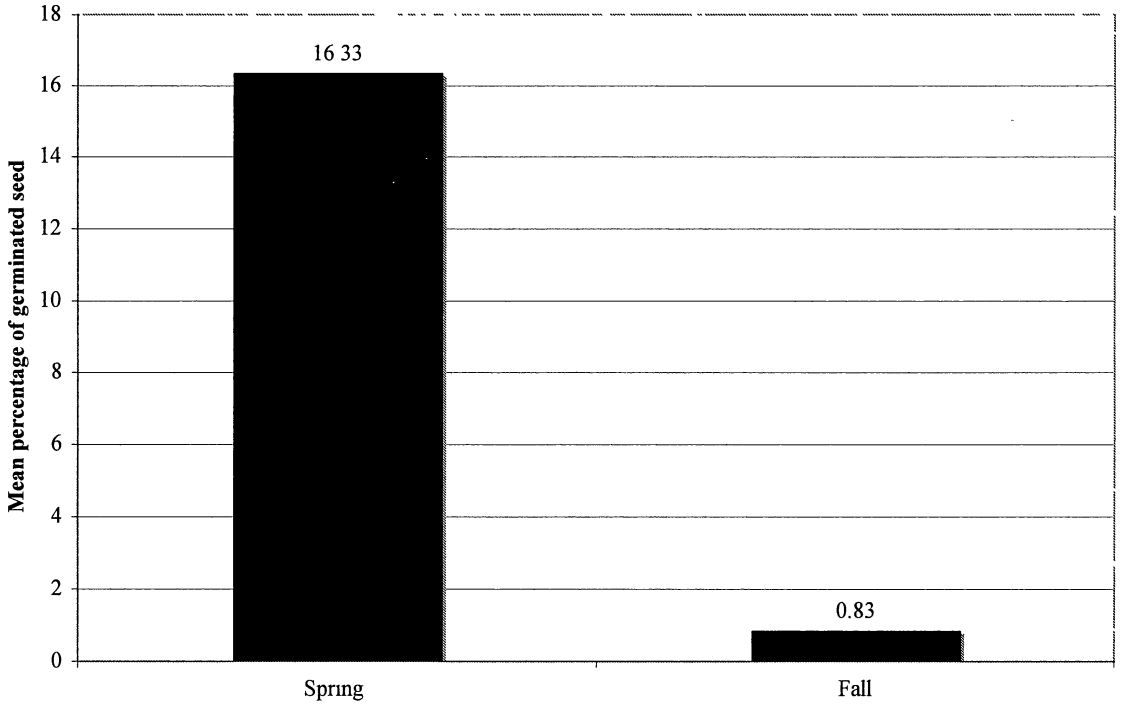

Figure 9. Mean germination percentage of seed planted in spring and fall at experimental property 1 (seed planted in 2006, data collected in spring 2007), p-value = <0.000001; t = 11.36; df = 5.

