FLORAL BIOLOGY AND BREEDING SYSTEM OF *ANGADENIA BERTEROI* (APOCYNACEAE): WHY DO FLOWERS OF THE PINELAND GOLDEN TRUMPET PRODUCE FEW FRUITS?

Beyte Barrios[1],* and Suzanne Koptur[2],*

*Department of Biological Sciences, Florida International University, Miami, Florida 33199, U.S.A.

Angadenia berteroi is a tropical perennial subshrub with large yellow flowers that sets very few fruits in its native pine rockland habitat. To learn more about the breeding system of this rare species, we collected seeds from two populations and grew plants for greenhouse study. The flowers open before sunrise. They have a complex floral arrangement (the anthers form a conical structure surrounding the stigma, which has a secondary pollen presentation) that promotes outcrossing from visits by long-tongued pollinators. Hand-pollinations show that *A. berteroi* is mostly self-incompatible, with greatest fruit set, fruit length, and seedling emergence resulting from crosses between unrelated individuals. The low fruit set observed in natural populations may be due to low visitation by pollinators, matings between closely related individuals, or both.

Keywords: breeding system, *Angadenia berteroi*, Apocynaceae, self-incompatibility, flowers, fruits, seeds, pollination.

Introduction

Studies of the reproductive biology and breeding systems of rare plant species are indispensable to successful conservation efforts (Anderson 1995). Many rare species are threatened by habitat destruction and habitat fragmentation, both of which may reduce population size and the abundance and effectiveness of pollinators (Aizen and Feinsinger 1994). Without effective pollinators, many species of flowering plants may go extinct.

The pine rockland ecosystem is unique in the United States and is considered an imperiled habitat (Koptur 2006). 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), which bears many large yellow flowers but rarely produces fruits. Because pine rockland plants may have had their reproductive success diminished by various disturbances such as fire and habitat fragmentation, it is necessary to determine the floral biology and breeding system of this species in a controlled environment before interpreting patterns of natural reproductive success and results of field experiments.

Pineland golden trumpet (*A. berteroi*, Apocynaceae) is a perennial subshrub listed as threatened by the Florida Department of Agriculture and Consumer Services, Division of Plant Industry (FDACS, DPI; Gann et al. 2002). The genus *Angadenia* includes two species (sensu Mabberley 2008) and is part of the tribe Echiteae, which includes 21 genera. Members of this tribe are vines, woody lianas, or, less frequently, shrubs,

with latex, opposite leaves, and extremely variable corollas (Endress and Bruyns 2000). *Angadenia berteroi* is placed in the New World clade, most species of which are endemic to the Americas (Livshultz et al. 2007).

The Apocynaceae are a diverse and species-rich family in the order Gentianales. The family has a widespread distribution throughout tropical and temperate regions. Species in this group are characterized by the presence of tissues containing laticifers and sap (usually milky) and radially symmetrical flowers with five connate petals and connivent stamens forming a ring that encloses the stylar head. The fruit consists of two terete follicles or berries (Judd et al. 2002).

Recent phylogenetic studies support the recognition of a single family, Apocynaceae sensu lato. This new classification places the species into 424 genera distributed among five subfamilies (Endress and Bruyns 2000). Apocynaceae exhibit a stepwise accumulation of floral characters, from more simple flower structures in early divergent clades of the Apocynaceae to highly complex flowers in Asclepiadoideae members (Endress 1994).

Little attention has been given to the pollination biology of the nonasclepioid members of the family Apocynaceae. Most of the literature about this family is related to the Asclepiadoideae. The presence of a corona, gynostegium, or pollinia is a distinctive characteristic of the floral morphology of Asclepioids (Endress 1994; Wyatt and Broyles 1994). The complex flower structure of the nonasclepioid 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). The complex pollination apparatus of the Apocynaceae represents a very efficient mechanism to avoid self-pollination (Darrault and Schlindwein 2005; Pinto et al. 2008), but the evolution of the agglutinated pollen in Apocynaceae can also be explained as an adaptation to

[1] E-mail: bbarr006@fiu.edu.

[2] Author for correspondence; e-mail: kopturs@fiu.edu.

reduce the probability of receiving mixed loads of self- and cross-pollen. A mixed pollen load is extremely costly because Apocynaceae s.l. possess a postzygotic self-incompatibility system that results in the abortion of cross-pollinated ovules after self- and cross-pollen reach the stigma (Wyatt et al. 2000; Wyatt and Lipow 2007).

