

FINAL REPORT

As Required by

THE ENDANGERED SPECIES ACT

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GRANT NUMBER E-3-1

ENDANGERED RESOURCES BRANCH

Project 44: Large-Fruited San Verbena (*Abronia macrocarpa*) Monitoring and Management Study

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**FINAL REPORT**

**State:** Texas

**Grant Number:** E-3-1

**Grant Title:** Endangered and Threatened Species Conservation

**Project Title:** Large-fruited San Verbena (*Abronia macrocarpa*) Monitoring and Mangement Study

**Contract Period:** September 1, 1995 through August 31, 1996

**Project Number:** 44

**Objective:** Protect known populations from existing and future threats. Maintain a reserve germ bank/cultivated population at the SWTSU Natural Science Center. Gather information necessary for protective management/restoration.

**PREFACE**

The attached Final Report entitled "Large-Fruited Sand-Verbena Monitoring and Management Study" by Paula S. Williamson resulted from this objective and is submitted in fulfillment of the report requirement.

**Prepared by:** Gena K. Janssen  
Conservation Scientist

**Date:** November 26, 1996

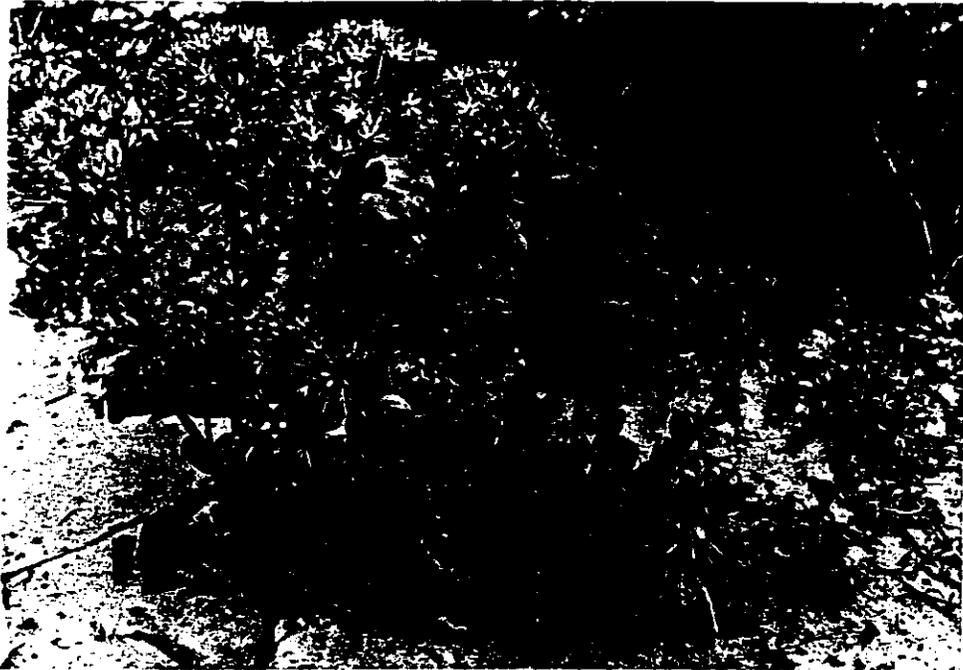
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**Date:** December 31, 1996

**FINAL REPORT**

**Large-Fruited Sand-Verbena Monitoring and Management Study**

**September 1992 - August 1996**



**prepared for**

**Texas Parks and Wildlife Department**

**submitted by**

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**November 20, 1996**

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## ABSTRACT

*Abronia macrocarpa*, large-fruited sand verbena, received listing as federally and state endangered in 1988. Potential threats to the species include oil exploration, resort/residential development, off-road vehicle use, the agricultural industry, browsing by deer and other wildlife, and fire suppression (U. S. Fish and Wildlife Service, 1992). An investigation of the biology of this species was initiated in 1992 to: 1) protect known populations from existing and future threats; 2) establish and maintain a genetically diverse germ bank/cultivated population; and 3) gather biological information necessary for protective management and restoration. This report includes data collected from 1992-1996.

This investigation is multifaceted and includes the following studies. Surveys were conducted in 1994 and 1995 to search for new populations in Leon, Robertson, Freestone, and Caldwell Counties. Fluctuation in population size has been monitored at the Hilltop Lakes population site in Leon County since 1992. Climatic features of the habitat, including precipitation and temperature, have been characterized. A genetic repository of *A. macrocarpa* plants and seed has been established at Southwest Texas Sate University and seed has been sent to Mercer Arboretum to supplement a repository of this species located at that facility. Plant phenology, floral morphology and maturation, analyses of stigmatic secretions and nectar, pollen viability, microgametogenesis, pollination biology, the breeding system, the barrier to selfing, reproductive capacity, fruit/seed set, seed viability, and seedling recruitment have been examined. Aspects of community ecology including extent of herbivory has been studied and the occurrence of a mycorrhizal association has been documented. Associated species (n=81) have been collected and identified. A program to educate the public about the importance of conserving this endangered species was initiated and has included presentations at scientific meetings by the principal investigator and graduate assistants, publication in scientific journals, and contributions to photographic exhibitions and magazine articles.

*Abronia macrocarpa* is known to occur in three counties (Freestone, Leon, and Robertson) in the Post Oak Savannah Woodlands region of eastern, central Texas. When the taxon was originally described in 1972 only one population was known. The type locality is a sand-dune area, approximately nine miles northwest of Normangee, Texas. Surveys conducted by Yantis and Williamson have identified nine additional populations, three more in Leon County, one in Freestone County, and five in Robertson County. The plant appears to be restricted to deep sandy soils of the Arenosa series and Padina series.

A greater number of plants (over 8,000) than previously documented was observed in the Hilltop Lakes population (Leon County) in March, 1995. A steady increase in the size of the population has been observed over the course of this study, despite a severe disturbance of the population resulting from oil well construction in September, 1992. The most likely factors contributing to this observed increase in population size are a ban of off-road vehicles and a modification in mowing regime. Off-road vehicles have been considered a threat since the population was first discovered in 1970. These vehicles are now banned at Hilltop Lakes. Based on data collected in the first segment of this study, a short-term management plan was implemented to postpone mowing the population site until mid to late May after plants have completed anthesis and above ground parts have died back. The management is continuing this practice in a cooperative effort to maintain a large, viable population at the Hilltop Lakes site. Population size was lower in 1996 than in 1995. This reduction in number is likely due to the drought Texas suffered in 1996.

Large-fruited sand verbena, is characterized by thin-walled, papery anthocarps that are larger than those of other species. The plant is an herbaceous perennial with a long taproot, glandular-pubescent leaves, flowers grouped into a head, and achene fruits. Plants form a rosette of leaves in October and overwinter in this stage. Increase in height coupled with the onset of anthesis begins in February, with peak flowering typically occurring in March. The above ground parts of the plants die back in late April and May, with a few lasting into June. The plant survives the hot, dry summer with the taproot and shoot apex buried in the sand.

The inflorescence is a capitulum or head typically composed of 25-48 individual flowers. The average number of flowers per inflorescence is 31. Five bracts subtend the inflorescence. The perianth consists of five pink sepals fused around the ovary and extending to form a narrow floral tube averaging 2.0 cm in length. The flower is apetalous. Nectar is secreted at the base of the floral tube. Sugar content of the nectar varies from 25% to 29%. The stamens are adnate to the floral tube. Anthers occur positioned approximately 1.0 cm above the stigma. The gynoecium consists of a single carpel with one basal ovule. The ovary is superior. The stigma is linear, 0.1-0.2 cm long (averaging 0.14 cm), covered with papillae. Stigmatic secretions contain lipids, proteins, and sugars.

Flowers open at 3:00 - 4:00 pm and close at approximately 9:00 - 10:00 am. A sweet floral odor is present once the flowers begin opening, increases in intensity towards dusk and remains strong during the night. Stigmas are most receptive approximately four to five hours after the flower opens on the first and second day of blooming. The study of pollination biology revealed that *A. macrocarpa* is reliant upon hawk moths and noctuid moths to bring about cross-pollination for successful fruit set. The peak period of stigma receptivity corresponds to peak activity of the crepuscular moths that serve as pollinators.

The breeding system was examined by experimentally crossing plants autogamously, geitonogamously, and xenogamously then monitoring for development of fruit. Flowers that were not manipulated served as controls. Development of achenes signifies that fertilization resulted from a particular type of pollination. If successful pollination and fertilization does not occur the flowers wither and abscise from the plant. No fruit was set in any cross involving autogamy, geitonogamy, or in the controls. Fruit developed only from xenogamous crosses. The plant is therefore an obligate outcrosser.

Flowers were experimentally self- and outcrossed then pollen germination and relative growth of pollen tubes were examined using fluorescent microscopy to assess the barrier to selfing. Both self- and outcross pollen readily adhere to the stigma and germinate. Following germination, growth of self-pollen tubes is arrested at the stigma surface due to the formation

of extensive callose deposits in the tubes. Cross-pollen tubes are able to penetrate the stigmatic tissue and continue to grow down the style reaching the ovule within 48-72 hours following hand-pollination. These results indicate that a pre-fertilization (self-incompatibility system) prevents seed set following self-pollination. Two general types of self-incompatibility mechanisms are known. The gametophytic self-incompatibility system, characterized by flowers with wet stigmatic papillae and binucleate pollen grains, results in inhibition of pollen tube growth within the style. The sporophytic self-incompatibility system, characterized by flowers with dry stigmas covered with a cuticle and trinucleate pollen grains, results in inhibition of pollen tube growth at the stigma-pollen interface. Flowers of *A. macrocarpa* possess a dry papillate type of stigma, the pollen is trinucleate, and self-pollen tube growth is blocked at the stigma surface. These data provide evidence that a sporophytic self-incompatibility system prevents seed set in self-pollinated flowers of this taxon.

The percentage of viable pollen is relatively high (ca. 94%) yet achene set is low (28% - 66%), suggesting a reduced fecundity. The reproductive capacity of the plant may also be limited by a low seed viability (56%) in addition to the low seed set. Browsing of vegetative parts of the plant is not extensive. More frequently, inflorescences are grazed. Herbivory may, therefore, also reduce reproductive capacity.

Seedlings (n=233) were numbered and tagged by placing a plastic plant stake next to them. Twenty-six seedlings were marked in 1992 and 207 seedlings were marked in 1993. Seedlings have been periodically monitored to assess seedling recruitment. Presence of vegetation above ground, number of leaves, leaf length and width, plant height, and presence of reproductive organs were recorded. Discounting the seedlings with unknown fate, the population exhibited a seedling recruitment rate of 27% with 73% seedling mortality. Seedling development data suggest that the plant may spend several years in a vegetative state prior to entering a reproductive stage. Much energy is devoted to production of a substantial taproot during the early growth phase, with less effort spent in leaf development and virtually no effort spent in reproduction.

## INTRODUCTION

*Abronia* is a western North American genus in the Nyctaginaceae or four o'clock family. Sixteen species occur in the United States (Galloway, 1975). *Abronia ameliae* Lundell, *A. angustifolia* Greene, *A. carletonia* Coult. and Fish., *A. macrocarpa* Galloway, and *A. fragrans* Hook. occur in Texas (Correll and Johnston, 1970; Galloway, 1975) (Figure 1 from Corlies, 1991). *Abronia ameliae* and *A. macrocarpa* are endemic to Texas (Galloway, 1975).

*Abronia macrocarpa* is restricted in distribution to eastern, central Texas. When *A. macrocarpa* was originally described only one population was known. *Abronia macrocarpa* 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). Due to the low number of occurrences globally and in Texas the plant's status was designated a G1, S1 status (Resource Protection Division, 1991).

The type locality is a sand-dune area, approximately nine miles northwest of Normangee, Texas in Hilltop Lakes Resort (Galloway, 1972). At the time the recovery plan was written, two more populations had been identified, one in Freestone County near Snyder Lake and one in Robertson County at Camp Creek Sand Barrens (Kennedy, et al., 1990; U. S. Fish and Wildlife Service, 1992). Recent surveys have identified additional populations in both Leon County and Robertson County. Currently a total of ten populations are known.

The species is not considered abundant and the number of individuals per population fluctuates. The plants are restricted to deep sandy soils in unstable openings and disturbed areas of Post Oak Savannah Woodlands (Galloway, 1972, 1975; Turner, 1983; Poole and Riskind, 1987; Bridges, 1988).

*Abronia macrocarpa*, commonly called Large-fruited sand verbena, was described and named in the early 1970's (Galloway, 1972). The species is characterized by thin-walled, papery anthocarps that are larger than those of other species (Galloway, 1972). An anthocarp

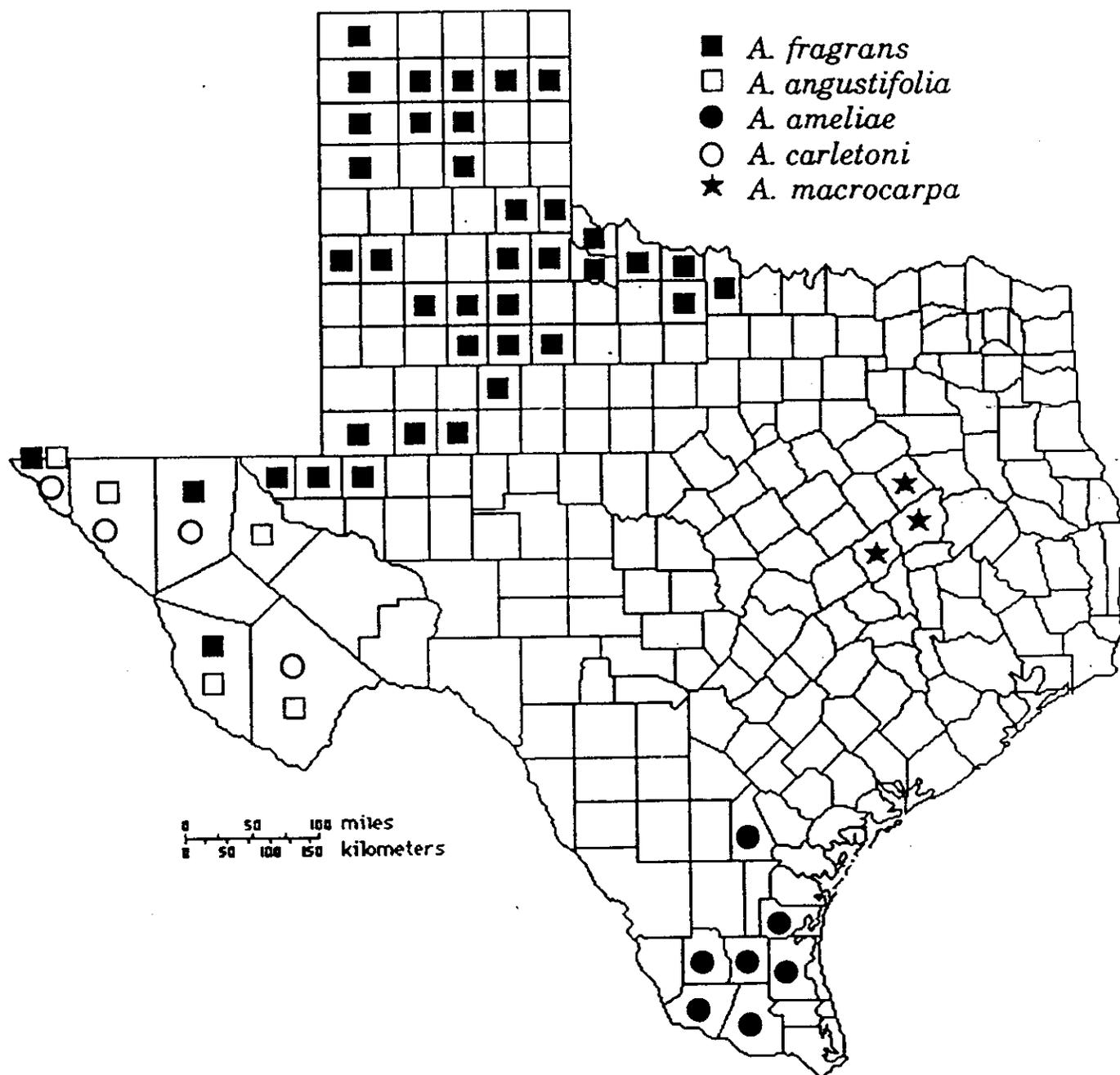


Figure 1. Map of Texas showing distribution of *Abronia fragrans*, *A. angustifolia*, *A. ameliae*, *A. carletoni*, and *A. macrocarpa*.

is defined as a structure in which the fruit proper is united with the perianth (Munz, 1974). The plant is an herbaceous perennial with a long taproot, glandular-pubescent leaves, flowers grouped into a head, and achene fruits (Figure 2).

The *A. macrocarpa* 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 grass species for range improvement and soil stabilization. Clearing and fire repression have also contributed to habitat modification. Additional impacts by man include oil exploration and development, resort and residential development, and recreational activities such as off-road vehicles, hiking, horseback riding, and wildflower picking. Browsing, possibly by livestock and deer and insect damage also pose threats to the species. Recommended actions include the protection, monitoring, and management of existing populations. Specific management and restoration needs, examination of reintroduction potential, and a cultivation and education program have been advised (U.S. Fish and Wildlife Service, 1992). Since the recovery of an endangered species depends on the whole ecosystem, studies of the habitat have been advised in addition to studies of the species biology.

Prior to the onset of this investigation, the only scientific publications on *A. macrocarpa* were the species description (Galloway, 1972) and another paper by Galloway (1975) on the systematics of North American species of *Abronia*. Virtually nothing was known about the biology of the species. Therefore, this study was initiated to examine aspects of the biology of *A. macrocarpa*. The specific objectives of this study are to: 1) protect known populations from existing and future threats; 2) establish and maintain a genetically diverse germ bank/cultivated population; and 3) gather biological information necessary for protective management and restoration (Appendix V). This research will improve our understanding of the taxon's biology in general, assess reproductive and other factors which may be limiting abundance of the species, and provide information on reproductive biology that will be essential in propagating plants for potential reintroduction of this endangered species.

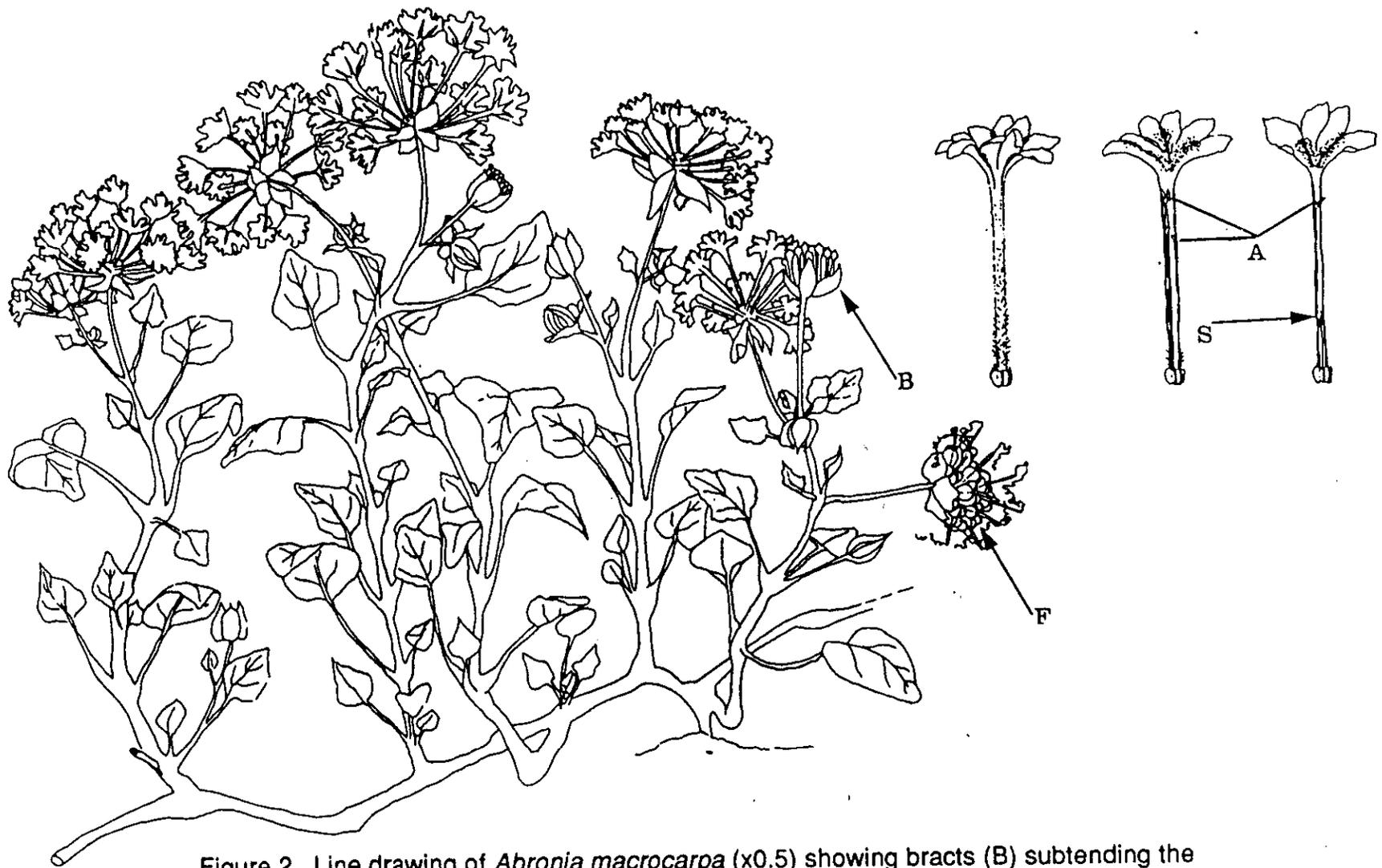


Figure 2. Line drawing of *Abronia macrocarpa* (x0.5) showing bracts (B) subtending the inflorescence, anthocarps (F), and flower (x2) showing location of anthers (A) and stigma (S).

## MATERIALS AND METHODS

Plants were studied in the field at the Hilltop Lakes population site in 1992, 1993, 1994, 1995, and 1996 (see Appendix I). Plants were also transplanted from the field to the greenhouse and to an outdoor bed at the Southwest Texas State University Biology greenhouses (SWT). Plants grown at SWT were used to examine the breeding system, self-incompatibility system, and to study reproductive capacity.

### Surveys for Additional Populations

Surveys to locate new populations were made in Leon, Robertson, Freestone, and Caldwell Counties in March and April, 1994. Sites to survey were chosen from the suggested sites listed in the 1990 Status Report. Potential population sites, located on private land, were surveyed from the road using binoculars. Surveys were also conducted during 1995 in collaboration with Mr. Jim Yantis of the Texas Parks and Wildlife Department. Additionally, Mr. Yantis was able to conduct an extensive survey of Robertson County in 1994 while searching for Wild Turkeys. New populations in Robertson County discovered by Yantis were verified by Williamson. Williamson identified potential population sites in Leon County using soil survey maps. The plant appears to be restricted to deep sandy soils of the Arenosa series and Padina series. The Arenosa series, formed in deep beds of sand, consisting of deep gently sloping to undulating, somewhat excessively drained soils on uplands; the solum ranges from 80 to more than 100 inches in thickness (U. S. Department of Agriculture, 1989). The Padina series consists of deep, gently sloping to moderately steep, well drained soils on uplands; they formed in thick beds of sandy material with the solum ranging from 70 to more than 80 inches in thickness (U. S. Department of Agriculture, 1989). Landowners were identified from data at the County Tax Assessor's Office in Centerville, Texas. Landowners were contacted by telephone for permission to survey their land. Only property to which permission for access was granted were surveyed in Leon County. Williamson and Yantis visited the populations in

Robertson and Leon Counties in March of 1995 and made estimates of population sizes. Leon, Robertson and Freestone Co. populations were visited by Williamson and Gena Janssen, of the Texas Parks and Wildlife Department, in early April of 1996.

### Population Monitoring and Mapping

The Hilltop Lakes population has been monitored on a regular basis (at least monthly) from September, 1992 to August, 1995, with the exception of January 1994 and 1995, and was monitored six times in year four. The following dates were spent in the field collecting data:

YEAR 1 (92-93)	YEAR 2 (93-94)	YEAR 3 (94-95)	YEAR 4 (95-96)
Sept. 27, 1992	Sept. 18, 1993	Sept. 17, 1994	Nov. 10, 1995
Oct. 31, 1992	Oct. 16, 1993	Sept. 23-25, 1994	March 14-14, 1996
Nov. 22, 1992	Nov. 7, 1993	Oct. 27, 1994	April 4-6, 1996
Dec. 17, 1992	Nov. 16, 1993	Nov. 12, 1994	April 28-29, 1996
Jan. 30, 1993	Dec. 20, 1993	Nov. 17-18, 1994	May 25, 1996
Feb. 19-20, 1993	Feb. 6, 1994	Dec. 30, 1994	June 23, 1996
Feb. 28, 1993	March 14, 1994	Feb. 27-28, 1995	
March 12, 1993	March 29, 1994	March 1, 1995	
March 18-19, 1993	April 2-3, 1994	March 12, 1995	
April 2-4, 1993	April 15-16, 1994	March 20-24, 1995	
April 9-10, 1993	April 26, 1994	March 26, 1995	
April 18, 1993	May 18, 1994	April 8, 1995	
April 30, 1993	June 17-18, 1994	April 11-13, 1995	
May 1-2, 1993	July 20, 1994	April 19-20, 1995	
May 11-13, 1993	Aug. 19-21, 1994	May 13, 1995	
May 17, 1993	Aug. 27, 1994	June 17, 1995	
June 11, 1993		July 23, 1995	
June 25-26, 1993		Aug. 5, 1995	
July 2, 1993			
July 10-11, 1993			
Aug. 6-7, 1993			

The population site encompasses a total area of approximately 25 acres. The site was arbitrarily divided into nine areas, designated as Areas A - I (Appendix II). Area A consists of a flat, open, grassy area at the south east end of the site adjacent to the intersection of Kingston Lane and Hilltop Blvd. Area B consists of open foredunes. Area C is an open area located at the top of the dunes. Area D consists of a grassy area interspersed with shrubs which slopes

from the top dunes down to Live Oak Drive. Area E consists of an open, wallow area surrounded by dunes. Area F consists of low foredunes with dense grass. Area G consists of a flat, grassy area located at the west end of the site along Hilltop Blvd. near Jamaica Lane. As a result of construction of an oil well, Area H now consists of a flat, open area bordered by shrubs located in the central area of the population site adjacent to Live Oak Drive. Area I consists of a small, flat, grassy area adjacent to Hilltop Blvd.

