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Pollination, Herbivory, and Habitat Fragmentation: Their Effects on the Reproductive Fitness of *Angadenia berteroi*, a Native Perennial Plant of the South Florida Pine Rocklands

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

POLLINATION, HERBIVORY, AND HABITAT FRAGMENTATION: THEIR
EFFECTS ON THE REPRODUCTIVE FITNESS OF *ANGADENIA BERTEROI*, A
NATIVE PERENNIAL PLANT OF THE SOUTH FLORIDA PINE ROCKLANDS

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

BIOLOGY

by

Beyte Barrios Roque

2015

To: Dean Michael R. Heithaus
College of Arts and Sciences

This dissertation, written by Beyte Barrios Roque, and entitled Pollination, Herbivory, and Habitat Fragmentation: Their Effects on the Reproductive Fitness of *Angadenia berteroi*, a Native Perennial Plant of the South Florida Pine Rocklands, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Date of Defense: March 27, 2015

The dissertation of Beyte Barrios Roque is approved.

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Florida International University, 2015

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DEDICATION

Le dedico esta tesis de doctorado a la memoria de mis abuelos por todo su cariño y por siempre estar a mi lado.

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ABSTRACT OF THE DISSERTATION

POLLINATION, HERBIVORY, AND HABITAT FRAGMENTATION: THEIR
EFFECTS ON THE REPRODUCTIVE FITNESS OF *ANGADENIA BERTEROI*, A
NATIVE PERENNIAL PLANT OF THE SOUTH FLORIDA PINE ROCKLANDS

by

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Florida International University, 2015

Miami, Florida

Professor Suzanne Koptur, Major Professor

Angadenia berteroi is a tropical perennial subshrub of the pine rocklands with large yellow flowers that set very few fruits. My dissertation seeks to elucidate the factors that affect the reproductive fitness of *Angadenia berteroi* a native species of the south Florida pine rocklands. I provide novel information on the pollination biology of this native species. I also assess the effects of herbivory on growth and the reproductive success of *A. berteroi*. Finally, I elucidate how habitat fragmentation and quality are correlated with reproductive fitness of this native perennial plant.

Using a novel experimental approach, I determined the most effective pollinator group. I used nylon fishing line of widths corresponding to proboscis diameter of the major groups of visitors to examine pollen removal and deposition. In the field, I estimated visitation frequency and efficacy of each pollinator type. Using potted plants, I exposed flowers to single visit from different types of pollinators to measure fruit set. I performed artificial defoliation with scissors on plants growing in the greenhouse to assess the effects of defoliation before flowering as well as during flowering.

Additionally, I used structural equation modelling (SEM) to elucidate how *A. berteroi* reproductive fitness was affected by habitat fragmentation and quality.

My experiments provide evidence that *Angadenia berteroi* is specialized for bee pollination; though butterflies, skippers and others also visit its flowers, *A. berteroi* is exclusively pollinated by two native bees of the South Florida pine rocklands . This research also demonstrated that herbivory by the oleander moth may have direct and indirect effects on *Angadenia berteroi* growth and reproductive success. The SEM results suggested that habitat quality (litter depth and subcanopy cover) may favor reproduction in native species of the South Florida pine rocklands that are properly maintained by periodic fires and exotic control. Insights from this threatened and charismatic species may provide impetus to properly manage remaining pine rocklands in South Florida for this and other endemic understory species.

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INTRODUCTION

Habitat destruction and fragmentation may modify ecological interactions between species, such as pollination and herbivory (Laurence 2002). Fragmentation results in an increased proportion of “edge” habitat, and resultant changes in microclimate and community structure at various distances from the edge (Margules and Pressey 2000). Such changes in habitat quality may have even greater effects on the reproductive biology and population dynamics of the species in the remaining fragments. Additionally, fragmentation alters the composition, abundance, and distribution of herbivorous insects, affecting herbivory rates (Didham et al. 1996); these changes may have a great influence on the growth, fruit production, and survival of plants (Marquis 1984, Strauss 1997).

Plant reproductive success is negatively affected by habitat fragmentation and degradation, regardless of the type of habitat, ecological interactions, and life history traits (Aguilar et al. 2006). A decrease in fragment area may cause significant reduction in pollination (Rathcke and Jules 1993, Aizen and Feinsinger 1994) while the distance between fragments may have adverse effects on the distribution and abundance of insect species (Artz and Waddington 2006); Both can result in a decline in fruit and seed set (Aizen and Feinsinger 1994, Hendrix and Kyhl 2000, Hobbs and Yates 2003).

Habitat fragmentation reduces species richness and the genetic variability of species as well as increases the population’s extinction risk through inbreeding depression and genetic drift (Young et al. 1996, Frankham 2005, Vargas et al. 2006). Fragmented populations often exhibit reduced population size, and there is reduced gene flow among plants in the remnant fragments compared with those in intact habitat (Aizen and Feinsinger 1994, Young et al. 1996). Low population sizes can result in Allee effects,

defined as positive (inverse) density dependence at low densities, where the fitness of individuals is lower than expected at low numbers (Courchamp et al 1999). Plants in small populations experience an Allee effect as a result of pollen limitation (Coombs et al. 2009); for animal-pollinated plants, a small population density may reduce pollination visits because of the reduced floral display and rewards. But pollen quality may also decrease at low plant densities as a result of cross-pollinations between related individuals (Lamont et al. 1993, Coombs et al. 2009, Levin et al. 2009). In addition, in fragmented habitat, the disturbance regime has been altered (Cochrane 2001).

The pine rockland ecosystem is unique in the United States and is considered an imperiled habitat. Pine rocklands are dominated by a single canopy species, *Pinus elliottii* var. *densa*, the south Florida slash pine. Subcanopy development is rare in pine rockland, but a great variety of species of shrubs, hardwood taxa and palms may be present. Many of these species are part of the tropical flora of the West Indies. Pine rockland flora is a mixture of tropical and temperate taxa, with many endemic species and endangered species (Snyder et al. 1990; US Fish and Wildlife Service, 1999). Especially, the herb stratum is diverse, with more than 250 species, a combination of grasses, ferns, sedges, and forbs: many endemic, some threatened and rare. Fire is a vital force that is essential to the existence of the pineland ecosystem. Burnings influence the vegetation structure and species composition of the understories (Snyder et al. 1990; US Fish and Wildlife Service, 1999).

Pine rockland landscapes have undergone dramatic changes because of human population growth over the last 100 years, leading to much destruction of pine rockland habitat; outside of Everglades National Park only 2 percent of the original pine rocklands

remain, and these sites occur in form of many small fragments (Snyder et al. 1990, Koptur 2006). Proximity to human habitats leads to fire exclusion. Within two decades of such exclusion, the pine rockland can become a closed canopy tropical hardwood forest (known as “hammock”), and the pine trees and native herbaceous flora disappear (Snyder et al. 1990).

My dissertation seeks to elucidate the factors that affect the reproductive fitness of *Angadenia berteroi*, a native species of the South Florida pine rocklands. By investigating the influence of various interacting factors on flowering and fruit set, I provide novel information on the pollination biology of this native species. I assess effects of herbivory on growth and reproductive success of *A. berteroi*. My overall objective is to elucidate how habitat fragmentation and quality are correlated with the reproductive fitness of this threatened endemic plant. An outline of the dissertation follows.

Chapter I elucidates the flower visitors and the effective pollinators of *Angadenia berteroi*. We study the diversity and abundance of animals visiting the flowers and determine the effectiveness of each type of visitors in terms of pollen removal and fruit set. The results of this chapter demonstrate the importance of pollen removal and fruit set in determining the most effective pollinators, rather than visitor abundance. Chapter 1 is currently in review by the American Journal of Botany.

Chapter II establishes the effects of simulated folivory and florivory on growth, flower production, and reproductive success of *Angadenia berteroi*. Results indicate that damage to foliage negatively affects growth and reproductive success of this native species, suggesting that *A. berteroi* compensates for leaf tissue lost by allocating

resources to production and maintenance of new vegetative tissues which in turn provide photosynthates for flower production. I plan to submit Chapter II to *Annals of Botany* and it has been formatted accordingly.

Chapter III investigates the effect of habitat fragmentation and quality on abundance and plant reproductive fitness of this pollination-dependent native species. I use a structural equation modeling to assess the influence of interactions among various factors affecting seedling density, flowering, and fruit set. Results indicated that habitat quality is very important, and that both canopy cover and litter depth have a strong negative effect on the reproductive fitness of *A. berteroi*. I plan to submit Chapter III to *Journal of Ecology* and it has been formatted accordingly.

Angadenia berteroi is a charismatic wildflower species, one of the most beautiful flowers native to South Florida pine rocklands. Each of the chapters helps us to understand the pollination biology of this endemic species, and how habitat fragmentation and herbivory affect its reproductive success. This attractive species can serve as a model system, representative of pine rockland plants (most which are perennials, some of which are self-incompatible, and many of which are rare/threatened) and the effects of anthropogenic changes in habitat on their biology and conservation.

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CHAPTER I
POLLINATION OF *ANGADENIA BERTEROI* (APOCYNACEAE) IN SOUTH
FLORIDA PINE ROCKLANDS: WHY BEES ARE BETTER THAN BUTTERFLIES.

Abstract

Angadenia berteroi is a tropical perennial subshrub with large yellow flowers that set few fruits in its pine rockland habitat. The flowers open early in the morning and have a complex floral arrangement that promotes outcrossing from visits by long-tongued pollinators. A variety of bees and butterflies visits the flowers, but low fruit set indicates that many are ineffective pollinators. In this study, we determined the most effective pollinator group, and estimated the visitation frequency and efficacy of each pollinator type. Using potted plants, we exposed flowers to single visits from different types of pollinators to measure fruit set. Our observations showed skipper butterflies to be the most frequent visitors, followed by bees and larger butterflies. Although we expected that butterflies and skippers, with their long mouthparts, would be the best pollinators, bees carried the largest quantities of pollen on the proboscises, apparently because they had the widest mouthparts. The width of the proboscis of the pollinators correlates with pollen transfer efficiency, as demonstrated experimentally with fishing lines of varying diameters. Furthermore, flowers visited in the field (then bagged and observed in the greenhouse) set fruit only when they were visited by bees, indicating bees are the most effective pollinators of this species. Our results demonstrate the importance of pollen removal and fruit set in determining the most effective pollinators, rather than visitor abundance. The distinctive morphology of these flowers, with a large bell and a narrow, short tube, suggests that other flowers of this shape may similarly benefit more from visitors with shorter mouthparts than previously considered optimal.

Key Words: Apocynaceae; Floral visitors; Lepidoptera; Hymenoptera; Pine rocklands; Pollen transfer efficiency; Pollination. *Megachile*, *Melissodes*

Introduction

Most flowering plants rely on animals to accomplish pollination, and the evolution and diversification of the perianth has been correlated with animal attraction (Proctor et al., 1996; Inouye and Kearns, 1997; Richards, 1997). Convergent groups of floral traits present in unrelated plants that share similar pollinators are known as pollination syndromes: floral morphology, phylogenetic position, and floral reward characteristics are all important in predicting what pollinates a given plant species (Johnson and Steiner, 2000; Etcheverry and Aleman, 2005; Ollerton et al., 2007). Within communities, the majority of plant species are visited by various pollinator groups, but visitation does not necessarily imply pollination; not all flower visitors are important and effective pollinators (Waser et al., 1996; Fenster et al., 2004). A pollinator's importance to the plant is its relative contribution to the plant's reproduction, and involves pollinator efficacy (successful dispersal of pollen grains deposited on conspecific stigmas), visitation rate, and pollinator abundance (Waser et al., 1996). These parameters, however, are independent, and sometimes the most frequent visitors may not be the most effective pollinators (Mayfield et al., 2001; Fenster et al., 2004).

The pine rockland ecosystem of South Florida is unique in the United States and is considered an imperiled habitat (US Fish and Wildlife Service, 1999). Pine rocklands are characterized by a diverse understory of flowering plants (Snyder et al., 1990; US Fish and Wildlife Service, 1999), including the pineland golden trumpet, *Angadenia berteroi* (A.DC.) Miers (Apocynaceae, Apocynoideae). South Florida has undergone dramatic human population growth over the past 100 years, leading to much destruction of pine rockland habitat. Outside of Everglades National Park, only 2 percent of the

original pine rocklands remain, and these sites occur as many small fragments (Snyder et al., 1990; Koptur, 2006).

Angadenia berteroi is a perennial subshrub listed as threatened by Florida Department of Consumer Services, Division of Plant Industry (Gann et al., 2002). The Apocynaceae *sensu lato* (i.e., including Asclepiadaceae) is a diverse family in the order Gentianales, which includes 424 genera distributed among five subfamilies, and is distributed worldwide (Endress and Bruyns, 2000). The genus *Angadenia* includes two species (Mabberley, 2008) and is part of the tribe Echiteae, which includes 21 genera in the New World clade, most species of which are endemic to the Americas (Livshultz et al., 2007).

The floral structure and pollination biology of asclepioid members of the family Apocynaceae *sensu lato* have been studied by many, but little attention has been given to the non-asclepioid members. These latter taxa do not have pollinia but are characterized by a complex floral structure and pollination mechanism favoring cross pollination (Darrault and Schlindwein, 2005). In most non-asclepioid species, the anthers are adnate to the corolla and form a conical structure surrounding the style head (stigma) (Galletto, 1997; Lipow and Wyatt, 1999; Darrault and Schlindwein, 2005). The style head is divided into three regions: the sterile apical portion, a medial secretory region, and a receptive area at the base (Lipow and Wyatt, 1999; Darrault and Schlindwein, 2005). Secondary pollen presentation results when the anthers deposit the pollen on the apical portion of the stigma (Yeo, 1993). Searching for nectar, an insect inserts its tongue into the flower tube. As the mouthparts are retracted, exogenous pollen is captured from the receptive area of the style head, and then the tongue is covered with the mucilaginous

substance from the medial secretory region, removing pollen grains from the flower as it passes through the apical pollen chamber (Darrault and Schlindwein, 2005; Pinto et al., 2008).

The complex flower structure of the non-asclepioid members of the Apocynaceae and secondary pollen presentation linked with herkogamy (spatial separation between anthers and stigma) point to a specialized pollination system (Yeo, 1993; Torres and Galetto, 1999). Flowers in the Apocynaceae may have evolved to attract insects with mouthparts long enough to reach the nectar in the base of the tubular flowers (Endress, 1994; Proctor et al 1996; Darrault and Schlindwein, 2005; Pinto et al., 2008) and lengths of the proboscises of the pollinators are related to the lengths of the floral tubes (Proctor et al., 1996).

Pollination syndromes in the family Apocynaceae are diverse. Insects represent the major floral visitors of Apocynaceae *s.l.* (Endress, 1994), with reports of beetles, butterflies, hawkmoths, flies, wasps, and bees pollinating species of this family (Haber, 1984; Lopes and Machado, 1999; Darrault and Schlindwein, 2005; Moré et al., 2007; Theiss et al., 2007; de Moura et al., 2011; Wiemer et al., 2012; Stoepler, 2012). In the Asclepiadoideae, hymenopteran and dipteran pollinators are common, whereas species of the non-asclepioid members of the Apocynaceae possess attributes that suggest bee and butterfly pollination syndromes (Endress, 1994). A functional specialization, defined by Waser et al. (1996) as a specialization to a functional group of pollinators, has been found in various members of the Asclepiadoideae (Ollerton and Liede, 1997; Wolff et al., 2008).

Angadenia berteroi bears large, showy, yellow flowers, which have a complex floral arrangement: the anthers form a conical structure surrounding the stigma (Barrios and Koptur, 2011). The flowers have no notable fragrance but offer viscous nectar as a pollinator reward. The sugar concentration of the nectar ranges from 30 – 67% on a wt/wt basis, in the higher range of butterfly-pollinated flowers, and well within the range of bee-pollinated flowers (Barrios and Koptur, 2011). Flowering begins in early April in South Florida and continues until late June; the flowers open early in the morning (prior to sunrise) and last less than 24h (Barrios et al., 2011, Barrios and Koptur, 2011). Our field observations revealed that the natural level of fruit set in *A. berteroi* is low: population average fruit set was 16.6%, ranging from 3.3% to 26.4%, over six pine rockland fragments (Barrios and Koptur, 2011). This species relies on pollinators to set fruit, and evidently, low fruit set is the result of inadequate pollination. Fruit set from self-pollination is unlikely to occur because *A. berteroi* is predominantly self-incompatible; in controlled self- and cross-pollinations, greatest fruit-set, fruit length, and seedling emergence result from crosses between unrelated individuals (Barrios and Koptur, 2011). The low fruit-set observed in natural populations may be due to low visitation by pollinators, mating between closely related individuals, or both (Barrios and Koptur, 2011). Though visitors to flowers are observed infrequently, Lepidoptera are the most commonly observed.