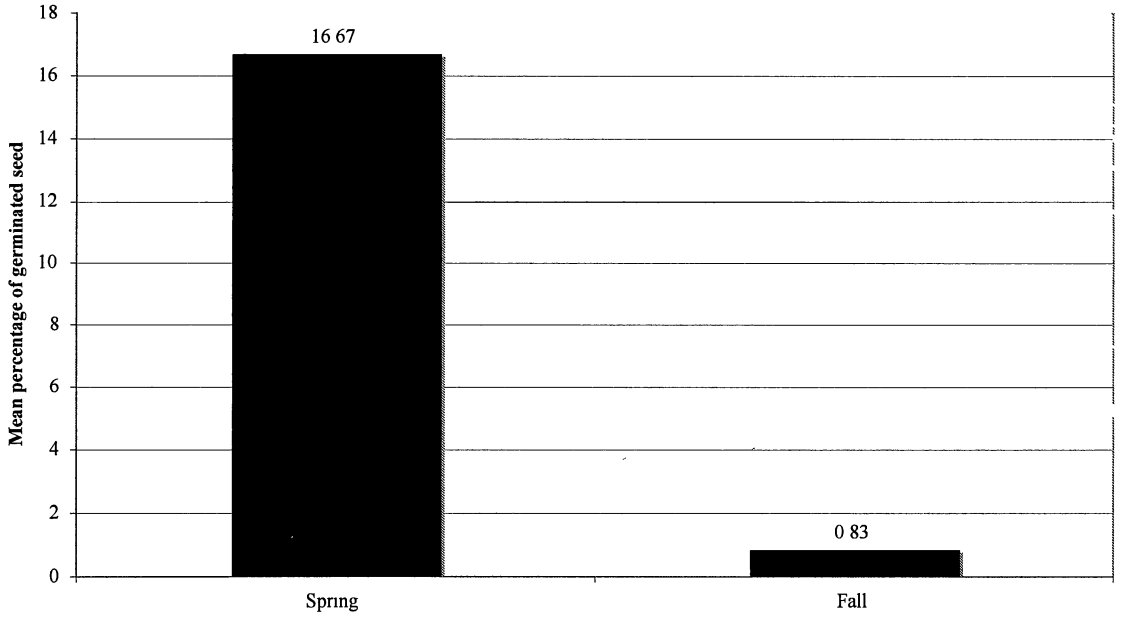

Figure 10. Mean germination percentage of seed planted in spring and fall at experimental property 2 (seed planted in 2006, data collected in spring 2007), p-value = <0.01036; t value = 3.99; df = 5.

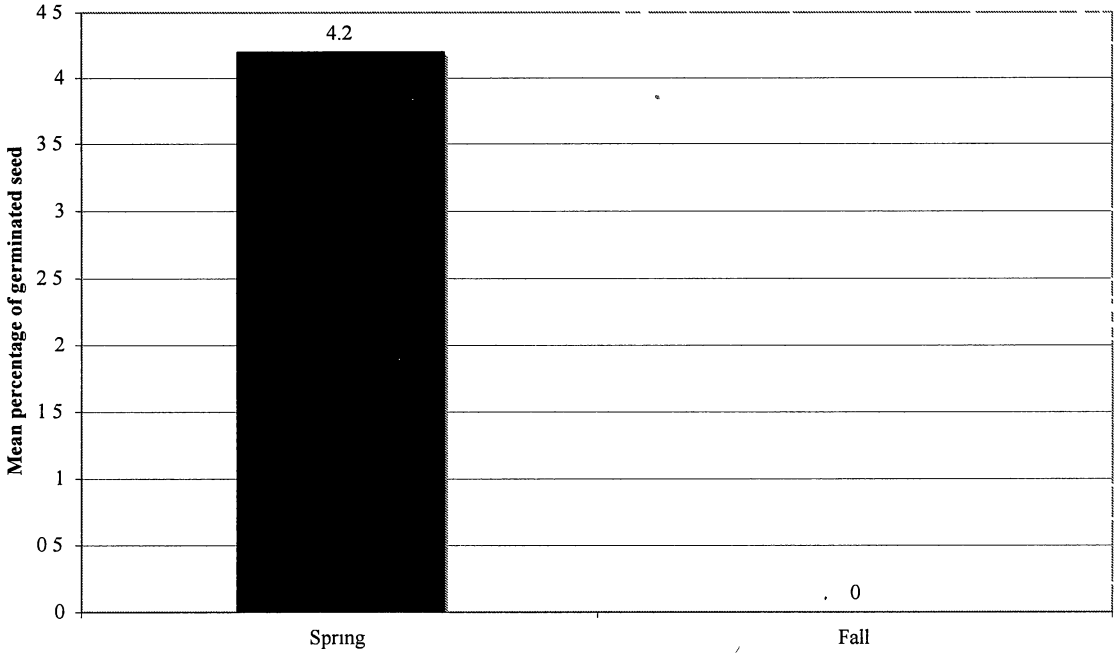

Figure 11. Mean germination percentage of seed planted in spring and fall seed at experimental property 3 (seed planted in 2006, data collected in spring 2007), p-value = 0.3632, t = 1, df = 5.