The number of *A. macrocarpa* plants in the population was determined by counting the plants growing in each mapped Area. Plants in the population were counted on the following dates:

YEAR 1 (92-93)	YEAR 2 (93-94)	YEAR 3 (94-95)	YEAR 4 (95-96)
Sept. 27, 1992	Sept. 18, 1993	Sept. 17, 1994	
Oct. 31, 1992	Oct. 16, 1993	Oct. 27, 1994	
Nov. 22, 1992	Nov. 16, 1993	Nov. 12, 1994	
Dec. 17, 1992	Dec. 20, 1993	Dec. 30, 1994	
Jan. 30, 1993			
Feb. 20, 1993	Feb. 6, 1994	Feb. 28, 1995	
March 18, 1993	March 14, 1994	March 21, 1995	March 14, 1996
April 30, 1993	April 26, 1994	April 19, 1995	April 28, 1996
May 12, 1993	May 18, 1994	May 13, 1995	
June 25, 1993	June 17, 1994	June 17, 1995	
July 10, 1993	July 20, 1994	July 23, 1995	
Aug. 6, 1993	Aug. 27, 1994	Aug. 5, 1995	

### Characterization of Climatic Features Associated with the Habitat

Initially, temperature and soil moisture readings were taken when the population was monitored. This procedure provided very limited information because data were collected only once a month. In an effort to obtain a more meaningful characterization of climatic features of the habitat, precipitation and temperature data was obtained from the National Climatic Data Center. Data from Madisonville, Texas was obtained since data was not available for Hilltop Lakes. Madisonville is located approximately 20 miles from Hilltop Lakes and is the closest site for which the National Climatic Data Center has available data. Monthly and annual mean maximum temperature, mean minimum temperature, mean temperature,

and precipitation for 1992, 1993, and monthly data from January through August of 1994 were obtained.

### Reserve Germ Bank/Cultivated Population

During the April to August, 1992 segment of this project a repository for seed and cultivated plants was established at the SWT Biology greenhouses, located on the campus of Southwest Texas State University, San Marcos. Plants transplanted from the field and grown from seed are housed in pots in the greenhouse at SWT. These plants are maintained and monitored by research and greenhouse staff.

Seventeen *A. macrocarpa* plants, in the seedling stage, were collected from the field in October 1992, transferred to pots containing sand from the Hilltop Lakes population site, and moved to SWT. These plants were then transplanted to the outdoor bed.

Seed collected from the field and seed produced by plants grown in the greenhouse and outdoor bed are stored under refrigerated conditions (5°C) or room temperature at SWT.

The following steps are being taken to maintain a genetically diverse genome: 1) the existing plants are from two different populations (Leon County and Freestone County); 2) seed resulting from cross pollination between different plants of a single population is then germinated to increase the number of plants under cultivation; 3) to conserve ecotypic and local population diversity plants from different populations are not inter-crossed; 4) vegetative propagation is kept to a minimum; 5) other species of *Abronia* will not be introduced into the SWT holdings so that the genetic identity of *A. macrocarpa* will not be eroded by hybridization; 6) if new populations are discovered, plants from the new sites will be added to the refugium which will assure an even greater variability of genome types.

Mercer Arboretum did not collect plant specimens (achenes, plants, or cuttings) from wild populations of *A. macrocarpa* in 1993 or 1994 (per. telephone conversation with Mr. Greg Wieland, botanist at Mercer Arboretum). On April 20, 1995 Mr. Wieland met with Williamson at the Hilltop Lakes population to collect plant cuttings. Approximately 12 small stem cuttings

were taken and are under cultivation at Mercer Arboretum. Achenes collected from the field in this study have been sent to Mercer Arboretum to increase the genetic repository of *A. macrocarpa* maintained at that facility. Deposition of plant material obtained in this study with Mercer Arboretum will prevent duplication in efforts and overcollection of specimens from the field (see Appendix IV).

### **Associated Species**

Plants growing in association with *A. macrocarpa* at the Hilltop Lakes population site have been collected and identified (Correll and Johnston, 1979). Herbarium specimens were prepared and the voucher specimens are deposited in the Southwest Texas State University Herbarium (SWT).

### **Floral Morphology and Maturation**

Plants were monitored in the field to document floral morphology and maturation. Stigmas were tested to determine period of receptivity using neutral red stain (Vogel, 1962) and with a 3% hydrogen peroxide solution (Kearns and Inouye, 1993). Neutral red stain in DI water (1:10,000) was applied to stigmas of flowers opening from the first through the fifth day of blooming to determine the length of time stigmas remain receptive. Receptive stigmas stain red using this technique. Hydrogen peroxide tests were performed to determine more exact timing of peak periods of stigma receptivity. The 3% hydrogen peroxide was applied to stigmas on an periodic basis after flowers opened on the first and second days a flower bloomed. The stigmas were observed with light microscopy for the presence of bubbling action on the stigmas to detect the peak time of receptivity. Bubbling action on the stigma provides evidence of peroxidase activity and is used as indication of inferred receptivity.

The number of flowers per inflorescence ( $n = 54$  inflorescences) was counted. Flowers ( $n = 250$ ) were measured to determine floral tube length, position of stamens and stigma, stigma

length, and style length. Measurements were taken of anthocarp ( $n = 400$ ) length and width and achene ( $n = 244$ ) length and width.

### Stigmatic Secretions

Stigmatic secretions were chemically analyzed to detect presence of carbohydrates, lipids, and proteins. The presence of carbohydrates in the secretions was tested by placing wet stigmas on microscope slides, adding a few drops of concentrated  $H_2SO_4$  and then a few drops of 5% phenol (Schemske et al., 1978; Casper and La Pine, 1984). The solution will turn orange if sugars are present. To test for the presence of lipids, stigmas wet with stigmatic secretions, were placed on microscope slides, drops of Sudan black B solution, which will stain lipids black, were applied and the stigmas were examined using light microscopy (Baker and Baker, 1975; Ciampolini et al. 1990). A spot staining technique was used to test for the presence of proteins (Baker and Baker, 1975). Stigmas with secretions were placed on Whatman number 1 chromatography paper and 0.1% bromophenol blue in methanol was applied and left for one hour. Papers were then rinsed in 5% acetic acid for 15 to 30 minutes and dried. Development of a blue color is used as indication of the presence of protein.

### Type of Stigma

Stigma surface proteins of the pellicle were examined to determine if they form a continuous hydrated layer indicative of a dry stigma or occur as a component of the exudate denoting a wet stigma (Shivanna and Sastri, 1981). Fresh stigmas, stained and whole mounted in 50% glycerin, were used for cytochemical localization of stigma-surface non-specific esterases. Non-specific esterase activity was detected using alpha-naphthyl acetate as the substrate in a coupling reaction with fast blue B salt (Pearse, 1972). Distribution of the reaction product provides an accurate indication of the location of the enzyme. Controls were run, omitting the substrate from the reaction mixture.

### Nectar Analysis

Nectar was collected from flowers of ten plants and sugar content analyzed using a Fisher High Contrast Hand Refractometer.

### Pollen Viability

The pollen viability of five *A. macrocarpa* plants grown in the greenhouse was tested during the April - August, 1992 segment of the study. The pollen viability of five *A. macrocarpa* plants from the field was tested during the September 1992 - August, 1993 segment of the study. The pollen from two to five flowers of each plant was placed on a slide and immersed in a drop of 1% aniline blue in lactophenol stain (Radford, et al., 1974). The slides were stained for two to three hours then examined using an AO light microscope. Pollen that stained blue was considered viable. For the greenhouse plants, twenty random counts were made from each slide to determine the percentage of viable pollen. Each random count recorded the number of viable pollen grains and the number of non-viable pollen grains present in the field of view at 40X magnification. For the field plants, all pollen on each slide was observed and recorded as viable or non-viable.

### Microgametogenesis

Ontogeny of the microgametophyte was examined by studying the nuclear details of preserved pollen grains to determine the developmental stage (binucleate or trinucleate) at which the pollen is shed. The flowers were first cleared in a 10% solution of chloral hydrate for 48 to 72 hours. Pollen grains were then mounted in a drop of acetocarmine, warmed gently over a flame, and examined using a Zeiss light microscope.

### Pollination

*Abronia macrocarpa* plants, at anthesis, were observed in the field during May of 1991, March and April of 1992, and March, April and May of 1993. Flowers were observed prior to

opening, during opening until several hours after dark and again just prior to dawn until flowers closed early in the morning. A total of 28 days were spent in observation with two to four observers in different areas of the population during each observation period. Visitation and movements of floral visitors was recorded. Potential pollinators were captured and transported to the Department of Entomology at Texas A&M University for identification. Floral visitors were examined for the presence of pollen using light microscopy.

### **Breeding System**

Greenhouse plants were experimentally crossed in one of the following ways (Faegri and van der Pijl, 1979):

1. Autogamy - Plants were self-pollinated by transferring pollen from the anther to the stigma of the same flower.
2. Geitonogamy - Plants were pollinated by transferring pollen from one flower to the stigma of another flower in the same inflorescence on the same plant or by transferring pollen from one flower to the stigma of another flower in a different inflorescence on the same plant.
3. Xenogamy - Plants were cross-pollinated by transferring the pollen from one plant to the stigma of a different plant.

Flowers were pollinated using the following technique. The portion of the floral tube to which the anthers are adnate was removed by cutting the tube 0.6-1.0 cm from the base with scissors. This exposed the stigma. Next pollen from the same or a different flower, as outlined above, was smeared onto the receptive stigma. Plants were monitored for development of fruit. Development of achenes signifies that fertilization resulted from a particular type of pollination. If successful pollination and fertilization does not occur the flowers wither and abscise from the plant.

## Barrier to Selfing

A study was conducted to determine the barrier to selfing operating in *A. macrocarpa*. Plants were experimentally self- and outcrossed and the pollen and pollen tubes were examined using fluorescence microscopy.

Flowers of five greenhouse grown plants and twenty-nine bagged plants growing in the field were hand-pollinated. Field plants with developing floral buds were covered with bags constructed of a double layer of bridal veil material. The plants were enclosed in tomato cages with the bags placed over the cages and secured at the base to exclude potential pollinators. Bagging was not deemed necessary for greenhouse grown plants since potential pollinators are excluded from the greenhouses.

The following three types of experimental crosses were made:

1. Autogamous cross - Plants were self-pollinated by transferring pollen from the anther to the stigma of the same flower.
2. Geitonogamous cross - Plants were pollinated by transferring pollen from one flower to the stigma of another flower on the same plant.
3. Xenogamous cross - Plants were cross-pollinated by transferring the pollen from one plant to the stigma of a different plant.

It was necessary to emasculate the flowers to preclude self-pollination and thereby control the type of cross. This was accomplished by removing the portion of the floral tube to which the anthers are adnate. Then pollen from the same or a different flower, as indicated above, was smeared onto the receptive stigma. Following pollination, flowers were fixed for use in studying pollen germination and pollen tube growth.

Flowers were fixed at 1, 2, 3, 6, 12, 24, 48, and 72 hours following hand-pollination in Carnoy's solution (Smith, 1991; Waser and Price, 1991) or in 70% ethanol (Mulcahy and Mulcahy, 1982; Aizen et al., 1990). Fixed tissues were treated with 1N NaOH at room temperature for approximately one hour to soften and clear the styles (Kho and Baër, 1968). A 0.01% decolorized aniline blue solution was prepared by dissolving aniline blue dye in

$K_2HPO_4$ , which after one or two hours at room temperature becomes colorless (Currier, 1957). Tissues were rinsed in water and stained for 24 to 48 hours in the decolorized aniline blue. Then tissues were mounted in a drop of the stain on a microscope slide and squashed with a coverslip. Tissues were viewed under a Zeiss epifluorescent microscope using a blue excitation, yellow transmittance filter. Pollen grains and pollen tubes with callose plugs, deposited periodically as the pollen tubes grow down the style, fluoresce a yellow color using this particular filter combination. The number of pollen grains adhering to the stigma, number germinating to form pollen tubes, lengths of pollen tubes, and the region of the carpel where the tubes stop growing were recorded. Photomicrographs were taken using a Minolta X700 35mm camera with Kodak Ektachrome Tungsten film (ASA 64).

Statistical analyses were performed using the Statistical Analysis System (SAS) General Linear Models (GLM) package. Differences in percentage pollen germination among the three treatments (autogamous, geitonogamous, and xenogamous crosses) within each of the two locations of the plants (greenhouse and field) and between the locations were tested for significance by ANOVA. Differences in mean pollen tube length among autogamous, geitonogamous, and xenogamous crosses within the field plants and between autogamous and xenogamous crosses within the greenhouse plants were also tested for significance by ANOVA. Mean pollen tube length data from geitonogamous crosses of greenhouse grown plants was not included in the statistical analysis because the data set was incomplete. After ANOVA means were compared with REGWF Multiple F comparison test for variable mean percentage germination and for mean tube length at the 0.05 level.

### Reproductive Capacity

Fifteen plants in Areas A and B were randomly selected, tagged, and monitored from March until August, 1992. Monitoring continued on three of these plants from September 1992 through August 1993. One of the plants continued to be monitored through August 1995. The remaining plants could not be identified because mowing or other factors resulted in removal

of the plant tags. Additionally, twenty-one new plants were randomly selected, tagged and monitored each month from October 1992 through August 1995. The growth and reproductive capacity of each plant was studied by measuring height of the plant and counting number of leaves, number of inflorescence buds, number of inflorescences at anthesis, and number of inflorescences with fruits.

During the blooming season of 1993, 1994, 1995, and 1996 twenty-five plants in each of six areas were randomly selected. The number of inflorescences at the following developmental stages: buds, flowers near anthesis, flowers at anthesis, post anthesis but before development of enlarged anthocarps, post anthesis but withered without fruit set, and with fruit set produced by each of the 150 plants was counted. Data was collected on the following dates:

YEAR 1 (1993)	YEAR 2 (1994)	YEAR 3 (1995)	YEAR 4 (1996)
March 19, 1993	March 14, 1994	February 27, 1995	March 14, 1996
April 3, 1993	March 29, 1994	March 22, 1995	April 28, 1996
April 10, 1993	April 26, 1994	April 19, 1995	
April 16, 1993	May 19, 1994	May 13, 1995	
May 2, 1993			
May 13, 1993			

The percentage of individuals in the population at the reproductive stage was examined monthly during the 1994-1995 growing season. The total number of plants producing inflorescences was counted and the percentage calculated by dividing the number of reproductive plants by the total number of plants in population then multiplying by 100.

### Fruit/Seed Set

Intact, mature anthocarps were collected from plants distributed throughout the population in May of 1991 (1,630 anthocarps collected), May of 1992 (901 anthocarps collected), April of 1993 (4,780 anthocarps collected), May of 1993 (1,927 anthocarps collected), April of 1994 (2,016 anthocarps collected), and April of 1995 (1,008 anthocarps collected). The papery part of the anthocarps were dissected and the number containing achenes was recorded. An

achene is a simple, dry fruit. The seed is attached to the pericarp by the funiculus. The fruit is indehiscent, therefore fruit set and seed set are used synonymously.

Three thousand achenes were retained for studies on seed viability and germination, and to maintain a genetic repository at both SWT and Mercer Arboretum. The remaining achenes (n=3,848) were returned to the field to minimize impact to the species. Anthocarps (n=11,703) were also collected from plants experimentally crossed in the greenhouse and from plants grown in the outdoor bed at SWT, dissected and the number containing achenes was recorded.

The number of seed (n = 4,008 total from all field collections 1992 - 1995) retained falls well below the 10% rule-of-thumb upper limit as advised by the U.S. Fish and Wildlife Service. The plants (n = 17 plants) grown in the outdoor bed at SWT produced 4,934 achenes in 1993, an average of 290 seed per plant. These numbers can serve as an estimate of reproductive potential. Plants in the field, however, may not reach full reproductive potential due to competition with associated species, browsing damage, and seed predation. Considering these factors and given a seed viability of 50%, it is estimated that a population of 2,000 individuals would produce well over 30,000 viable seed in a given year. Hence, the 3,000 seed retained fits within recommended guidelines.

### Seed Viability

One hundred achenes, collected from the Hilltop Lakes population in 1993 and stored at room temperature for two months, were used to test for seed viability using a 1% Tetrazolium stain (Grabe, 1970; Copeland, 1981). The achenes were scarified with a one part bleach to four parts water solution for 15 minutes. This procedure removed the pericarp wall. Seeds were then placed in an open faced petri dish, covered with nylon, and placed in running tap water overnight. The seeds were then rinsed with distilled water. The seeds were left in the distilled water overnight. To test for viability, seeds were cut in half and treated with tetrazolium stain prepared by adding 1gm of tetrazolium to one hundred milliliters of tap water at pH 7.0.

Seeds were left in the stain for two and one half to three hours. Embryos that stained red were considered viable.

### Seedling Recruitment

Seedlings in undisturbed areas of the population were numbered and tagged by placing a plastic plant stake next to them. Twenty-six of these seedlings were marked on 10 April 1992, 207 seedlings were marked within two weeks from 2 May 1993 to 13 May 1993, yielding a total of 233 marked individuals. Presence of vegetation, number of leaves, leaf length and width, plant height, and presence of reproductive organs were recorded on the following dates:

Year 1 (1992)	Year 2 (1993)	Year 3 (1994)	Year 4 (1995)
May 14, 1992	April 30, 1993	February 5, 1994	February 28, 1995
May 13, 1993	March 14, 1994	March 22, 1995	
April 26, 1994	April 9, 1995		
May 19, 1994			
June 17, 1994			
October 28, 1994			
December 30, 1994			

During the study, some markers were pulled out of the soil as a result of mowing activity, hikers, deer or other animals, etc., consequently, measurements were obtained only from plants with intact markers. In cases where the plant stakes were missing/pulled out of the soil in the summer, fall, or winter, seedlings were considered dead if non-emergent during the previous March and April. When insufficient data were obtained (i.e. missing markers during March or April) the seedling survivorship/mortality for these individuals was inconclusive and, therefore, not used in the seedling survivorship calculation.

The seedling survivorship ratio was calculated using the equation:

$$l_x = \frac{N_x}{T_x}$$

where:  $l_x$  = seedling survivorship  
 $N_x$  = number of individuals surviving  
 $T_x$  = number of total individuals

Growth rates of seedlings were examined by calculating vegetative measurements such as mean number of leaves/plant, mean seedling height, mean leaf length and width, and also reproductive measurements such as presence of buds, inflorescences at anthesis, and inflorescences with fruit were also noted. Data were compiled using Microsoft EXCEL for the Macintosh Version 4.0.

Standard deviation of a sample of the population was calculated using the following equation:

$$s = \sqrt{\frac{n \sum x^2 - (\sum x)^2}{n(n-1)}}$$

where:  $s$  = standard deviation  
 $n$  = size of the sample  
 $x$  = measurement in the sample or datum value

Standard error (sample standard error of the mean) was calculated using the equation:

$$s_{\bar{x}} = \frac{s}{\sqrt{n}}$$

where:  $s_{\bar{x}}$  = standard error  
 $s$  = standard deviation  
 $n$  = sample size

### Herbivory

The extent of herbivory was assessed by recording number of leaves, branches, and inflorescences which had been grazed on the plants used to study reproductive capacity.

### **Mycorrhizal Association**

Roots were collected, fixed in FAA, stained using trypan blue, and examined with light microscopy to determine if the plant exhibits a mycorrhizal association.

### **Educational Program**

A program to educate the public about the importance of conserving this endangered species was initiated. The program includes presentations at scientific meetings by the primary investigator and graduate assistants, publication in scientific journals, and contributions to photographic exhibitions and magazine articles.

## **RESULTS**

### **Surveys for Additional Populations**

*Abronia macrocarpa* is known to occur in three counties (Freestone, Leon, and Robertson) in the Post Oak Savannah Woodlands region of eastern, central Texas (Figure 1). When the taxon was originally described in the early 1970's (Galloway, 1972), only one population was known. The type locality is a sand-dune area, approximately nine miles northwest of Normangee, Texas. Surveys conducted by Orzell in 1988 (Kennedy, et al., 1990), Yantis in 1990 (Kennedy, et al., 1990) and 1994, and Williamson in 1995 have identified nine additional populations, three more in Leon County, one in Freestone County, and five in Robertson County (Appendix III).

Nine of the ten known populations were visited in March of 1995 and estimates of the population sizes were made (Table 1). Six populations in Leon Co. and the Freestone Co. population were visited by Williamson and Janssen in early April, 1996. March to early April is the opportune time to accurately determine population size since studies of phenology show the largest number of plants to be present at that time of the growing season. It is expected, based on studies of reproductive capacity, that approximately 10% of the individuals in a

population tend to be in the reproductive stage in March. It was not feasible to count each individual plant therefore the number of individuals producing inflorescences was counted and the total population size was extrapolated using the 10% figure.

Table 1. Estimated size of the ten known populations of *A. macrocarpa*. Estimation of size made in March 1995 unless otherwise noted.

<u>Population (year discovered)</u>	<u>Current number of plants in population</u>
No. 1 Hilltop Lakes, Leon Co. (1970 by Correll)	8,500
No. 2 Leon Co. (1994 by Yantis)	1,000
No. 3 Leon Co. (1995 by Williamson)	500
No. 4 Leon Co. (1995 by Williamson)	5,000
No. 5 Camp Creek Barrens, Robertson Co. (1988 by Orzell)	650
No. 6 Robertson Co. (1994 by Yantis)	2,000
No. 7 Robertson Co. (1994 by Yantis)	600
No. 8 Robertson Co. (1994 by Yantis)	10,000
No. 9 Robertson Co. (1994 by Yantis)	5,000
No. 10 Snyder Lake, Freestone Co. (1990 by Yantis)	2,000-3,000 (est. in 1992)

### Population Monitoring and Mapping

The number of *A. macrocarpa* plants in the Hilltop Lakes population was counted from September, 1992 to May, 1995 (Figures 3, 4) and in March and April, 1996. During the first year of observation, the highest population count ( $n=2,531$ ) was recorded in February, 1993; in the second year, the population reached a peak size ( $n=3,560$ ) in March, 1994. During 1995, the population reached a size of 8,382 in March. This is the largest size ever documented for the population (Table 2). Population size was 4,668 in March, 1996. Three of the areas (A, D and G) were subjected to mowing in 1992, but not in 1993. More plants were present in each of these areas in 1993 than in 1992 (Figures 5 and 6), indicating that mowing early in the spring (March - April) adversely impacts population numbers.

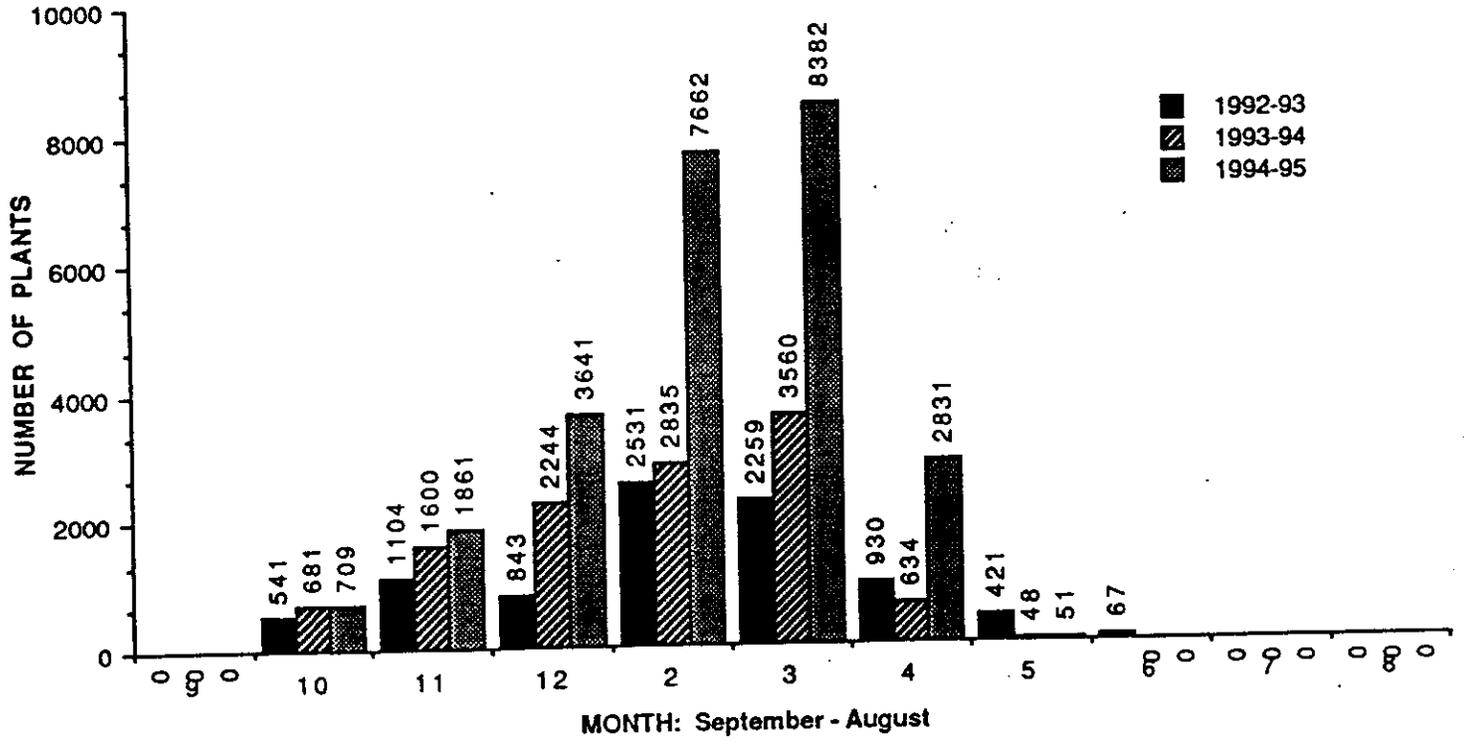


Figure 3. Number of *A. macrocarpa* plants counted in the Hilltop Lakes population monthly from September, 1992 through May, 1995.