We undertook this study to elucidate the flower visitors and the effective pollinators of *Angadenia berteroi*. We asked: 1) What is the diversity and abundance of animals visiting the flowers; and 2) Are the most common visitors the best pollinators?

We used a variety of methods to determine the effectiveness of each type of floral visitor in terms of pollen removal, and fruit set.

Materials and Methods

We studied *Angadenia berteroi* and its habitat variables in four pine rockland fragments and one fire management unit in Everglades National Park (ENP) (Table 1). We chose the sites on the basis of their size and degree of isolation from the continuous pine rocklands in ENP (Barrios et al., 2011), as well as the presence of many individuals of the study species.

Flower visitors —We conducted pollinator watches weekly for a total of three hours from 9:00 am to 12:00 pm (in 15 min periods) at each of the five study sites during the flowering period (April to June). Flower visitors were counted and some representatives of each group were captured at all sites using aerial nets. We identified each visitor to pollinator group or species when possible; vouchers were deposited at Florida International University. Pollen grains were collected from the insect bodies to see if visitors carried *A. berteroi* and/or other pollen. Foraging behavior, time spent on the flower, and visitation frequency of floral visitors were estimated by counting visits of foraging insects to the *A. berteroi* flowers and following their movements. Pollination watches were performed to monitor flower visitors on plants in the four fragments of different sizes, as well as Everglades National Park (Table 1). Floral visitors were divided into four taxonomic groups (Table 2) and the groups were compared.

Pollinator effectiveness —We placed 15 greenhouse-grown potted plants in the field to quantify pollination at Site 3 each day for over 20 days of observations, at site 3, the site with the highest visitation rates. We determined pollination success and compared

the effectiveness of the different pollinator groups by observing visits to individual flowers on the potted plants, then bagging and following those flowers after they were visited. Flowers that were ready to open were bagged while in bud, to exclude visitors; bags were removed and flowers exposed to foraging insects, and were observed continuously to record any and all visits. Flowers visited by different pollinators were tagged, bagged to exclude subsequent visitors, and followed to see if they produced fruit (when the potted plants brought back to the greenhouse). Fruit set (the ultimate measure of pollination success) was compared among flowers visited by different kinds of pollinators. We recorded a total of 69 visits for over 300 plants (each plants have one to three open flowers) over 20 days of observations. Pollination efficacy of different insect visitors was assessed by estimating success in producing fruits.

To determine the relationship between the numbers of pollen grains removed by the different types of flower visitors and the thickness of their proboscises, we first measured the length and width of the proboscis of each captured flower visitor using a dissecting microscope (Leica MZ12 5). We simulated flower visits using 4-cm lengths of premium monofilament nylon fishing line of four different diameters (4-lb, 0.20-mm diameter; 6-lb, 0.23-mm diameter; 8-lb, 0.28-mm diameter; 25-lb, 0.53-mm diameter), chosen to match the size of the mouthparts of each group of visitors. A single piece of fishing line was introduced into the corolla tube of a fresh flower, and the adhering pollen grains were counted; 50 replications of each diameter introduction were performed. To test for a possible relation between the thickness of the proboscis of the different groups of flower visitors with pollen deposition, we hand pollinated fresh flowers using fishing line of four different diameters (see above), inserting each into a fresh flower to collect

pollen after staining the fishing line with methylene blue. The flowers were carefully dissected, and the length of the stigmatic surface that was stained with methylene blue was recorded. This measure (length of the stigmatic surface stained blue) indicated the portion of the stigmatic surface touched by the fishing line, as well as indicating potential pollen deposition on the stigma. We performed 23 replicates of each diameter introduction.

On the basis of our observations, bees (Apidae and Megachilidae) and metallic bees (Halictidae) were the only two pollinator groups that consistently entered the bell of the flower to insert their proboscis into the pollen tube. To estimate how far these two types of pollinators could get in to the corolla tube, we measured the distance from the apical part of the pollen chamber to the corolla walls in 30 individual plants (63 flowers total) using a Bausch & Lomb measuring magnifier (Figure 1) We measured the width of the head of the two bee groups (n = 4 metallic bees, n = 17 bees) using a dissecting microscope (Leica MZ12 5).

We used pollination efficiency to evaluate the effectiveness of each visitor group, whereas visitation frequency helped us to identify the most common visitor of *A. berteroi*. These variables allowed us to rank the significance of each visitor species to the reproduction of *A. berteroi*, and to determine the most effective pollinator.

Pollination and flowering plant density —To determine if a positive relationship exists between pollinator visitation and flower density, a field experiment was conducted involving two treatments: 1) plants with a single flower and, 2) plants with up to 5 flowers, placed at least 5 meters apart from another flowering plant in the field. We placed 5 replicates with at least two greenhouse-grown potted plants of each treatment at

least 10 meters apart. The plants were left at the site for 24 h to allow maximum visitation to open flowers. At each replicate, 10 min floral visitor watches were performed for a total of 2 hours per period. We recorded pollinator visits and length of visits per treatment. Visited flowers were tagged, labeled and followed in the greenhouse to see if they produced fruit; open flowers that were exposed in the field (and perhaps visited when we were not watching) were also tagged. Fruit and seed set were compared between treatments. As a measure of visitation rate, we carefully collected the stigma of each fallen flower to compare the numbers of pollen grains deposited at the base of the stigma.

Statistical Analysis —We used the Kruskal-Wallis test to detect differences among visitor groups for the average length of the visit, as data were not normally distributed; and then used the Mann-Whitney test (post-hoc) to determine differences among the groups. Analysis of variance (ANOVA) was used to test for differences among visitor groups for proboscis length and width, and length of the stigmatic surface stained with methylene blue, as the assumptions of ANOVA were satisfied; *post hoc* tests were conducted using Tukey HSD (Honestly Significant Difference) to test for differences between pairs of visitor groups. Pollen loads on the fishing line were compared using the Kruskal-Wallis test, with the Mann-Whitney test (post-hoc) to determine differences in pollen loads between fishing line sizes, as the data were not normally distributed. Fruit set and pollen on the stigma were analyzed with chi-square (contingency table analysis) to compare single and grouped flowers. We also evaluated the differences between the two groups' head widths using Student's t-test. We performed correlation analyses using Spearman's coefficient to investigate the

relationship between pollen on the stigma and fruit set (using the terminology of Zar 1999, Green and Salkind 2007). We used the Bonferroni method to control type I error for all pairwise comparisons. Statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) version 21 (SPSS, 2014).

Results

Flower visitors — we observed a total of 153 insect visits to *A. berteroi* flowers of which 56 insect visitors were captured at the four sites belonged to 12 species in four visitor groups (Table 2, 3). Skippers (Hesperiidae) were the most frequent visitors (Table 3). Butterflies and skippers were observed to return to the same flower more frequently than bees (Table 3). Furthermore, bees were much less likely than butterflies and skippers to visit a consecutive *A. berteroi* flower (8–15 % of the total observed bee visits showed a bee visiting a flower on the same plant, versus around 30 % of butterfly visits). The length of the visits differed significantly among pollinator groups (Kruskal-Wallis test, $X^2_3 = 12.3$, $P = 0.006$, $n = 153$, Table 3), with skippers spending approximately 10 seconds in each flower, while visits from the other groups were one or two seconds shorter (Table 3). All visitors seemed to prefer to visit plants with more than two flowers. Though we expected butterflies and skippers to be the best pollinators, bees were the visitors that carried large quantities of pollen on the proboscises (Table 4). Overall, bees and skippers were the most common floral visitors in all the study sites (Table 5).

Pollination efficiency — Proboscis width and length differed significantly among visitors groups ($F_{3, 51} = 41.11$, $P < 0.0001$; $F_{3, 44} = 85.3$, $p = 0.002$, respectively, Figure 2). Butterflies and skippers had the longest proboscises, while metallic bees (Halictidae) had the shortest (Figure 2). Bees were the visitor group with the widest proboscises (Figure

2); between the two species of bees collected (*Megachile georgica* and *Melissodes communis communis*), proboscis width and length did not differ significantly ($t = 1.12$, $df = 15$, $P = 0.26$; $t = -1.24$, $df = 11$, $P = 0.24$ respectively). In the visitation simulations using nylon fishing line of different diameters, pollen quantity significantly differed among diameters (Kruskal-Wallis test, $X^2_3 = 20.7$, $P < 0.001$, $n = 172$, Figure 3), with the widest fishing line having the greatest pollen load. Sizes 0.20 mm, 0.23 mm, 0.28 mm, which represent the proboscises of the skippers, butterflies, and metallic bees, respectively, had significantly lower numbers of pollen grains than 0.53 mm fishing line (proboscis size of bees).

In the pollen deposition simulation experiment, the length of the stigmatic surface stained with methylene blue was influenced by diameter of the fishing line ($F_{3,51} = 14.19$, $P < 0.0001$); size 0.53 fishing line touched a significantly larger proportion of the stigmatic surface than the smaller diameter lines (Figure 4). The mean (\pm SD) distance from the apical portion of the pollen chamber and the corolla walls was 2.6 (± 0.4) mm; and the mean (\pm SD) width of the bees' heads was 3.9 (± 0.22) mm, while that of metallic bees' heads was 1.8 (± 0.07) mm (Figure 1).

The final test of pollinator effectiveness --whether flowers visited by the different pollinators set fruit--gave clear cut results. Placing potted plants in the field, observing visits, and bagging, tagging, and following subsequent fruit set showed that only flowers visited by bees set fruit. Of the 44 flowers visited by bees, 36.4% set fruit. None of the flowers visited by any of the other groups (4 by butterflies, 19 by skippers, and 2 by metallic bees) produced fruit. No statistical analysis was employed as the difference was so dramatic.

Pollination and flowering plant density — Even though our field observations showed a positive relationship between flower visitors and flower density, as visitors were only observed on flowers present in groups of five or more, we did not see differences in either pollen deposition ($X^2_{1, n=71}=0.31$, $P = 0.58$) or fruit set ($X^2_{1, n=71}=2.1$, $P = 0.14$) in flowers on plants with single or multiple flowers placed in the field for 24 hours. Fruit set was positively correlated with pollen on stigma and quantity of pollen ($r = 0.4$, $P = 0.001$, $r = 0.5$, $P < 0.0001$ respectively).

Discussion:

The unusual flowers of the Apocynaceae vary in floral mechanisms, and many attract diverse visitors with appropriate body size or behavior (Haber, 1984; Lopes and Machado, 1999; Darrault and Schlindwein, 2005; Moré et al., 2007; Theiss, 2007; de Moura et al., 2011; Wiemer et al., 2012). The complex pollination apparatus in the Apocynaceae, with large numbers of ovules and pollen aggregation whereby large numbers of pollen grains can be removed and deposited collectively onto a single stigma, has likely been selected because these features increase the probability of producing fruit after a single visit (Harder and Johnson, 2008). We have taken advantage of this apparatus to evaluate pollination effectiveness.

The complex flower morphology of *Angadenia berteroi* is similar to the morphology described for other Apocynaceae (Barrios and Koptur, 2011). The yellow, campanulate flowers of *A. berteroi* restrict access to only those visitors with mouthparts long enough to reach the nectar. Secondary pollen presentation and the position of the receptive stigmatic surface further limit the receipt of pollen for fruit and seed set (Barrios and Koptur, 2011). Furthermore the sugar concentration of the nectar (30–67 %)

is within the range of values reported for flowers pollinated by bees (approximately 40%; Proctor et al., 1996). Although Pascarella et al. (2001) stated that *A. berteroi* is visited exclusively by lepidopterans, our field observations showed bees and skippers to be the most common floral visitors, with skippers the most frequent and constant visitors, often visiting numerous flowers of the same species in a row.

We have observed that skippers and butterflies often revisit the same flowers, while bees rarely return to a previously visited flower. Insects revisiting the same flowers could have negative consequences, as *A. berteroi* has a late-acting self-incompatibility mechanism (Barrios and Koptur, 2011). In many self-incompatible Apocynaceae, flower revisitation increases the probability that self-pollen is deposited onto the stigma, leading to ovule and fruit abortion (Wyatt et al., 2000; Wyatt and Lipow, 2007; Lipow and Wyatt, 1999, 2000); abortion interferes with ovules in those fruits developing from cross-pollination and wastes those potential progeny (Lipow and Wyatt, 1999; Lopes and Machado, 1999).

Pollination efficiency is a function of multiple interacting characters and behaviors, including flower shape and size and animal behavior and morphology (e.g., proboscis shape) (Ollerton et al., 2007). On the basis of visitation frequency and foraging activities, we anticipated that skippers would be good pollinators of *A. berteroi*. We also expected that butterflies and skippers, with their long mouthparts, would be the best pollinators. Endress (1994) proposed that flowers in the Apocynaceae tend to be pollinated by long-tongued pollinators with many reports of butterflies and hawkmoths, pollinating species of this family (Haber, 1984; Darrault and Schlindwein, 2005; Sugiura and Yamazaki, 2005; Moré et al., 2007). We found, surprisingly, that skippers and

butterflies did not carry much pollen on their proboscises, nor did they deposit pollen on stigmas: therefore, they were acting as nectar thieves. Skippers have been described as nectar thieves (Adrienne et al., 1985) because they carry pollen loads too small for fruit set, and/or they do not tend to contact the female parts of the flowers (although they can serve as pollinators in narrow-tubed flowers). Hoc and Garcia (1999) found that Lepidopterans are nectar robbers for *Phaseolus vulgaris*. Castro et al. (2013) reported that for flowers of *Polygala vayredae* several species of Lepidoptera behave as nectar thieves.

Comparing pollination performance, frequency and effectiveness of the flower visitors has been a recurrent problem in pollination biology studies (Ne'eman et al., 2010). Pollination efficiency has been defined in terms of visitation rate, pollen deposition, and pollen removal and consequent seed set (Herrera, 1987; Kearns and Inouye, 1993; Waser et al., 1996; Mayfield et al., 2001; Fenster et al., 2004). The pollination syndromes concept implies specialization on a functional group of pollinators (Waser et al., 1996; Fenster et al., 2004) and has been associated with pollination efficiency (Rosas-Guerrero et al., 2014). Many studies have used visitation frequency as an estimator of pollination (Waser et al. 1996; Fenster et al. 2004), but this may lead to misinterpretations, as visitation does not always imply pollination (Waser et al., 1996; Fenster et al., 2004; Ne'eman et al., 2010). In some cases, the most frequent visitor is the most important pollinator (Motten et al., 1981; Stone, 1996; Olsen, 1997), but often the most common visitors are poor pollinators, while less common, but morphologically or behaviorally appropriate visitors appear to be the best pollinators (Horvitz and Schemske, 1984; Mayfield et al., 2001; Fenster et al., 2004; Ollerton et al., 2007; Watts et al., 2012).

Several studies also reported that the body structure of floral visitors, especially the feeding apparatus associated with the dimensions and the morphology of the flowers, is what determines which visitors can effectively function as pollinators (Inouye, 1980; Waser et al., 1996; Alexanderson and Johnson, 2002; Castellanos et al., 2004; Ibanez, 2012; Moré et al., 2012, Miller et al., 2014). Proboscis length is an important determinant of pollination efficiency during foraging for bumblebees (Inouye, 1980; Dohzono et al., 2004; Arbulo et al., 2011). In hawkmoth-pollinated plants, floral tube length is associated with pollen transfer; hawkmoths with tongues too short (or too long) will not pick up pollen effectively (Alexandersson and Johnson, 2002 Anderson et al., 2010; Moré et al., 2012). Flower width has also been correlated with pollen transfer and pollination efficiency in hummingbird- and bumblebee- pollinated flowers (Galen, 1989; Campbell et al., 1996).