Self-incompatibility appears to be a widespread characteristic in the subfamily Asclepiadoideae (Wyatt and Broyles 1994; Shuttleworth and Johnson 2008), but little is known about the nonasclepioid members of the Apocynaceae s.l. Although a few cases of self-compatibility have been reported (Lin and Bernardello 1999; Torres and Galetto 1999), most species of Apocynaceae appear to be self-incompatible (Lipow and Wyatt 1999; Lopes and Machado 1999; Darrault and Schlindwein 2005). Reports of self-pollen tubes entering ovules suggest a late-acting self-incompatibility mechanism (Lipow and Wyatt 1999; Lopes and Machado 1999). The consequences of late-acting self-incompatibility are abortion of ovaries or fruits from self-pollinated flowers. This mechanism therefore tends to be much less efficient than sporophytic systems (in which recognition and rejection of self-pollen occurs before pollen germination; Seavey and Bawa 1986; Richards 1997). Studies in *Asclepias exaltata* show that late-acting self-incompatibility is controlled by a highly polymorphic single gene (S-gene); the rejection of self-fertilized ovules occurs when two plants share at least one allele of the S-gene (Lipow and Wyatt 2000). In late-acting self-incompatibility systems, self-pollen tubes compete with cross-pollen tubes, preventing them from penetrating the style (Wyatt and Broyles 1994). Aborted ovules from self-pollination can lead to fruit abortion, interfering with ovules in those fruits developing from cross-pollination, wasting those potential progeny and leading to the low levels of fruit set observed for many species of this family (Lipow and Wyatt 1999; Lopes and Machado 1999). In some nonasclepioid members of the Apocynaceae s.l., the pollen may be aggregated in tetrads or simply in masses that are clumped together by a sticky secretion of the style head (Harder and Johnson 2008); this mass transfer of the pollen reduces the likelihood that mixed self- and cross-pollen load reach the stigmatic surface (Wyatt and Lipow 2007). The gynoecium of this family consists of two free carpels in the ovule-bearing region (Judd et al. 2002). Apocarpy may also reduce the wasteful effect of a mixed pollen load because there are two independently fertilized ovaries, giving the possibility for one to mature even if the other one receives a mixed pollen load (Wyatt and Lipow 2007).

Because of the complex pollination apparatus in Apocynaceae s.l., self-pollination occurs only when pollinators visit the flowers at least two consecutive times (Darrault and Schlindwein 2005); automatic self-pollination is prevented. Consecutive pollination within flowers may be unlikely (Darrault and Schlindwein 2005), but geitonogamy (transfer of pollen between flowers on the same individual plant) also can have negative effects on fruit production (Lopes and Machado 1999; Pinto et al. 2008).

In most nonasclepioid species, the anthers are adnate to the corolla and form a conical structure surrounding the stigma. The pistil comprises a fleshy stigma, slender styles, and a bicarpellate ovary, with nectaries located at the base of the corolla, surrounding the ovary (Galetto 1997; Lipow and

Wyatt 1999). Secondary pollen presentation results when the anthers transfer pollen to the apical portion of the stigma (Yeo 1993). The stigma is divided into three regions (as in fig. 1C): apical sterile parts that receive self-pollen from the anthers and form the pollen chamber, a middle secretory area that produces sticky mucilage that aids self-pollen adhesion, and a receptive area at the base that receives pollen from mouthparts of the flower's visitors (Lipow and Wyatt 1999; Darrault and Schlindwein 2005). Searching for nectar, an insect inserts its tongue into the flower tube. Upon retraction of the mouthparts, exogenous pollen is captured at the receptive area of the style head, and then the tongue is covered with the mucilage, removing the pollen grains from the flower as it passes through the pollen chamber (Darrault and Schlindwein 2005; Pinto et al. 2008).

Pollination syndromes in the Apocynaceae are diverse. In the Asclepiadoideae, hymenopteran and dipteran pollinators are common, whereas the majority of species of the nonasclepioid members of the family Apocynaceae s.l. 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). Because of the distinctive floral morphology and the complex pollination mechanism, flowers in the Apocynaceae tend to be pollinated by long-tongued and small-sized pollinators (Endress 1994); length of the proboscises of the pollinators is related to the length of the floral tube (Proctor et al. 1996). Insects represent the major floral visitors of Apocynaceae s.l. (Endress 1994), and there are reports of butterflies, hawk moths, and bees pollinating species of this family (Haber 1984; Lopes and Machado 1999; Darrault and Schlindwein 2005).