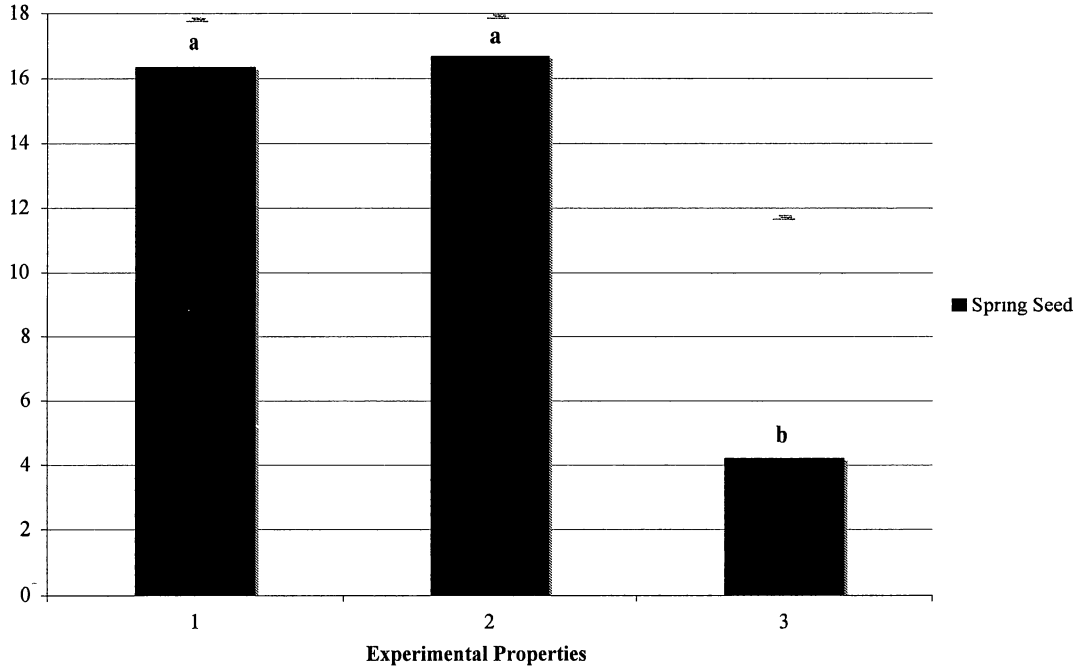

Figure 12. Mean percentage of germinated seed planted in spring (seed planted 2006, data collected 2007) at experimental properties 1, 2, and 3, p-value = 0.01303; F = 5.88; df = 2.

CHAPTER IV

DISCUSSION

According to Pavlik (1997) conserving rare plants is a five-step process: The first step is inventory of the relative abundance and richness of the taxon; The second step is survey and assessment of the populations, this often requires population census data; Habitat preservation is the third step, this usually involves landowner contact programs and land purchase and transfer to federal agencies; The fourth step monitoring, is useful in determining changes or trends in the population structure; The final step involved is the recovery of the plant species at risk. Previous studies of *Abronia macrocarpa* have addressed steps 1 to 4 (Williamson et al., 1994; Williamson and Bazeer, 1997; Williamson and Werth, 1999; Meredith, 2006). Since the recovery of a rare plant often involves augmentation or reintroduction of the diminishing populations this study focused on testing the potential for reintroduction of *A. macrocarpa.*

Depending on the species, seed may undergo a period of dormancy prior to germination. Various treatments are known to break dormancy. A study conducted on rare plants in the Pacific Northwest found that cold stratification promoted germination in the five rare plant species tested (*Erigeron decumbens*, *Horkelia congesta*, *Aster curtus*, *Lomatium bradshawii*, and *Lupinus sulphureus* ssp. *kincaidii*, Kaye and Kuykendall, 2001). Seed dormancy may be broken by physical or physiological features, or a combination of both (Baskin and Baskin, 2004).