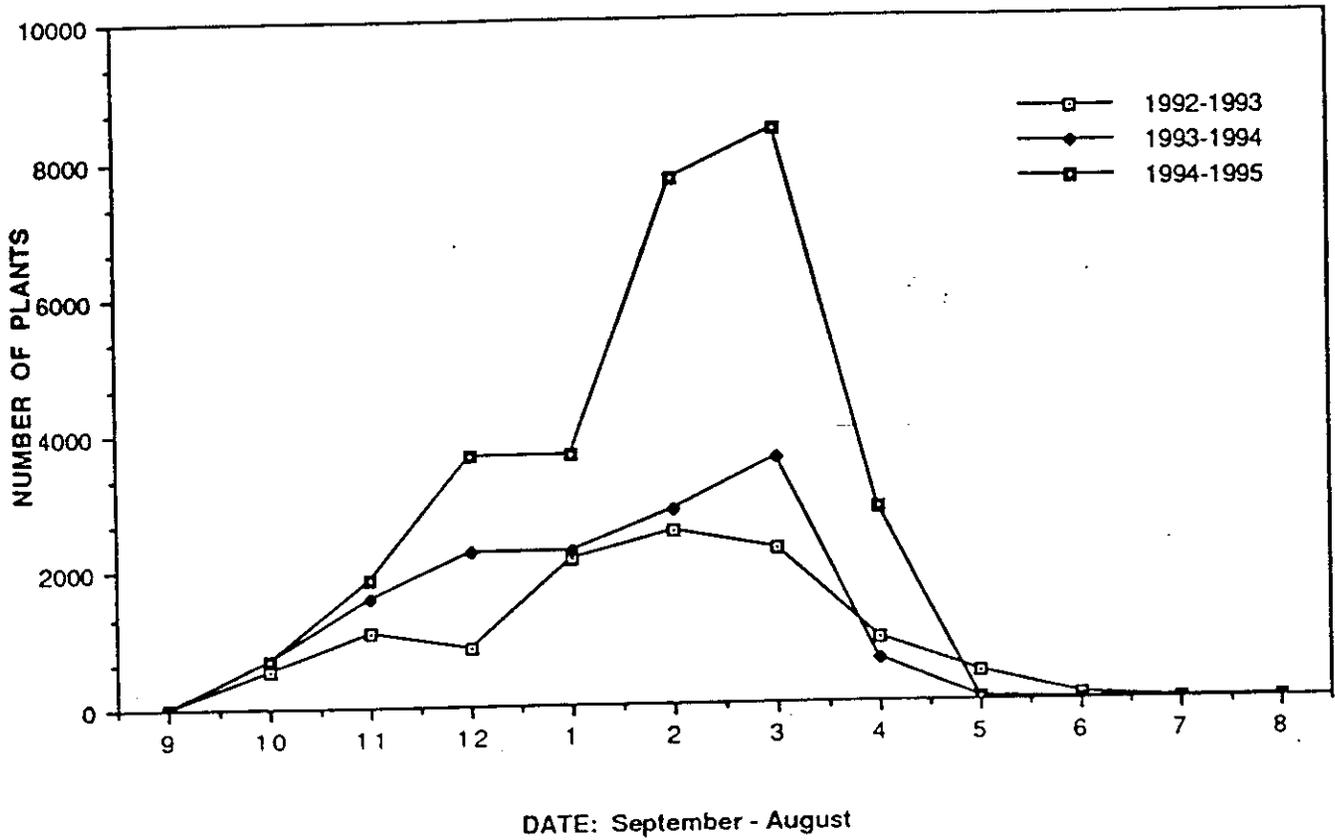


Figure 4. Number of *A. macrocarpa* plants counted in the Hilltop Lakes population monthly from September, 1992 through May, 1995.

Fig. 5. Number of *Abronia macrocarpa* plants in Areas A, D and G counted in the Hilltop Lakes population during selected months in 1992 and 1993.

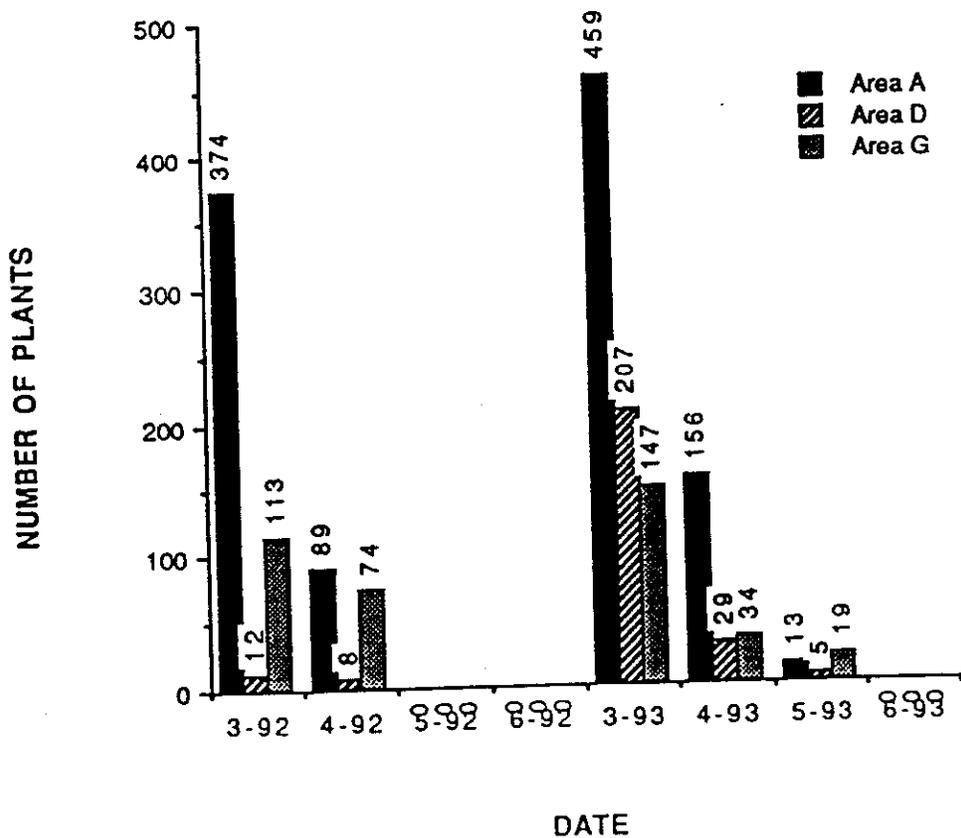
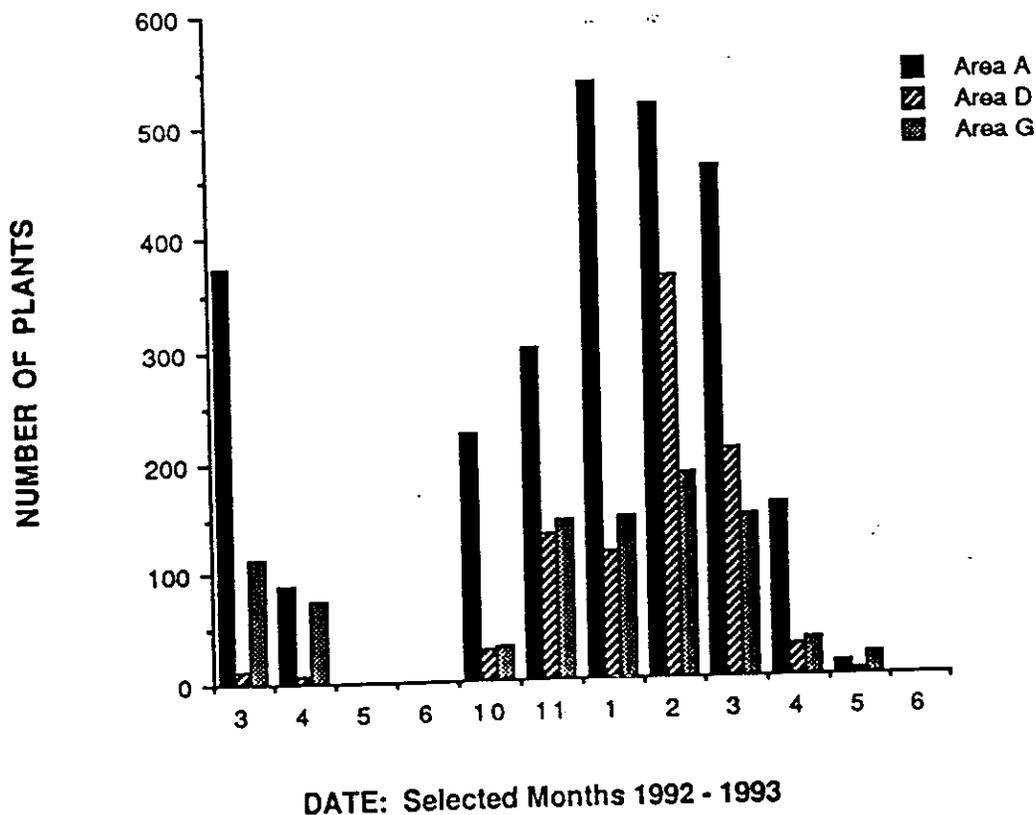


Fig. 6. Number of *Abronia macrocarpa* plants in Areas A, D and G counted in the Hilltop Lakes population March - June, 1992 and March - June, 1993.

Table 2. Fluctuation in population numbers at Hilltop Lakes - area of ca. 25 acres

Year	(Observer)	Number of plants in Population
1971	(Galloway)	fewer than 200
1979	(Turner)	about 500
1984	(Ajilvsgi)	none
1985	(Poole)	15 in early June
1986	(Orzell)	about 56
1988	(Orzell)	several hundred in early May
1989	(Orzell)	none
1990	(Young)	215
1991	(per. obser.)	approximately 100 in early May
1992	(per. obser.)	over 1,799 in mid March
1993	(per. obser.)	2,531 in mid February
1994	(per. obser.)	3,560 in mid March
1995	(per. obser.)	8,382 in mid March
1996	(per. obser.)	4,668 in mid March

### Characterization of Climatic Features Associated with the Habitat

Monthly and annual mean maximum temperature, mean minimum temperature, mean temperature, and precipitation for 1992, 1993, and monthly data from January through August of 1994 obtained from the National Climatic Data Center are shown in Figures 7, 8 and 9. Temperature data was recorded in °F. Temperatures were relatively constant among the years (Figures 7, 8). Annual mean temperature was 68.3 in 1992 and 67.6 in 1993. The annual mean maximum temperature was 79.2 in 1992 and 78.5 in 1993. The annual mean minimum temperature was 57.3 in both 1992 and 1993. Temperature was slightly higher overall in 1992 (0.5 departure from normal) than in 1993 (-0.4 departure from normal).

Total precipitation was 53.71 inches in 1992 and 53.72 inches in 1993. Both years received more precipitation than normal (12.65 departure from normal in 1992 and 12.10 departure

from normal in 1993). Precipitation from January through August of 1994 has been 28.02 inches. The rainfall patterns varied among years (Figure 9). Comparison among the years shows that precipitation was higher in February, July, November and December of 1992, higher in January, March, April, May, June, and October of 1993, and higher in August of 1994.

### Reserve Germ Bank/Cultivated Population

Seeds are currently maintained in storage and plants are in cultivation in an outdoor planting bed at SWT. The plants are healthy, with a pollen viability of approximately 95%, and readily set viable seed when cross pollinated. Mortality of greenhouse plants (N = 25) following an aphid infestation may have been the result of chemical spraying to eliminate the insects. There has also been mortality of greenhouse plants (N = 15) apparently by natural aging and senescence. Phenology studies of field plants provides some evidence to suggest that the species may be a relatively short-lived perennial. This refugium will continue to be maintained.

Achenes collected from the field in this study have been deposited at Mercer Arboretum to increase the genetic repository of *A. macrocarpa* maintained at that facility. Mercer Arboretum is also studying vegetative propagation via stem cuttings. Efforts to work in concert with Mercer Arboretum have been successful in preventing duplication in efforts and overcollection of specimens from the field.

### Associated Species

Plants growing in association with *A. macrocarpa* at the Hilltop Lakes population site are listed in Table 3. Percent cover and density (Barbour, et al., 1987) have not been calculated, however all of the species listed in Table 3 appear to be relatively common. This list of associated species represents those species collected during September 1992 through August 1995 for which herbarium specimens have been prepared.

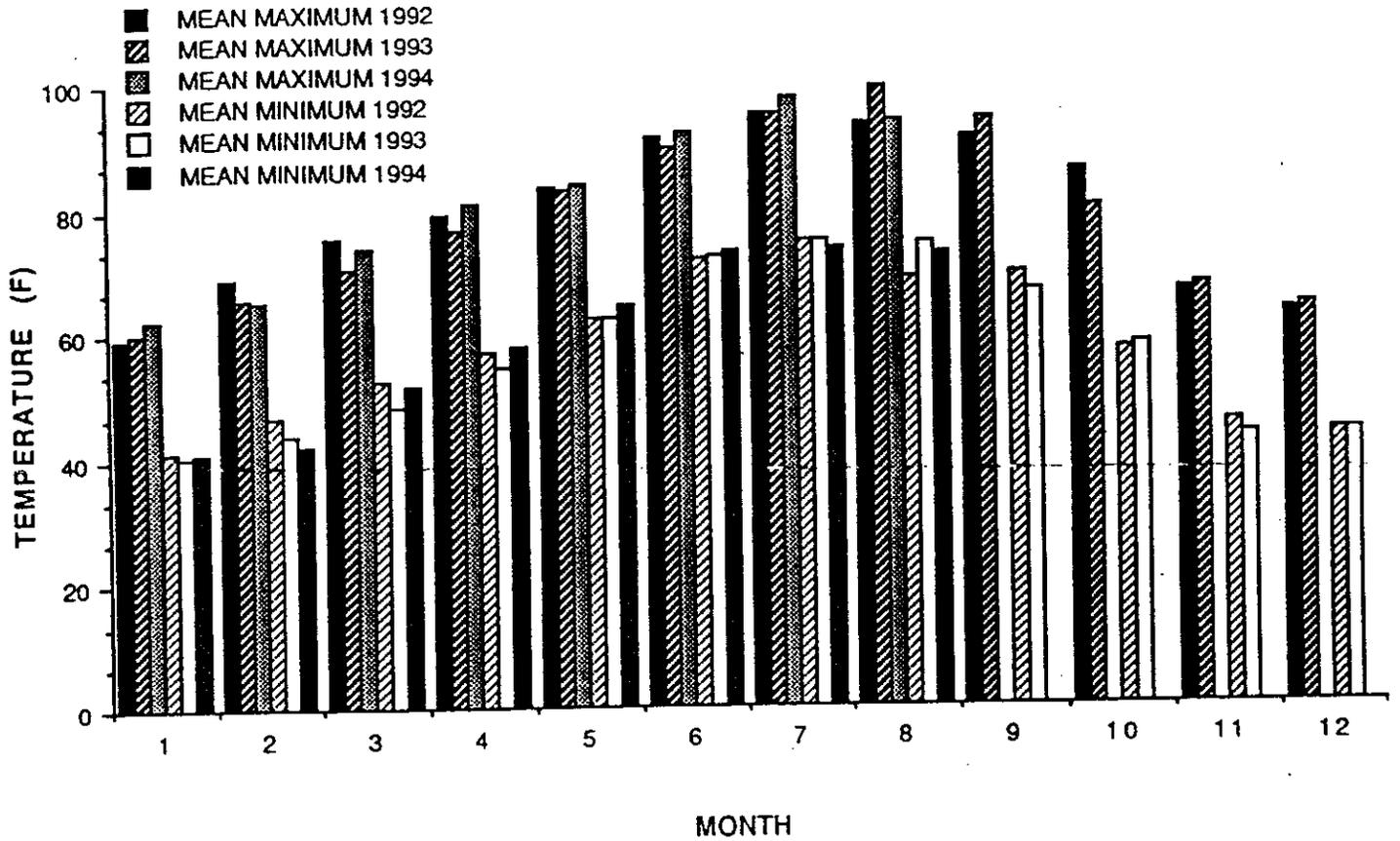


Figure 7. Mean monthly temperatures recorded at Madisonville, Texas.

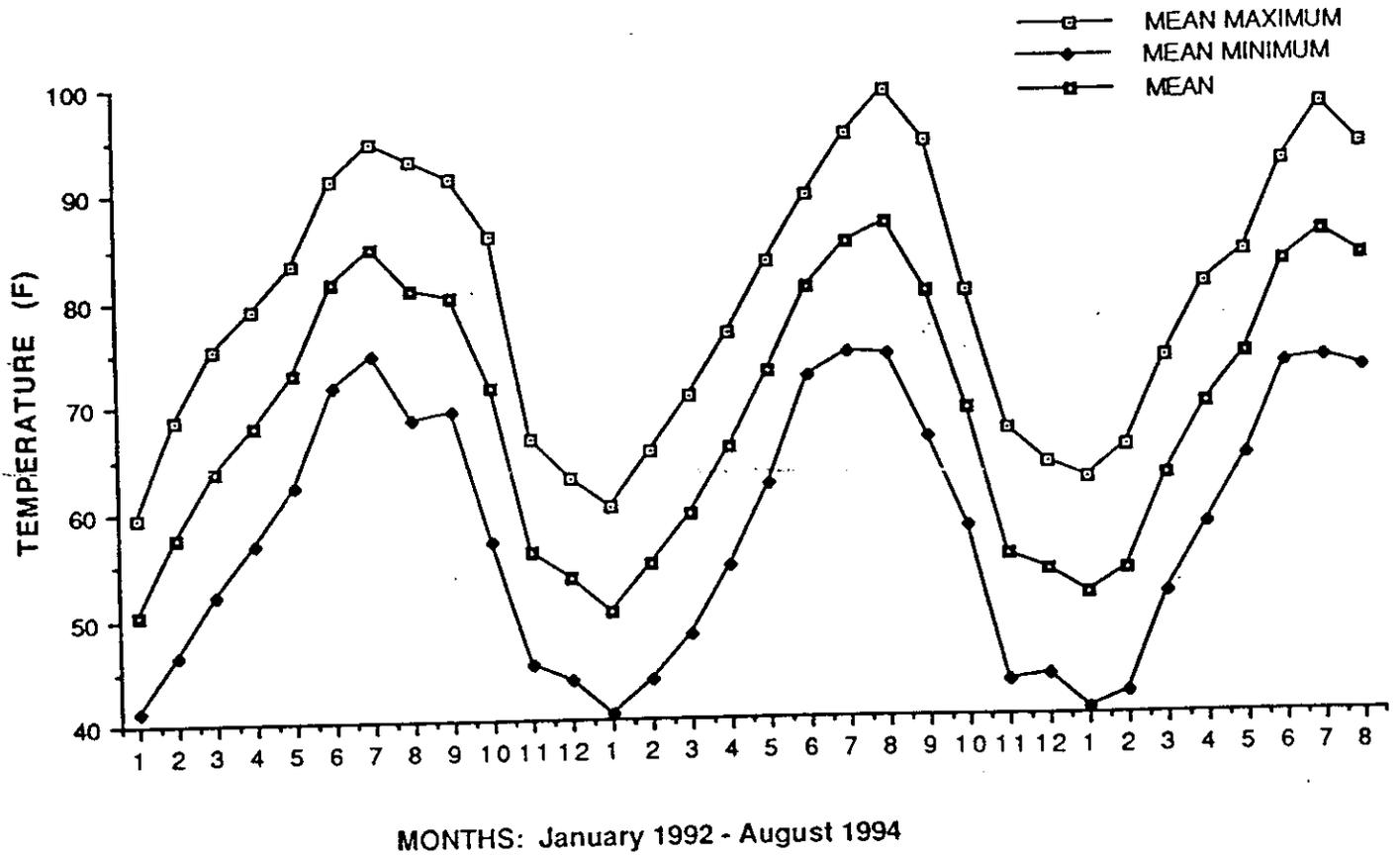


Figure 8. Mean monthly temperatures recorded at Madisonville, Texas.

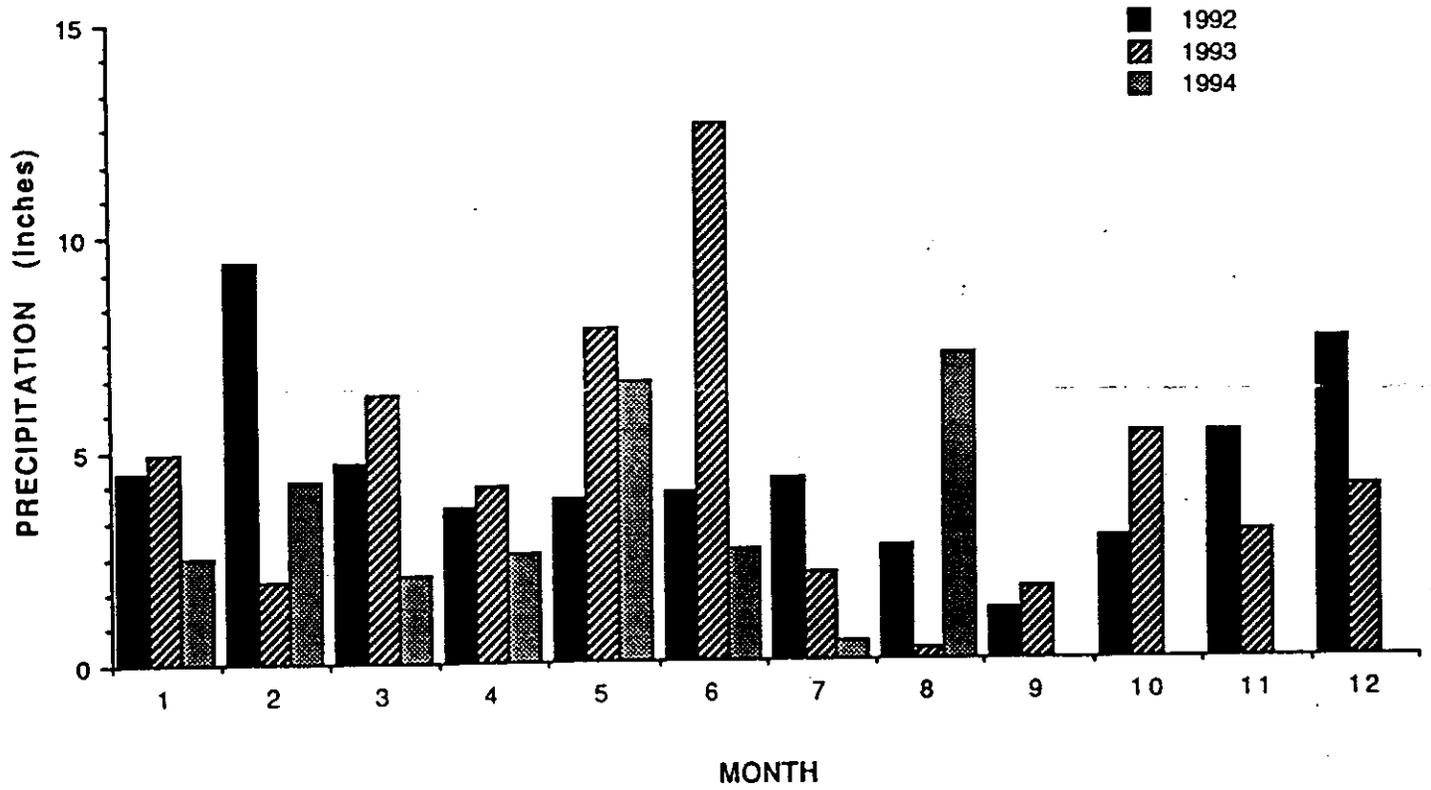


Figure 9. Monthly precipitation recorded at Madisonville, Texas.