The goals of our study were to elucidate the breeding system of *A. berteroi* and to determine if this species depends on pollinators for sexual reproduction. Specifically, we looked at the compatibility relationships between closely related individuals and how mating between siblings versus nonrelatives affects seed production, seedling emergence, and progeny vigor. If *A. berteroi* is a self-incompatible species that depends on pollinators for sexual reproduction, we expect that crosses between unrelated individuals will perform better than selfing or crosses between related individuals.

Methods

Study Organism

Angadenia berteroi has opposite, coriaceous, entire leaves and flowers borne in lateral scorpioid inflorescences. The flowers have no detectable fragrance (to humans). The striking corolla is yellow and has a 2–3-cm-long tube that contains nectar. Its five stamens are agglutinated to the stigma like other Apocynaceae, and the two carpels of the ovary are fused at their apex. The V-shaped fruit is made up of two terete follicles up to 5 cm long at maturity (Long and Lakela 1971).

Pineland golden trumpet is present in south Florida, the Bahamas, and Cuba. In south Florida it grows in the pine rocklands, rockland hammocks, and marl prairies in Miami-
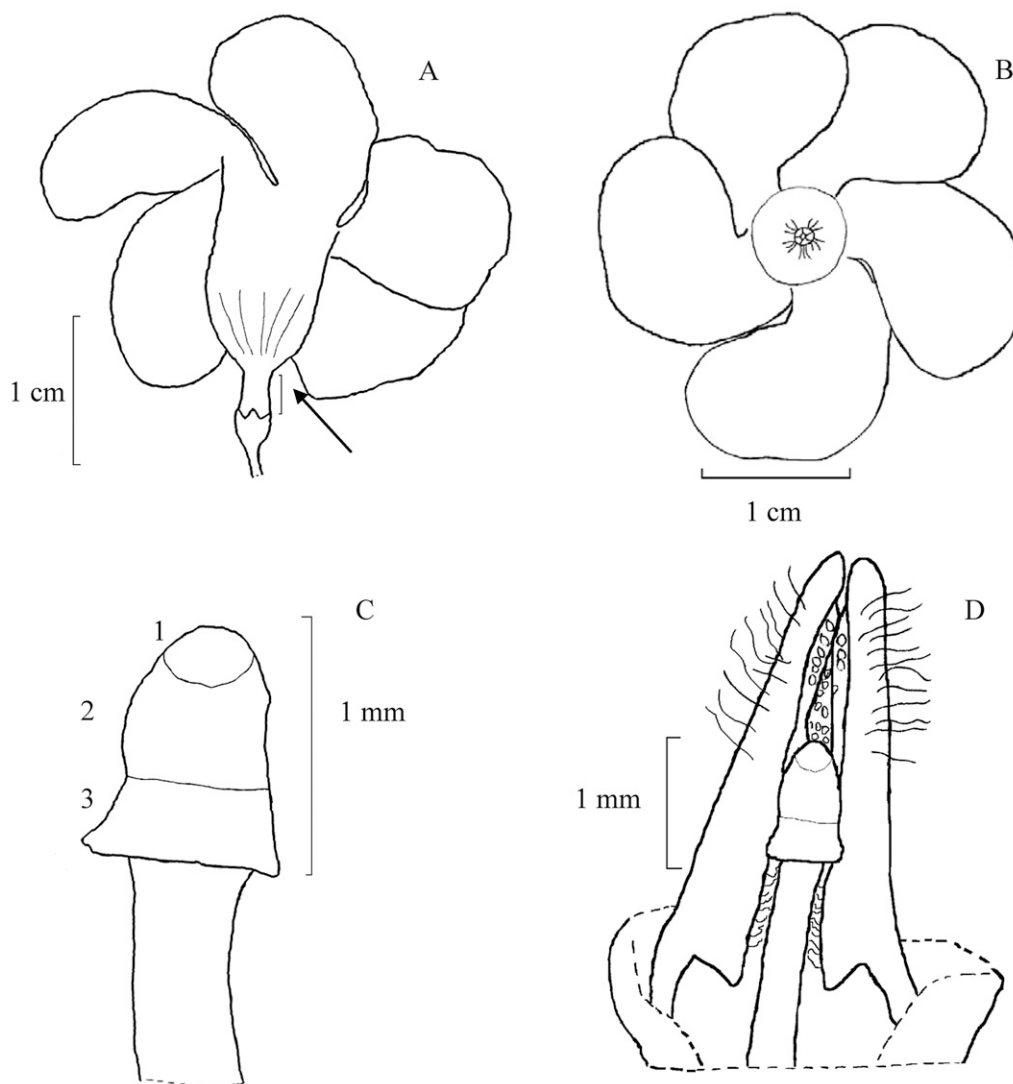
Fig. 1 Flower of *Angadenia berteroi*. *A*, Lateral view of the whole flower. Arrow points to floral tube constriction. *B*, Abaxial view of the whole flower. *C*, Style head. *1* = apical part, *2* = medium secretory area, *3* = receptive area. *D*, Flower dissected to reveal the anther after dehiscence deposited pollen in the style head, forming the pollen chamber, from which all petals and two anthers have been removed.