In the present study the principal pollinators of *Angadenia berteroi* are bees, specifically two native species of the pine rocklands of south Florida: *Megachile georgica* and *Melissodes communis communis*. Bee pollination (mostly *Euglossine* bees) has been previously reported for this family (Lopes and Machado, 1999; de Moura et al., 2011). Even though their proboscises are slightly shorter (4.9 mm) than the height at which the floral tube is constricted (6 mm, Barrios and Koptur, 2011), bees carry large quantities of pollen on their proboscises. Evidently, bees push their mouthparts in and pick up more pollen on the wide proboscis base than the narrow, longer mouthparts of Lepidoptera that miss the reproductive parts of the flowers. The fact that only flowers visited by bees set fruit contradicts the classic assumption that the tubular flowers in the Apocynaceae family limit visitors to long-tongued pollinators with proboscises long

enough to reach the base of the flower tube to collect nectar (Endress, 1994; Proctor et al., 1996).

In the pollination apparatus of members of the family Apocynaceae (especially in the subfamilies in which the anthers are postgenitally united with the style-head), many studies have highlighted a close match between the length of the flowers and pollinator mouthparts (Endress, 1994; Proctor et al., 1996; Lopes and Machado, 1999; Darrault and Schlindwein, 2005; Moré et al., 2007). In contrast, we observed no correlation between the lengths of the proboscis of the pollinators with pollen removal, but we did find a correlation between the widths of the mouthparts of the pollinator with pollen transfer efficiency. Our results contrast with the findings of Moré et al. (2007) and de Araujo et al. (2014), who reported that flowers of *Mandevilla* sp. were pollinated exclusively by pollinators with long, thin proboscises. *Angadenia* and *Mandevilla* are closely related in the subfamily Apocynoideae, and are located in the New World clade (Livshultz et al., 2007), so we might have expected similar pollinator requirements. The floral morphology of *A. berteroi*, however, with a relatively wide and large throat (“bell”) and a short floral tube constriction, allows short-tongued visitors to enter in the flowers, touching the pollen and the receptive portion of the stigma, functioning as pollinators. In our study, the width of the bees’ heads was wider than the apical portion of the pollen chamber, allowing the bees to touch the reproductive parts of the flowers; metallic bees, with thinner heads, entered more deeply into the corolla, missing the gynostegium. The thicker fishing line used to replicate the size of the mouth parts of the bees removed twice as much pollen as the thinner fishing lines, and we observed a similar pattern with our pollen deposition experiments, in which thicker fishing line touched the stigmatic surface

much more extensively than thinner ones. Using a similar approach, Darrault and Schlindwein (2005) observed that proboscis width played an important role in pollen transfer efficiency in *Hancornia speciosa* (Apocynaceae).

Even though many studies have reported negative effects of low plant density on plant reproductive success (Lamont et al., 1993; Coombs et al., 2009; Levin et al., 2009; Essenberg 2012), our potted plant experiment found no effects of flower density on plant reproductive success. Despite the fact that all groups of visitors appeared to be more abundant in areas with more flowers, the effective pollinators of *A. berteroi* rarely returned to a previously visited flower, normally moving to a nearby (or distant) conspecific flower. Perhaps because overall visitation to flowers of this species is low, the density effects shown in other species were not reflected in this system.

Similar studies in others families and pollinator systems highlight the importance of measuring quantity and quality components of pollination effectiveness such as pollen removal, deposition, morphology and behavior of the visitors, because visitation rates alone were not a good indicator of pollinator effectiveness. Liu and Koptur (2003) reported that flowers of *Chameacrista keyensis* (Fabaceae) were visited by seven species, but only two— and not the most frequent—visitors were effective pollinators with the right size and ability to buzz-pollinate the flowers. Similar results were reported for *Chamerion angustifolium* (Onagraceae) (Ollerton et al., 2007), *Phaseolus vulgaris* (Fabaceae) (Hoc and Garcia1999), and even *Ipomopsis aggregata* (Polemoniaceae), whose red tubular flowers are consider adapted to hummingbird pollination (Mayfield et al. 2001). Watts et al. (2012) found that the main pollinators of *Duranta mandonii* (Verbenaceae) were large *Bombus* spp., although hummingbirds were the most abundant

visitors. Likewise, Miller et al. (2014) found that nocturnal visitors of *Clarkia breweri* (Onagraceae) were more effective pollinators than the more frequent diurnal visitors, including hummingbirds.

Conclusion — We have provided evidence that *Angadenia berteroi* is specialized for bee pollination. It is exclusively pollinated by *Megachile georgica* and *Melissodes* spp., two native bees of the pine rocklands of South Florida. Although many other species visit the flowers, the ones that are the most frequent and constant (skippers) do not carry or deposit pollen. Many studies of other plant-pollinator systems provide evidence that the mechanical match between the pollination apparatus and the proboscis is associated with pollination effectiveness. Our results show that the size of the proboscis of the pollinators, especially the width of the proboscis, correlates with pollen transfer efficiency in the flower visitors of *A. berteroi*. By looking more closely into the mechanics of pollen removal and deposition, and by allowing single visits by the various guilds of visitors, we discovered that pollination was not as it first appeared and that the pollination of this species was much more specialized than previously assumed.

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Tables

Table 1. Pine rockland study sites in Miami-Dade County, Florida, used for *Angadenia berteroi* study. Site names, number designation, area, and landscape type.

Site	Site	Area (acres)	Fragment
Rockdale Preserve	1	26.70	fragment
Nixon Smiley Park	2	127.00	fragment
Larry & Penny Thompson Park	3	270.00	fragment
Navy Wells Preserve	4	353.17	fragment
Everglades G	5	794.45	continuum

Table 2. Flower visitors of *Angadenia berteroi* collected in the study sites.

Visitor Group	Scientific name	Site collected	Pollen on the proboscis
Bees	<i>Megachile georgica</i> Cresson	4	Yes
Bees	<i>Melissodes communis communis</i> Cresson	2,3,4	Yes
Metallic bees	<i>Augochlorella gratiosa</i> Smith	2,3,4	No
Metallic bees	<i>Augochloropsis anonyma</i> Cockerell	4	No
Butterflies	<i>Ascia monuste phileta</i> Fabricius	4	No
Butterflies	<i>Agraulis vanillae nigrior</i> Michener	2,4	No
Skippers	<i>Asbolis capucinus</i> Lucas	2,3	No
Skippers	<i>Cymaenes tripunctus</i> Herrich-Schäffer	2	No
Skippers	<i>Hylephyla phyleus</i> Drury	4	No
Skippers	<i>Lerema accius</i> Abbot & Smith	2,3	No
Skippers	<i>Polites baracoa baracoa</i> Lucas	2,3,4	No
Skippers	<i>Wallengrenia otho</i> Abbot & J.E. Smith	2,3,4	No

Table 3. Percentage of visits and foraging behavior of *Angadenia berteroi* visitors.

Visitor Group	n	Percentage of total visits	Percentage returned to the same flower	Percentage moved to another <i>A. berteroi</i> flower	Average length of visit (sec)
Bees	45	29.4	0	15.6	7.8 ± 4.9 ^a
Metallic Bees	26	16.9	3.8	7.6	6.7 ± 3.3 ^a
Butterflies	15	9.8	26.7	33.3	9.0 ± 14.4 ^a
Skippers	67	43.8	14.3	26.9	10.0 ± 5.9 ^b

Table 4. Percentage of flower visitors with pollen on the proboscis.

Visitor Group	n	Percentage with pollen on the proboscis	Percentage with large pollen load (> 50 pollen grains)
Bees	17	76.5	100
Metallic Bees	6	0	0
Butterflies	5	40.0	0
Skippers	26	0	0

Table 5. Flower visitors of *Angadenia berteroi* per study site.

Site	Number of visits				Total (%)
	Bees	Metallic bees	Butterflies	Skippers	
1	4	0	0	1	5
2	10	15	3	20	48
3	12	4	4	37	57
4	18	5	8	8	39
5	1	2	0	1	4

Figures

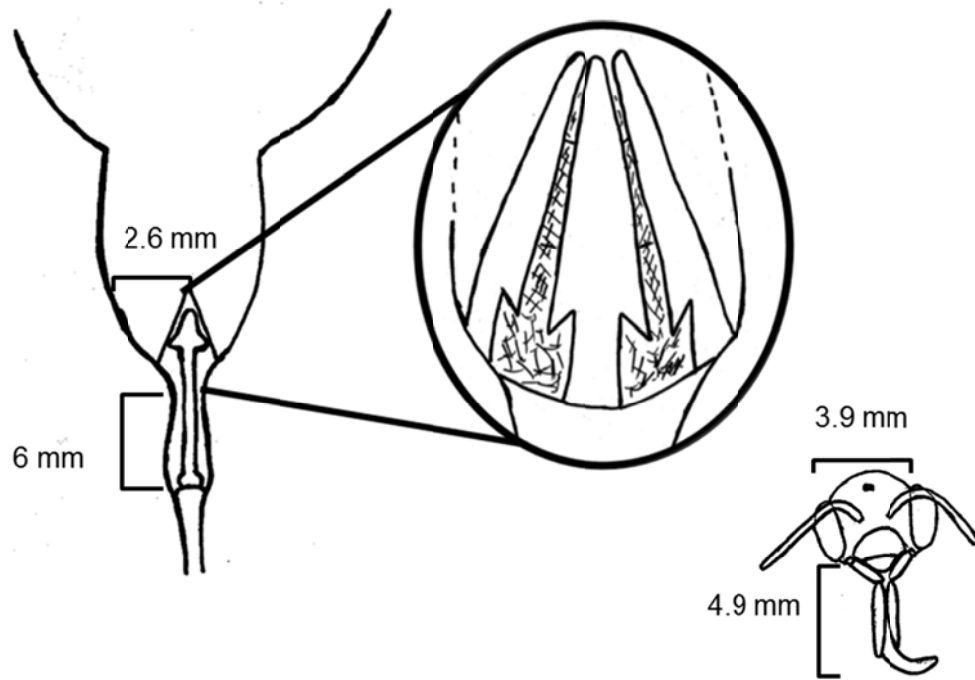


Figure 1. Morphology of *Angadenia berteroi* flower and the Bees' mouth parts. (A) Longitudinal section of the flower (B) Details of the anthers. (C) Details of the head of the bees with the mean width of the head and the proboscis length.

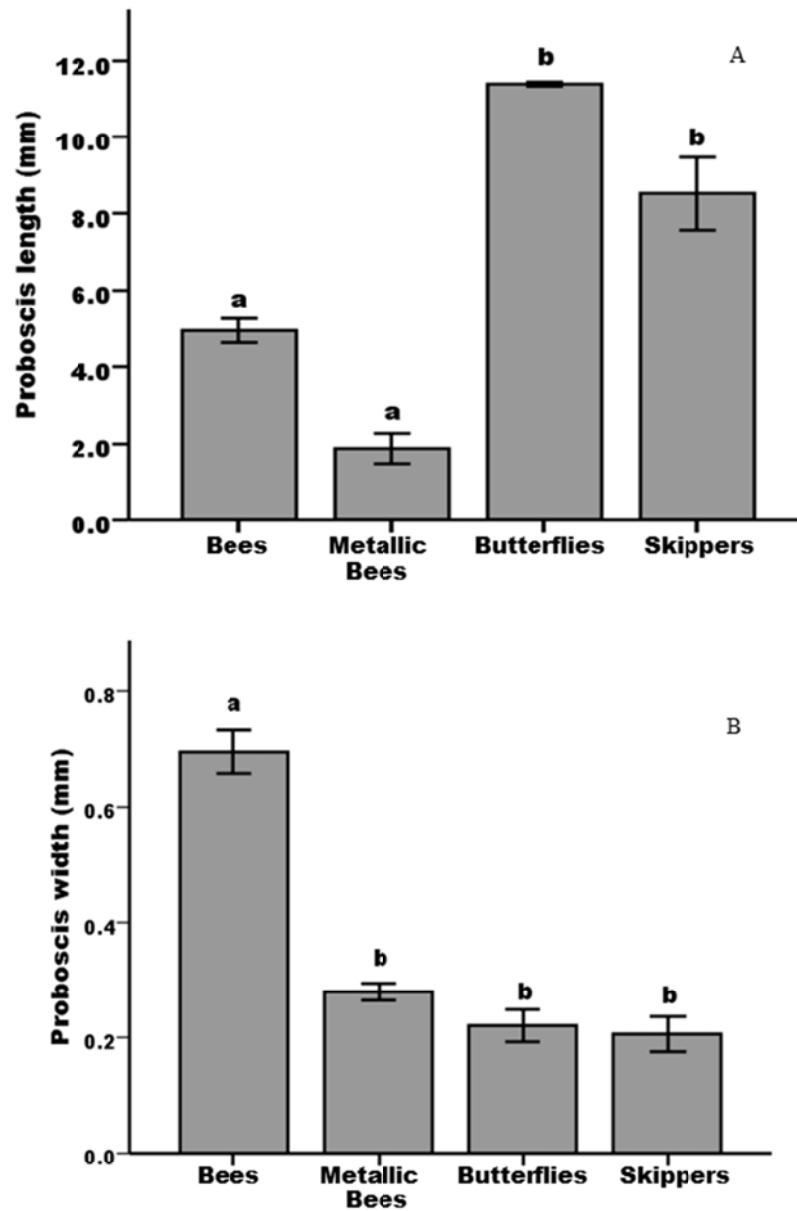


Figure 2. Mean and standard error of the mean of the proboscis measurements of the *Angadenia berteroi* flower visitors. . (A) Proboscis width (B) Proboscis length. The letters indicate significant differences at the 0.05 significance level

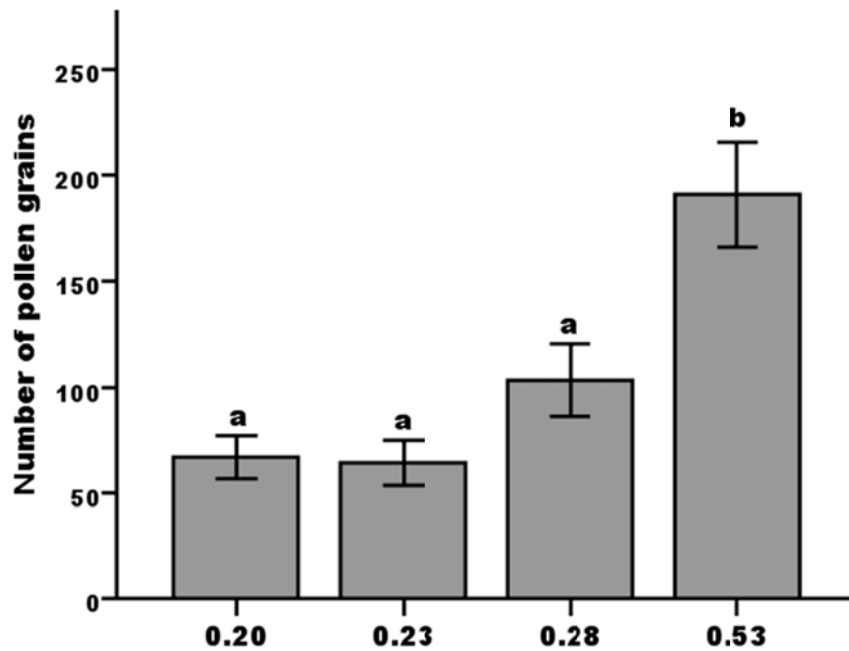


Figure 3. Mean and standard error of the number of pollen grains on fishing line of increasing width. The diameter of 0.20, 0.23, 0.28 mm and 0.53 represents diameter of the proboscis of skippers, butterflies, metallic bees and bees, respectively. Diameters with the same letters are not significantly different with pair-wise Mann-Whitney test.