Table 3. List of associated species collected at the Hilltop Lakes population site

Scientific Name	Common Name	Family Name
<i>Froelichia drummondii</i> Moq.	Snake-cotton	Amaranthaceae
<i>Spermolepis echinata</i> DC.	Scale-seed	Apiaceae
<i>Apocynum cannabinum</i> L.	Indian Hemp	Apocynaceae
<i>Ilex vomitoria</i> Ait.	Yaupon	Aquafoliaceae
<i>Asclepias amplexicaulis</i> Sm.	Butterfly-weed	Asclepiadaceae
<i>Asclepias tuberosa</i> L.		Asclepiadaceae
<i>Matelea cynanchoides</i> (Raf.) Woods.		Asclepiadaceae
<i>Ambrosia artemisiifolia</i> L.	Short Ragweed	Asteraceae
<i>Ambrosia psilostachya</i> DC.	Western Ragweed	Asteraceae
<i>Aphanostephus ramosissimus</i> DC.	Lazy Daisy	Asteraceae
<i>Coreopsis tinctoria</i> Nutt.	Tick-seed	Asteraceae
<i>Gallardia pulchella</i> Foug.	Indian Blanket	Asteraceae
<i>Helenium amarum</i> Raf.	Bitterweed	Asteraceae
<i>Heterotheca latifolia</i> Buckl.	Camphor Weed	Asteraceae
<i>Heterotheca pilosa</i> Nutt.		Asteraceae
<i>Hymenopappus artemisiaefolius</i> DC.		Asteraceae
<i>Palafoxia Hookeriana</i> T.&G.		Asteraceae
<i>Rudbeckia hirta</i> L.		Asteraceae
<i>Senecio ampullaceus</i> Hook.	Texas Groundsel	Asteraceae
<i>Betula nigra</i> L.	River Birch	Betulaceae
<i>Opuntia compressa</i> (Salisb.) Macbr.	Eastern Prickly Pear	Cactaceae
<i>Polanisia erosa</i> Nutt.	Clammy-weed	Capparidaceae
<i>Arenaria serpyllifolia</i> L.	Thyme-leaved Sandwort	Caryophyllaceae
<i>Cerastium glomeratum</i> Thuill.	Mouse-ear	Caryophyllaceae
<i>Paronychia Drummondii</i> T.&G.		Caryophyllaceae
<i>Stellaria media</i> (L.) Cyr.	Common Chickweed	Caryophyllaceae
<i>Chenopodium ambrosioides</i> L.	Epazote	Chenopodiaceae
<i>Tradescantia occidentalis</i> (Britt.) L.		Commelinaceae
<i>Cuscuta</i> sp. L.	Dodder	Convolvulaceae
<i>Stylisma Pickeringii</i> (Torr.) Gray		Convolvulaceae
<i>Cornus florida</i> L.	Flowering Dogwood	Cornaceae
<i>Juniperus virginiana</i> L.	Eastern Red Cedar	Cupressaceae

<i>Carex</i> sp. L.		Cyperaceae
<i>Cyperus</i> sp. L.	Flatsedge	Cyperaceae
<i>Diospyros virginiana</i> L.	Common Persimmon	Ebenaceae
<i>Vaccinium arboreum</i> Marsh.	Farkleberry	Ericaceae
<i>Cnidioscolus texanus</i> (Muell. Arg.) Small.	Bull nettle	Euphorbiaceae
<i>Croton argyranthemus</i> Michx.	Silver Croton	Euphorbiaceae
<i>Croton Lindheimerianus</i> Scheele.	Croton	Euphorbiaceae
<i>Croton capitatus</i> Michx.	Woolly Croton	Euphorbiaceae
<i>Crotonopsis linerus</i> Michx.	Rush-foil	Euphorbiaceae
<i>Euphorbia cordifolia</i> Ell.	Spurge	Euphorbiaceae
<i>Stillingia sylvatica</i> L.		Euphorbiaceae
<i>Astragalus Nuttallianus</i> A. DC.		Fabaceae
<i>Baptisia Nuttalliana</i> Small.	Wild Indigo	Fabaceae
<i>Cassia fasciculata</i> Greene	Partridge Pea	Fabaceae
<i>Medicago lupulina</i> L.	Black medick	Fabaceae
<i>Medicago lpolymorpha</i> L.	Bur-clover	Fabaceae
<i>Sesbania vesicaria</i> (Jacq.) Ell.	Bagpod	Fabaceae
<i>Vicia</i> sp. L.	Vetch	Fabaceae
<i>Quercus incana</i> Bartr.	Sand Jack	Fagaceae
<i>Corydalis curvisiliqua</i> Engelm.		Fumariaceae
<i>Carya texana</i> Buckl.	Black Hickory	Juglandaceae
<i>Monarda citriodora</i> Cerv.	Lemon Beebalm	Lamiaceae
<i>Rhododon ciliatus</i> (Benth.) Epl.		Lamiaceae
<i>Allium Drummondii</i> Regel.	Wild Onion	Liliaceae
<i>Nothoscordum bivalve</i> (L.) Britt	Crow Poison	Liliaceae
<i>Smilax</i> sp. L.		Liliaceae
<i>Oenothera laciniata</i> Hill.	Cutleaved Primrose	Onagraceae
<i>Argemone</i> sp. L.	Prickly Poppy	Papaveraceae
<i>Phytolacca americana</i> L.	Pokeweed	Phytolaccaceae
<i>Plantago aristata</i> Michx.	Buckthorn	Plantaginaceae
<i>Plantago hookeriana</i> Fisch. & Mey.	Tallow Weed	Plantaginaceae
<i>Plantago patagonica</i> (Nutt.) Gray	Plantain	Plantaginaceae
<i>Bromus unioloides</i> H.B.K.	Rescue Grass	Poaceae
<i>Cenchrus incertus</i> M.A. Curtis.	Coast Sandbur	Poaceae
<i>Dactyloctenium aegyptium</i> (L.) Beauv.	Crowfoot	Poaceae
<i>Dichanthelium</i> sp. Willem.		Poaceae
<i>Stipa</i> sp. L.	Needlegrass	Poaceae
<i>Vulpia</i> sp. C.C. Gmel.		Poaceae

<i>Ipomopsis rubra</i> (L.) Wherry. <i>Phlox Drummondii</i> Hook.	Standing Cypress Phlox	Polemoniaceae Polemoniaceae
<i>Eriogonum multiflorum</i> Benth. <i>Eriogonum</i> sp. Michx. <i>Rumex hastatulus</i> Ell.	Buckwheat Buckwheat Heart Sorrel	Polygonaceae Polygonaceae Polygonaceae
<i>Anagallis arvensis</i> L.	Scarlet Pimpernel	Primulaceae
<i>Rubus trivialis</i> Michx.	Southern Dewberry	Roasaceae
<i>Diodia teres</i> Walt.	Rough Buttonweed	Rubiaceae
<i>Callicarpa americana</i> L. <i>Verbena bipinnatifida</i> Nutt.	American Beautyberry Dakota Vervain	Verbenaceae Verbenaceae
<i>Vitis mustangensis</i> Buckl.	Mustang Grape	Vitaceae

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## Floral Morphology and Maturation

The inflorescence is a capitulum or head typically composed of 25-48 individual flowers (Figure 2). The average number of flowers per inflorescence is 31. Galloway (1972) describes the inflorescence as containing up to 75 flowers, however an inflorescence that large has not been observed in this study. Five greenish-pink bracts subtend the inflorescence (Figure 2). The flowers open centripetally. The perianth consists of five sepals that are pink and look like petals. The flower is apetalous. The sepals are fused, enclosing the ovary at the base and extending to form a narrow floral tube averaging 2.0 cm in length (Figure 2). Floral tube length ranges from 1.1-3.0 cm. There are five free limbs or sepal lobes distally, each is emarginate (Figure 2). Nectar is secreted at the base of the floral tube. There are five stamens positioned above the stigma (Figure 2), approximately 1.9 cm (range of 0.9-2.5 cm) from the base of the floral tube. Filaments are fused to the floral tube. Pollen dehiscence begins the first day the flower is open. The gynoecium consists of a single carpel with one basal ovule. The ovary is superior (Figure 2). Combined stigma and style length ranges from 0.5-1.0 cm, averaging 0.87 cm. The stigma is linear, 0.1-0.2 cm long (averaging 0.14 cm) and covered with papillae.

Neutral red staining reveals that stigmas are most receptive the first day the flower is open. Stigmas continue to be receptive through the fifth day of blooming. Use of 3% hydrogen peroxide applied to stigmas indicates that stigmas are most receptive approximately four to five hours after flowers open on the first day of blooming. The relative amount of bubbling action on the stigma is indicative of the level of receptivity. The extent of bubbling action was greatest from 7:00 to 9:00 pm. The amount of bubbling decreased around 10:00 pm. Very little bubbling activity was observed by 8:00 am. Flowers closed at approximately 9:00 am. The intensity of bubbling action was again high between 7:00 and 9:00 pm the second day a flower opened.

If the flower has been successfully pollinated and fertilized the characteristic anthocarps develop. Anthocarps are initially greenish, turn pink, and at maturation are papery and

brown in color. The base of the calyx, surrounding the ovary develops into the papery part of the anthocarp. Anthocarps are five winged and turbinate (Figure 2). The fruit proper is an achene. The anthocarps measure 0.6-1.7 cm in length, and are 0.2-1.2 cm wide. Average anthocarp length is 1.0 cm and width is 0.6 cm. Achenes are 0.25-0.4 cm long and 0.1-0.2 cm wide. Average achene length is 0.35 cm and width is 0.12 cm.

Flowers open at approximately 3:00 - 4:00 pm (1500-1600 hours) Central Standard Time. A faint floral odor is present once the flowers begin opening. The odor, reminiscent of honeysuckle, increases towards dusk and remains strong during the night. Flowers close at approximately 9:00 - 10:00 am (0900-1000 hours), although they may remain open until noon (1200 hours) on overcast days.

### **Stigmatic Secretions**

Results of chemical analyses indicate that carbohydrates, lipids, and proteins are constituents of the stigmatic secretions. Stigmas tested positive for the presence of sugars by turning orange when treated with concentrated  $H_2SO_4$  and 5% phenol. Stigmas stained black when Sudan black B solution was applied demonstrating the presence of lipids. Stigmas subjected to the spot staining technique developed a blue color indicating that proteins are also present in the secretions.

### **Type of Stigma**

Intense esterase activity was observed on the surface of stigmas subjected to the reaction mixture containing substrate, whereas control stigmas showed no non-specific esterase staining. A thin, continuous hydrated layer of pellicle over the surface of the cuticular layer of the stigmatic papillae was detectable. These features are characteristic of a dry stigma (Heslop-Harrison and Shivanna, 1977; Shivanna and Sastri, 1981).

### Nectar Analysis

Analysis of the nectar using a hand-held refractometer reveals that the sugar content varies from 25% to 29%.

### Pollen Viability

The percentage of viable pollen taken from greenhouse plants (n=6,869 pollen grains observed) ranged from 89% to 100%, with an average viability of 93% (Table 4). The percentage of viable pollen taken from plants in the field (n=7,370 pollen grains observed) ranged from 92% to 98% and averaged 94% (Table 5).

### Microgametogenesis

Examination of pollen grains revealed that in development of the microgametophyte the generative cell divides to produce two sperm cells prior to dehiscence. Hence the pollen is shed in the trinucleate stage with two sperm and a vegetative nucleus.

Table 4. Analysis of pollen viability in five plants of *A. macrocarpa* grown in the greenhouse. The results from examining five flowers per plant during the April to August 1992 segment are shown

Plant	Viable	Nonviable	Percent Viable
Y1	963	130	90
Y2	1,921	98	95
Y3	1,001	153	87
Y4	865	120	86
11	1,408	10	98

Table 5. Analysis of pollen viability in five plants of *A. macrocarpa* from the field. The results from examining five flowers per plant in the spring of 1993 are shown

Plant	Viable	Nonviable	Percent Viable
1	1,381	22	98
2	955	43	96
3	704	106	87
4	558	34	94
5	564	68	89

### Pollination

Diurnal and nocturnal floral visitors were observed (Table 6). Crepuscular and nocturnal moths were observed visiting flowers of *A. macrocarpa* plants on May 10, 1991 at 8:05 pm (2005 hours), March 14, 1992 at 6:20 pm and 6:45 pm (1820 and 1845 hours), April 4, 1992 at 7:10 pm and 7:15 pm (1910 and 1915 hours), March 12, 1993 at 6:30 pm (1830 hours), March 18, 1993 at 6:45 pm (1845 hours), April 3, 1993 at 7:15 pm (1915 hours), April 9, 1993 at 7:30 pm (1930 hours), and May 12, 1993 at 7:30 pm (1930 hours).

The moths were seen in flight visiting several flowers of an inflorescence in rapid succession, moving from flower to flower between plants and were seen to insert their proboscis into the floral tubes. Most moth visitors hovered above the flowers while feeding. Occasionally moths were observed landing on the inflorescence.

Several of these floral visitors were captured and identified as sphinx moths (hawk moths) in the family Sphingoidea. Members of this family are characterized by a well-developed and

functional tongue (Hodges, 1971). Three species were identified: *Dolba hyloeus* (Drury), *Deidamia inscripta* (Harris) and *Erinnyis obscura* (Fabricius). The length of the proboscis of each moth was: *Dolba hyloeus* 3.9 cm, *Deidamia inscripta* 1.5 cm, and *Erinnyis obscura* 2.6 cm. *Dolba hyloeus*, the Black Alder or Paw Paw Sphinx, is known to be a dusk flyer and to frequent deep-throated flowers (Hodges, 1971). This species is found in flight from March to September (Hodges, 1971). Hodges (1971) indicates that the larvae of this species feed on Yaupon (*Ilex vomitoria*) and Paw Paw (*Asimina*). *Deidamia inscripta*, the Lettered Sphinx, is known to be in flight from early spring until late June (Hodges, 1971). This species visits flowers and although it flies by day or night is often observed flying just before dawn (Hodges, 1971). The larvae feed on grape (*Vitis*), Virginia Creeper (*Parthenocissus*), and *Ampelopsis* (Hodges, 1971). *Erinnyis obscura* (Fabricius), the Obscure Sphinx, is common throughout much of the southern part of the United States (Hodges, 1971). Milkweed is known to be a food plant for the larvae; adults are attracted to flowers (Hodges, 1971). All moths captured were found to be carrying *A. macrocarpa* pollen grains on their proboscis. Yaupon, grape and milkweed, known to be food sources for the moth larvae (Hodges, 1971), occur at the population site.

A fourth species was collected and identified as *Hypsoroph monilis* (Fabricius), the Large Necklace Moth, in the Noctuidae family. The proboscis measured 1.2 cm in length. Noctuid moths typically do not hover, rather they use their legs when alighting on a blossom (Faegri and van der Pijl, 1979). *Abronia macrocarpa* pollen was found on the moth's proboscis and legs.

Diurnal floral visitors included bees (*Bombus* and *Apis*), sweat bees, syrphid or flower flies and plant bugs of the family Miridae which are general feeders on plants (Table 6). The bees were seen foraging for pollen after the flowers opened 3:00 pm (1500 hours) until near sunset. The size of the bees prohibited their moving down the narrow floral tube, and the length of their proboscis (*Bombus pennsylvanicus pennsylvanicus* 0.81 cm and *Apis mellifera* 0.38 cm) would prevent direct contact with the stigma in all but the shortest flowers. Presumably, some of the pollen carried by these insects could fall down the floral tube, reach the stigma and result in incidental pollination.

Table 6. List of insect visitors to *A. macrocarpa* flowers

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Floral Visitor
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LEPIDOPTERA

  Sphingoidea

*Dolba hyloeus* (Drury)

*Deidamia inscripta* (Harris)

*Erinnyis obscura* (Fabricius)

  Noctuidae

*Hypsoroph monilis* (Fabricius)

HYMENOPTERA

  Apidae

*Bombus pennsylvanicus pennsylvanicus* (Degeer)

*Apis mellifera* Linn.

  Halictidae

    Unidentified sweat bees

DIPTERA

  Syrphidae

*Eupeodes volucris* Osten Sacken

HEMIPTERA

  Miridae

*Polymeris basalis* (Reuter)

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## Breeding System

Greenhouse pollination experiments tested the capacity of *A. macrocarpa* plants to undergo self-pollination or autogamy (pollen to stigma of same flower), geitonogamy involving transfer of pollen from one flower to a different flower on the same plant, and cross-pollination between flowers on two plants (xenogamy). Flowers that were not manipulated served as controls. Results of the crosses are shown in Table 7. No fruit was set in any cross involving autogamy or geitonogamy. Fruit was produced in cross-pollination experiments. Of 1,194 flowers cross-pollinated, 767 developed anthocarps (64%) and 374 of the anthocarps contained achenes (49% fruit/seed set).

Table 7. Experimental crosses to determine breeding system in *A. macrocarpa*. Non-manipulated flowers serve as controls

Cross	No. Flowers	No. Anthocarps	No. Achenes	% Fruit/Seed Set
Autogamous	1038	0	0	0
Geitonogamous	1031	0	0	0
Xenogamous	1194	767	374	49
Control	1012	0	0	0

## Barrier to Selfing

Fluorescent microscopic examination of stigmas following experimental crosses of greenhouse grown plants (G) and field plants (F) indicates that both self- and cross-pollen readily adhere to the stigmatic surface and germinate forming pollen tubes. The mean number of pollen grains present on the stigma over the time intervals at which hand-pollinated flowers were fixed ranged from 43-98 (G) and 31-72 (F) in autogamous crosses, 10-79 (G) and

50-84 (F) in geitonogamous crosses, and 19-56 (G) and 26-65 (F) in xenogamous crosses. Mean pollen germination was 62% (G) and 58% (F) in autogamous crosses, 54% (G) and 52% (F) in geitonogamous crosses, and 60% (G) and 35% (F) in xenogamous crosses (Table 8). Statistical analyses of greenhouse data indicated no significant difference in percentage germination between autogamous, geitonogamous, and xenogamous crosses (Table 8). However, statistical analyses of field data (Table 8) showed a significant difference in percentage germination between cross-pollen (xenogamous crosses) and the two types of self-pollen (autogamous and geitonogamous crosses). Germination percentages were higher in self-pollinated flowers. There was no significant difference in percentage germination between autogamous and geitonogamous crosses of field plants (Table 8).

Initial rates of pollen tube growth (Figure 10) were similar for self- and cross-pollen (Williamson, et al., 1996). However, differences in subsequent growth of the pollen tubes are notable and attributable to termination of pollen tube growth following self-pollination due to the development of extensive callose deposits along the length of the pollen tube and a callose plug at the tip of the tube. Mean pollen tube length (Table 9) was 0.08 mm (G) and 0.12 mm (F) in autogamous crosses, and 0.11 mm (F) in geitonogamous crosses. Self-pollen tubes do not penetrate the stigmatic tissue. Cross-pollen tubes, on the other hand, penetrate the stigmatic tissue and continue to grow through the carpel tissue. Mean pollen tube length (Table 9) in xenogamous crosses was 1.52 mm (G) and 4.81 (F). Statistical analyses of greenhouse and of field data indicate a significant difference in mean pollen tube length between outcross pollen and the two types of self-pollination (Table 9). There was no significant difference between autogamous and geitonogamous crosses within field plants (Table 9).

In control flowers the number of pollen grains that had fallen onto the stigma ranged from 0-5 (mean of 1) the first day a flower opened, 0-24 (mean of 6) in second day flowers, and 0-25 (mean of 7) on the third day of blooming. Pollen tube length ranged from 0.06 to 0.15 mm with a mean of 0.10 mm. All of the pollen tubes formed extensive callose deposits throughout their lengths and stopped growing at the level of the stigma.

Table 8. Mean percentage pollen germination (standard deviation) in the three treatments autogamous, geitonogamous and xenogamous crosses within the two locations G=greenhouse and F=field. Means sharing the same superscript are not significantly different ( $p>0.05$ )

Location	Autogamous	Geitonogamous	Xenogamous
G	62(22) <sup>A</sup> (n=63)	54(24) <sup>A</sup> (n=11)	60(21) (n=54)
F	58(15) <sup>A</sup> (n=20)	52(23) <sup>A</sup> (n=20)	35(20) <sup>B</sup> (n=18)

Table 9. Mean pollen tube length (standard deviation) in the three treatments autogamous, geitonogamous and xenogamous crosses within the two locations G=greenhouse and F=field. Means sharing the same superscript are not significantly different ( $p>0.05$ )

Location	Autogamous	Geitonogamous	Xenogamous
G	0.08(0.02) <sup>B</sup> (n=53)	N/A	1.52(1.75) <sup>A</sup> (n=53)
F	0.12(0.03) <sup>B</sup> (n=20)	0.11(0.03) <sup>B</sup> (n=20)	4.81(2.4) <sup>A</sup> (n=20)

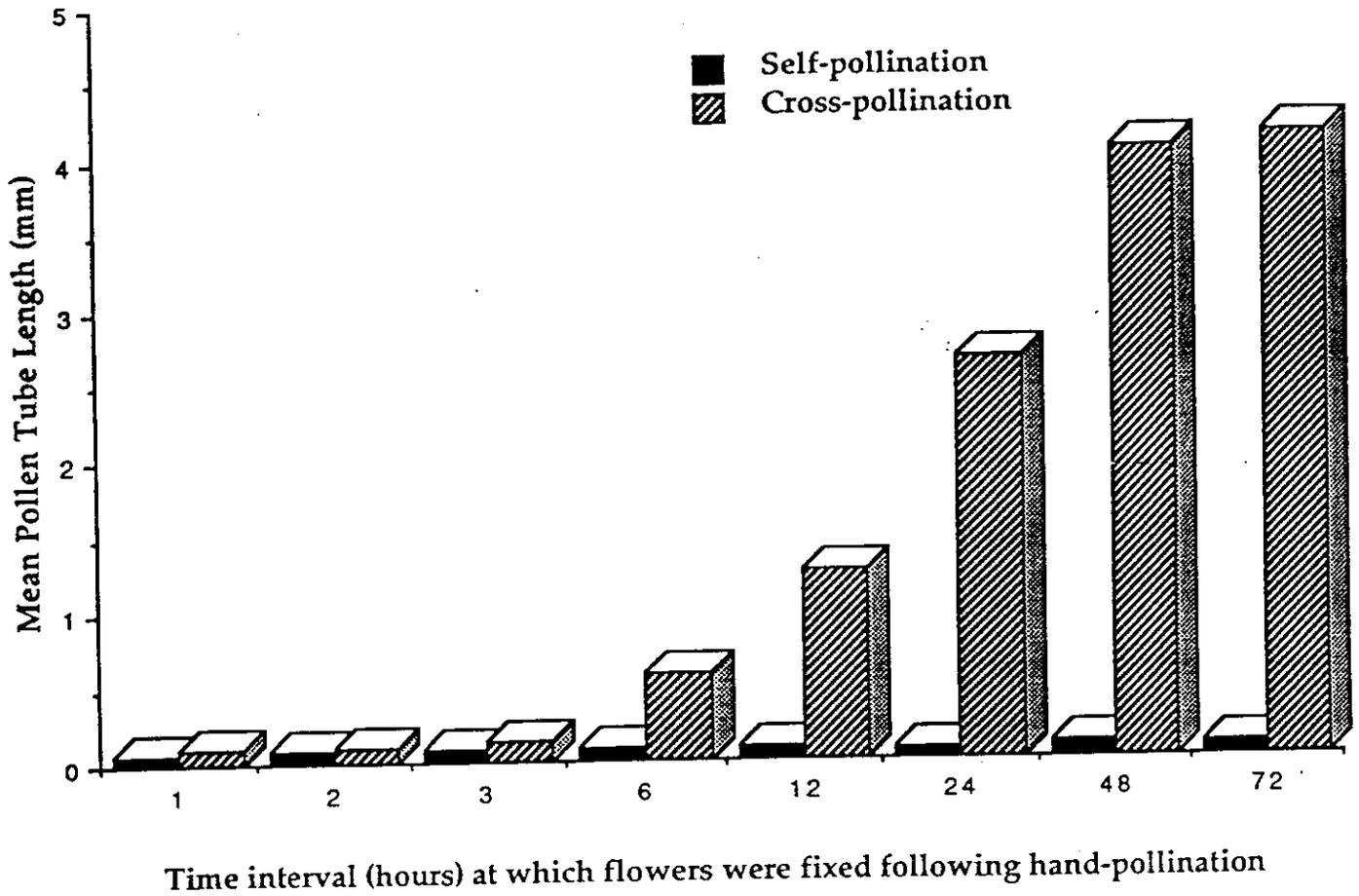


Figure 10. Mean pollen tube length (mm) of self- and cross-pollen at various time intervals following hand-pollination of *Abronia macrocarpa* flowers.

## Reproductive Capacity

The number of inflorescences at different developmental stages produced by 25 randomly selected plants in eight arbitrarily selected areas (n=200 plants) at Hilltop Lakes during the study period (1993 and 1994) were counted (Figures 11, 12, 13, 14). Results combining data from all areas over the study period are shown in Figures 15 and 16. The results show that more than 50% of the buds abort before they reach anthesis. In 1993, the number of buds that reached anthesis in different areas ranged from 21% to 68%. In 1994, the buds that reached anthesis ranged from 18% to 34%. Most of the plants produced flower buds and flowers at anthesis during March and April. By May most of the plants had set fruit. The highest number of inflorescences at bud stage was in March 1994, average of 14.25 per plant; and the lowest in May 1994, average of 2.55 per plant. The number at anthesis was highest in April 1993, average of 5.55 per plant and lowest in May 1994, average of 0.33 per plant. The number of inflorescences with developing fruits in the population was shown to be lowest (0.02 per plant) in March 1993 and highest in April 1993 (5 per plant).

Of the 25 plants monitored in Area A, on April 3, 1993, a total of 174 inflorescences in bud stage, 14 at a near anthesis stage, 49 at anthesis, 14 at post anthesis, 12 post and withered, and 23 with developing fruits were observed. This is in contrast to observations made on April 5, 1992 at which time no plants in Area A were found to have inflorescences. It was speculated that mowing was responsible for the absence of inflorescences on these plants in 1992. Other sites of the population impacted by mowing in 1992 were Area D and Area G. As a short-term management procedure, an agreement with the Hilltop Lakes management was made to postpone mowing of the population site until after anthesis in 1993. The data obtained in 1993 confirms that early mowing reduced the reproductive potential of the plants. Since this alteration in mowing regime appears to have positive results, the management has continued to postpone mowing of the population site until after anthesis.

Area H had the highest reproductive capacity among the areas. As shown in Figure 17, area H had the highest number of buds for both 1993 and 1994; area D, in contrast, had the

lowest number of buds. Anthesis stage was highest in area H in 1993 and area G in 1994; area D still had the lowest number of inflorescences with flowers at anthesis (Figures 17, 18, 19). In 1993, area E (Figure 20) produced the highest number of inflorescences with developing fruits and area H still had the highest number of inflorescences with developing fruit in 1994. Area F and D had the lowest number of fruit set for the year of 1993 and 1994 respectively (Figure 21). Data from Areas A, B and C are shown in Figures 22, 23, and 24.

In 1993, April was the month most of the buds were produced (Figure 15). In 1994, however, most buds were produced in March (Figure 15). Not all the buds reached anthesis. The number of inflorescences that reached anthesis was highest in April 1993, while the highest number in 1994 was in March (Figure 15). In general most of the fruits developed in April in both 1993 and 1994 (Figure 15).

The population produced more inflorescences later in the season in 1993 than in 1994. The number of inflorescences at each reproductive stage was greater in 1993 than in 1994 (Figure 16). The plants continued to produce more inflorescences later in the season in 1993 than in 1994 (Figure 15). In May 1994, fewer plants at anthesis were found in the entire population. In some areas (C, D, F, and G) no plants were found, they died back by May 1994. In May 1993, there were still a few plants found in all areas although there were less than 25 plants per area.

Differences in phenology can also be gleaned by comparing 1992 and 1993 data. The population exhibited a greater reproductive capacity later in the season in 1993 than in 1992. The number of inflorescences at each reproductive stage was higher in April of 1993 than in April of 1992. Additionally, the period of anthesis was extended by several weeks in 1993. When the population was surveyed on May 1, 1992 fewer than 25 plants at anthesis were found in the entire population. The population had died back by late May. On May 2, 1993 the average number of inflorescences per plant in each developmental stage were 6.17 in bud, 0.4 near anthesis, 1.4 at anthesis, 0.74 at post anthesis, 0.28 withered at post anthesis, and 1.8 with developing fruit. Several hundred plants were observed in reproductive stages on May

13, 1993. A few plants (67) were still growing, although most were past anthesis, as late as June 25, 1993.

In addition to data collection in the field, the number of inflorescences at different developmental stages (buds, anthesis, and post-anthesis with fruits) per plant were counted in the outdoor bed at SWT. In 1994, the plants reached the reproductive stage in mid-November, but in 1993, the reproductive stage began in late February. In 1993, more than 65% of the buds reached the anthesis stage and only 54% were fertilized and set fruits. In 1994, on the other hand, an average of 58% of the buds reached anthesis and 57% set fruits (Table 10). Twenty-six seedlings were produced from the mature plants of the previous year (1993). Twelve of the 26 seedlings reached the mature stage and produced flowers and fruit. Of the flowers produced by these seedlings, 33% of the buds produced reached anthesis, and 80% set fruits (Table 11).

During the 1994-95 growing season, the total number of plants in the population and the number of those plants in the reproductive stage (producing inflorescences) was counted (Figure 25). These data show that a low percentage (1-13%) of the plants in the population are at the reproductive stage in a given month of the growing season. Approximately 1% of the population were reproductive in December, 3% were reproductive in February, and 6% were reproductive in May. The greatest number of reproductive individuals was observed in March (13%) and April (12%). No reproductive individuals were observed during other months of the year.