Dade and Monroe counties (Gann et al. 2002; Wunderlin and Hansen 2003). There is no information about its breeding system. Because our field observations suggested that natural levels of fruit set are low (see below), it is likely that *A. berteroi* not only relies on pollinators but also is self-incompatible and therefore requires pollen from unrelated conspecifics to set fruit. In addition, experimental results from other species suggest the possibility of self-incompatibility in the Apocynaceae. Although Pascarella et al. (2001) stated that *A. berteroi* is visited exclusively by lepidopterans, our preliminary observations in the field confirm that both bees and butterflies visit *A. berteroi*, though visitation was infrequent at the midmorning hours when we observed.

In our study, we made observations and performed measurements and experiments on plants in the field (six sites) and plants grown from seeds collected from two pine rockland sites in Miami-Dade County (Larry and Penny Thompson

Park and Navy Wells Preserve) and raised in the greenhouse at Florida International University. Seeds from the same fruit by definition shared a mother and so gave rise to plants that were siblings.

Floral Biology

Using calipers, we measured length and diameter of the corolla, constriction diameter and length of the floral tube, and length of the style head (fig. 1) in 50 flowers, using two flowers from 25 plants. Nectar volume was measured using micropipettes, and sugar concentration was measured using a handheld refractometer that measured percent sugar on a weight-to-weight basis (Kearns and Inouye 1993), from plants in both the field and the greenhouse. We measured the length of both follicles of each fruit to the nearest 1 mm using a ruler. We counted seeds in 17 mesh-bagged fruits collected in

the field to explore the relationship between fruit length and number of seeds. Flower opening and closing times were determined by monitoring 15 potted plants over 15-min intervals during predawn and early morning hours for a period of 1 mo during peak flowering. Fruit maturation time was estimated on fruits produced by hand-pollination (see below) from the day of pollination to the day the fruits released seeds.

Natural Levels of Fruit Set

The total number of plants that produced flowers and fruits was recorded from six sites: four pine rockland forest sites in Miami-Dade County and two burn units in pine rocklands of Everglades National Park where *A. berteroi* is present (Barrios et al. 2011). Data were recorded for a period of 4 mo (April through July 2009), from the first days of the flowering period to fruit maturity. The fruit set per site was estimated from the variables measured, as a percentage of the total number of fruits produced per plant during the flowering season over the total number of flowers produced per plant, averaged for individuals monitored at each site.

Breeding System

We performed controlled hand-pollinations to determine whether plants are self-compatible and to understand patterns of fruit set observed in plants in the field. Four hand-pollination treatments were performed on 300 plants growing in the greenhouse at Florida International University: (1) self-pollination within the same flower, (2) cross-pollination among siblings (plants grown from seeds from the same mother), (3) cross-pollination among unrelated individuals, and (4) no pollination (control). We simulated flower visits using a 4-cm length of premium 14-lb nylon fishing line to simulate the proboscis of a lepidopteran. A single piece of fishing line was introduced into the corolla tube three times for the self-pollination treatment and then was used on another flower for another pollination treatment (Darrault and Schlindwein 2005). All four treatments were performed on different flowers of the same plant, over time, to control for genetic effects (e.g., different individuals may have different levels of self-incompatibility). Fruit production was recorded for each flower for all treatments. Mature fruits from the experiment were collected after they released their seeds, to assure that the seeds were mature. The length of mature fruits was recorded for cross-pollinations (treatments 2 and 3) and self-pollinations (treatment 1) as an indicator of the number of seeds produced, as fruit length is positively correlated with the number of seeds per fruit in *A. berteroi* (reported below).