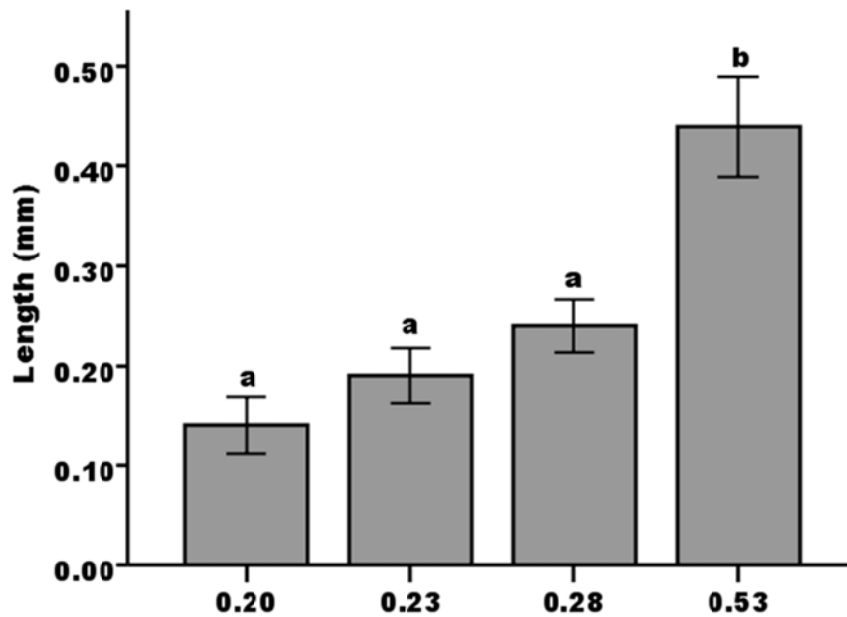


Figure 4. Mean and standard error of the length of the stigmatic surface stained with methylene blue. The diameters of 0.20, 0.23, 0.28 and 0.53 mm represent diameter of the proboscis of skippers, butterflies, metallic bees and bees, respectively. Diameters with the same letters are not significantly different (Tukey HSD post-hoc test)

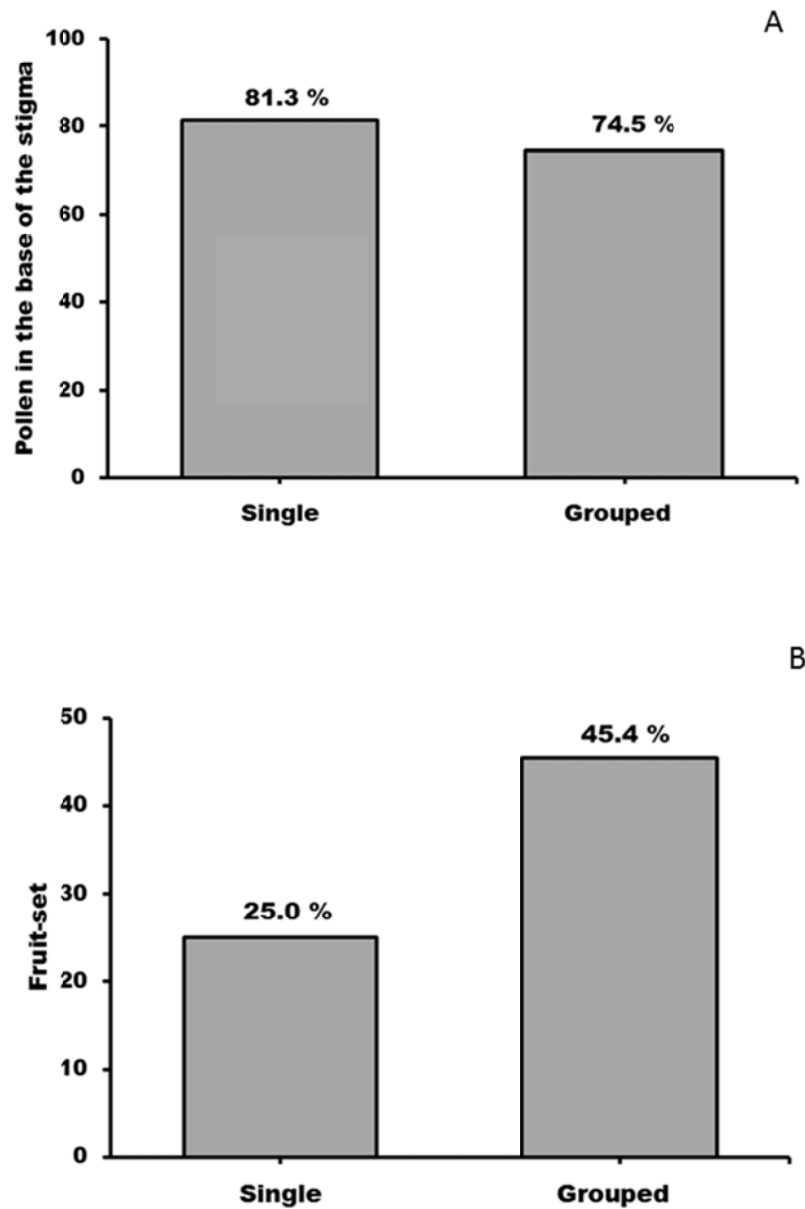


Figure 5. Percentage of (A) Pollen at the base of the stigma (B) Fruit set, for single and grouped flowers presented to pollinators in the field for 24 hours.

CHAPTER II

DOES HERBIVORY AFFECT PLANT GROWTH AND REPRODUCTION: AN
EXPERIMENTAL STUDY USING SIMULATED DAMAGE TO LEAVES AND
FLOWERS OF *ANGADENIA BERTEROI* (APOCYNACEAE)

Abstract:

Herbivores can have large direct or indirect effects on plant fitness, as herbivory damage to both vegetative and reproductive tissues can negatively influence the reproductive success of the plant. A plant's response to herbivore damage may be determined by its ability to compensate and depends on the intensity and frequency of the damage, the types of structures damaged, and at what plant developmental stage the damage is experienced. *Angadenia berteroi* is a tropical perennial subshrub native to the pine rockland habitat in southern Florida. *Syntomeida epilais* is the primary herbivore of *A. berteroi*, with caterpillars often consuming all the foliage and flowers of a plant.

Angadenia. berteroi is capable of tolerating moderate levels of artificial damage with no detriment to growth, leaf, or flower production. But severe damage (artificial defoliation of 100% of the leaf area) negatively affects growth and reproductive success, with a reduction in growth and production of leaves and flowers during the subsequent few months. Severely damaged plants are able to compensate in terms of biomass four months after defoliation. A field experiment with potted plants showed that flowers with simulated (partial) florivore damage received pollen only half as often as intact flowers and set half as many fruits. Our results suggest that *A. berteroi* compensates for leaf tissue lost to severe damage by allocating resources to production and maintenance of new vegetative tissues, which in turn provide photosynthates for flower production. The same response that allows these fire-adapted pine rockland perennials to regrow after fire may permit recovery from severe herbivory events.

Key Words: compensation, leaf herbivory, artificial damage, plant fitness, fire, floral herbivory, pine rocklands.

Introduction

Herbivory is an important component in the life history of most plant species. It may result in reduced growth and fitness, in addition to having a detrimental effect on reproductive biology and population dynamics (Louda, 1984; Strauss, 1997). A plant's response to herbivory damage may be determined by its compensatory abilities (Belsky, 1986) and depends on many things, including the intensity and frequency of the damage, the types of structures damaged, plant developmental stage, and nutrient and light availability (Maschinski and Whitham, 1989). Plants may also evolve mechanisms that allow them to tolerate herbivory damage with low fitness cost and frequently show phenotypic responses to damage such as altered morphology and physiology (Stowe et al., 2000; Juenger and Bergelson, 1997). Tolerance can be defined as the degree to which plant fitness is reduced by herbivory damage relative to the fitness of undamaged plants (Strauss and Agrawal, 1999; Agrawal, 2000). Herbivory-mediated response can be categorized as either under-, exact, or over-compensation of biomass or fitness (Belsky, 1986). Additionally, response to herbivory damage is constrained by resource allocation trade-offs, which are regulated by source-sink relationships (Stowe et al., 2000). Plant tolerance to herbivory can depend on the plant's habit: woody and herbaceous plants exhibit different compensatory responses determined by differences in the biology of the plants, their architecture, and the types of herbivory that they commonly experience (Obeso, 1993; Haukoikja and Koricheva, 2000; Hawkes and Sullivan, 2001). Annual and perennial plants may also differ in their response to herbivory based on the differences in the life history strategies (Rosenthal and Dirzo, 1997; Blossey and Hunt-Joshi, 2003). Tropical plants may suffer higher levels of herbivory than plants in the temperate zone

and may be selected for a higher tolerance and investment in anti-herbivore defenses compared to temperate zone plants (Coley and Barone, 1996).

Folivory, i.e. damage reducing the photosynthetic area of leaves, gives plants less energy to put into fruit and seed production. Folivory can therefore influence the growth, flowering, fruit production, and survival of a plant species (Karban and Strauss, 1993; Krupnick et al., 1999). Plants can make up for tissue lost to herbivory via compensatory photosynthesis of remnant photosynthetic tissues (Thomson et al., 2003; Zhu et al., 2014). Defoliation can also negatively affect reproductive success by reducing resources available for reproductive structures such as flowers (Krupnick and Weis, 1999; Puentes and Ågren, 2012). On the other hand, some studies have shown that herbivory may also increase plant productivity and fitness, since some levels of herbivory may result in overcompensatory growth (Thomson et al., 2003; Zhu et al., 2014). Overcompensation has been defined as damaged plants having higher fitness relative to fitness of plants in the undamaged state (Agrawal, 2000). A plant's compensatory ability depends on its resource allocation patterns, the activation of dormant meristems that increase branching, and the mobilization of stored resources (Whitham et al., 1991; Strauss and Agrawal, 1999; Stowe et al., 2000; Pilson and Decker, 2002).

Herbivory may reduce reproductive success by affecting female reproductive traits (ovule and seed production), male reproductive traits (pollen production or size of the pollen grains), or both (Strauss et al., 2001; Thompson et al., 2004; Narbona and Dirzo, 2010), as well as secondary sexual characteristics such as floral characters (e.g. corolla size or nectar production), flower number, and floral displays (Strauss, 1997; Karban and Strauss, 1993; Krupnick et al., 1999). Changes in floral display and number

of flowers may influence pollinator attraction and efficiency (Root, 1996; Krupnick et al., 1999; Strauss and Irwin, 2004; Cardel and Koptur, 2010), and thus plant reproductive success (Mothershead and Marquis, 2000). Changes in plant architecture and phenology can also affect the plant's relationship with its pollinators (Juenger and Bergelson, 1997).

The effects of herbivory on reproduction can be assessed with either natural damage that occurs in the field or with artificial damage, mimicking herbivore damage using a mechanical device such as scissors (Tiffin and Inouye, 2000). Natural herbivory experiments may be biased because resistance to herbivory varies among genetic families, as well as the unmeasured environmental factors that affect both plant fitness and herbivore density and preferences (Tiffin and Inouye, 2000). Though it has been shown in some systems that damage from real herbivores may elicit different responses in plants than damage by artificial means (Baldwin, 1990; Tiffin and Inouye, 2000; Ehrlén, 2003; Zvereva and Kozlov, 2014). Artificial damage to plants grown in a common environment has been often used to assess the effect of herbivory on plant fitness (Strauss et al., 2001; Thompson et al., 2004; Narbona and Dirzo, 2010; Puentes and Ågren, 2012), to avoid the different response to herbivory among genotypes, and to obtain a true estimate of the tolerance (Tiffin and Inouye, 2000) while controlling for genetic differences and growing conditions. In this experiment, we used this approach, to determine the effects of damage to leaves and to flowers of a perennial plant growing in habitats frequently affected by fire.

Angadenia berteroi (A.DC.) Miers (Apocynaceae) is a tropical perennial subshrub native to pine rocklands, rockland hammocks, and marl prairies in Miami Dade and Monroe Counties (Gann et al., 2002; Wunderlin and Hansen, 2011). In southern Florida

A. berteroi is more abundant in the largest pine rockland fragments with higher fire frequency; its flowering and fruit production, however, are not fire-dependent (Barrios et al., 2011). *Angadenia berteroi* flowers open early in the morning (prior to sunrise), last a single day (often falling off by the next morning), and have a complex floral arrangement (Barrios and Koptur, 2011) that results in a specialized pollination system (Barrios et al., in review). Our field observations suggested that *Syntomeida epilais* (Walker) (Lepidoptera: Arctiidae; the oleander moth caterpillar) is the primary herbivore of *A. berteroi*, with caterpillars often consuming all the foliage and flowers of stems they encounter (Barrios et al., 2011). Damage to flowers and leaves is also caused by orthopteran and coleopteran nymphs (Barrios et al., 2011).

In this research we sought to establish the effects of simulated folivory and florivory on growth, flower production, and reproductive success of *Angadenia berteroi*. We asked the following question: 1) Does damage to foliage decrease growth and plant sexual reproduction, reducing flowering of the damaged plants? and 2) Does damage to flowers decrease visits of pollinators, resulting in lower fruit set?

Materials and Methods

Foliar herbivory

For this study, plants were grown from seed in the greenhouse to create four full-sibling families to control for genotypic differences. We performed artificial defoliation with scissors on plants growing in the greenhouse to assess the effects of defoliation pre-flowering as well as during flowering on growth and reproductive fitness.

Pre-flowering defoliation

We randomly assigned one of three treatments to each of 167 plants used in the experiment: (1) Control (undamaged); (2) moderate damage; and (3) severe damage. Damage levels were chosen on the basis of natural levels of herbivory observed on plants in the field, where oleander moth caterpillars may partially damage or completely obliterate a leafy stem of this plant. Randomization of treatment replicates was made with each sibling family; we use two sibling families to test for differences in pre-flowering defoliation. Plants within a sibling family were undamaged or subjected to the removal of 50% (moderate damage) and 100% (severe damage) of their leaves. Herbivory treatments were applied in February, before the flowering season, which is March-June (Barrios et al. 2011). Vegetative growth was estimated from plant height. We also estimated the production of new leaves, shoot and root biomass and flower production. Plant height, total number of leaves, and number of flowers, were measured at the beginning of the experiment, then one, and two months later. Growth was estimated by subtracting the initial measurements (height) from the final values, and standardized by dividing them by the initial values. New leaves were counted beyond the last leaf defoliated. The number of flowers produced per plant was counted one, two, three and four months after defoliation (March through June). In June, four months after defoliation, we harvested all the plants to estimate shoot and root biomass, removing the plants from their pots and placing them in plastic trays filled with water. We floated the roots with water and carefully rinsed the remaining soil from the roots. We separated the roots from the shoots and dried plants for at least 72 h in a drying oven at 40 °C. We then weighed to the nearest 0.1 g all root and shoot materials, separately, to estimate root and shoot biomass

for each plant. Total biomass was estimated by the sum of the shoot and the root biomass. Shoot to root ratios were calculated by dividing the shoot biomass by the root biomass. Multiple flower measurements (corolla length and diameter, nectar sugar concentration, and pollen grain size) were made on at least ten flowers per plant to determine the effects of defoliation on both female and male components. To test how pre-flowering herbivory influenced the female reproductive success of this native species, new flowers were randomly selected to perform hand-cross-pollinations within defoliation treatments, using unrelated undamaged plants as pollen donors, to compare fruit set among defoliation treatments.

Post-flowering defoliation

A second set of defoliation experiments that used the same methods as the earlier experiments was conducted to assess the effect of leaf damage during flowering on reproductive success. Defoliation treatments were applied during the flowering season, in April. In this experiment, 36 plants within a sibling family were randomly assigned to one of three treatments per plant: Control (undamaged), moderate damage, and severe damage (see above). Biomass measurements were taken in June, two months after the treatments were applied.

Damage to flowers and visitation

During the flowering months, we placed potted plants in the field to quantify the effects of simulated floral herbivory on pollinator activity at flowers. Field studies were conducted in Larry and Penny Thompson Park, one of the sites with high natural herbivory rates to flowers (Barrios et al. 2011). As individual plants may open from zero to three flowers on a given day, we placed at least eight plants per day. Each plant was

placed in the field only one time. Each day we artificially damaged one or two flowers per plant with scissors, to simulate damage by larval orthopterans, for a total of at least five flowers damaged; we left intact an equal number of flowers on different plants. Although florivores in the field might eat flowers completely, our simulated damage was only to the large outer bell of the corolla, not damaging the reproductive parts of the flower at the mouth of the tubular constriction at the base of the corolla bell. We left those parts intact to allow measurement of pollen deposition and potential fruit development.