Physical dormancy can be broken by scarification (nicking the seed coat or rubbing the seed with sand paper) or soaking seeds in a weak acid solution (Baskin and Baskin, 2004). Cold or warm stratification and gibberellic acid (GA_3) are treatments both known to break physiological dormancy (Baskin and Baskin, 2004). These treatments, however, did not result in high rates of germination in *A. macrocarpa*. The gibberellic acid treatment and the warm stratification treatment each resulted in 0% germination and cold stratification resulted in only 1% or 7% germination, with the higher rate achieved when the achene was removed from the anthocarp. Maximum germination of *A. macrocarpa* was achieved when seeds were scarified followed by warm and cold stratification. This treatment may mimic the conditions required to effectively break dormancy under natural conditions. Seed set occurs in the spring, seed are first exposed to a period of warmth (summer temperatures) followed by cold (winter temperatures) then germinate the following spring. *Abronia macrocarpa* occurs in sandy soils, which naturally exposes the seed to scarification therefore weakening the seed coat. The need for warm followed by cold stratification coupled with scarification may explain the higher percentage of germination of seed planted in the spring vs. the fall. Fall planted seed would not have been exposed to warm stratification.

Physiological dormancy is the only type of dormancy previously reported in Nyctaginaceae (Baskin and Baskin, 1998). A study conducted by Kaye (1999) on the endangered *Abronia umbellata* ssp. *breviflora* found cold stratification broke seed dormancy in this taxon and that germination reached approximately 90% after 2 weeks of stratification. Since both stratification and scarification were necessary to achieve high rates of germination in *A. macrocarpa* this may indicate that seed undergo physical and

physiological dormancy. Techniques for propagating rare species are seldom well developed, and thus pose a challenge to restoration projects (Kaye and Kuykendall, 2001). The improved understanding of seed dormancy and propagation of *A. macrocarpa*, resulting from this study, will be important in formulating a reintroduction plan for this rare species.

If a reintroduction plan proves to be successful, delisting of *A. macrocarpa* may be possible by 2015 (U. S. Fish and Wildlife Service, 1992). This may be a realistic goal considering *Abronia umbellata* subsp. *breviflora* has recently been successfully reintroduced in beach dune habitat in Oregon (McGlaughlin et al., 2002). Pavlik et al. (1994) pointed out the need to identify suitable habitat for a reintroduction program.

Most endangered species have specific habitat requirements and a narrow geographic range (Falk, Millar, and Olwell, 1996). White and Drozda (2006) describe endangered white-haired goldenrod (*Solidago albopilosa*) as a species with a narrow geographic range and extremely particular habitat requirements. *Solidago albopilosa* is only known to occur in three adjoining counties in Kentucky where it occupies partial shade behind the dripline of sandstone rockshelters (White and Drozda, 2006). In a study of *Cirsium pitcheri*, Rowland and Maun (2001) found that the plant requires an open habitat with full sunlight for maximum survival and growth. *Abronia macrocarpa* populations are associated with distinct community characteristics.

The principal component analysis conducted by Meredith (2006) showed a strong correlation in the presence of *Rhododon ciliatus, Plantago* sp. and *Croton argythamnia* in plant communities supporting *A. macrocarpa.* These particular species may be strong indicators of suitable habitat. *Plantago* sp. and *Croton argythamnia* were present at all

three experimental properties. Germination percentages were significantly higher at experimental properties 1 and 2, which had *R. ciliatus* as a member of the associated plant community. *Rhododon ciliatus* was not represented in the community composition of experimental property 3, which had significantly lower percentage germination. The absence of *R. ciliatus* at experimental property 3 may indicate the habitat is not suitable to support *A. macrocarpa.*

Selecting reintroduction sites that are ecologically similar to the source populations will aid in the recovery success of restored populations (Montalvo and Ellstrand, 2000). Experimental properties 1 and 2 both had higher coefficient of community index values and a higher percentage of germination than experimental property 3 when compared to that of the source population. This supports the "home-site advantage" hypothesis (Montalvo and Ellstrand, 2000), that experimental properties that are more environmentally similar to the source population have higher success rates. Populations are adapted to specific habitats and selective pressures; therefore, ecological similarity between source and introduced populations is a critical factor when choosing the origin of source material (Guerrant, 1996).