Seedling Emergence

We conducted a study of seedling emergence and seedling vigor using seeds produced in the breeding system experiment. We weighed each seed to the nearest 0.001 g and calculated the mean seed mass of 15–20 seeds per fruit from each of the different treatments that produced seeds. We planted 1028 seeds from 49 fruits from different treatments in 9-pack trays and monitored seed germination weekly. Each group of seeds from one fruit was considered an independent sample ($n = 49$). We counted the number of seedlings at days 15, 35, and 56 after planting. Seedling emergence was measured as the percentage of total seeds that emerged by day 15. Percentage of seedlings present was calculated at days 35 and 56 as the total number of emerged seedlings divided by the total number of planted seeds. Seedling vigor was estimated from plant height at day 56 (Kearns and Inouye 1993). Percentage seedling emergence, percentage seedlings present, and plant height were compared across the different pollination treatments.

Analysis

Arcsine square-root transformations were performed on the seedling emergence data, and logarithmic transformation was performed on fruit length and plant height to normalize distributions (using the terminology of Zar 1999; Green and Salkind 2007); when the data could not be normalized, we used the nonparametric test. Fruit set among treatments (proportions) was compared using the Kruskal-Wallis test. We used the Mann-Whitney test (post hoc) to determine differences between treatments. Analysis of variance (ANOVA) was used to test for differences among treatments for seed weight and fruit length. Post hoc tests were conducted using Tukey HSD (honestly significant difference) to test for differences among treatments. We also evaluated differences between treatments using Student's *t*-test for seedling emergence, seedlings present, and plant height. We used the Bonferroni method to control Type I error for all pairwise comparisons. We performed correlation analyses using Pearson's and Spearman's coefficient to investigate the relationship between number of seeds and fruit length (using the terminology of Zar 1999; Green and Salkind 2007). Statistical analyses were performed using SPSS (Statistical Package for the Social Sciences), version 17 (SPSS 2009).

Results

Floral Biology

The flowers of *Angadenia berteroi* are conspicuous, with a yellow corolla that is long (2.2 cm [SE = 0.04]) and broad (2.6 cm [SE = 0.06]), having a campanulate form with connate, contorted petals. The floral tube constriction is short (mean = 0.6 cm, range 0.4–0.7 cm) and wide (mean = 1.7 mm, range 2.3–1.1 mm). Stamens (mean length = 4 mm, range 3–5 mm) are inserted above the level of the style head, and the stigma is ~6.8 mm long (SE = 0.8; fig. 1). The majority of the flowers presented viscous nectar with an average volume of 1.5 mL (SE = 0.3) and sugar concentrations ranging from 30% to 67% (mean = 43.8%, SE = 1.1) on a weight-to-weight basis. Field and greenhouse measurement of nectar concentration did not differ significantly from each other ($t = 0.4$, $P = 0.7$). Floral buds were ~2.3 cm long the day before opening.

Flowers opened before sunrise, at ~5:30 a.m. The closing time varied among flowers but was always after sunset (8:00 p.m.) on the day they opened. The flowers lasted less than 24 h. The mature fruits were brown in color and had two follicles (mean length 9.0 cm [SE = 0.4]). The fruits opened ~80 d after pollination, releasing, on average, 33.4 (SE = 3.8) tan
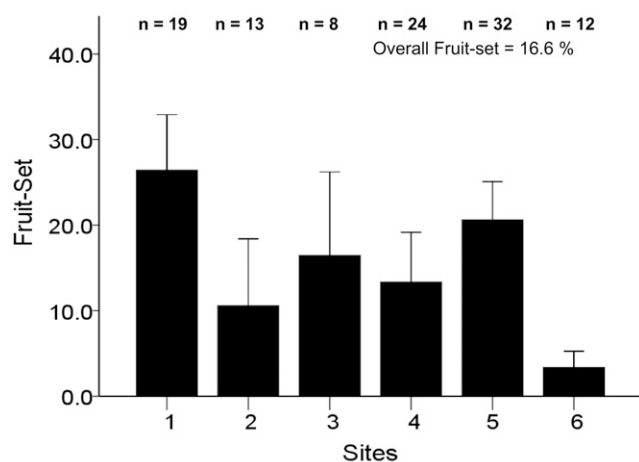
plumed seeds that weighed, on average, 1.08 mg (SE = 0.02). A positive correlation exists ($r = 0.769$, $P < 0.0001$, $n = 17$) between number of seeds and fruit length.