We placed potted plants with these experimental flowers around the study site, at least 5 m away from another flowering individual of the species. We left plants in the field for 24 hours to detect any visits by pollinators they might receive over floral life. The flowers were tagged after their exposure in the field. Later, plants were returned to the FIU greenhouse and fruit set was monitored. We put plants out on 10 different days between March 31, 2014 and April 16, 2014, exposing over 50 flowers of each type (damaged and intact) to potential visitation. Final sample sizes were 58 damaged and 62 undamaged flowers; with 47 of each group monitored for fruit set. We lost some of the flowers because subsequent natural herbivory or plant damage that occurring during transportation.

We compared fruit set between damaged and undamaged flowers. As a measure of visitation rate, we collected the flowers the following day in the field. Then we collected the stigma of each flower, mounted it in basic fuchsin gel (Kearns and Inouye 1993), and examined it under a microscope to see if pollen had been deposited in the receptive surface at the base of the stigma (Barrios and Koptur 2011). Pollen grains on

the receptive area at the base of the stigma were counted to determine the number of flowers with pollen on the stigma. Percentage of flowers that produced fruit as well as percentage of flowers with pollen on the stigma was compared among treatments.

Statistical Analysis

Data were checked for normality and equal variances before conducting statistical analyses. Nested analysis of variance (ANOVA) was used to test for differences among treatments for growth, biomass, and floral measurements (corolla length, corolla diameter, and pollen grain size). We pooled sibling families in treatments since there were not significant differences among sibling families for growth, biomass, and floral measurements. Post *hoc* tests were conducted using Tukey HSD (Honestly Significant Difference) to test for differences among treatments. The Mann-Whitney test was used to determine differences among sibling families for non-parametric data (number of leaves, total number of flowers produced per plant, and sugar concentrations of nectar). In all cases there were no significant differences among sibling families for each defoliation experiment; therefore the replicates for each treatment were combined. Number of leaves, total number of flowers produced per plant, and sugar concentrations of nectar were compared using the Kruskal-Wallis test, with the Mann-Whitney test (post-hoc) to detect differences among treatments. Fruit set among defoliation treatments was analyzed with contingency table analysis. Percentage of flowers with fruit and with pollen on the stigma was analyzed with chi-square to compare damaged and undamaged flowers. We used the Bonferroni method to control type I error for all pairwise comparisons. Statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) version 21 (SPSS, 2014).

Results

Pre-flowering defoliation

Our estimates of growth (increase in height) for each treatment revealed significant differences among treatments at both one (Figure 1A) and two (Figure 1B) months after defoliation ($F_{2, 161} = 17.78$, $P < 0.0001$, $F_{2, 161} = 7.78$, $P = 0.001$). Growth of control and moderately damaged plants was significantly higher than the growth of severely damaged plants one month after defoliation (Figure 1A), but only control and severely damaged plants were different two months after defoliation (Figure 1B). The number of new leaves significantly differed among treatments both one and two months after defoliation (Kruskal-Wallis test, $\chi^2 = 7.650$ df = 2, $P = 0.022$, $n = 167$, $\chi^2 = 8.743$ df = 2, $P = 0.013$, $n = 167$ respectively; Figure 2 A, B). Moderately damaged plants had the highest number of leaves one month after defoliation, while control and severely damaged plants did not significantly differ in both one or two months after defoliation. No measure of biomass, such as shoot, root, and total biomass, as well as shoot to root ratios, differed among treatments ($F_{2, 109} = 0.38$, $P = 0.687$, $F_{2, 109} = 2.12$, $P = 0.125$, $F_{2, 109} = 1.90$, $P = 0.83$, $F_{2, 109} = 0.18$, $P = 0.84$, respectively, Table 1).

The total number of flowers produced differed significantly among treatments at one, two, and three months after defoliation (Kruskal-Wallis test, $\chi^2 = 16.126$, df = 2, $P < 0.0001$, $n = 167$; $\chi^2 = 43.691$, df = 2, $P < 0.0001$, $n = 167$; $\chi^2 = 14.430$, df = 2, $P = 0.001$, $n = 152$, respectively; Figure 3). Within this period, severely defoliated plants had significantly fewer flowers than did control and moderately defoliated plants (Figure 3).

The total number of flowers, however, did not significantly differ among treatments 4 months after defoliation (Kruskal-Wallis test, $\chi^2 = 1.46$, $df = 2$, $P = 0.481$, $n = 106$, Figure 3).

Flowering for both control and the moderate damage treatment began one month after defoliation, with a marked increase in the number of flowers two months after defoliation in April (Figure 3). Flower production for the severely damaged plants was delayed in comparison with the other two treatments; flowering began in April (two months after defoliation), with its peak in May (Figure 3).

Floral measurements did not differ significantly among treatments (Table 1). Corolla length and diameter showed no significant differences ($F_{2, 121} = 1.98$, $P = 0.143$, $F_{2, 121} = 2.42$, $P = 0.092$ respectively, Table 2); neither did sugar concentration of nectar nor pollen grain size (Kruskal-Wallis test, $\chi^2 = 3.98$, $df = 2$, $P = 0.137$, $N = 175$, $F_{2, 2115} = 2.36$, $P = 0.094$). Even though control plants produced a much higher percentage of fruit after hand cross-pollination (27.7 % Table 3) than the two defoliation treatments (16.4 & 14.3 %), the differences were not significant ($X^2_2 = 2.15$, $P = 0.341$).

Defoliation after flowering

Estimates of growth (in height) differed significantly among treatments one month after defoliation ($F_{2, 102} = 3.68$, $P = 0.029$, Figure 1C). The control group had the highest growth and was significantly higher than the severely damaged group but was not significantly different from moderately damaged (Figure 1C). The number of new leaves did not significantly differ among treatments one month after defoliation (Kruskal-Wallis test, $\chi^2 = 0.84$, $df = 2$, $P = 0.959$, $n = 106$; Figure 2C). Shoot and root biomass, as well as total biomass and shoot to root ratios, did not differ among treatments two months after

defoliation ($F_{2, 82} = 0.63$, $P = 0.535$, $F_{2, 82} = 0.16$, $P = 0.853$, $F_{2, 82} = 0.09$ $P = 0.91$, $F_{2, 82} = 0.60$, $P = 0.55$ respectively, Table 1).

Total number of flowers significantly differed among treatments one month after post-flowering defoliation (Kruskal-Wallis test, $\chi^2 = 20.04$, $df=2$, $P < 0.0001$, $n = 106$; Figure 4). Control and moderate damage treatments had the highest numbers of flowers, while severely damaged plants had a significantly lower number of flowers. Two months after defoliation, however, the total number of flowers did not differ significantly among treatments (Kruskal-Wallis test, $\chi^2 = 2.16$, $df=2$ $P = 0.339$, $n = 82$; Figure 4).

Flower measurements

Floral parameters measured did not differ significantly among treatments (Table 1). Corolla length and diameter were similar among treatments ($F_{2, 133} = 1.39$, $P = 0.254$, $F_{2, 133} = 2.20$, $P = 0.115$ respectively; Table 2). Likewise, sugar concentration of nectar and pollen grain size also did not differ among treatments (Kruskal-Wallis test, $\chi^2 = 0.68$, $df = 2$, $P = 0.710$ $n = 63$, $F_{2, 1972} = 0.095$, $P = 0.909$). Fruit production after hand-cross-pollination also did not differ significantly among treatments ($X^2_2 = 1.16$, $P=0.438$, Table 3).

Floral herbivory

Plants with undamaged flowers were more likely to receive pollen on the stigma and to produce more fruits (28.1 % and 12.28 %, respectively; Table 4). However, fruit set between damaged and undamaged flowers was not significantly different ($X^2_1 = 0.29$, $P=0.24$), and pollen at the base of the stigma was only marginally significant ($X^2_1 = 3.139$, $P=0.059$), with undamaged flowers placed in the field for 24 hours receiving more pollen (i.e, more pollinator visits) than artificially damaged flowers.

Discussion

Our artificial defoliation experiment demonstrated that leaf damage had significant negative impacts on both growth and reproduction. In addition, timing of damage had no significant impact on plant fitness, since pre-flowering and flowering defoliation had similar effects on both growth and reproduction. Negative effects of defoliation on fitness have been reported in other species (Parra-Tabla et al., 2004; Narbona and Dirzo, 2010; Maguire et al., 2011; Irwin and Brody, 2011; Puentes and Ägren, 2012; Zhu et al. 2014), where growth and reproduction were reduced as the percentage of defoliation increased.

Our results showed that plants suffering leaf damage pre-flowering and during flowering were able to compensate in terms of biomass. In both cases, the plants compensated the damage by producing new leaves. Plant reproduction also suffered, and in both cases flower production was diminished and delayed. These results contrast with other studies (Maschinski and Whitham, 1989; Thomson et al., 2003) that found plants are more capable of compensating if damage occurs before the reproductive phase, since plants have time to recover before reproduction.

Angadenia berteroi is capable of tolerating moderate levels of herbivory (50 % of the leaves removed) with no cost to growth, or leaf and flower production. On the other hand, artificial defoliation removing 100 % of the leaf tissue (severe herbivory) negatively affected both plant growth and production of flowers. Similar results were reported by Dominguez and Dirzo (1994), where vegetative growth and flowering of *Erythroxylum havanense* (Erythroxylaceae) were significantly reduced in 100% defoliated plants.

Bergelson et al. (1996) also reported that high levels of herbivory reduced plant height and decreased flowering in *Ipomopsis aggregata* (Polemoniaceae).

Root and shoot biomass were similar among *Angadenia berteroi* treatments, contrasting with Karban and Strauss (1993) where root biomass was more affected by defoliation than shoot biomass for the perennial herb *Erigeron glaucus* (Asteraceae). Our experiment also demonstrated that severely defoliated plants were capable of compensating for tissue loss by producing new leaves and slightly increasing growth after defoliation; this ability to compensate for defoliation has been reported in many other species (Strauss and Agrawal, 1999; Agrawal, 2000). Parra-Tabla et al. (2004) reported that *Cnidoscolus aconitifolius* (Euphorbiaceae) compensated in leaf growth after herbivory damage, suggesting strong resource allocation to re-establish photosynthetic capacity and increase plant growth and survival. Narbona and Dirzo (2010) also reported that *Croton suberosus* (Euphorbiaceae) defoliated plants compensated for tissue loss after defoliation by producing new leaves.

Severely defoliated *Angadenia berteroi* plants produced significantly fewer flowers than the other treatments, and their flowering was delayed by one month in comparison with plants in the control and moderate defoliated groups in which flowering began in March (Figure 3). Several studies have also found that herbivory can negatively affect reproductive success by delaying flowering and decreasing floral production (Krupnick and Weis, 1999; Strauss et al., 2001; Narbona and Dirzo, 2010; Irwin and Brody, 2011; Puentes and Ågren, 2012). Our field observations indicated that flower number may increase pollinator visitations since visitors tend to be more abundant in areas with more flowers (Barrios et al., in review). Both delayed flowering and reduction

in flower number, may reduce the attractiveness of the floral display and the efficiency of the pollinators, thus affecting plant reproductive success (Strauss et al., 1996; Krupnick et al., 1999; Cardel and Koptur 2010).

Experimental defoliation did not affect the selected reproductive traits we measured in *Angadenia berteroi*. This absence of effects is in contrast with several other studies demonstrating negative effects of herbivory on the same parameters we measured, such as decreased flower size (Mothershead and Marquis, 2000; Thompson et al., 2004, Parra-Tabla and Herrera, 2010), reduced nectar production (Irwin and Brody 2011), decreased pollen quality (Strauss et al., 1996; Parra-Tabla and Herrera, 2010), or altered floral sex ratios (Thompson et al., 2004; Avila-Sakar and Stephenson, 2006; Narbona and Dirzo, 2010; Parra-Tabla and Herrera, 2010). We did not measure every parameter (e.g., pollen number), however, so our conclusions must be regarded as provisional.

From these data it is apparent that *A. berteroi* compensates for leaf tissue lost by allocating resources to production and maintenance of new vegetative tissues, which in turn provide energy for flower production. *Angadenia berteroi* has a great capacity to resprout after a fire, or after total defoliation by caterpillars in the field (Barrios per. obs), indicating that plants accumulate reserves in the roots. Many studies have shown that plants can compensate depending on the photosynthetic capacity of remnant tissues or by increasing photosynthetic efficiency (Thomson et al., 2003; Zhu et al., 2014), as well as by mobilizing resources from storage tissues like roots or stems (Whitham et al., 1991).

The effect of florivory on plant reproductive success in *Angadenia berteroi* was not clearly established in this study, we found an apparent, but not statistically significant difference in fruit set between undamaged and damaged flowers, and only marginally

significant differences in pollen deposition. Several studies have shown that floral herbivory can reduce directly plant reproductive fitness, since damaged flowers receive fewer visits than undamaged flowers, and subsequently, have lower fruit and seed set (Karban and Strauss, 1993; Root, 1996; Krupnick et al., 1999; Krupnick and Weis, 1999; Cardel and Koptur, 2010). In the case of *Angadenia berteroi*, flowers are often consumed completely by florivores and herbivores, rendering those flowers entirely useless in sexual reproduction, thus negatively affecting the reproductive success.

Herbivory may have direct and indirect effects on *Angadenia berteroi* growth and reproductive success. Although defoliation decreases growth and leaf production during the first one-two months, severely damaged plants were able to compensate in terms of biomass four months after defoliation. Plants flowering after the population peak (as would result from severe defoliation) may experience lower visitation and increased likelihood of selfing, thereby reducing fruiting success in this mostly self-incompatible species, thus indirectly affecting reproductive success. Our results suggest that fire-adapted subtropical *A. berteroi* is able to compensate leaf tissue lost to severe damage by the allocation of resources to production and maintenance of new vegetative tissues, which in turn provide photosynthate for flower production; however, delaying flowering may have a reproductive cost.

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Tables

Table 1. Mean (\pm SD) biomass of *Angadenia berteroi* plants defoliated in the greenhouse before flowering or during flowering. Treatments were no defoliation (Control), 50% defoliation (moderate) and 100% defoliation (severe). Biomass was collected four months after pre-flowering defoliation and two months after defoliation during flowering. Treatments with the same letter are not significantly different with Tukey HSD post-hoc test.

	Root biomass	Shoot biomass	Total biomass	Shoot/ root ratio
Pre-flowering				
Control	0.90 ± 0.47^a	2.26 ± 1.31^a	3.16 ± 1.54^a	2.87 ± 1.85^a
Moderate	0.94 ± 0.46^a	2.17 ± 0.86^a	3.12 ± 1.04^a	2.89 ± 1.66^a
Severe	0.91 ± 0.53^a	1.90 ± 0.79^a	2.81 ± 1.15^a	2.54 ± 1.23^a
During flowering				
Control	1.01 ± 0.36^a	2.12 ± 0.81^a	3.13 ± 0.96^a	2.32 ± 1.22^a
Moderate	0.90 ± 0.29^a	2.15 ± 0.68^a	3.05 ± 0.79^a	2.59 ± 1.19^a
Severe	0.91 ± 0.40^a	2.11 ± 0.73^a	3.02 ± 0.98^a	2.56 ± 1.23

Table 2. Flower measurements \pm standard deviation of *Angadenia berteroi* plants defoliated in the greenhouse before flowering or during flowering. The values in parenthesis are the number of flowers. Treatments were no defoliation (Control), 50% defoliation (moderate) and 100% defoliation (severe). Floral characters were measured two months after treatments were applied. Treatments with the same letter are not significantly different with Tukey HSD post-hoc test and pair-wise Mann-Whitney test.

	Control	Moderate	Severe
	Mean \pm SD (n)	Mean \pm SD (n)	Mean \pm SD (n)
Pre-flowering			
Corolla length (mm)	22.33 \pm 4.09 ^a (53)	21.43 \pm 3.67 ^a (50)	22.89 \pm 3.87 ^a (18)
Corolla Diameter (mm)	28.74 \pm 5.07 ^a (53)	26.98 \pm 4.65 ^a (50)	28.15 \pm 3.90 ^a (18)
Pollen Size (μm)	39.81 \pm 3.49 ^a (870)	39.48 \pm 3.52 ^a (959)	39.78 \pm 2.99 ^a (288)
Nectar (Sugar concentration)	44.54 \pm 19.47 ^a (16)	42.05 \pm 17.82 ^a (21)	48.33 \pm 19.29 ^a (9)
During flowering			
Corolla length (mm)	23.90 \pm 3.10 ^a (64)	24.10 \pm 3.08 ^a (40)	24.82 \pm 3.03 ^a (29)
Corolla Diameter (mm)	28.53 \pm 4.56 ^a (64)	28.27 \pm 3.17 ^a (40)	29.70 \pm 2.91 ^a (29)
Pollen Size (μm)	39.95 \pm 3.01 ^a (964)	39.94 \pm 2.82 ^a (558)	40.16 \pm 3.30 ^a (450)
Nectar (Sugar concentration)	39.67 \pm 4.68 ^a (6)	37.13 \pm 17.97 ^a (6)	41.25 \pm 1.26 ^a (4)

Table 3. Percentage of crosses that produce fruit among defoliation treatments pre-flowering and during flowering. Number in parenthesis represents the number of plot used in the experiment. Treatments with the same letter are not significantly different with chi-square test.