Plant 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 juvenile and anthesis stages in the first year of growth. Experimental property 2 also had the greatest plant survivorship, which may be attributed to higher potassium levels promoting root longevity through the dry summer months. A study by Lofflin and Kephart (2005) comparing seedling establishment of rare and common varieties of *Silene douglasii* found that the rare variety (*S. douglasii* var. *oraria*) produced significantly fewer juveniles indicating seedling survival limits plant establishment. 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.

Reintroduction efforts should focus on establishing new populations in suitable habitat to meet recovery objectives. In the case of *A. macrocarpa*, edaphic features and community composition are particularly important to consider when selecting suitable habitat. The *A. macrocarpa* recovery plan (U.S. Fish and Wildlife, 1992) recommends a minimum population size of population of 600 individuals. Given the percentage germination and plant survivorship found in this 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 study has increased our understanding of criteria to use in selecting reintroduction sites and provided important knowledge of seed germination and

survivorship rates. Incorporation of the information generated in this study in future reintroduction efforts will assist in achieving recovery of *A. macrocarpa*.

LITERATURE CITED

Baskin, C.C. and J.M. Baskin. 1998. Seeds: ecology, biogeography, and evolution of dormancy and germination. Academic Press. San Diego, California.

Baskin, C.C. and J.M. Baskin. 2004. Determining dormancy-breaking requirements and germination requirements from the fewest seeds. In Ex Situ Plant Conservation: Supporting Species in the Wild. (eds. Guerrant, E.O., K. Havens, and M. Maunder), pp. 162–179. Island Press: Washington, DC.

Cheetham, A.H. and J.E. Hazel. 1969. Binary (presence-absence) similarity coefficient. *Journal of Paleontology* 23: 1130–1136.

Crawford, N.M. 1995. Nitrate: nutrient and signal for plant growth. *The Plant Cell* 7: 859–868.

Correll, D.S. and M.C. Johnston. 1979. Manual of the Vascular Plants of Texas. University of Texas Dallas. Richardson, TX.

Deyn, G.B., C.E. Raaijmakers, and W.H. Van Der Putten. 2004. Plant community development is affected by nutrients and soil biota. *Journal of Ecology* 92: 824–834.

Diamond, D.D., D.H. Riskind, and S.L. Orzell. 1987. A framework for plant community classification and conservation in Texas. *The Texas Journal of Science* 39: 203–221.

Egilla, J.N., F.T. Davies, Jr., and M.C. Drew. 2001. Effect of potassium on drought resistance of *Hibiscus rosa-sinesis* cv. Leprechaun: Plant growth, leaf macro- and micro-nutrient content and root longevity. *Plant and Soil* 229: 213–224.

Falk, D.A. and K.E. Holsinger. 1991. Genetics and Conservation of Rare Plants. New York: Oxford University Press.

Falk, D.A., C.I. Millar, and M. Olwell (editors). 1996. Restoring Diversity: Strategies for Reintroduction of Endangered Plants. Center for Plant Conservation. Island Press. Washington, D. C.

Fielder, P.L. 1991. Mitigation related transplantation, relocation and reintroduction projects involving endangered and threatened, and rare plant species in California. Final report to California Department of Fish and Game. Sacramento: Endangered Plant Program.

Falk, D.A. and P. Olwell. 1992. Scientific and policy considerations in restoration and reintroduction of endangered species. *Rhodora* 94: 287–315.

Friar, E.A., D.L. Boose, T. LaDoux, E.H. Roalson, and R.H. Robichaux. 2001. Population structure in the endangered Mauna Loa silversword, *Argyroxiphium kauense* (Asteraceae), and its bearing on reintroduction. *Molecular Ecology* 10: 1657–1663.

Galloway, L. 1972. *Abronia macrocarpa* (Nyctaginaceae): a new species from Texas. *Brittonia* 24: 148–149.

Guerrant, E.O. 1996. Designing populations: demographic, genetic, and horticultural dimensions. In Restoring Diversity: Strategies for Reintroduction of Endangered Plants. (eds. Falk, D.A., C.I. Millar, and M. Olwell), pp. 171–207. Island Press, Washington, DC.