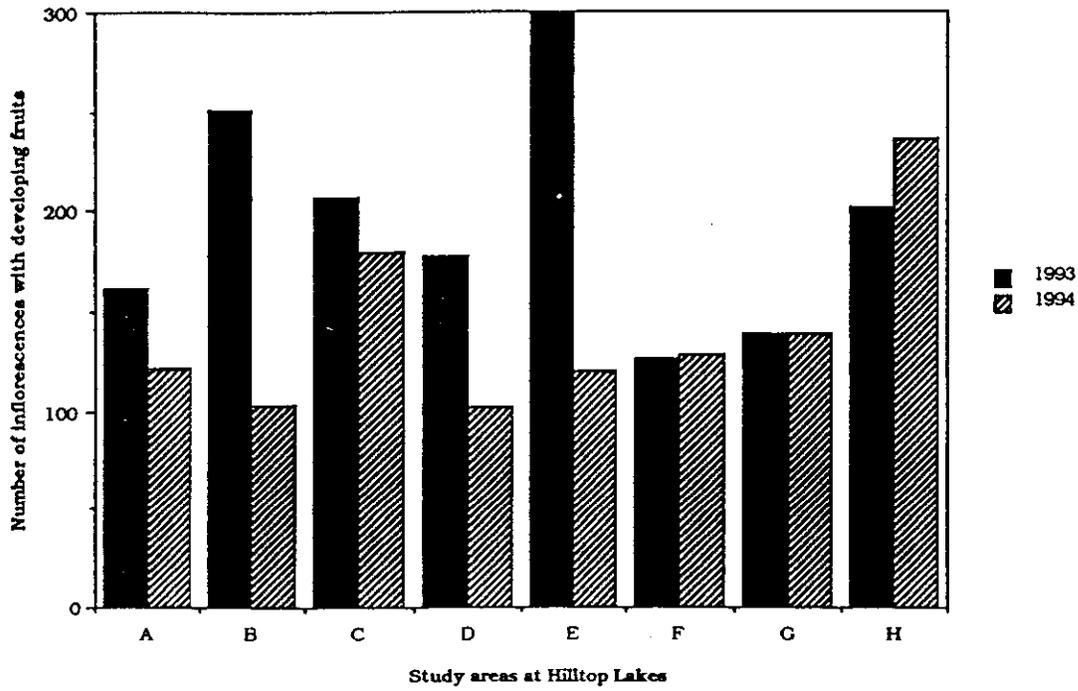
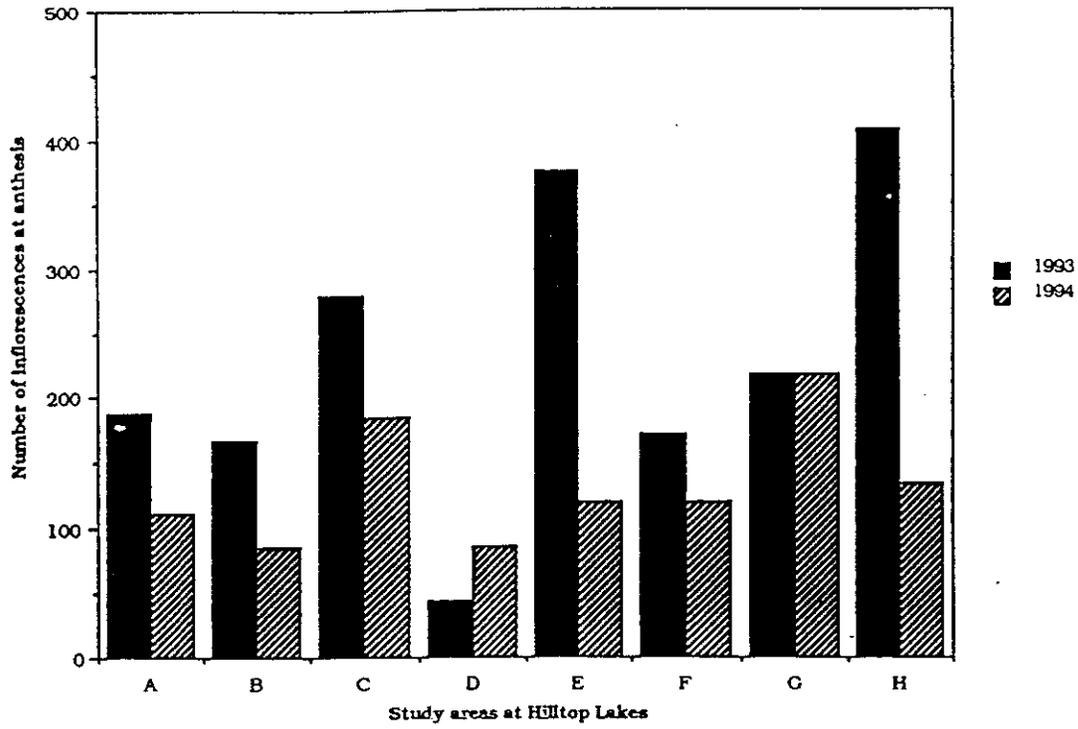


Figure 11. Total number of inflorescences with flowers at anthesis (top graph) and with developing fruits (bottom graph) in each area collected during the study period.

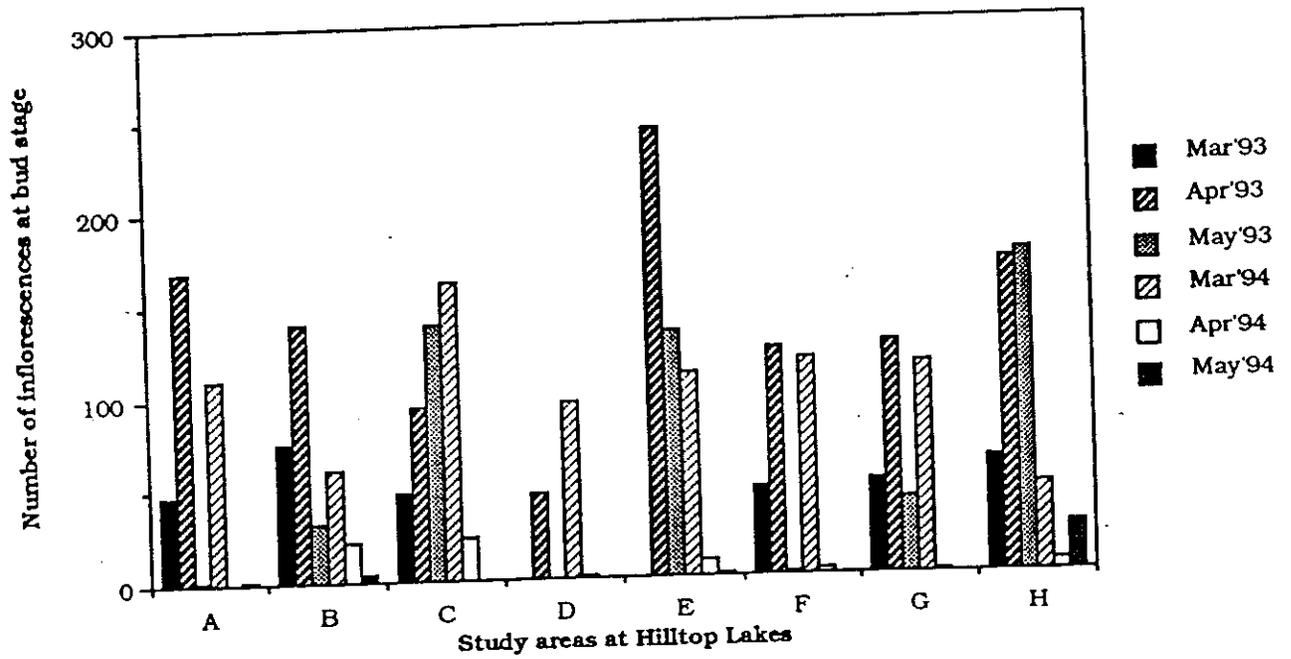


Figure 12. Total number of inflorescences in the bud stage counted in each area.

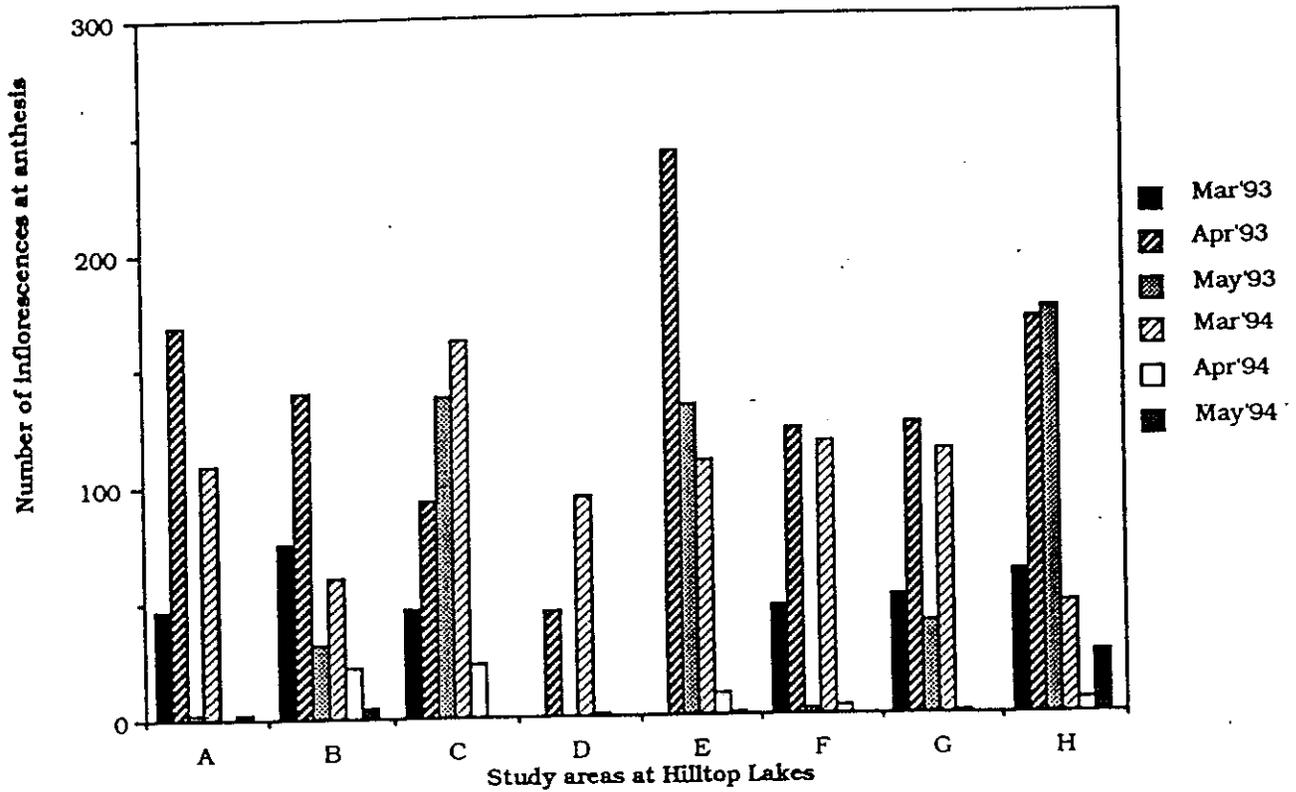


Figure 13. Total number of inflorescences with flowers at anthesis counted during the study period.

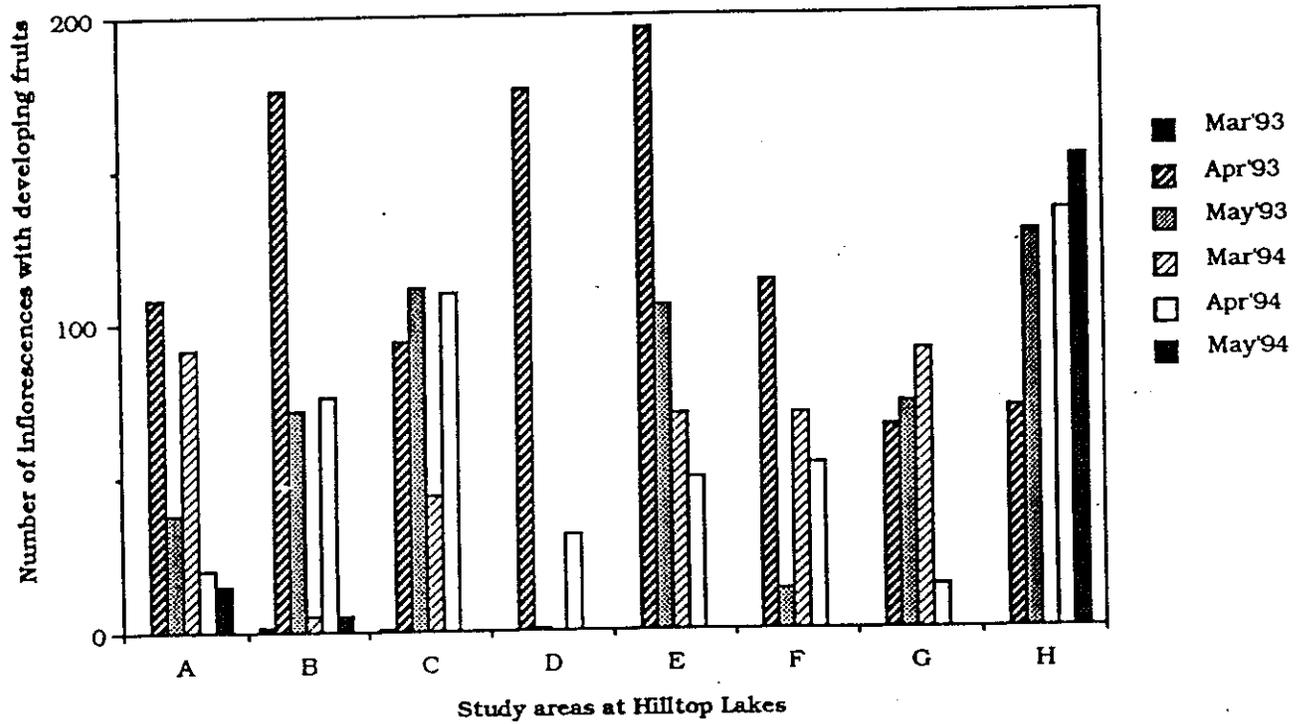


Figure 14. Total number of inflorescences with developing fruits counted during the study period.

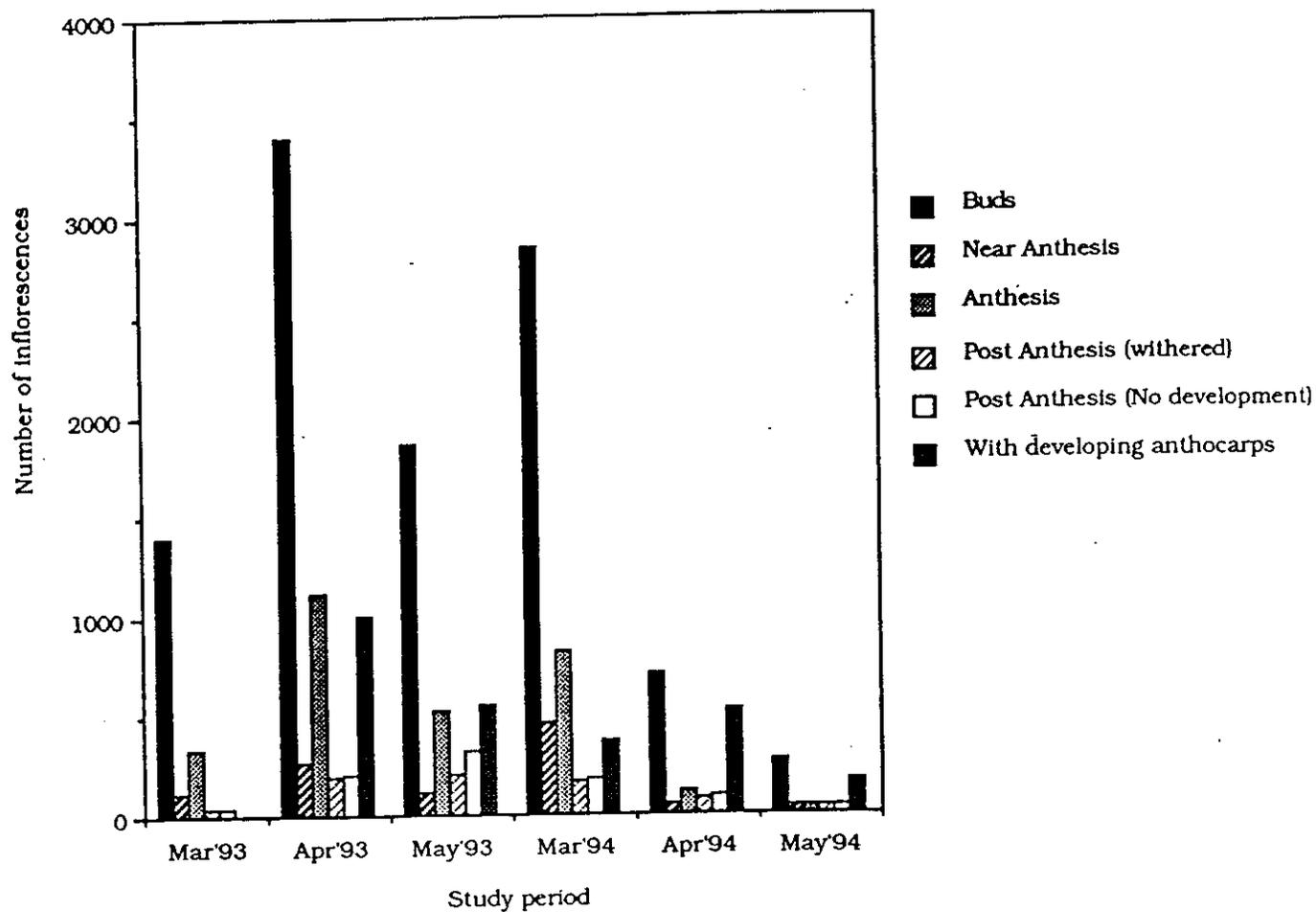


Figure 15. Total number of inflorescences at each of the different developmental stages counted during the study period (March 1993 - May 1994) at Hilltop Lakes.

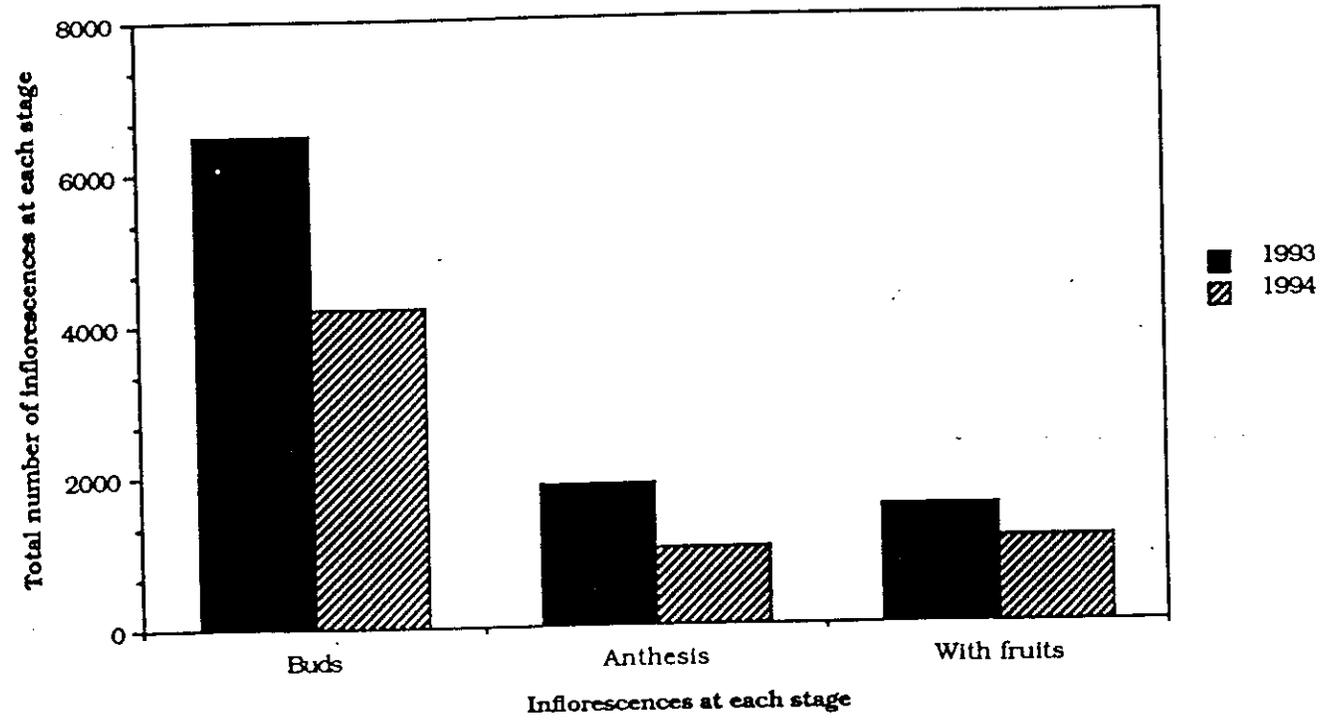


Figure 16. Total number of inflorescences at each different stage counted from March 1993 - May 1994.

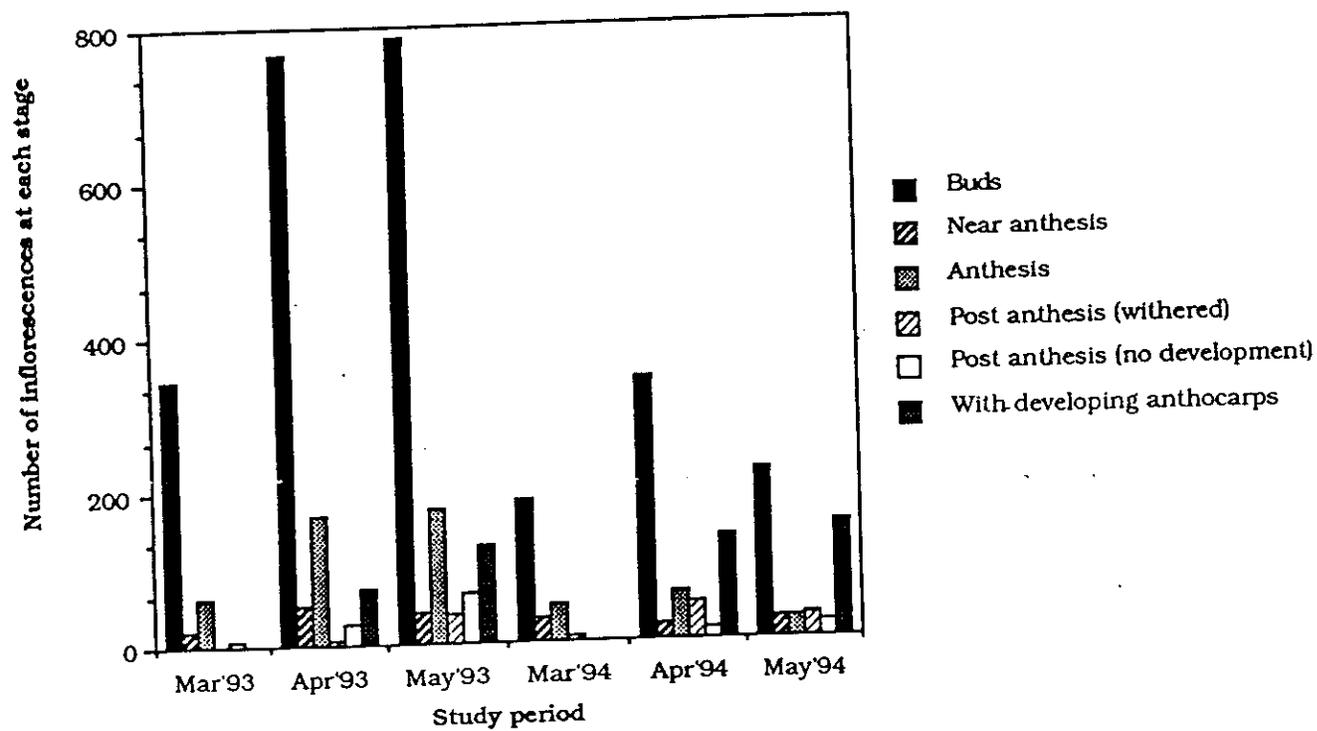


Figure 17. Total number of inflorescences at each different developmental stage collected from area H at Hilltop Lakes during the study period.

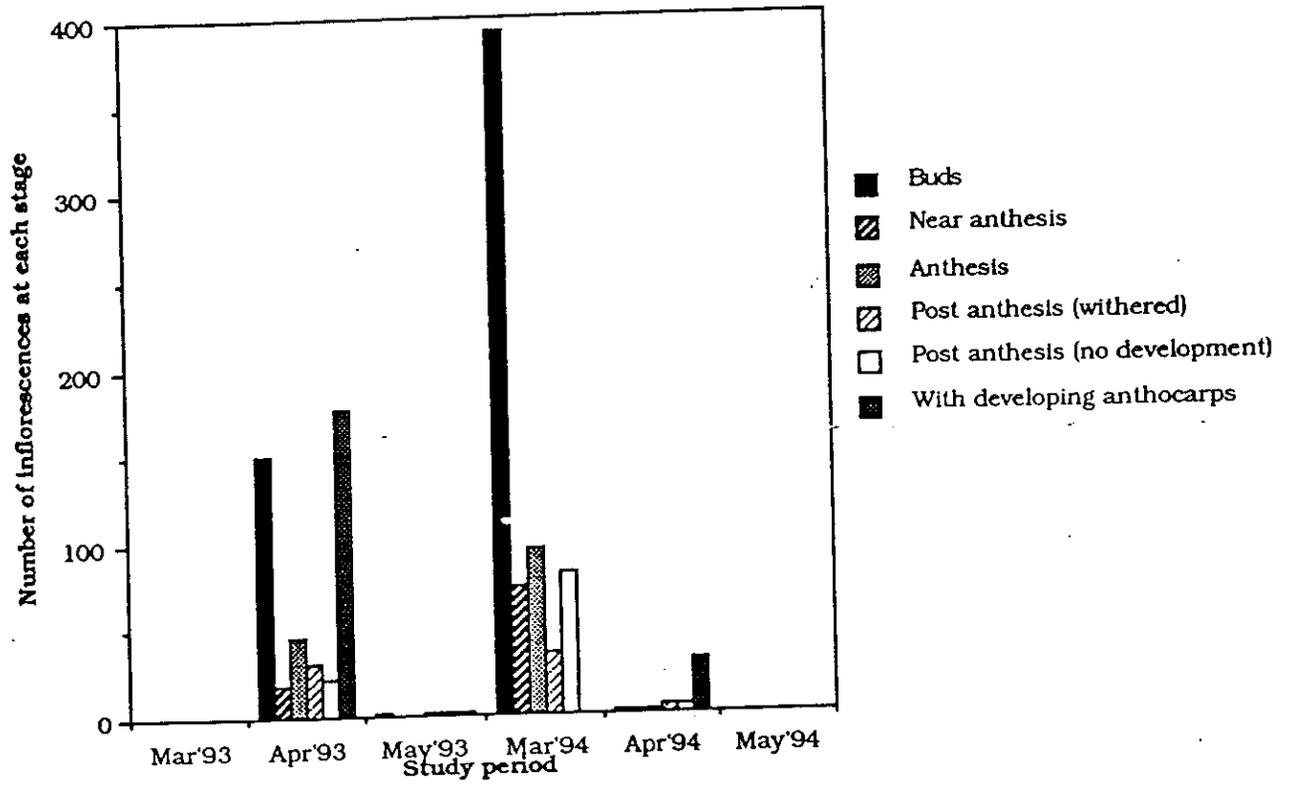


Figure 18. Total number of inflorescences at each of the different developmental stages counted from area D at Hilltop Lakes during study period.