Treatment	Pre-flowering season	flowering season
	Percentage	Percentage
Control	27.7 ^a (47)	12.5 ^a (40)
Moderate	16.4 ^a (55)	18.2 ^a (33)
Severe	14.3 ^a (7)	5.6 ^a (18)

Table 4. Percentage of fruit set, and pollen deposited at the base of the stigma in undamaged and damaged flowers on potted plants placed in the field for one day. Treatments with the same letter are not significantly different with chi-square test.

Treatment	Fruit	Pollen at the base of the stigma
	Percentage	Percentage
Damage	6.4 ^a (47)	15.5 ^a (58)
Undamaged	12.8 ^a (47)	29 ^a (62)

Figures

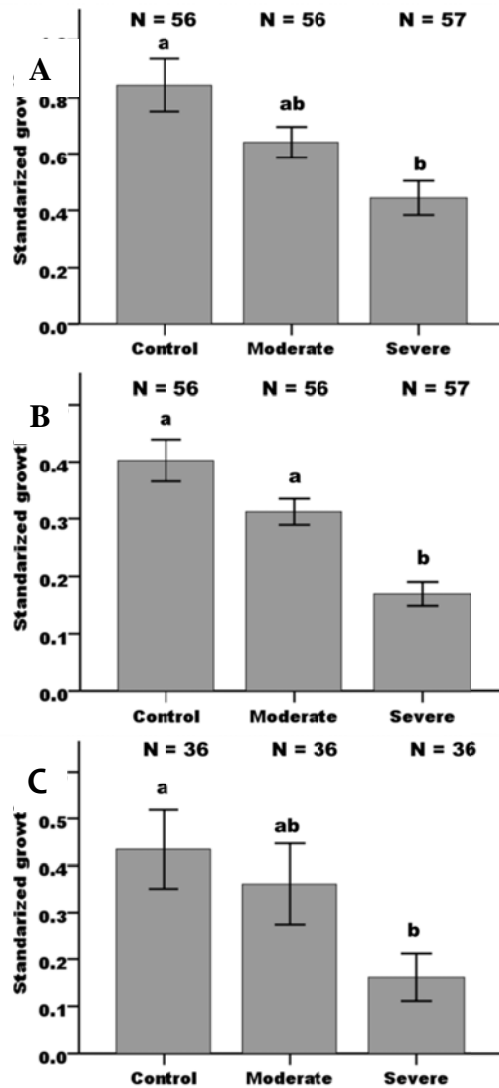


Figure 1. Mean (\pm SE) standardized growth of *Angadenia berteroi* plants defoliated before flowering (A-B), during flowering (C). Treatments were no defoliation (Control), 50% defoliation (moderate) and 100% defoliation (severe). A. Growth one month after pre-flowering defoliation. B. Growth two months after pre-flowering defoliation. C. Growth one month after defoliation during flowering. Treatments with the same letter above the bar are not significantly different with Tukey HSD post-hoc test.

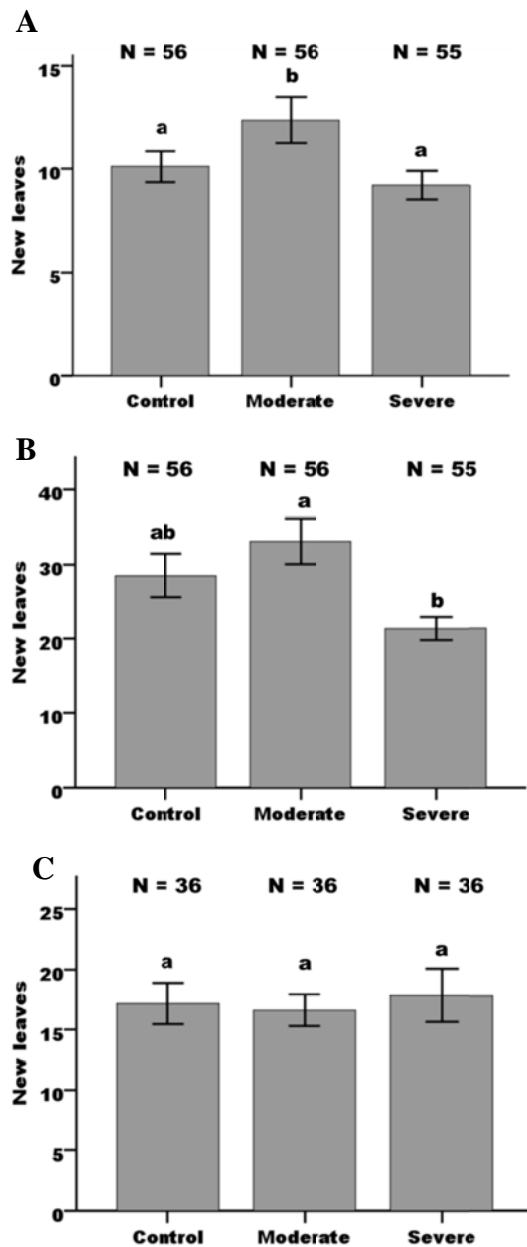


Figure 2. Mean (\pm SE) number of new leaves of *Angadenia berteroi* plants defoliated before flowering (A-B), during flowering (C). Treatments were no defoliation (Control), 50% defoliation (moderate) and 100% defoliation (severe). A. One month after pre-flowering defoliation. B. Two months after pre-flowering defoliation. C. One month after defoliation during flowering. Treatments with the same letter above the bar are not significantly different with pair-wise Mann-Whitney test.

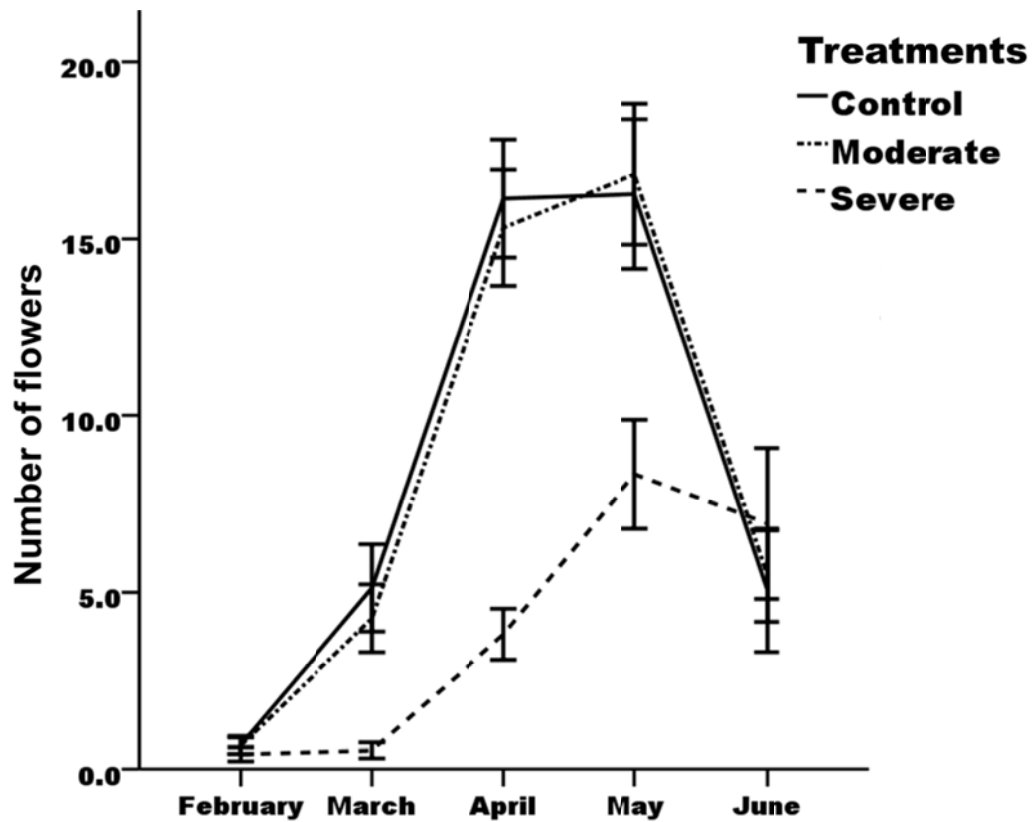


Figure 3. Monthly mean (\pm SE) number of flowers on *Angadenia berteroi* plants defoliated before flowering. Treatments were no defoliation (Control), 50% defoliation (moderate) and 100% defoliation (severe).

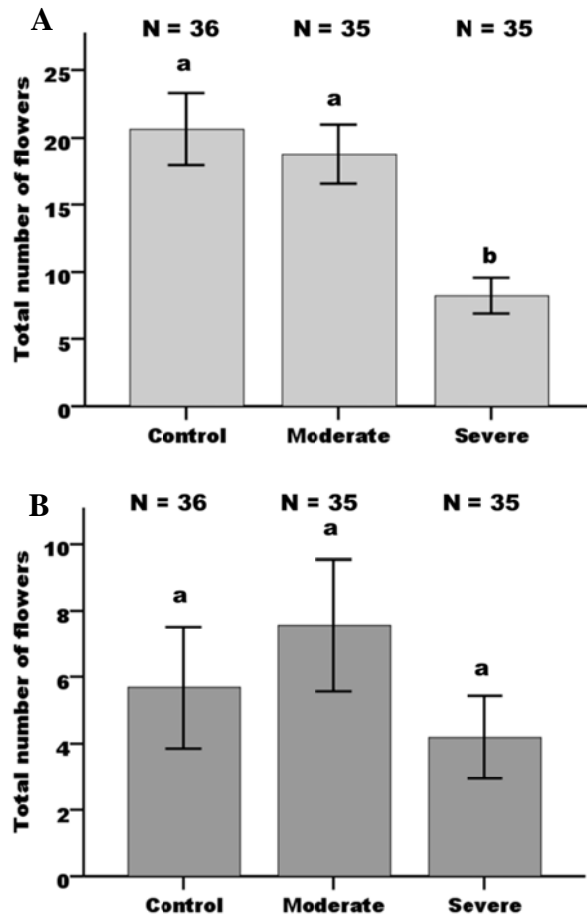


Figure 4. Mean (\pm SE) number of flowers on *Angadenia berteroi* plants defoliated during flowering. A. one month after defoliation; B. two months after defoliation. Treatments were no defoliation (Control), 50% defoliation (moderate) and 100% defoliation (severe). Treatments with the same letter above the bar are not significantly different with pair-wise Mann-Whitney test.

CHAPTER III

THE EFFECTS OF HABITAT FRAGMENTATION ON THE REPRODUCTIVE FITNESS AND ABUNDANCE OF *ANGADENIA BERTEROI*, A NATIVE PERENNIAL PLANT OF THE SOUTH FLORIDA PINE ROCKLANDS

Abstract

The pine rocklands of southern Florida are a fire-dependent forest associated with outcroppings of limestone. Pine rockland plants have several adaptations to fire, and for many species burns increase plant growth, flowering, and seedling establishment. The pine rockland forest has been reduced and fragmented in recent decades. Outside of Everglades National Park, only two percent of the original pine rocklands are left, and they are in the form of small fragments. We investigated the effects of fragmentation on *Angadenia berteroi* (A.DC.) Miers, a threatened species of the southern Florida pine rockland. We estimated the density of plants using a stratified random sampling design, and reproductive fitness (in terms of percentage of plants with flowers and fruit) using a random walk in an array of habitat fragments of different sizes and degrees of isolation as well as in continuous habitat. Structural equation modelling (SEM) was employed to demonstrate how *A. berteroi* reproductive fitness was affected by fragmentation and habitat quality. Habitat fragment size was correlated with the density of *Angadenia berteroi*, and it did not have a great impact on reproductive success of this native species. However, habitat quality represented by litter depth and subcanopy cover had strong negative effects on the reproductive fitness of *A. berteroi*, suggesting that increased light availability and low litter cover resulted from recent fires may favor reproduction of this native species of the south Florida pine rocklands. Insights from this threatened species may provide impetus not only to conserve, but to properly manage remaining pine rocklands in south Florida.

Key words: Fragmentation, isolation, Fire, Structural equation modeling, reproductive fitness, abundance.

Introduction

Habitat destruction and fragmentation are the principal reasons for decrease in biodiversity. Habitat fragmentation affects the reproductive biology of a species (Yates & Ladd 2005), and can reduce species richness as well as genetic variability (the diversity of genotypes and alleles present in species or populations; Vargas et al. 2006). In plants, such a reduction in genetic variability affects pollen quality as well as seed production (Aspinwall & Christian 1992; Vargas et al. 2006). Usually, there is reduced gene flow among plants in the remnant fragments compared with those in intact habitat (Aizen & Feinsinger 1994; Young et al. 1996). Thus, reductions in genetic variability may also increase the population's extinction risk through inbreeding depression, ultimately diminishing the population's ability to respond to environmental disturbances (Frankham 2005). The genetic consequences of habitat fragmentation may result in fitness decline and increase the isolation of populations occupying the remaining fragments, consequently causing reduced population size in the fragments (Frankham 2005; Young et al. 1996). Low population sizes can result in Allee effects, defined as positive (inverse) density dependence at low densities, where the fitness of individuals is lower than expected at low numbers (Courchamp et al. 1999). However, for some herbs, habitat fragmentation does not affect the abundance of the species, probably because the plants can disperse among fragments, or the fragments are larger than the minimum critical size for population maintenance (O'Brien 1998).

Both destruction and fragmentation of the habitat may modify ecological interactions between species such as pollination and herbivory (Laurence 2002). Plants that rely on insects for pollination are at a disadvantage in fragmented habitats, because

size of fragments as well as the distance between fragments may have effects on the distribution and abundance of insect species (Artz & Waddington 2006).

The pine rockland ecosystem is unique in the United States and is considered an imperiled habitat (Koptur 2006). Pine rockland flora is a mixture of tropical and temperate taxa, with very diverse understory (Snyder et al. 1990, US Fish and Wildlife Service 1999). Many of these understory species are endemic, some are threatened, and some are rare. The high levels of endemism in pine rockland ecosystems may be explained by the presence of unique limestone outcropping and calcareous, phosphorus-limited soils. The calcium-rich loams and high pH, along with the presence of iron and manganese, contrasts with the acidic quartz sand found in pine forests of northern Florida (O'Brien 1998).

Landscapes where pine forests were once dominant have experienced dramatic human population growth over the last 100 years, leading to much destruction of pine rockland habitat. For instance, outside of Everglades National Park, only two percent of the original pine rocklands are left and that also in many small fragments (Snyder et al. 1990, Koptur 2006). Even the remaining Pine rockland habitats are threatened because they are primarily fire maintained systems but, pineland fragments embedded within the urban landscape do not get burned as frequently as they once were. Within two decades of fire exclusion, pine rockland can become a closed canopy tropical hardwood forest (known as "hammock"), resulting in the disappearance of pine trees and rich native herbaceous flora (Snyder et al. 1990). In addition, litter layer accumulation, caused by fire suppression, adversely impact understory species richness and diversity (Kirkman et al. 2001). In this ecosystem, leaf litter represents a physical barrier to plant growth and

inhibits seed germination and establishment (Hiers et al. 2007; Wendelberger & Maschinski 2009). Litter modifies the physical and chemical environment of the forest floor, ultimately affecting soil moisture and nutrient availability (Hiers et al. 2007).