Janack, E.F. and E.L. Griffin. 2002. Soil Survey of Freestone County, Texas. Natural Resources Conservation Service.

Kaye, T.N. 1999. Propagation of endangered species: variable germination of pink sand verbena from Pacific Coast beaches. Combined Proceedings of the International Plant Propagators Society 49: 617–621.

Kaye, T.N. and K. Kuykendall. 2001. Germination and propagation techniques for restoring rare Pacific Northwest prairie plants. In Conservation of Washington's Native Plant Society. (eds. Reichard, S.H, P.W. Dunwiddie, J.G. Gamon, A.R. Kruckeberg, D.L. Salstrom), pp. 213–224. Washington Native Plant Society, Seattle, Washington.

Lofflin, D.L. and S.R. Kephart. 2005. Outbreeding, seedling establishment, and maladaptation in natural and reintroduced populations of rare and common *Silene douglasii* (Caryophyllaceae). *American Journal of Botany* 92: 1691–1700.

McGlaughlin, M., K. Karoly, and T. Kaye. 2002. Genetic variation and its relation to population size in reintroduced populations of pink sand verbena, *Abronia umbelleta* subsp. *breviflora* (Nyctaginaceae). *Conservation Genetics* 3: 411–420.

Meredith, C.G. 2006. Response to disturbance and habitat characterization of *Abronia macrocarpa* (Nyctaginaceae). Unpublished Masters Thesis. Texas State University-San Marcos.

Montalvo, A.M. and N.C. Ellstrand. 2000. Transplantation of the subshrub *Lotus scoparius*: testing the home-site advantage hypothesis. *Conservation Biology* 14: 1034–1045.

Morse, L.E. 1996. Plant rarity and endangerment in North America. In Restoring Diversity: Strategies for Reintroduction of Endangered Plants. (eds. Falk, D.A., C.I. Millar, and M. Olwell), pp. 7–22. Island Press, Washington, DC.

Neitsch, C.L., J.J. Castille, and M.R. Jurena. 1998. Soil survey of Leon County, Texas. United States Soil Conservation Service.

Pavlik, B.M. 1994. Demographic monitoring and the recovery of endangered plants. In: Restoration of Endangered Species. (eds. Bowles, M.L. and C.J. Wheelan), pp-322–350. Cambridge University Press, New York, NY.

Pavlik, B.M. 1996. Defining and measuring success. In Restoring Diversity: Strategies for Reintroduction of Endangered Plants. (eds. Falk, D.A.,C.I. Millar, and M. Olwell), pp. 127–155. Island Press, Washington, DC.

Pavlik, B.M. 1997. Perspectives, tools, and institutions for conserving rare plants. *Southwestern Naturalist* 42: 375–383.

Rowland, J. and M.A. Maun. 2001. Restoration ecology of an endangered plant species: establishment of new populations of *Cirsium pitcheri*. *Society for Ecological Restoration* 9: 60–70.

U.S. Fish and Wildlife Service. 1988. Endangered and threatened wildlife and plants: determination of endangered status for *Abronia macrocarpa* (large-fruited sand-verbena). *Federal Register* 43: 37975.

U. S. Fish and Wildlife Service. 1992. Large-fruited sand verbena (*Abronia macrocarpa*) recovery plan. Albuquerque, New Mexico.

White, D.L. and N.C. Drozda. 2006. Status of *Solidago albopilosa* Braun (White-haired Goldenrod) [Asteraceae], a Kentucky endemic. *Castanea, The Journal of Southern Appalachian Botanical Society* 71: 124–128.

Williamson, P.S., L. Muliani, and G. Janssen. 1994. Pollination biology of *Abronia macrocarpa* (Nyctaginaceae), an endangered Texas species. *Southwestern Naturalist* 39: 336–341.

Williamson, P.S. and S.K. Bazeer. 1997. Self incompatibility in *Abronia macrocarpa*. *Southwestern Naturalist* 42: 409–415.

Williamson, P.S. and C.R. Werth. 1999. Levels and patterns of genetic variation in the endangered species *Abronia macrocarpa* (Nyctaginaceae). *American Journal of Botany* 86: 293–301.