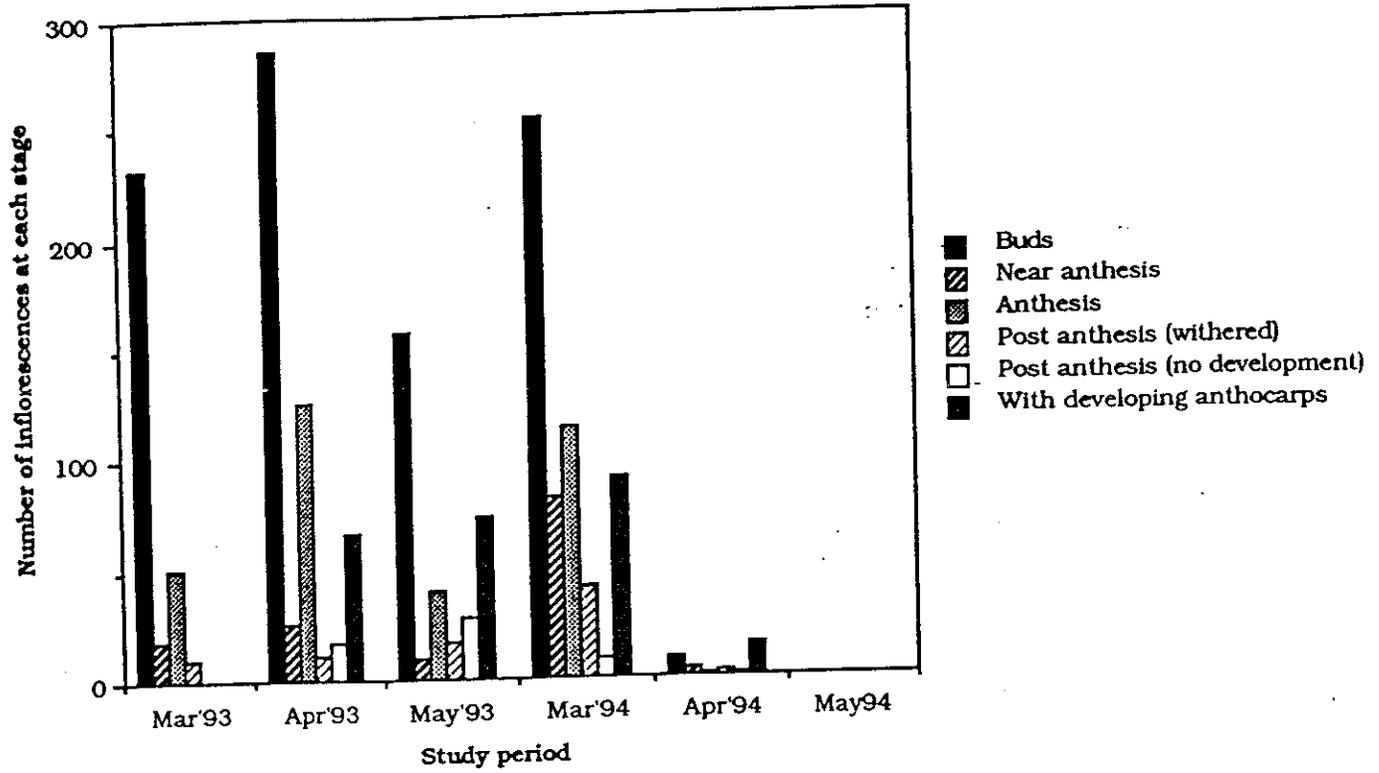


Figure 19. Total number of inflorescences at each of the different developmental stages counted from area G at Hilltop Lakes during study period.

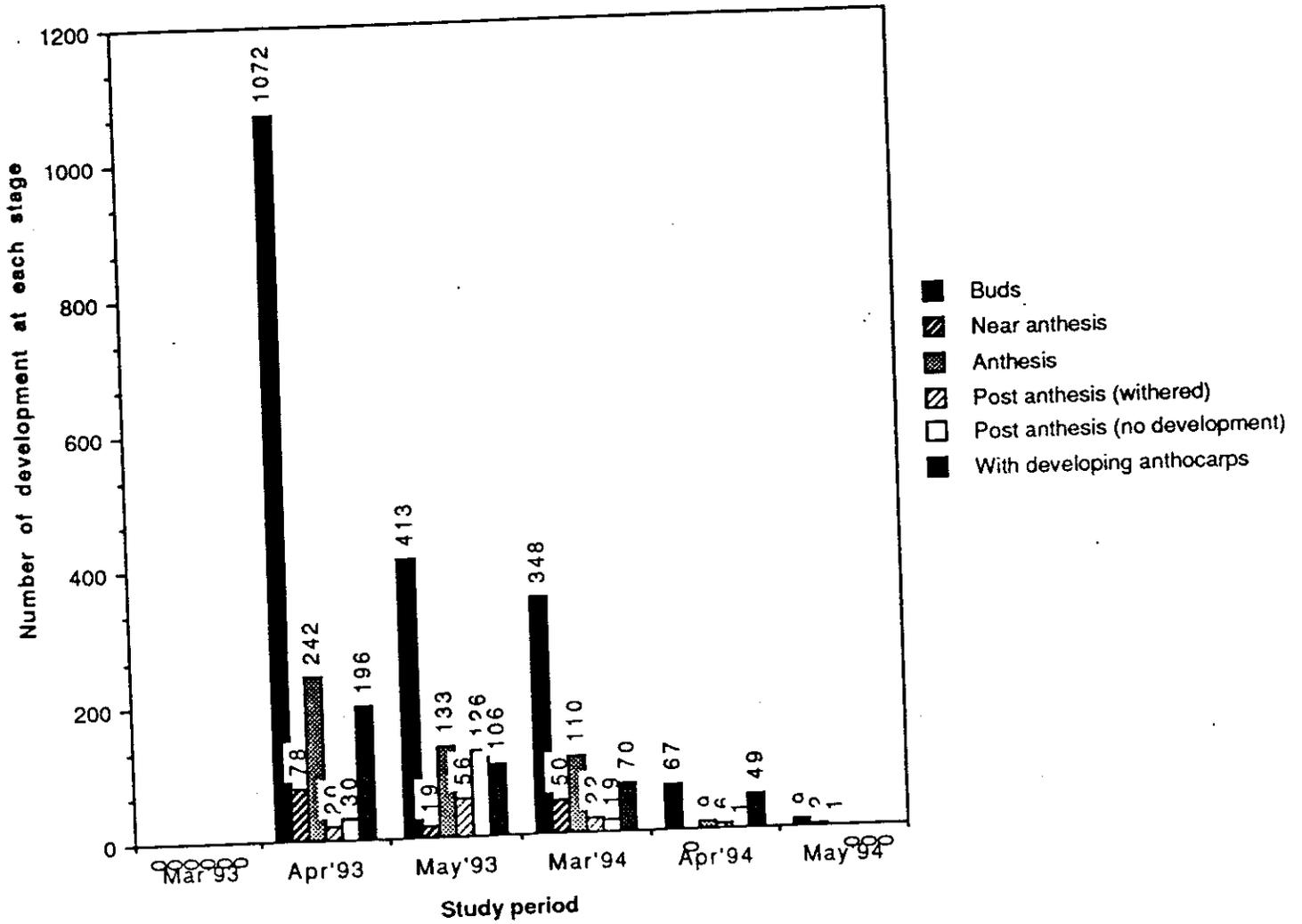


Figure 20. Total number of inflorescences at each different developmental stage collected from area E at Hilltop Lakes during the study period.

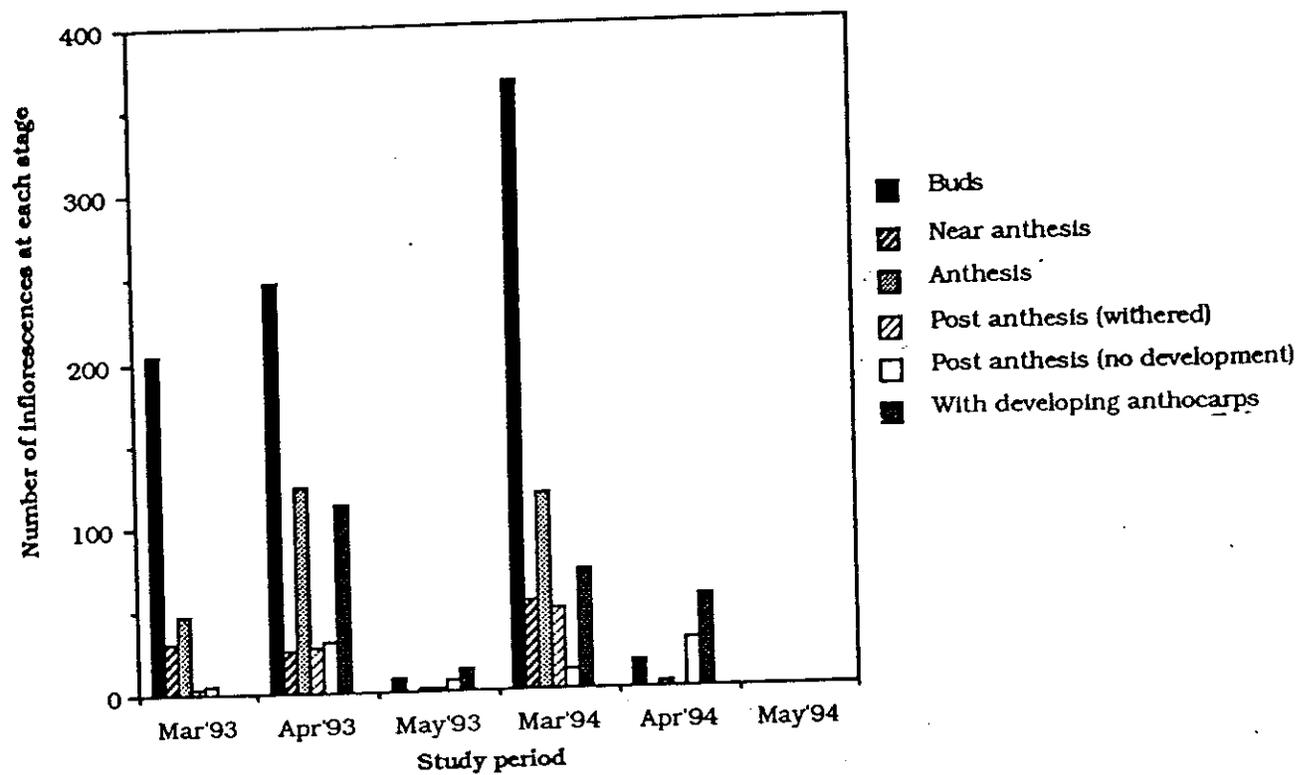


Figure 21. Total number of inflorescences at each of the different developmental stages counted from area F at Hilltop Lakes during study period.

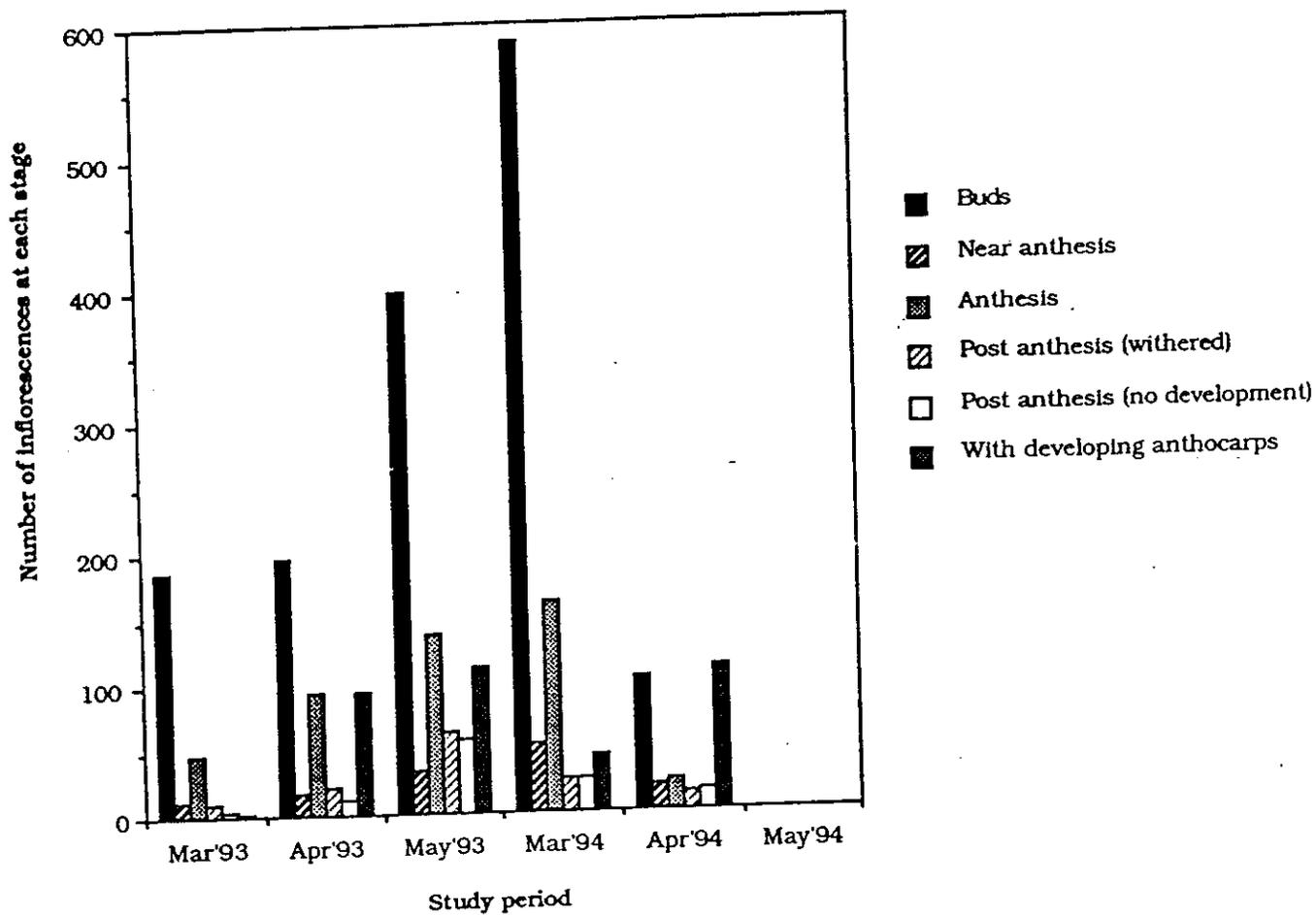


Figure 22. Total number of inflorescences at each of the different developmental stages counted from area C at Hilltop Lakes during study period.

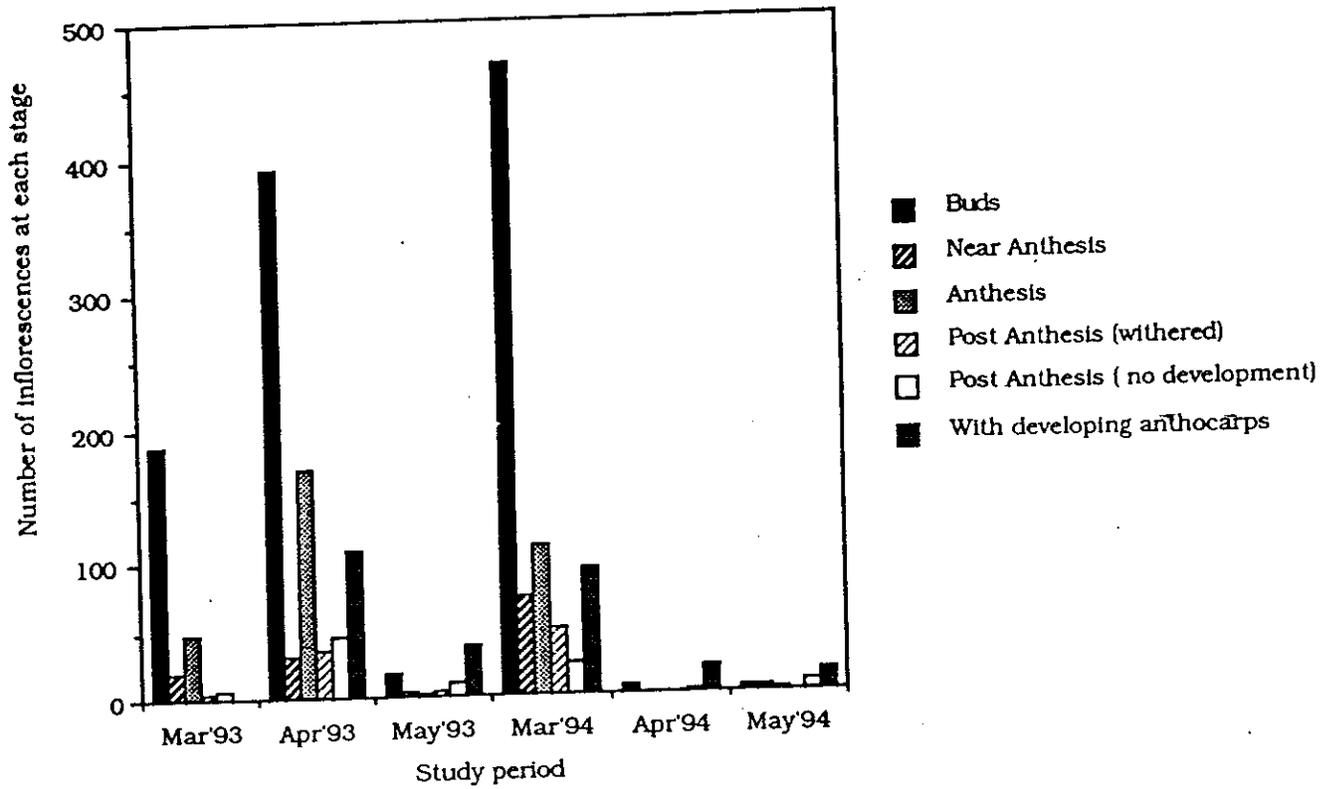


Figure 23. Total number of inflorescences at each of the different developmental stages counted from area A at Hilltop Lakes during study period.

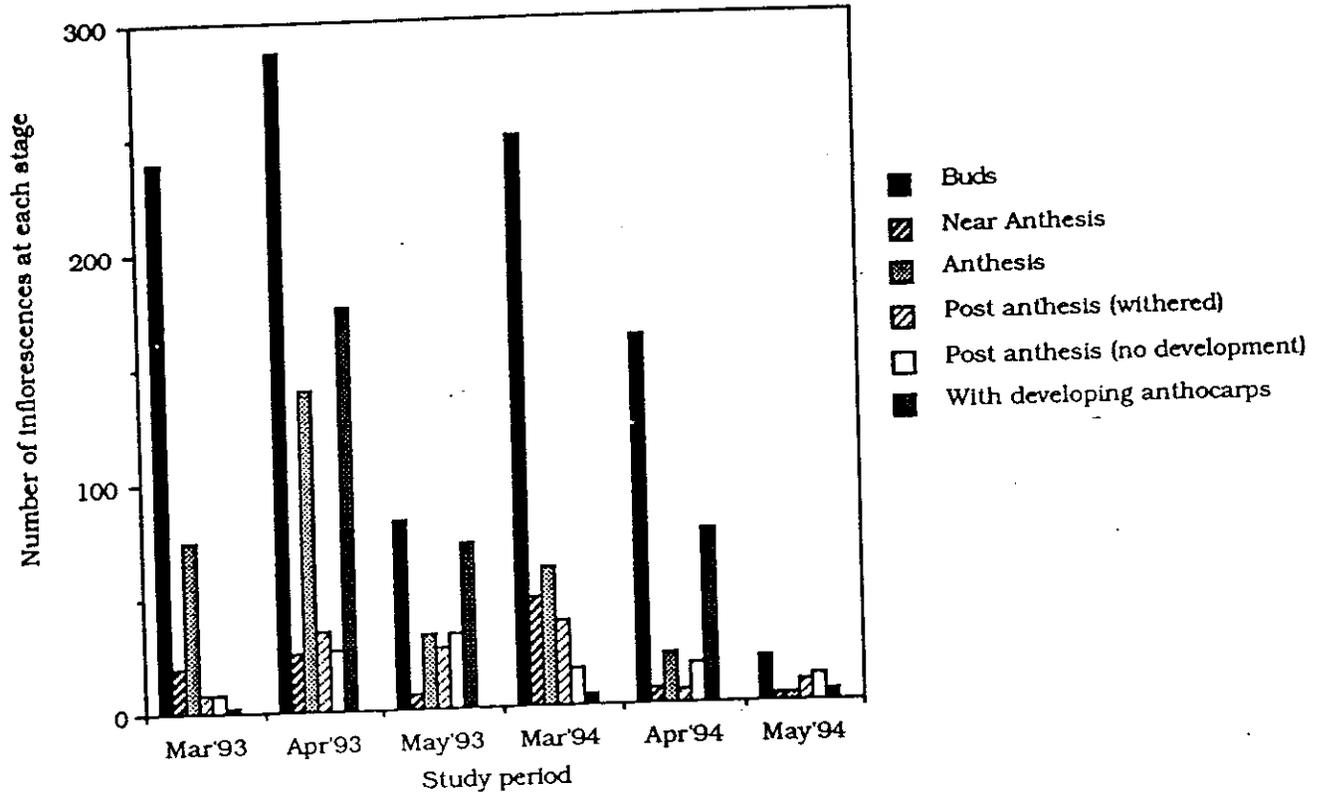


Figure 24 Total number of inflorescences at each of the different developmental stages counted from area B at Hilltop Lakes during study period.

Table 10. Developmental stages and fruit set of mature *A. macrocarpa* plants grown in the outdoor bed at SWT in 1994

Plants	Number of Inflorescences			Number Flowers	Number Anthocarps	Number Achenes	% Fruit Set
	Buds	Anthesis	with Fruits				
1	23	12	1	399	31	10	32
2	41	23	3	616	89	66	74
3	30	13	1	366	40	2	5
4	85	46	5	1,310	134	39	29
5	50	22	5	622	147	87	59
7	70	48	21	1,342	586	398	68
8	88	47	12	1,406	320	162	51
9	51	40	8	1,298	209	79	38
11	33	17	3	577	88	62	70
12	101	53	14	1,716	382	251	66
13	37	36	6	840	173	117	68
14	17	13	2	423	47	37	79
16	70	42	4	1,237	106	34	32
17	58	23	5	577	146	82	56

Table 11. Developmental stages and fruit set of *A. macrocarpa* plants grown from seeds produced by the plants grown in the outdoor bed at SWT in 1993

Plant	Number of Inflorescences			Number Flowers	Number Anthocarps	Number Achenes	% Fruit Set
	Buds	Anthesis	with Fruits				
S1193-1	6	1	0	32	0	0	0
S1193-4	3	1	0	30	0	0	0
S1193-5	5	0	0	0	0	0	0
S1193-10	2	1	0	28	0	0	0
S1193-11	4	1	0	30	0	0	0
S1193-12	6	3	1	60	31	28	90
S1193-15	3	1	1	27	24	15	63
S1193-16	3	1	1	25	24	21	88
S1193-17	2	1	0	30	0	0	0
S1193-18	5	3	0	96	0	0	0
S1193-19	2	0	0	0	0	0	0
S1193-26	6	3	0	0	0	0	0

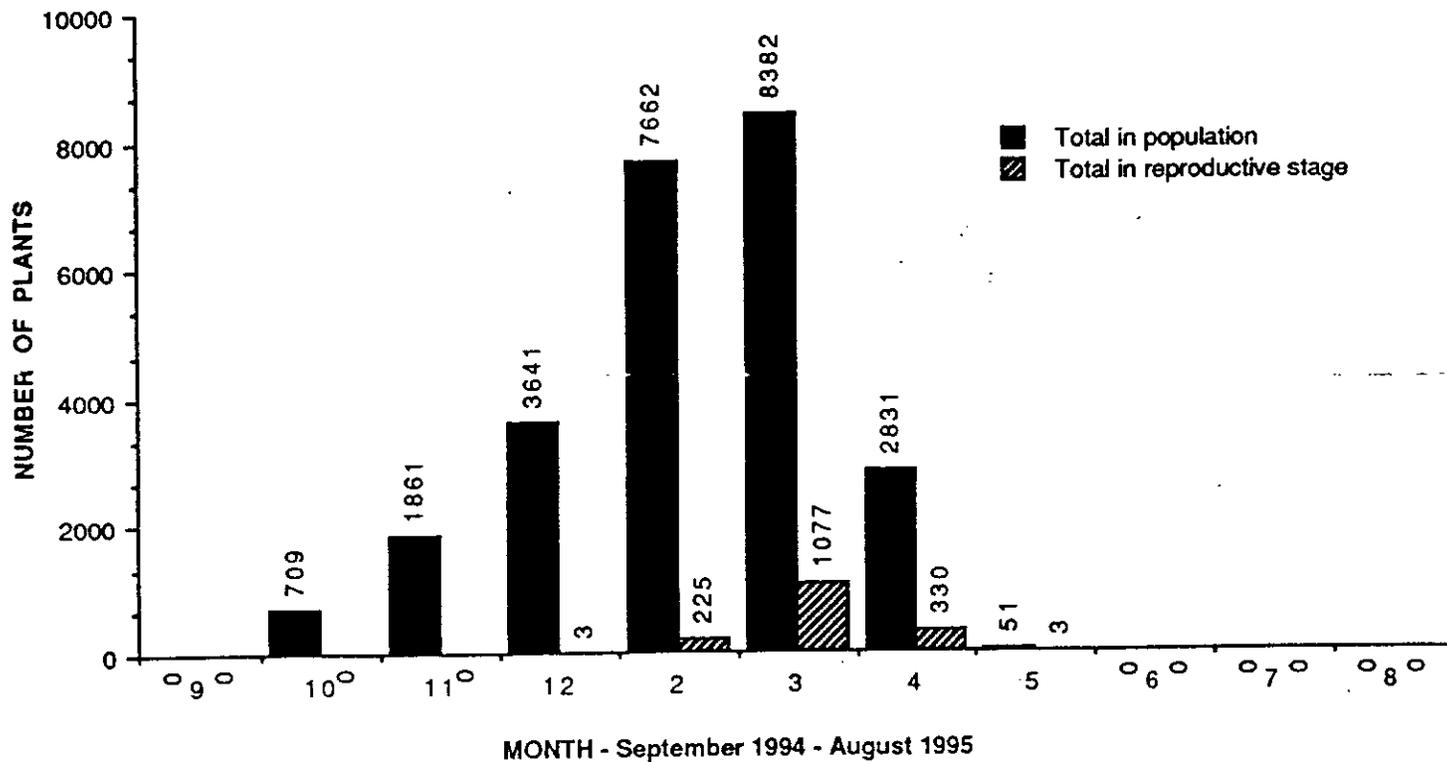


Figure 25. Total number of plants and number at the reproductive stage in the Hilltop Lakes population from September 1994 through August 1995.