Angadenia berteroi (A.DC.) Miers (Apocynaceae, Apocynoideae) is a tropical perennial subshrub with large yellow flowers, and its distribution is south Florida, the Bahamas, and Cuba. In south Florida, *A. berteroi* grows in the pine rocklands, rockland hammocks, and marl prairies in Miami Dade and Monroe Counties (Gann et al. 2002; Wunderlin & Hansen 2011). In southern Florida *A. berteroi* is more abundant in the largest pine rockland fragments with higher fire frequency (Barrios et al. 2011).

Angadenia berteroi flowers open early in the morning (prior to sunrise) and have a complex floral arrangement (Barrios & Koptur 2011) that results in a specialized pollination system (Barrios et al. in review). Natural levels of fruit set in *A. berteroi* are low (Barrios and Koptur 2011). In *A. berteroi*, fruit-set from self-pollination is unlikely to occur because the species is predominantly self-incompatible (Barrios and Koptur 2011). Thus, the low fruit-set in natural populations of *A. berteroi* is usually due to low visitation by pollinators, mating between closely related individuals, or both (Barrios and Koptur 2011).

Objectives

The widespread distribution of *Angadenia berteroi*, nearly ubiquitous in pine rockland sites, makes it an ideal species to study how fragmentation affects the reproductive fitness of a pollination-dependent native species. The objective of this research is to establish the effect of habitat fragmentation and habitat quality on

abundance and plant reproductive fitness of *A. berteroi*. We also assess the influence and interactions of various factors affecting seedling density, flowering, and fruit set.

Materials and Methods

Study Sites

We measured reproductive traits of *Angadenia berteroi* and its habitat variables in 13 pine rockland forest sites scattered along the Miami Rock Ridge, including fire management units within Everglades National Park (ENP) (Table 1). I have chosen the forest sites based on their size and degree of isolation from the continuous pine rocklands in ENP (Figure 1, Table 1).

Habitat structure and fragmentation

Fragment size and distance to the near fragment were determined using geographic information system (GIS) data provided by the Fire History database from Everglades National Park. The GIS data generated by Florida Natural Areas Inventory (Public Lands-June 2008 shape files), for the Miami-Dade County fragments. Distance to the nearest fragment was determined by point to point distance between the centers of each fragment. We measured several habitat variables: litter depth, canopy closure, and sub-canopy closure. These variables were considered as the representations of habitat quality, microhabitat structure and physical indicators of time since the last fire, as both increases with time since the last fire (Snyder et al. 1990). Litter depth was measured with a rigid wire and a rule scale to the nearest 0.5 cm in three points across the plot diameter at each plot (see below). To estimate canopy and sub-canopy closure, we counted how many squares of a forestry densiometer were occupied by canopy image,

looking to four cardinal directions at the center of the plot at a height of 1.0 m and 0.5 m, respectively at each plot (see below).

Fragmentation, abundance and reproductive fitness

To quantify the density of *Angadenia berteroi* at each site, we used a stratified random sampling design. From one to seven 30 x 30 m blocks were selected at each site, avoiding edges of the sites. At each site, one to six blocks were used to ensure that 5 % of the site was surveyed. In each block, we sampled 10, 1-m radius circular plots. In each plot we recorded the number of adults and seedlings of *A. berteroi*. We then calculated the density of adults and seedlings as individual per m⁻². Each separate aerial stem was considered a separate individual and we define seedling as individuals whose height was less than 10 cm.

To examine the effect of fragmentation on reproductive fitness, we determined the number of plants with flowers and fruits and the density of seedlings within each fragment. We random-walked for two hours at each site during the flowering peak in May; and counted the total number of adult plants. The number of flowers per plant was recorded for each flowering individual. We repeated these measurements in June during peak fruiting (Barrios et al 2011), when we recorded the total number of plants and the number of fruits per plant. The percentage of flowering and fruiting plants were estimated by dividing the number of flowering and fruiting plants by the total number plants counted. The mean percentage of plants with flowers and fruits were used to estimate reproductive success of *Angadenia berteroi* at each site. Both reproductive success and seedling density were used to estimate the reproductive fitness of *A. berteroi*.

Seedling emergence and vigor

At least 5 mature fruits from seven sites (Site 2, 3, 8, 10, 11, 12, and 13) were collected when they turned dark, i.e. ready to dehisce to release their seeds, to assure that the seeds were mature. Seed quality (seed mass), and offspring fitness (seedling germination and survivorship) was calculated for each site. Each seed was weighed to the nearest 0.1mg, and the mean seed mass of 15 to 20 seeds per fruit was calculated for each fruit. From each site a total of 330 seeds were weighed. Later, seeds from each site were mixed to eliminate genetic differentiation among fruits and a total of 274 seeds per site were planted singly in 6-pack trays. The trays were placed on a mist bench for two weeks, and then from the third we monitored seedling emergence weekly for next four weeks. We counted the number of seedlings present three weeks after planting. Seedling emergence was measured as the percentage of total seedlings that emerged by week three, when percentage of seedlings present was calculated as the total remaining number of emerged seedlings divided by the total number of planted seeds. Seedling vigor was estimated from plant height at week 5 and 9 (Kearns & Inouye 1993). Percentage of seedlings present and plant height were compared across the different sites.

To see whether seed mass had an effect on germination success, we collected at least 5 mature fruits from five sites (sites 9, 10, 11, 12, 13). Weighed seeds (573 in total) were soaked for 5 minutes in 5% bleach solution to sterilize the seeds, and then rinsed thoroughly with distilled water. Seeds were placed in well plates that were filled with distilled water, with one seed per well. Five replicates were performed per site and 12 seeds per replicate were used. The seeds were germinated in a growth chamber at 25 °C,

60 % humidity, in a 12/12 h of light/dark. We monitored seed germination daily for 2 weeks.

Statistical Analysis

Our goal was to investigate the effect of habitat fragmentation on reproductive output using an integrative approach. For this we used structural equation modelling (SEM) to explore the direct and indirect effect of predictors on response variables, taking into account the causal effects among variables (Grace 2006; Grace et al. 2014). SEM models represent network hypotheses and typically involve multiple regression equations. The SEM analysis starts by building an analytical model representing all the hypothetical causal links between predictors and response variables, based on previous studies of the ecological system. We sought to evaluate the direct and indirect effects of landscape variables (fragment size and distance to the nearest fragment) and site quality variables (litter depth and sub-canopy closure) on *Angadenia berteroi* density (adult and seedling) and reproductive output (percentage of flowers and fruit) at the site level. For this analysis, adults and seedling density as well as the reproductive measurements and the habitat quality parameters were averaged for each of the 13 sites. The SEM Model includes two latent variables. Habitat fragmentation and reproductive success: habitat fragmentation was measured in this model as a function of area of the fragment and point to point distance to the center of the nearest fragment; reproductive success was measured in this model as a function of the mean percentage of plants with flowers and fruits. The hypothetical model for the causal relationships among the variables is given Figure 2.

We conducted a SEM analysis in R (R Core Team 2012) using the Lavaan package, “latent variable analysis” (Rosseel 2012). Direct effects were measured by standardized regression coefficients between the predictor and response variables, while the indirect effects were calculated as the sum of the products of all standardized regression coefficients over all paths between the predictor and the response variable. The maximum likelihood chi-square value was used to estimate the fit of model, and the final model was chosen on the basis of the Akaike Information Criterion (AIC) (Bollen et al. 2014). A non- significant goodness of fit test indicated that there is no significant discrepancy between model and data. Since our design considered each site as an experimental replicate, to accurately characterize landscape processes our sample size was small (N =13). Thus,, we also tested the goodness of fit by Haughton’s BIC test (HBIC) (Bollen et al. 2014). Prior to analysis, square root transformations were performed to achieve normality if the data were not normally distributed.

Data were checked for normality and equal variances before conducting statistical analyses. Analysis of variance (ANOVA) was used to test for differences among sites for seed mass, percentage of seedlings present, and plant height, as data were normally distributed, and post hoc tests were conducted using Tukey HSD (Honestly Significant Difference) to test for differences among sites. We performed correlation analyses using Spearman’s coefficient to investigate the relationship between seed mass and germination success (using the terminology of Zar 1999; Green & Salkind 2007). Statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) version 21 (SPSS 2014).

Results

Fragmentation, abundance and reproductive fitness

The structural equation model (Figure 2) containing all significant relationships found in the multiple regression analyses showed a good fit between the model and the data, indicated by a chi-square ($\chi^2_{17} = 15.05$, $P = 0.592$, AIC = -23.6, HBIC = 2.69).

The SEM model revealed that adult density was significantly affected by habitat fragmentation and subcanopy cover. The area of a fragment had a positive effect on adult density, while distance to the nearest fragment, and subcanopy cover were negatively correlated with adult density (Figure 2). Seedling density was directly affected by adult density and litter depth (Figure 2): positively by adult density, but negatively by litter depth. As expected, subcanopy cover had positive effects on litter depth. Neither habitat fragmentation nor subcanopy cover had direct effects on seedling density. However, both habitat fragmentation and subcanopy cover had an indirect effect on seedling density. While habitat fragmentation had positive effects through adult density, subcanopy cover also had an indirect effect on seedling density through its negative effect through adult density and litter depth. Reproductive success, represented by number of flowers and fruits, was negatively influenced by subcanopy cover, but we found no significant relationship between fragmentation or adult density and reproductive success (Figure 2, Table 2).

Seedling emergence and Vigor

Seed weight differed significantly among sites ($F_{6, 330} = 27.88$, $P < 0.0001$, Table 3). Site 2 and 13 were the sites with higher seed weight, while sites 10 and 3 had lower seed weight (Table 3). Despite those differences, the percentage of seedlings present did

not differ significantly among sites ($F_{6,45} = 2.05$, $P = 0.083$). Additionally, seed germination was positively correlated with seed weight ($r = 0.34$, $P < 0.0001$). Plant height differed significantly among sites at both five and nine weeks ($F_{6,168} = 8.08$, $P < 0.0001$; $F_{6,168} = 7.38$, $P < 0.0001$ respectively, Table 3) after planting. In both cases seeds from larger sites (10, 11, 12, and 13) produced significantly shorter plants than seeds from site 3. Plants from sites 2 and 8 had intermediate values (Table 3).

Discussion

In this study we used a structural equation modelling to explore how *A. berteroi* reproductive fitness was affected by fragmentation and habitat quality. The SEM model indicated that *A. berteroi* does best in large natural areas that are close to other pine rockland sites. We also found that *A. berteroi* is more abundant in fragments with low subcanopy cover; these results are in accordance with our previous studies, where we reported that *A. berteroi* is more abundant in larger fragments with higher fire frequency (Barrios et al. 2011). Other studies (Possley et al. 2008) have also reported that fragment size had a positive influence on understory species richness, and *A. berteroi* is one of the species with the greatest mean coverage in sites with high fire frequency in that study as well. Our results also showed seedling density to be negatively correlated with litter depth and indirectly correlated to subcanopy cover. These were different measurements of habitat quality, as well as indicator of microhabitat structure and physical indicators of time since the last fire, since both increases with time since the last fire (Snyder et al. 1990). Increased canopy cover contributes to greater litter development and as well as reducing light availability for understory plants (Hiers et al. 2007). Increased litter depth and light reduction due to fire suppression alter the physical and chemical properties of

the forest floor, where seedling germination and establishment is inhibited (Hiers et al. 2007; Wendelberger & Maschinski 2009). Some species (e.g.; *Trifolium sp.*, Harrod & Halpern 2005; 2009) have better germination in burned plots due to the removal of the inhibitory effects of the litter accumulation, as well as the reduction in abundance of competitive plants and increased light availability. Time since the last fire was also negatively correlated with population growth rates of *Chamaecrista keyensis*, another endemic herb of the pine rocklands, with the result of reduced density in pine rockland of the Florida Keys that are more than 15 years postburn (Liu et al. 2005). Not all species are negatively affected by fragment size or isolation. For instance, *Galactia sp.*, another endemic species in pinelands, was not affected by the fragment size; although the abundance of that species was also negatively affected by plant cover. Competition for light, nutrients, and space were suggested as causes for the negative correlation (O'Brien 1998).

Lack of fire in pine rockland fragments may promote the number and growth of exotic species (O'Brien 1998). The introduction of exotic species also plays an important role affecting plant abundance and seedling germination of rare native plants (Yates & Ladd 2005). Even though we do not have data to examine the correlation between non-native species and the abundance of *A. berteroi*, the increase in canopy cover due to the high incidence of non-native species reported in the remaining pine rockland fragments (O'Brien 1998; Possley et al. 2008), and the negative correlation between *A. berteroi* density and subcanopy cover suggest that the introduction of exotics also has the negative effects on this pine rockland species. The fact that seedling density at a site was not correlated with the mean number of flowers and fruit per plant in the SEM models,

contrary to our expectations, indicates that habitat quality (litter depth and subcanopy cover) plays a strong role in controlling seedling germination and establishment of *A. berteroi*.

We also found *A. berteroi* to be more abundant in less isolated fragments. These results concur with Lienert & Fischer (2003) who reported that both fragment size and isolation had negative effects on the abundance of *Primula farinosa* mainly because of the combined effects of inbreeding depression and lower genetic diversity in more isolated populations. Habitat loss and isolation are accumulating consequences of habitat fragmentation (Digiovinazzo et al. 2010), where isolation has a negative effect on species richness because it negatively affects migration between fragments. Although we do not have data to examine seed dispersal between fragments, *A. berteroi* seeds are wind dispersed, suggesting that dispersion of seeds to nearby fragments may likely to occur (Barrios pers. obs). Bruna (2003) reported that dispersal between nearby fragments can ameliorate the negative effects of fragmentation on population growth rate and reproduction. More work on this aspect is indicated, especially on the possibility of long distance dispersal with extreme weather events such as hurricanes.

Contrary to our expectation, we found no significant relationship between adult density or fragmentation and reproductive success. Other studies have shown focal species' reproduction to be unaffected by fragmentation (Bruna & Kress 2002; Yates & Ladd 2005); however, seed germination and establishment of *Heliconia acuminata*, a herbaceous perennial plant, was negatively affected by fragmentation, resulting in reductions of recruitment (Bruna 2002; 2003). Researchers have reported that plant density had no effect on reproductive success, but habitat fragmentation and isolation had

a negative effect on the reproductive success of *Cestrum parqui* (Solanaceae) as plants in small isolated populations were more likely to be pollination or pollen-limited than plants in larger populations (Aizen & Feinsinger 1994; Aguilar & Galetto 2004; Aguilar et al. 2006).

Our results also indicate that habitat quality plays an important role in the reproductive success of *Angadenia berteroi* with increased reproductive output in the sites with low canopy cover. Our early work suggested that greater light availability has a great positive impact on the reproductive success of *A. berteroi* (Barrios et al. 2011) and the SEM results confirm this. Yates & Ladd (2005) reported similar results, with increased reproduction and germination of *Verticordia fimbriilepis* on roadsides or in disturbed areas with little plant cover. Harrod & Halpern (2009) reported that flowering appears to be stimulated by increased light availability and low litter cover as the results of recent fires.

The effects of habitat fragmentation on seed germination and seedling fitness were not clearly established in this study. However, our results are in concordance with observations of Eisto et al. (2000) who have reported that population size in the perennial herb *Campanula cervicaria* had no effect on its seed germination or seedling growth. These results were surprising since we might expect that plants in small populations may have reduced fitness as a result of the effects of inbreeding depression and lower genetic diversity caused by fragmentation (Honnay & Jacquemyn 2007). The seed germination and seedling fitness of the *A. berteroi* populations sampled in this study might not be affected by fragmentation, as the fragments were large enough to maintain the minimum

critical population size, or the fragments were not completely isolated from other sites thus allowing seed or pollen dispersion between fragments.

The major result of this study is the strong negative effect of habitat quality on the reproductive success and seedling abundance of *Angadenia berteroi*, rather than fragment area. Many studies found that altered local environmental conditions within remaining habitat can significantly alter the growth rate and reproduction of plant species (Hobbs & Yates 2003; McKechnie & Sargent 2013). In addition to fragment quality, connectivity and landscape characteristics in which the habitats are embedded may influence population survival and reproduction (Tschardt & Brandl 2004). The habitat of *A. berteroi* has suffered from severe anthropogenic disturbance, both urbanization and agricultural intensification in the recent decades (Snyder et al. 1990). Agricultural and urban surrounding matrix may change habitat conditions that greatly affect pollinator diversity and composition (Ahrne et al. 2009; Frankie et al. 2009; Carre et al. 2009), thus affecting the reproductive success of the plants in the remaining fragments (Aguilar et al. 2006; Ferreira 2013; Newman et al. 2013).

In conclusion, the aim of this study, to establish the effect of habitat fragmentation and quality on abundance and plant reproductive fitness of *Angadenia berteroi*, met with mixed results. Although habitat fragmentation did not have a great impact on reproductive success of this native species, litter depth and subcanopy cover had strong negative effects on both the reproductive success and fitness of *A. berteroi*. The increased light availability and low litter cover as the results periodic fires favor reproduction of this native species of the south Florida pine rocklands. These results

emphasize the importance of fire as a tool for the habitat management and the conservation of this and other endemic species in South Florida pinelands.

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Tables

Table 1. Pine rockland study sites in Miami-Dade County, Florida, used for *A. berteroi* study. Site names, number designation, area, and distance to the nearest fragment (as figured from central points).

Sites	Site	Area (Acres)	Distance to the near fragment (km)
Pine Shore Preserve	1	7.8	2.09
Ludlam Pineland	2	10.2	0.9
Ingraham Pineland	3	10.4	1.34
Ned Glenn nature Preserve	4	11.0	0.43
West Biscayne Pineland	5	15.1	1.01
Palm Drive Pineland	6	20.0	1.59
Silver Palms Groves	7	20.4	1.22
Florida City Pineland	8	23.5	0.96
Rockdale Pineland	9	37.1	1.83
Nixon Smiley Pineland Preserve	10	120.0	1.2
Larry and Penny Thompson park	11	270.0	0
Navy wells Pineland	12	353.2	1.44
Long Pine Key	13	12,322.2	0

Table 2. Population parameters used on the Structural Equation model.

Sites	Adults density (Number of individuals/ m²)	Seedlings density (Number of individuals/ m²)	Litter depth (cm)	Shrub cover (%)	% of plants with flowers	% of plants with fruit
1	0.02 ± 0.08	0.06 ± 0.18	1.99 ± 1.69	22.27 ± 19.04	10.00	5.66
2	0.66 ± 0.88	0.66 ± 1.01	6.41 ± 6.16	19.99 ± 25.71	17.80	10.37
3	0.55 ± 0.89	0.34 ± 0.78	5.32 ± 5.56	16.64 ± 20.45	65.82	11.02
4	0.42 ± 0.63	0.04 ± 0.16	7.30 ± 8.10	14.72 ± 17.22	21.24	9.55
5	0.11 ± 0.33	0.21 ± 0.57	18.58 ± 7.81	24.15 ± 17.52	9.38	2.44
6	0.13 ± 0.27	0.00	14.94 ± 8.52	72.39 ± 28.40	5.88	0.00
7	0.08 ± 0.22	0.00	18.29 ± 5.91	50.50 ± 27.98	13.33	0.00
8	0.06 ± 0.18	0.00	12.07 ± 5.78	39.47 ± 25.57	10.53	10.98
9	0.24 ± 0.40	0.03 ± 0.14	10.63 ± 5.15	45.73 ± 24.61	18.79	3.65
10	0.08 ± 0.22	0.03 ± 0.17	9.77 ± 10.51	35.16 ± 34.91	23.23	5.51
11	0.35 ± 0.52	0.45 ± 1.16	1.23 ± 2.70	27.59 ± 22.35	21.89	6.45
12	0.39 ± 0.61	0.16 ± 0.36	10.11 ± 9.04	10.42 ± 11.16	59.62	10.99
13	0.95 ± 1.33	0.38 ± 0.96	9.18 ± 7.40	20.77 ± 27.62	33.89	5.78

Table 3. Mean and standard deviation of seed mass, seedling emergence and plant height. Sites with the same letter are not significantly different with Tukey HSD post-hoc test.

Site	Seed mass (mg)	Seedlings emergence (%)	Height (m) week 5	Height (m) week 9	
2	1.24 ± 0.40 ^a	53.70 ± 21.70 ^a	1.24 ± 0.48 ^{ab}	1.88 ± 0.61 ^{ab}	
3	0.70 ± 0.34 ^b	62.96 ± 33.10 ^a	1.33 ± 0.60 ^b	2.51 ± 1.33 ^a	
8	0.95 ± 0.39 ^c	61.11 ± 53.58 ^a	1.04 ± 0.41 ^{abc}	1.91 ± 0.92 ^{ab}	
10	0.57 ± 0.50 ^b	77.78 ± 13.61 ^a	0.91 ± 0.38 ^{ac}	1.65 ± 0.87 ^a	
11	0.96 ± 0.33 ^c	41.67 ± 34.56 ^a	0.73 ± 0.23 ^c	1.34 ± 0.41 ^a	
12	1.01 ± 0.29 ^c	63.89 ± 34.02 ^a	0.94 ± 0.34 ^c	1.32 ± 0.56 ^a	
13	1.30 ± 0.20 ^a	91.67 ± 9.13 ^a	0.75 ± 0.20 ^{ac}	1.46 ± 0.37 ^a	

Figures

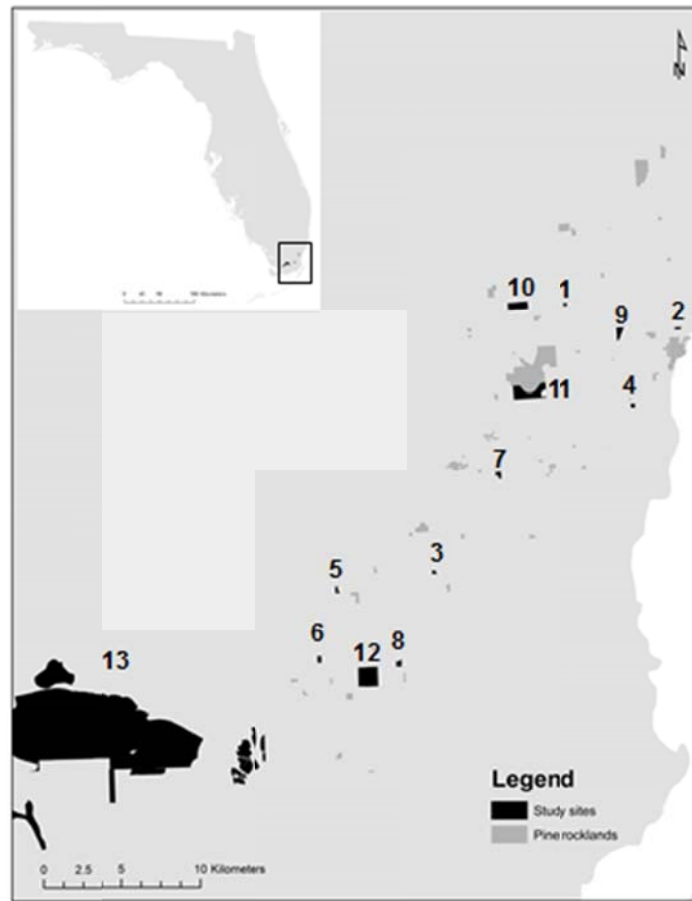


Figure 1. Map of the pine rocklands habitat in southern Florida. Study Sites shown here are described in Table 1.

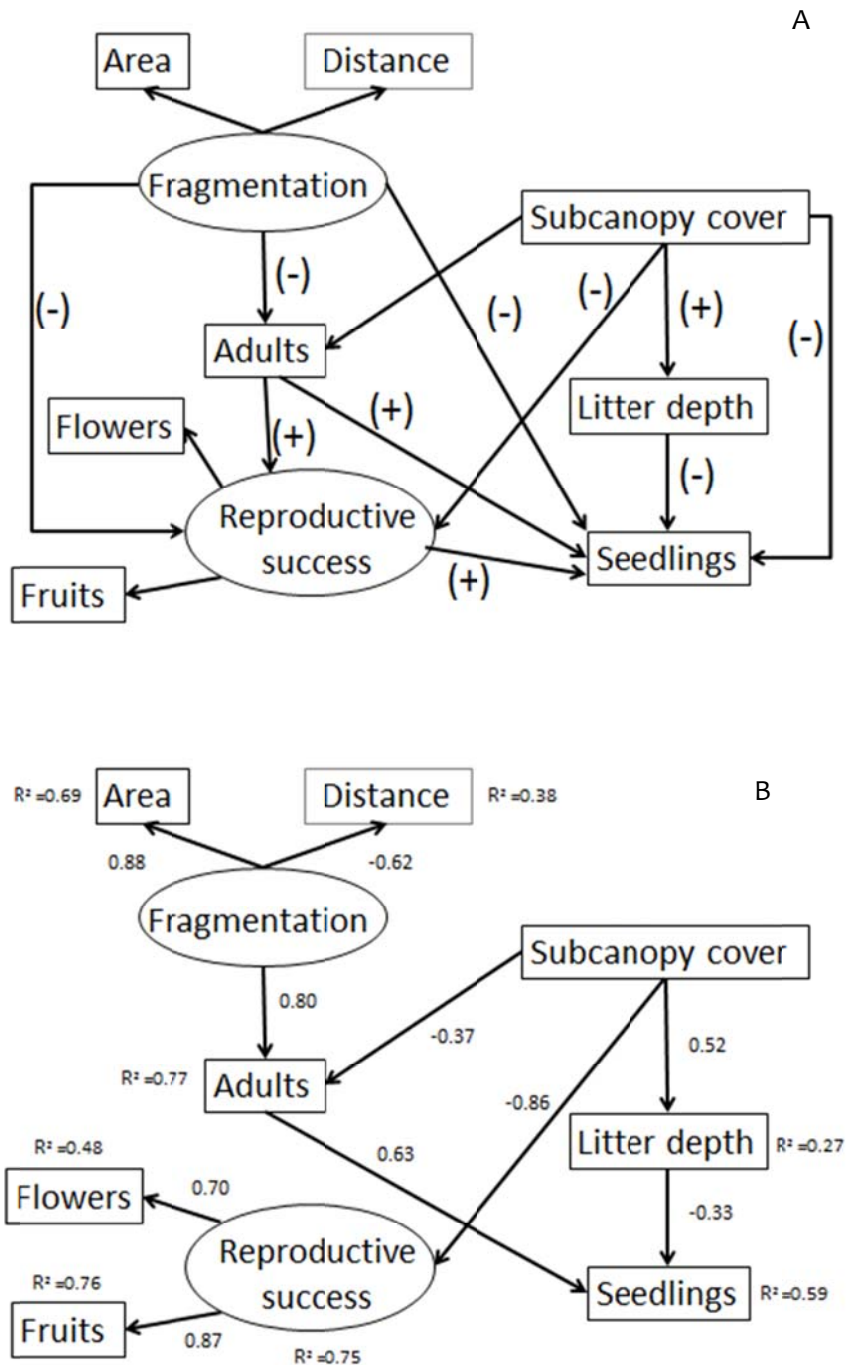


Figure 2. Structural equation model illustrating the interactions between habitat fragmentation and quality with the reproductive fitness of *Angadenia berteroi*. Single arrows indicate directional relationships between the variables. A: A priori basic model including all the variables and the potential relationships among them. B: Actual model where solid lines indicate significant relationships. Numerals near each path indicate standardized regression coefficient. Oval boxes are latent variables and square boxes are observed variables.

CONCLUSION AND FUTURE DIRECTIONS

The pine rockland ecosystem is unique in the United States and is considered an imperiled habitat (Koptur 2006). The pine rockland ecosystem in subtropical south Florida is associated with outcroppings of limestone. The Miami Ridge, which is the largest outcrop, is more or less continuous from Miami to Homestead and Long Pine Key in Everglades National Park (Snyder et. al. 1990; US. Fish and Wildlife Service 1999). Pine rocklands are characterized by a diverse understory of flowering plants (Snyder et al. 1990; US Fish and Wildlife Service, 1999), including the pineland golden trumpet, *Angadenia berteroi* (A.DC.) Miers (Apocynaceae, Apocynoideae).

In the last century, most of this rockland habitat has been developed by humans, so only a small portion remains intact, scattered among many small patches. The most important effect of the destruction on pineland is great loss of biodiversity because of the reduction of the habitat availability, fire suppression, and exotic invasion (Snyder et. al. 1990). The underlying theme of my dissertation addresses the factors that affect the reproductive fitness of *Angadenia berteroi*, a native species of the south Florida pine rocklands.

Chapter I provided evidence that *Angadenia berteroi* is specialized for bee pollination. It is exclusively pollinated by *Megachile georgica* and *Melissodes* spp., two native bees of the pine rocklands of South Florida. Based on visitation frequency and foraging activities, we anticipated that Skippers (Hesperiidae) would be the best pollinators of *A. berteroi*, because they were the most frequent and constant visitors of this native species. However, we found that skippers and butterflies were acting as nectar thieves, because they did not carry much pollen on their proboscises, and did not deposit pollen on stigmas. Additionally we also found a correlation between the size of the

proboscis of the pollinators, especially the width of the proboscis, and pollen transfer efficiency in the flower visitors of *A. berteroi*. Our results demonstrate that bees carry large quantities of pollen on their proboscises, as bees push their mouthparts in and pick up more pollen on the wide proboscis base than do the narrow, longer mouthparts of Lepidoptera that miss the reproductive parts of the flowers. Furthermore, flowers on potted plants that were visited in the field then bagged and observed for the following weeks in the greenhouse; set fruit only when visited by bees, indicating bees to be the most effective pollinators of this species.

Our results demonstrate the importance of pollen removal, pollen deposition, and fruit set, in determining the most effective pollinators, rather than simply visitor abundance. We discovered that pollination was not as it first appeared, and that the pollination of this species was much more specialized than previously assumed.

Chapter II demonstrated that herbivory may have direct and indirect effects on *Angadenia berteroi* growth and reproductive success. This plant species is capable of tolerating moderate levels of artificial damage (50% of the leaves removed) with no cost to growth, leaf, or flower production. But severe damage (artificial defoliation of 100% of the leaf tissue) negatively affected both growth and reproductive success, with a reduction in growth and production of leaves during the subsequent few months. Although defoliation decreased growth and leaf production during the first two months, severely damaged plants were able to compensate in terms of biomass four months after defoliation. Additionally, severe defoliation reduced flower production and delayed flowering. Lower flower number may reduce pollination visitation in this native plant, and fruit success because of plants flowering after the population peak may experience

lower visitation and increased likelihood of selfing in this mostly self-incompatible species: thus herbivory can indirectly affect reproductive success. A novel finding of my research is that fire-adapted subtropical *A. berteroi* may compensate leaf tissue lost to severe damage by the allocation of resources to production and maintenance of new vegetative tissues, which in turn provide photosynthate for flower production.

Chapter III used structural equation modelling (SEM) to demonstrate how *A. berteroi* reproductive fitness was affected by fragmentation and habitat quality. Habitat fragmentation, measured in this research as function of size of the fragment and point to point distance to the nearest fragment was correlated only with the density of *Angadenia berteroi*, and did not have a great impact on reproductive success of this native species. The results also indicated that habitat quality plays an important role on the reproduction success of *Angadenia berteroi* with increased reproductive output on sites with low canopy cover. Litter depth and subcanopy cover have strong negative effects on the seedling density and reproductive fitness of *A. berteroi*. The effect of mean number of flowers and fruit per plant on seedling density was not established with the SEM models, contrary to what we expected. This may also indicate that habitat quality (litter depth and subcanopy cover) play a much stronger role in controlling seedling germination and establishment for this native species. Our results suggest that increased light availability and low litter cover as the results of frequent fires will favor reproduction of this native species of the south Florida pine rocklands.

My research has shown that the reproductive fitness of *Angadenia berteroi* is affected by herbivory damage, adequate pollination, and proper habitat management. This research has raised a number of interesting questions in a plant that had many good

features for further research; a future study could investigate the effect of habitat fragmentation on the genetic diversity and the genetic structure of populations of *A. berteroi* within and among pine rockland fragments. It would also be interesting to know how seeds and pollen may disperse among sites, and whether sites are connected or isolated from each other for mating and immigration purposes.

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PUBLICATIONS AND PRESENTATIONS

Barrios, B., S. Pena, S. Koptur. Pollination biology in fragmented populations of *Angadenia berteroi* a native perennial plant of the south Florida pine rocklands? Contributed paper, Ecological section, BOTANY 2014 meetings, Boise, Idaho, July 2014

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