Growth and reproductive capacity was also analyzed by counting the height, number of leaves, and number of inflorescences at each developmental stage produced by 24 individual plants that had been tagged. Twenty-one of the plants were monitored from September, 1992 through August, 1993. Three of the 24 plants originally tagged could not be relocated. Tags for 17 of the 21 plants were relocated and monitored from September 1993 through August 1994. Four of the 21 tagged plants could not be relocated. Nine of these 17 plants came back up, the other plants had apparently died and did not produce any above ground plant parts. Eleven of the 17 tagged plants that had been monitored during the 1993-1994 growing season were relocated and monitored from September 1994 through August of 1995. Only one of these 11 plants was still alive during the growing season of 1994-1995.

Plants exhibit a seasonal pattern in phenology. No above ground parts of plants were observed in September. Plants were apparent and in a rosette stage by October. Plants remained in the rosette stage during December, January and February. Plants increased in height and entered a reproduction phase during late February and March. Anthesis and fruit set continued during April and May. Most plants had died back by mid-May and all had died back by June. The taproots could be found buried in the ground at depths of 1-12 cm.

### Fruit/Seed Set

Most flowers in a given inflorescence will form anthocarps, even if only a few of the flowers are actually cross pollinated. But, an achene will only form in those in which pollination and fertilization takes place. The anthocarps collected in 1991, 1992, 1993, 1994, and 1995 were opened to determine if achenes were present. After counts were made most of the achenes were returned to the field. A small percentage were retained for additional studies. Seed set of plants in the field was found to be 51% in 1991 (Corlies, 1991), 28% in 1992, 50% to 66% in 1993, 53% in 1994, and 58% in 1995 (Table 12). A lowest percentage seed set was found when anthocarps were collected relatively late in the growing season (28% on May 21,

1992). Anthocarps collected from plants grown in the outdoor bed at SWT were collected in 1993 and 1994 and opened to determine if achenes had been produced. Seed set of plants grown at SWT was found to be 54% in 1993 and 57% in 1994 (Table 13).

Table 12. Seed set in *A. macrocarpa*. The date refers to the time anthocarps were collected from the field

Date	No. Anthocarps	No. Achenes	% Seed Set
May 9, 1991	1630	830	51
May 21, 1992	901	250	28
April 17, 1993	4780	3153	66
May 2, 1993	1927	963	50
April 26, 1994	2016	1067	53
April 12, 1995	1008	585	58

Table 13. Seed set in *A. macrocarpa*. The date refers to year anthocarps were collected from the plants grown at SWT

Date	No. Anthocarps	No. Achenes	% Seed Set
1993	9126	4934	54
1994	2577	1490	57

### Seed Viability

Seeds which had been stored at room temperature for two months exhibited a viability of 56%.

### Seedling Recruitment

Twenty-six seedlings were tagged on April 10, 1993 and monitored on April 30 and May 14, 1993. Most of the seedlings produced only one or two small leaves during the time span from April 10 to May 14. Three of the seedlings produced three leaves. Leaf blades averaged 1 cm in length (range of 0.6 - 2 cm) and 0.5 cm in width (range of 0.2 - 1 cm). On April 30 two seedlings were excavated and the extent of root growth vs. leaf growth was measured. One of the two seedlings had produced two leaves, the stem measured 1.7 cm in length and the root measured 9.2 cm in length. The anthocarp was still attached and buried approximately 5 cm beneath the surface. The other seedling had two leaves, a stem 2.5 cm in length and a root 9.0 cm in length. The anthocarp was still attached and also at a depth of about 5 cm. Apparently the majority of vegetative growth following germination is directed to establishing a tap root that may survive the summer months buried in the sand, rather than production of leaf tissues. By May 14 the above ground parts of three of the seedlings had died back. Upon excavation, the shoot apices of the three seedlings were found to be buried at depths of 2.5 cm, 4 cm and 8 cm. The above ground parts of the remaining seedlings had died back by June.

An additional 207 seedlings were marked within two weeks from 2 May 1993 to 13 May 1993. To assess recruitment, tagged seedlings were classified into three groups: living, dead, and fate unknown depending on the status of the seedling as of 9 April 1995 (Table 14). Seedlings that exhibited vegetation above ground were considered alive those that did not were considered to be dead. In cases where tags were missing the fate of the seedling was considered unknown. Discounting the seedlings with unknown fate, the population exhibited a seedling recruitment rate of 27% with 73% seedling mortality. To study seedling development, the number of leaves, leaf length and width, plant height, and presence of

reproductive organs were recorded (Tables 15, 16). Seedling development data suggest that the plant may spend several years in a vegetative state prior to entering a reproductive stage. Much energy is devoted to production of a substantial taproot during the early growth phase, with less effort spent in leaf development and virtually no effort spent in reproduction.

Table 14. Status, as of April 1995, of seedlings tagged in 1992 and 1993 at the Hilltop Lakes population

Date marked	Living	Dead	Fate Unknown	Total individuals (n)
10 Apr. 1992	4	10	13	27
2-13 May 1993	18	48	141	207

Table 15. Mean ( $\bar{x}$ ), standard error (SE), and range of values of seedling leaf number, leaf length and width of seedlings tagged in 1992 and 1993 at the Hilltop Lakes population

Characteristics	May-92	Apr-93
$\bar{x}$ leaves	1.38	1.58
n =	21	25
SE	0.11	0.13
$\bar{x}$ leaf length (mm)	9.27	
SE	0.79	
$\bar{x}$ leaf width (mm)	5.10	
SE	0.40	

Table 16. Mean ( $\bar{x}$ ) values during the study period of seedling leaf number, height, number of buds, inflorescences, and post-anthesis inflorescences with accompanying standard error (SE) of plants tagged in 1993 at the Hilltop Lakes population

Characteristics	May-93	Feb-94	Mar-94	Apr-94	May-94	Jun-94	Oct-94	Dec-94
$\bar{x}$ leaves	2.28	6.95	9.11	2.60	1.60	0	2.25	5.31
n=	68	19	27	5	5	0	8	16
SE	0.106	0.807	1.21	0.509	0.245	-	0.251	0.567
$\bar{x}$ height (cm)			1.87				0.15	
SE			0.49				0.02	
$\bar{x}$ buds			3.67					
n=			6.00					
SE			0.21					
$\bar{x}$ inflorescences			1.50					
n=			6					
SE			0					
$\bar{x}$ inflorescences post-anthesis			2					
n=			1					
SE			N/A					

### Herbivory

The plants appear to be minimally impacted by herbivory of leaves. The glandular trichomes located in the leaf epidermal surface make the leaves very sticky, so sticky that sand adheres to the leaf surface. The sticky leaves and grittiness of the sand may serve to reduce herbivory. Inflorescences appear to be the main part of the plant that is grazed. Browsing of inflorescences would serve to reduce reproductive capacity of the plant. Deer appear to be the predominant grazers in this community.

### **Mycorrhizal Association**

Examination of roots indicates that a mycorrhizal association does occur. Extensive fungal hyphae occur on young roots. Hyphae are much less extensive on older, woody roots.

### **Educational Program**

A program to educate the public about the importance of conserving this endangered species was initiated. The program includes presentations at scientific meetings by the primary investigator and graduate assistants, publication in scientific journals, and contributions to photographic exhibitions and magazine articles:

Much of the research on pollination biology was presented at the annual meeting of the Texas Academy of Science, University of North Texas, Denton, Texas in March, 1993, at the annual meeting of the Southwestern Association of Naturalists in Springfield, Missouri in April, 1993, and at the Botanical Society of America meeting in Knoxville, Tennessee in August 1994. This research was also presented in a faculty seminar at Southwest Texas State University in February, 1993. The study of pollination biology has been published in the *Southwestern Naturalist* (Williamson et al., 1994). The breeding system and barrier to selfing study was presented at the Second Southwestern Rare and Endangered Plant Conference in September, 1995 with a brief paper published in the Proceedings of that meeting. This study was also presented at the Texas Academy of Science meeting in Galveston in March, 1996 and at the Botanical Society of America meeting in Seattle, Washington in August, 1996. A research seminar was also presented for the Department of Biology at Stephen F. Austin State University in October, 1996.

The educational outreach efforts included serving as a contact for Ms. Maryl C. Levine who has included photographs of *A. macrocarpa* in her photographic exhibit of rare and endangered plants of the United States and as a consultant for the California Academy of Sciences North American Species Project. The project Co-Directors, Ms. Susan Middleton and Mr. David Littschwager, photographed *A. macrocarpa* plants growing at SWT. A photograph appeared in

Life magazine (September 1994), in an article entitled Saving the endangered 100 and will also be included in the book entitled Witness: Endangered Species of North America. Both Ms. Levine and the California Academy of Sciences were provided with accurate biological information concerning the species and were cautioned and agreed to not provide specifics of the locations of populations of *A. macrocarpa* in order to deter possible collections of the plant from the wild.

Photographs of *A. macrocarpa* plants showing habit and habitat were supplied to the Texas Parks and Wildlife Department for use in their outreach programs with landowners and the public.

## DISCUSSION

Grant (1983) speculated that, based on floral morphology, 13 Nyctaginaceae species occurring in the southwestern United States are sphingophilous. Moth pollination, however, has not yet been documented for all of these species. North American genera, in the four o'clock family, that have been reported as hawk moth pollinated include *Acleisanthes longifolia* (Gregory, 1963-1964; Spellenberg and Delson, 1977), *Anulocaulis leiosolenus* (Spellenberg and Delson, 1977), *Mirabilis froebelii* (Baker, 1961; Grant and Grant, 1983), *M. longiflora* (Grant and Grant, 1983), *M. multiflora* (Cruden, 1970) and *M. nyctaginea* (Cruden, 1970). Keeler and Fredricks (1979) reported that *Abronia fragrans*, a white-flowered, night-blooming species, was pollinated by the Noctuidae moth *Nycterophaeta luna* (Morr.). Tillett (1967) indicated that the pink-flowered *A. umbellata* was pollinated by Noctuidae and Sphingidae moths, but was also visited by day-flying insects. *Abronia pogonantha*, an eastern species, is pollinated by both bombyliid flies by day and the hawk moth *Hyles lineata* by night (Grant, 1983).

*Abronia macrocarpa* exhibits a floral morphology typical of the moth pollination syndrome described by Faegri and van der Pijl (1979) and Grant (1983). The plant is night-blooming. The

floral odor is sweet and increases in intensity towards dusk. Flowers are light pink colored with a floral tube approximately 2 cm long, containing nectar at the base. Floral tube length falls within the range (2-7 cm) typical of hawkmoth flowers in the western United States (Grant and Grant, 1983). Of the floral visitors observed in this study, only moths were able to consistently contact anthers and stigma by inserting their proboscis deeply within the floral tube. The shortest moth proboscis measured was that of the noctuid moth (1.2 cm in length). The floral tube averages 2 cm in length with the stigma positioned approximately 1 cm from the base of the tube. The stigma is typically within reach of even the shortest moth proboscis. Peroxidase enzyme tests indicate that peak stigma receptivity occurs at 7:00 to 9:00 pm. Timing of peak stigma receptivity corresponds to peak activity of the crepuscular moths that serve as pollinators. ~~On the basis of the floral visitor activity observed, Noctuidae and~~ Sphingidae moths are considered to be effective pollinators. *Abronia macrocarpa* is apparently adapted to a sphinogophilous pollination syndrome and is not reliant upon a single species of moth.

Grant (1983) states that in a hawk moth pollination system, each partner in the system has a certain degree of ability to obtain nectar or set seeds, as the case may be, in the absence of the other. The moth may obtain nectar from bee or hummingbird flowers; pollen-collecting bees, ubiquitous visitors to hawk moth flowers in late afternoon and early morning, may serve as incidental pollinators. Bees have been observed visiting *A. macrocarpa* flowers and may effect some pollination. Neither species of bee (*Bombus pennsylvanicus pennsylvanicus* and *Apis mellifera*) observed in this study could effect reliable pollination because the proboscis (0.8 cm and 0.4 cm in length respectively) would not reach the stigma except in the few flowers with very short floral tubes. Only pollen carried from a different plant would result in seed set and the chances of that pollen reaching the stigma, positioned near the base of the slender floral tube, is slight. Peak stigma receptivity occurs near dusk. The bees are diurnal and are not active at the time the stigma is most receptive. Therefore, any pollen they carry, that might

land on the stigma, would land on it at a time that would not be optimal for germination of pollen tubes.

Grant (1983) further points out that many sphingophilous flowers are self-compatible and partially self-pollinating. *Abronia macrocarpa*, however, does not appear to be capable of selfing and therefore the presence and abundance of hawk moth pollinators is crucial to survival of the species.

Plants experimentally crossed autogamously and geitonogamously did not set fruit. Floral morphology readily allows autogamy; the anthers are located above the stigma and the stigma is receptive during pollen dehiscence. It's apparent that self-pollination does occur because in the greenhouse pollen has frequently been observed on the stigmas of unmanipulated plants. However, since unmanipulated plants and plants experimentally crossed autogamously and geitonogamously do not set fruit, *A. macrocarpa* appears to be incapable of selfing.

*Abronia macrocarpa* is an obligate outcrosser and does not set seed when experimentally self-pollinated (Williamson, et al., 1994). The plant is apparently reliant upon hawk moths and noctuid moths to bring about cross-pollination for successful seed set (Williamson, et al., 1994).

Obligate outcrossing breeding systems result from pre-fertilization or post-fertilization barriers to selfing that manifest in the inability of a plant to set seed following self-pollination. Examination of pollen germination and relative growth of pollen tubes following hand-pollination revealed that both self- and outcross pollen readily adhere to the stigma and germinate forming pollen tubes. Interestingly, results in pollen germination differed between field plants and greenhouse grown plants. No significant difference in pollen germination was noted among autogamous, geitonogamous, and xenogamous crosses in greenhouse grown plants. Pollen germination percentage was significantly higher in both types of self-pollination (autogamous and geitonogamous) than in cross-pollination in field plants. Environmental factors such as moisture levels, humidity, temperature, and nutrient levels are known to influence pollen vigor and germinability (Kearns and Inouye, 1993; Thomson et al., 1994). Differences in pollen germination observed in self- and outcrossed flowers in field plants may

be due to variation in environmental conditions that are typical of field situations, but controlled for in the greenhouse.

Subsequent pollen performance was similar in both greenhouse and field treatments. Growth of self- (autogamous and geitonogamous) vs. cross-pollen tubes differs significantly. Cross-pollen tubes penetrate the stigmatic tissue and continue to grow down the style. Within 48-72 hours following hand-pollination a cross-pollen tube is of sufficient length to reach the ovule where sperm can effect fertilization. Growth of self-pollen tubes is arrested at the stigma surface, due to the formation of extensive callose deposits in the tubes, precluding self-fertilization. The requirement for cross-pollination to yield seed is therefore the result of a pre-fertilization barrier to selfing.

Outcrossing results in greater genetic variability than does selfing. Flowering plants have developed diverse mechanisms to promote outcrossing and prevent self-fertilization. Examples include: dioeciousness, protogyny, protandry, and self-incompatibility systems. Self-incompatibility, which prevents the production of seed following self-pollination, may result from a variety of mechanisms. Self-pollen may not adhere to the stigma, adhere but not germinate, or germinate but be unable to penetrate the carpel or grown down the style (Richards, 1986).

Self-incompatibility systems are based on the inherited capacity of the flower to reject its own pollen, due to the recognition of identical gene products in pollen and carpel tissue (de Nettancourt, 1977; Thompson and Kirch, 1992; Sims, 1993). Self-incompatibility systems are broadly classified as gametophytic or sporophytic. Although considered unrelated, both systems are thought to have been derived from proteins produced in plants that are involved in defense against pathogens (Dickinson, 1994). Self-incompatibility systems are controlled by the S-locus which is multiallelic (de Nettancourt, 1977). The incompatibility reaction involves phenotypic expression due to S-alleles present in the carpel tissue and the pollen grain. If the pollen S-allele matches one of the carpel S-alleles rejection will occur. The two types of self-incompatibility systems differ in site of inhibition and genetic control.

In a gametophytic incompatibility system, the incompatibility reaction with the female tissue is mediated by the genotype of the haploid pollen grain (Kearns and Inouye, 1993). The incompatibility reaction occurs as inhibition of pollen tube growth within the style (Newbigin, et al., 1993). Plants exhibiting gametophytic systems typically have flowers with wet stigmatic papillae and binucleate pollen grains (Richards, 1986).

The incompatibility reaction with the female tissue is mediated by the genotype of the diploid anther that produced the pollen grain in sporophytic systems (Kearns and Inouye, 1993). The system involves proteins produced by the anther tapetal cells and deposited on the developing pollen grains. Rejection occurs at the stigma-pollen interface (Richards, 1986; Nasrallah and Nasrallah, 1993). The pollen may not adhere to the stigma, not germinate, or pollen tubes may not be able to penetrate the stigmatic tissue. Stigmatic inhibition of pollen tubes, known to be typical of trinucleate species and of sporophytic incompatibility, appears to be restricted to plants of the dry-stigma type (de Nettancourt 1977; Elleman, et al., 1992).

The control of self-incompatibility (gametophytic or sporophytic), site of pollen-tube inhibition (style or stigma), nature of the stigma (wet or dry), and developmental stage of the pollen at dehiscence (binucleate or trinucleate) are strongly correlated (Richards, 1986).

*Abronia macrocarpa* flowers exhibit trinucleate pollen grains, a dry stigma, and self-pollen tube inhibition at the stigma surface. These results indicate that the self-incompatibility system is sporophytic in this taxon.

Plants begin undergoing sexual reproduction by February or March. Observations in the field and greenhouse during the study period showed most bud initiation occurred early in the flowering season and declined as the plants started to initiate fruits. Plants will keep initiating buds and flowers as long as there is excess resource available. When the plants start to produce fruits, the number of buds and flowers produced declines due to the competition for resources (Stephenson, 1981).

The study showed that *A. macrocarpa* plants set more flowers than actually develop into fruits. Studies by Bawa and Webb (1984), Ehrlen (1991), Sutherland (1986, 1987), and Walker

and Whelan (1991) demonstrate that plants bearing excess flowers contribute to increased male fitness through pollen donation. Plants bear more flowers than can be matured into fruits to supply enough compatible pollen (Bawa and Webb, 1984). Male reproductive success (seed sired) will decrease while the female reproductive success (seed set) will increase (Campbell, 1989). The excess flowers also allow plants to abort fruits selectively and increase the average quality of offspring eventually produced (Stephenson and Winsor, 1986). This may explain the low percentage of seed set by *A. macrocarpa*. The timing of flowers opening during the season may result in variation in seed set. Zimmerman (1984) and Dieringer (1991) show that plants flowering in the early and middle part of the blooming season are able to produce more flowers and fruits than late flowering plants.

According to Bawa and Webb (1984), there are several hypotheses that can explain flower, fruit and seed abortion. First, pollinator limitation in which inadequate pollination is the major cause of abortion. Second, sexual selection which attributes abortion to conflicts inherent in the optimizing of male and female reproductive success. Finally, resource limitation in which fruits are aborted to adjust the number to available resources.

Phillip and Schou (1981) found that in *Anchusa officinalis* the low seed set was due to ovule inhibition after fertilization with the wrong alleles, and competition for limited resources between different metabolic sinks. Flowers are inhibited from setting fruits if other pollinated flowers and juvenile fruits are developing because they compete for limited maternal resources (Stephenson, 1981).

According to Sutherland (1987), several hypotheses can explain an observed low fruit set: (1) pollen limitation where fruit production is limited by the availability of pollen to fertilize ovule; (2) pollinator attraction where excess flowers will ensure the pollinator visitation rate be sufficient for adequate pollination; (3) bet-hedging where the excess flowers allows the plant to compensate for either resource availability for fruit maturation or pollen success due to variation in pollinator densities; (4) selective abortion where the plant can selectively abort some fruits and mature only those which have high quality; (5) pollen donation which can

contribute either female fitness (production of mature seed) and male fitness (seed sired). Competition for resources will reduce fruit/seed set. In some study areas where *A. macrocarpa* plants grow in association with other species, the reproductive success is lower compared to other areas. Although there are not many *A. macrocarpa* plants in area H, which is disturbed more than other areas, this area has the highest reproductive success compared to other areas. While area D, which has a diversity of other plant associations, has the lowest reproductive success. This may indicate that either intra- or interspecific competition will reduce the number of plants and seed produced.

Pollen viability of field plants has been shown to be 93%-94%. The percentage of viable pollen is relatively high yet achene set is low. Achene set was found to be 51% in 1991, 28% in 1992, ranged from 50% to 66% in 1993, was 53% in 1994, and was 58% in 1995. Now, based on five years of data, low seed set appears to be a strong trend rather than an isolated event that might be accounted for by a "bad" year. The causes of this trend are not yet clear, but may result from pollinator limitation.

A comparatively low seed set (28%) was observed in 1992. It may be that pollinators were more scarce or less active in 1992 which would result in a lower fruit set, or since studies have shown that some species are more likely to set fruit from flowers produced earlier in the blooming season (Stephenson, 1981) the lower fruit set observed in 1992 may be the result of collecting anthocarps very late in the season that year (collections were made on May 21, 1992). The hypothesis that flowers produced later in the blooming season are less likely to produce fruit is supported by 1993 data. A decline in percentage seed set over the season was found in 1993. Collections made on April 17, 1993 exhibited a 66% seed set, while collections made on May 2, 1993 exhibited a 50% seed set.

The low fruit set is either due to flowers not getting an adequate amount of pollen or because the fruits are aborted before they are mature. Many authors (Schemske, 1977; Schemske, et al., 1978; Willson and Schemske, 1980; Bierzychudek, 1981) imply that the major cause of fruit abortion is due to lack of successful pollination.

Studies of the breeding system show that *A. macrocarpa* is an obligate outcrosser. The population studied appears to be reliant upon hawk moths and noctuid moths to bring about cross-pollination for successful seed set. Pollinator presence and abundance, therefore, is crucial to survival of this endangered species.

The Hilltop Lakes population, that was studied intensively, has been subjected to several disturbances which may influence pollinator abundance and visitation rates. The site occurs in what is now a residential resort and the natural vegetation surrounding the population has been altered. Associated species known to be food plants for the moth larvae occur at the site. But, the natural vegetation has been greatly reduced from what it was in the past due to housing development and associated landscaping practices and may no longer be sufficient to support large moth populations. Application of pesticides around yards may result in a reduction in population sizes of the moths that serve as pollinators.

An oil well and associated ponds were constructed in the immediate vicinity of the population in September of 1992. This disruption may have impacted olfactory cues. Hawk moths have a good sense of smell and are attracted to flowers by their sweet odors. Alteration of olfactory cues may result in reducing pollinator visitation rates. The well has since been capped and the ponds filled in, however, the impact of this disturbance may be more long lasting.

This study of reproductive ecology has provided excellent background information, but we still have much to learn. The critical next step in understanding the reproductive ecology of this species is to examine potential pollinator limitation. For example, moth population size needs to be studied. Additional populations (a total of ten populations are currently known) are now known and accessible for study. Pollination biology of these populations needs to be investigated and compared with what was found at the Hilltop Lakes site.

Plants experimentally cross-pollinated in the greenhouse exhibited a fruit/seed set of 49%. Fruit set under ideal conditions, in which pollen is placed directly onto stigmas at the peak of receptivity, is still low. These data indicate that *A. macrocarpa* may have a diminished

fecundity. Consequently, even with a high pollinator abundance, the plant may still be unable to produce a greater number of fruit. But, low seed set in the greenhouse could be due to other factors such as pollen clogging during hand-pollination, reduced vigor of greenhouse-grown plants, and environmental conditions in the greenhouse (the SWT Biology greenhouses are constructed with tinted glass which diminishes light).

Insect predation on the plants might also lower the seed set because insects transmit pathogens or leave wounds where pathogens may enter which will eventually kill the plants. Insect predation has been observed in the field and the greenhouse, especially in 1994 where most of the greenhouse plants were infested by aphids. The flower and fruit set of the infested plants was greatly reduced. Mortality of greenhouse plants occurred following the insect infestation, which may have been the result of exposure to pesticides used to treat the infestation.

Studies of phenology and reproductive capacity indicate that a low percentage (1-13%) of the plants in the population are at the reproductive stage in a given month of the growing season. The greatest number of reproductive individuals was observed in March (13%) and April (12%). Studies of seedling development suggest that the plant may spend several years in a vegetative state prior to entering a reproductive stage. Much energy is devoted to production of a substantial taproot during the early growth phase, with less effort spent in leaf development and virtually no effort spent in reproduction. Plants tagged once they had entered the reproductive phase lived only one or two additional growing seasons. These data suggest that the species is a short-lived perennial. Continued monitoring of marked seedlings will yield additional insight into the life history of this species.

The manager of Hilltop Lakes, Mr. Lonnie Richards, was notified of the impact of mowing on population size and reproductive capacity as determined by surveys of the population in 1992. The importance of delaying mowing until the plants have time to set fruit was stressed. Mr. Richards verbally agreed that he would postpone mowing until notified that plants were past anthesis. I notified Mr. Richards on May 13, 1993 that the majority of plants in the areas

subjected to mowing were past anthesis and above ground parts had died back and indicated that the areas could be mowed. A longer waiting period prior to mowing could have presented a burden to the resort management as the grass was already very high and dense. Mr. Richards has continued the suggested mowing regime since this practice will likely lead to increased population numbers.

Presentations at scientific meetings, scientific publications, photographic exhibits, and articles in popular magazines are helping to contribute to developing a public concern for endangered species in general, and this species in particular.

The Hilltop Lakes population was severely impacted by construction of an oil well in September, 1992. Upon consultation with botanists at the Texas Parks and Wildlife Department and the U.S. Fish and Wildlife Service it has been decided that to minimize further impact to the species, certain planned research activities such as artificial shading experiments and pressure bomb experiments were not carried out.

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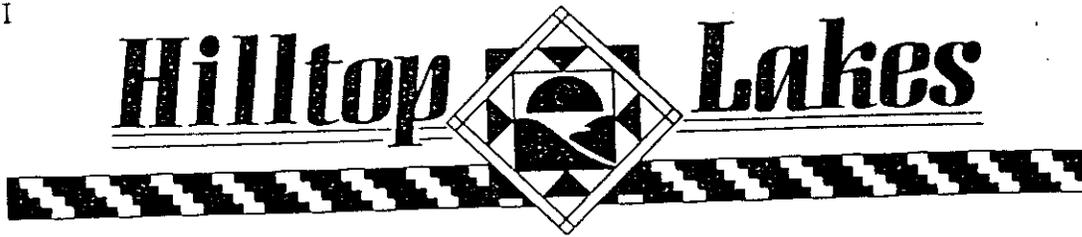
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Hilltop Lakes Resort/City  
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May 13, 1991

To Whom it May Concern:

Dr. Paula S. Williamson of Southwest Texas State University has permission to study the *Abronia Macrocarpa* (Large Fruited Sand Verbena) located in Hilltop Lakes.

~~Dr. Williamson has permission to study this plant for as long as it takes to complete her study.~~

Lonnie Richards  
General Manager

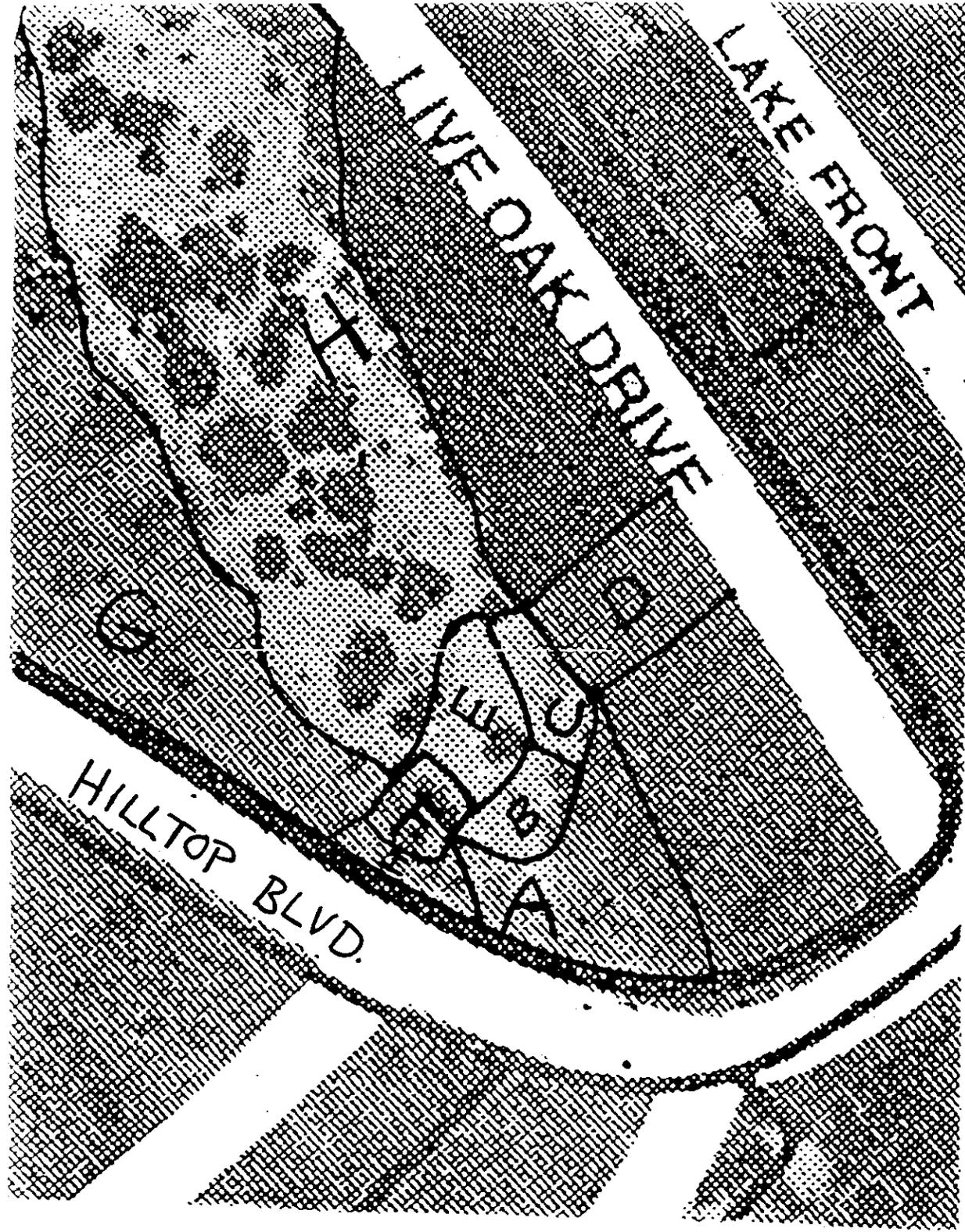
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AMERICA'S MOST COMPLETE RESORT

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APPENDIX II. Overview of the Hilltop Lakes population site. Enlarged photograph shows the designated regions



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APPENDIX II, continued. Enlarged aerial photograph showing the designated areas (Areas A - I) at the Hilltop Lakes population site.





# Annual Report on the Conservation Program at Mercer Arboretum and Botanic Gardens 1995

prepared by Greg Wieland, Botanist

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## Introduction

In 1995 the conservation program at Mercer Arboretum and Botanic Gardens continued to expand and increase its contribution to the conservation of native plants in the Mississippi Delta region. Advancements were made in preserving genetically representative samples of imperiled plant germplasm, in conservation-oriented and biological research, and in education and public involvement in preserving our botanical heritage.

## Personnel

Greg Wieland (Botanist) continued to manage the conservation program at Mercer under the direction of Doug Williams (Director) and with the assistance of Linda Gay (Horticulture Manager), and Dorothy Welch (Greenhouse Manager). Maggie Honig was the 1995 Conservation Intern.

Volunteers Gail Frances, Lee McCaffree, and Reba Morrill assisted with seed collection, cleaning, storage, and testing; maintaining the Endangered Species Garden; keeping records on the conservation collections and experiments; and making the tasks involved in the conservation program do-able and enjoyable.

## Funding

Thanks are in order to A.A. Biekle, the US Fish & Wildlife Service, the Garden Club of America, the Garden Club of Houston, Harris County Precinct Four Parks Department, the Kingwood Garden Club, the Margaret Cullinan Wray Charitable Lead Annuity Trust, the Mercer Arboretum Advisory Committee and the Mercer Arboretum Foundation, the National Park Service, the Rare Plant Group of the Garden Club of America, *Texas Gulf Coast Gardening*, and Tomball Ford for funds made available for projects, equipment, and an internship in 1995.

## Highlights

1. Endangered Species Garden

Forty plants (each propagated at Mercer from a cutting of a separate wild individual) of *Phlox nivalis* subsp *texensis* (Texas trailing phlox) were reintroduced near a historic site in the Big Thicket National Preserve on 13 December. This cooperative project involved Mercer, the the US Fish & Wildlife Service, Garden Club of America, the National Park Service, The Nature Conservancy of Texas, Sam Houston State University, and the Texas Regional Institute for Environmental Studies.

## **2. Guidelines for the Management of Orthodox Seeds**

The Center for Plant Conservation's "Guidelines for the Management of Orthodox Seeds," authored by Mercer's botanist, was published. This publication involved the cooperation of Mercer, other botanical gardens in the Center's network, the Center for Plant Conservation National Office, and seed experts in the U.S. and England.

## **3. Center for Plant Conservation Board of Trustees Quarterly Meeting**

Mercer hosted the February meeting of the Board of Trustees of the Center for Plant Conservation. Board members experienced southern hospitality at a barbeque lunch prepared only as Mercer's staff is capable, an elegant dinner at the lovely home of Sally Squires, and a field trip to East Texas's Big Thicket.

## **4. *Torreya taxifolia***

Mercer continued to maintain a genetic collection of *Torreya taxifolia* (Stinking cedar) plants and has maintained the records on *Torreya taxifolia* housed at institutions throughout the country for the Center for Plant Conservation. See Attachment C.

## **5. Development of Houston Region Native Grass Seedbank**

Mercer actively participated in an effort to collect and maintain a seedbank of local ecotypes of native grasses. The Houston Region Native Grass Seedbank project involved participants from Mercer, Armand Bayou Nature Center, US Fish & Wildlife Service, Harris County Flood Control District, Houston Lighting and Power, Phase One Technologies, Tenneco, Texas Parks and Wildlife, the University of Houston, other professionals, and a team of volunteers who are collecting and maintaining carefully documented samples of locally obtained native grass seeds. These seeds will be banked at Mercer and will be available for prairie restorations, flood control projects, research, and projects yet to be identified.

# ***Research***

## **1. Report on the ability of *ex situ* *Hymenoxys texana* to survive submerging**

Mercer performed inundation tests involving *Hymenoxys texana* (Prairie dawn) for the US Fish & Wildlife Service to accompany a report on the Corps of Engineer's plan to raise the flooding level of Addicks Reservoir in Harris County. See Attachment D.

## **2. Testing of seed viability and propagation requirements and characteristics of the conservation collection**

Taxa tested included *Abronia macrocarpa* (Large-fruited sandverbena), *Aquilegia hinckleyana* (Hinckley's columbine), *Aster puniceus* subsp *elliottii* var *scaberrimus* (Rough-stemmed aster), *Chloris texensis* (Texas windmill grass), *Cyperus Louisiana* (Louisiana nutsedge), *Fryxellia pygmaea* (Fryxell's pygmy mallow), *Hibiscus dasycalyx* (Neches River rosemallow), *Hymenoxys texana* (Prairie dawn), *Machaeranthera aurea* (Houston daisy), *Physostegia*

*correllii* (Correll's false dragonhead), *Silene subciliata* (Scarlet catchfly), and *Thalictrum texanum* (Houston meadowrue). Additional propagation information was acquired when attempts were made to propagate via cuttings *Abronia macrocarpa* (Large-fruited sandverbena), *Aster puniceus* subsp *elliottii* var *scabricalis* (Rough-stemmed aster), *Gaillardia aestivalis* var *winkleri* (White firewheel), *Hibiscus dasycalyx* (Neches River rosemallow), *Manihot walkerae* (Walker's manioc), *Phlox nivalis* subsp *texensis* (Texas trailing phlox), *Physostegia correllii* (Correll's false dragonhead), *Salvia penstemonoides* (Big red sage), and *Torreya taxifolia* (Stinking cedar). Propagation by division was attempted on *Chloris texensis* (Texas windmill grass) and *Isoetes louisianensis* (Louisiana quillwort). (See individual taxon reports below for conclusions.)

### 3. *Lesquerella pallida* germination studies

A draft report was prepared on germination studies involving *Lesquerella pallida* (White bladderpod) in which germination was compared of seeds exposed to a variety of environmental conditions. See Attachment E.

## Conservation Collection

### 1. Center for Plant Conservation National Collection of Endangered Plants

Several new taxa were added to Mercer's portion of the CPC's National Collection of Endangered Plants, including *Chloris texensis* (Texas windmill grass), *Gaillardia aestivalis* var *winkleri* (White firewheel), *Hibiscus dasycalyx* (Neches River rosemallow), and *Phlox nivalis* subsp *texensis* (Texas trailing phlox). These taxa were added to Mercer's portion of the National Collection which already included *Abronia macrocarpa* (Large-fruited sandverbena), *Aster puniceus* subsp *elliottii* var *scabricalis* (Rough-stemmed aster), *Cyperus louisianensis* (Louisiana nutsedge), *Hymenoxys texana* (Prairie dawn), *Lesquerella pallida* (White bladderpod), and *Physostegia correllii* (Correll's false dragonhead).

### 2. Field Collections

The genetic representation in Mercer's collections was augmented through collections from the wild of *Abronia macrocarpa* (Large-fruited sandverbena), *Chloris texensis* (Texas windmill grass), *Hymenoxys texensis* (Prairie dawn), *Machaeranthera aurea* (Houston daisy), and *Phlox nivalis* subsp *texensis* (Texas trailing phlox). (See reports on individual taxa below.)

**Selected Taxon Summaries** See Attachment F, a printout of the Status of the Conservation Collection at Mercer, for detailed inventory information.)

1. *Abronia macrocarpa* (Large-fruited sandverbena), part of the CPC National Collection at Mercer. Known from a handful of sites, only one of which is accessible. Our three wild-collected accessions were made from the same site in different years. One of these accessions, 93090002 (previously known as MERC #3), collected in 1989, is represented by 3 genetically identical plants resulting from one seed of the original collection, 128 second and third generation seeds (accessions 94110001, 94110002, 94110003, and 94110044) from 93090002 plants, and 2 plants from one of these second generation seed accessions, 94110002. The other two wild-collected accessions include 1175 seeds (950543) collected in 1994, and 242 seeds and 6 resulting plants (950232) collected in 1995.

Two sets of germination tests were performed in 1995:

Test #1 (initiated 29 March): 30 seeds from 93090002, collected from *ex situ* plants in 1992 and stored in a refrigerator, were rehydrated, soaked for 10 minutes in a 25% bleach solution, washed in running water for 8 hours,

stratified in moist paper towels in a zip lock bag in the refrigerator for 10 days, and sown 1/4 inch deep in Redi-Earth germination mix. Seven seeds germinated (28%) and two still survive.

Test #2 (initiated 9 May) was a preliminary test to determine if the bleach treatment helped or hurt and whether or not it was necessary or desirable to remove seeds from their anthocarps.

Treatment 1.	20 seeds, bleach treatment, anthocarp removed.	4 seedlings (20% germination)
Treatment 2.	20 seeds, no bleach, anthocarp removed.	3 seedlings (15% germination)
Treatment 3.	20 seeds, bleach treatment, in anthocarps.	0 seedlings (0% germination)
Treatment 4.	20 seeds, no bleach, in anthocarps.	0 seedlings (0% germination)

These preliminary tests suggest that soaking in a bleach solution has little effect on germination, but removing the seeds from their anthocarp may be helpful in promoting germination. Six of the resulting plants from this test still survive.

*Abronia macrocarpa* plants are found on sand dunes, where they develop carrot-like tap roots. The plants bloom in early spring, set seed, and die back to the root for the summer. Seeds sown in late May from the wild-collected accession 950232 produced little succulent tap roots, and survived the summer well. Cuttings, however, taken from wild plants in April rooted nicely but appeared to produce only fibrous roots and did not survive the summer. Cuttings taken at other times, however, have done well at Mercer, perhaps if taken earlier in the growing season the plants have enough time to develop sustainable tap roots.

2. *Aster puniceus* subsp. *elliottii* var. *scabricaulis* aka *A. scabricaulis* (Rough-stemmed aster), part of the CPC National Collection at Mercer. Of approximately 6 populations in the wild, we have two accessions (5600 seeds in 92110001 and 800 seeds in 92110003) from two sites collected in 1992. Two plants from seed accession 92110001, exhibited phenotypic differences and have been assigned accession numbers 92110001-1 and 92110001-2. Eighteen cuttings taken August 24 from each of these two plants rooted within two weeks (100% success) and still survive.

A germination test initiated 2 November 1994, of 100 seeds from 92110001, which were stored under sub-optimal conditions, resulted in 3 seedlings (3% germination) of which 2 plants survive (see above). Seeds were pressed onto soil surface (Redi-Earth germination mix) and placed under mist. Trays of ungerminated seeds were stratified twice in the hope that more germination would result; none did.

Our plants flowered, but each flower head contained a tiny larva which apparently ate all the seeds, thus no second generation seeds were collected.

3. *Chionanthus pygmaeus* (Pygmy fringe tree), a CPC National Collection taxon at Bok Tower Gardens. The commercially obtained plant in the Endangered Species Garden has performed well and survived the October, 1994, flood but did not bloom as profusely in 1995 as it did the previous year (because of the flood?). Again this year, no seeds were produced. Perhaps outcrossing is necessary. The three seedlings from Bok Tower seeds did well in the nursery this year and will probably be planted in the Endangered Species Garden this winter.

4. *Chloris texensis* (Texas windmill grass), newly approved for the CPC National Collection. We have fourteen wild-collected plant divisions from seven sites in what are probably three populations and wild-collected seed from four populations, leading to a total of five populations represented in our collection.

- Population 1: plants: 93120104, 93120105, 93120106, 93120107, 93120108, 93120109, 94110014, 94110017, 94110018; seed: 93120110
- Population 2: plants: 94110012
- Population 3: plants: 950918, 950920, 950921, 920922, 950923; seed 950828, 950924

Mercer Arboretum and Botanic Gardens - Status of Conservation Collection at Mercer as of 22 DEC 1995

Page 1

LAG	ACC.NO.	SET	PRIMARY SCIENTIFIC NAME	PROVINANCE	QUANT.	TYPE	LOCATION	BED
>C	93090002	33	<i>Abronia macrocarpa</i>	field collected	1	plant	Nursery	Square
>C	93090002	33	<i>Abronia macrocarpa</i>	field collected	1	plant	Conservation	Tall
>C	93090002	33	<i>Abronia macrocarpa</i>	field collected	1	plant	Conservation	Tall
>C	94110001	00	<i>Abronia macrocarpa</i>	in-house propagation 1991	28	seed	BIC	Laboratory
>C	94110002	00	<i>Abronia macrocarpa</i>	in-house propagation 1992	48	seed	BIC	Laboratory
>C	94110002	01	<i>Abronia macrocarpa</i>	in-house propagation 1992	2	plant	Conservation	Tall
>C	94110003	00	<i>Abronia macrocarpa</i>	in-house propagation of 93090002	6	seed	BIC	Laboratory
>C	94110044	25	<i>Abronia macrocarpa</i>	in-house propagation of 93090002	46	seed	BIC	Laboratory
>C	950232	00	<i>Abronia macrocarpa</i>	field collected	242	seed	BIC	Laboratory
>C	950232	01	<i>Abronia macrocarpa</i>	field collected	6	plant	Conservation	Tall
>C	950543	00	<i>Abronia macrocarpa</i>	field collected	1175	seed	BIC	Laboratory
***								
	93060019	01	<i>Aesculus parviflora</i>	commercial source	1	plant	East Gardens	Endangered Spe
***								
	950901	01	<i>Alophia drummondii</i>	private collection	1	clump	East Gardens	Endangered Spe
	950902	01	<i>Alophia drummondii</i>		1	clump	East Gardens	Iridaceae
***								
>C	94120031	01	<i>Aquilegia hinckleyana</i>	commercial source	2	plant	Central Garde	Little Triangl
>C	950068	01	<i>Aquilegia hinckleyana</i>	commercial source	17	plant	East Gardens	Endangered Spe
>C	950504	00	<i>Aquilegia hinckleyana</i>	in-house propagation 94110042, 9	25400	seed	BIC	Laboratory
>C	950504	01	<i>Aquilegia hinckleyana</i>	in-house propagation 94110042, 9	180	plant	Nursery	Shade
***								
>C	92110001	00	<i>Aster puniceus</i> subsp <i>elliottii</i> var <i>scabr</i>	field collected	5600	seed	BIC	Laboratory
>C	92110001	01	<i>Aster puniceus</i> subsp <i>elliottii</i> var <i>scabr</i>	field collected	7	plant	East Gardens	Endangered Spe
>C	92110001	01	<i>Aster puniceus</i> subsp <i>elliottii</i> var <i>scabr</i>	field collected	12	plant	Nursery	Square
>C	92110001	02	<i>Aster puniceus</i> subsp <i>elliottii</i> var <i>scabr</i>	field collected	13	plant	East Gardens	Endangered Spe
>C	92110001	02	<i>Aster puniceus</i> subsp <i>elliottii</i> var <i>scabr</i>	field collected	5	plant	Nursery	Square
>C	92110003	00	<i>Aster puniceus</i> subsp <i>elliottii</i> var <i>scabr</i>	field collected	800	seed	BIC	Laboratory
>C	950941	00	<i>Aster puniceus</i> subsp <i>elliottii</i> var <i>scabr</i>	in-house propagation from Endang	150	seed	BIC	Laboratory
***								
>C	93070361	01	<i>Chionanthus pygmaeus</i>	commercial source	1	plant	East Gardens	Endangered Spe
>C	94010132	00	<i>Chionanthus pygmaeus</i>	from another botanical instituti	3	plant	Nursery	Square
***								
>C	93120104	01	<i>Chloris texensis</i>	field collected	1	plant	Conservation	
>C	93120104	01	<i>Chloris texensis</i>	field collected	2	clump	East Gardens	Endangered Spe
>C	93120105	01	<i>Chloris texensis</i>	field collected	1	plant	Conservation	
>C	93120105	01	<i>Chloris texensis</i>	field collected	1	clump	East Gardens	Endangered Spe
>C	93120106	01	<i>Chloris texensis</i>	field collected	1	plant	Conservation	
>C	93120106	01	<i>Chloris texensis</i>	field collected	1	clump	East Gardens	Endangered Spe
>C	93120107	01	<i>Chloris texensis</i>	field collected	1	plant	East Gardens	Endangered Spe
>C	93120107	01	<i>Chloris texensis</i>	field collected	1	plant	Conservation	
>C	93120108	01	<i>Chloris texensis</i>	field collected	1	plant	Conservation	

APPENDIX V Research plan for September, 1992 through August, 1993.

- I. Protect known populations from existing and future threats.
  - A. Continue to work closely with landowners to establish short-term management practices adequate to protect the species.
  - B. Monitor populations monthly (September - January), bimonthly (February - June), and monthly (July & August) and map individual plants to assess population numbers and site requirements within a population (4).
- II. Maintain the reserve germ bank/cultivated population at SWT.
  - A. Maintain and monitor plants in the Greenhouse and outdoor planting area.
- III. Continue studies to gather information necessary for protective management/restoration.
  - A. Determine microclimate habitat requirements (4).
    1. Measure surface and subsurface temperature profiles using a hand-held digital thermometer.
    2. Measure plant water stress tolerance using a Scholander pressure chamber.
  - B. Community Structure:
    1. Continue to survey diagnostic/associated species and prepare voucher specimens.
  - C. Community dynamics/ecology:
    1. Monitor interactions with other species.
    2. Assess requirement for openings through artificial shading experiments.
  - D. Characterize phenology:
    1. Monitor plant development in the population over a twelve month period.
  - E. Determine reproductive biology including: Pollination biology, Seed production and dispersal, and Seedling recruitment (5; 6; 7; 8). February - August 1993.
    1. Reproductive Biology will be studied in the field and greenhouse as follows:
      - a) Field Studies (February - June 1993):
        - Survey for Potential Pollinators:
          - collect & identify floral visitors
          - determine pollen load using light and scanning electron microscopy
          - document visitor movements
          - bagging experiments to test effect of visitor exclusion
        - Investigate Floral Biology and Reproductive Capacity:
          - maturation of flower (anthesis, receptivity, dehiscence)
          - collect & analyze nectar using chromatography
          - determine pollen viability (aniline blue-lactophenol stain)
          - count number of flowers per plant
          - count number of fruits per plant
          - determine percentage of viable seed using tetrazolium
          - determine extent of fruit/seed dispersal
          - determine extent of seedling recruitment
      - b) Greenhouse Pollination Experiments:
        - Test methods of pollination:
          - Autogamy - transfer of pollen within a single flower.
          - Geitonogamy - transfer of pollen within the same plant but not the same flower.
          - Xenogamy - transfer of pollen from one plant to another within the same species.
  - F. Study cultivation requirements (9; 10):
  - G. Search/inventory potential habitats:
    1. For existing populations
    2. For existence/inventory of potential restoration sites
  - H. Develop public concern & support for the preservation and study of the species:
    1. Present paper(s) at scientific meeting(s).

## RESEARCH PLAN YEAR 2: (September 1, 1993 - August 31, 1994)

- I. Protect existing populations from existing and future threats.
  - A. Continue establishing working relationship with landowners to:
    1. Maintain protected sites
    2. Develop and implement long-term management/monitoring plans
  - B. Continue to monitor and map populations
  - C. Assess and revise management plans considering changes in population conditions
- II. Maintain a reserve seed bank/cultivated population at the SWT Natural Science Center.
  - A. Maintain maximum genetic diversity
  - B. Continue monitoring/management plan
  - C. Continue cultivation/restoration research efforts
- III. Continue studies to gather information to assess management/restoration plan
  - A. Continue evaluation of community structure
  - B. Continue study of community dynamics/ecology
    1. Examine temperature and soil moisture microclimate requirements
    2. Examine potential competition by associated species
    3. Examine extent of mycorrhizal associations
    4. Examine extent of herbivory
  - C. Continue monitoring phenology
  - D. Continue monitoring reproductive biology
  - E. Continue monitoring cultivation requirements
  - F. Continue search/inventory for potential habitat
  - G. Reassess restoration feasibility
    1. Establish a pilot program
    2. Reassess feasibility of re-introduction program
  - H. Maintain and expand public concern & support for the preservation and study of the species

## RESEARCH PLAN YEAR 3: September 1, 1994 - August 31, 1995

- I. Protect existing populations from existing and future threats.
  - A. Continue establishing working relationship with landowners to:
    1. Maintain protected sites
    2. Develop and implement long-term management/monitoring plans
  - B. Continue to monitor populations
  - C. Assess and revise management plans
- II. Maintain a reserve seed bank/cultivated population at the SWT Natural Science Center
  - A. Maintain maximum genetic diversity
  - B. Continue monitoring/management plan
  - C. Continue cultivation/restoration research efforts
- III. Continue studies to gather information to assess management/restoration plan
  - A. Continue evaluation of community structure
    1. Continue monitoring population fluctuations
  - B. Continue study of community dynamics/ecology as in year 2 and study
    1. Response to disturbance and agricultural practices through monitoring an area disturbed by oil drilling practices and removal of non-native species
  - C. Continue monitoring phenology
  - D. Continue monitoring reproductive biology
  - E. Continue monitoring cultivation requirements
  - F. Continue search/inventory for potential habitat
  - G. Reassess restoration/ re-introduction feasibility and pilot program established in year 2
  - H. Increase public concern & support for the preservation and study of the species