For plants followed at six field sites, the overall fruit set was low. Mean fruit set was 16.6% (SE = 2.5), ranging from 3.3% (SE = 1.9) to 26.4% (SE = 6.5; fig. 2).
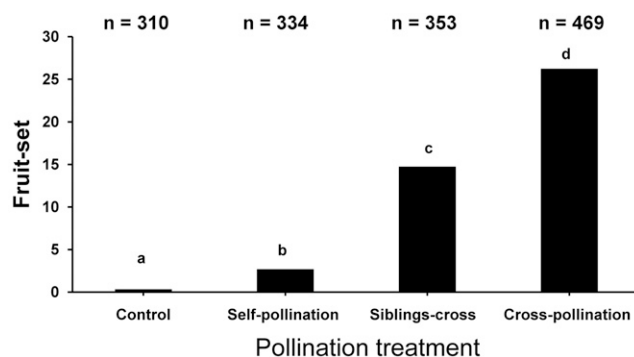
Breeding System

Results of the pollination experiment indicate that *A. berteroi* relies on pollinators to set fruits and that fruit set from self-pollination is unlikely to occur (fig. 3). In this study, only 8 of 146 individuals were self-fertile, and 95% of the individuals were self-sterile after self-pollination. Fruit set differed significantly among treatments (Kruskal-Wallis test, $\chi^2_4 = 152.97$, $P < 0.0001$, $n = 1599$, a total of 162 plants). Self-pollinations, as well as no pollination (treatments 1 and 4), produced fruits less than 3% of the time, whereas cross-pollinations (treatment 3) set fruits 26%, on average, differing significantly from self-pollination and no pollination. Cross-pollinations involving siblings (treatment 2) were midway between the other two groups and significantly different from both (fig. 3).

Seed weight differed significantly among treatments (treatments 1–3; $F_{2,353} = 18.56$, $P < 0.0001$; table 1). The mass of seeds from cross-pollinations was significantly greater than the other two treatments, whereas seeds from self-pollination treatments were black (in contrast to the normal tan color) and weighed the least, significantly differing from the other treatments. Mature fruit length differed significantly among treatments as well ($F_{2,52} = 3.56$, $P = 0.036$). Self-pollinated flowers produced shorter fruits that were not, however, significantly shorter than fruits produced by cross-pollination among siblings. Cross-pollinated flowers produced the longest fruits (mean = 9.98 cm, SE = 0.48), significantly longer than selfed fruits but not fruits from sibling crosses (table 1).

Fig. 3 Results of hand-pollination experiment with *Angadenia berteroi*. Fruit set (proportion of number of fruit produced over number of flowers pollinated) from five hand-pollination treatments with *A. berteroi*. *n* = number of flowers for that treatment. Treatments with the same letters are not significantly different with Kruskal-Wallis analysis.

Seedling Emergence

Seedling emergence from the cross- and sibling-treatment seeds did not differ significantly ($t = 0.44$, $P = 0.66$), but emergence rate for the self-pollination treatment was zero (table 2). Seedlings present from cross- and sibling-treatment seeds differed significantly at 35 d postplanting as well as at 56 d ($t = 2.2$, $P = 0.03$, and $t = 2.3$, $P = 0.03$, respectively; table 2). The progeny from the cross-pollination treatment were substantially more numerous than those from the other two treatments (table 2). The seedlings present from the self-pollination treatment at 56 d (6.25%) were two small seedlings that appeared at day 56 after planting (fig. 4), perhaps an experimental error in the greenhouse.

A *t*-test showed that seedlings from the self-pollination treatment were shorter than those from the other two treatments (table 1), but the sample size for seedlings from self-pollination treatments was too small for any statistical comparison. Seedlings from cross-pollinations were not significantly taller than those from sibling cross-pollinations ($t = 1.2$, $P = 0.22$; table 2).

Discussion

As in most other members of the Apocynaceae, the complex flowers of *Angadenia berteroi* restrict access to only those visitors with mouthparts adequate to reach the nectar. Secondary pollen presentation, a pollen "gluing" mechanism, and the position of the receptive stigmatic surface further limit the receipt of pollen for fruit and seed set. Studies of other species in the family can inform our understanding of results with this species.

The floral morphology of *A. berteroi* is similar to the morphology described for other Apocynaceae (Galetto 1997; Lipow and Wyatt 1999; Lopes and Machado 1999; Darrault and Schlindwein 2005). The complex floral structure captures outcross pollen from the visitor mouthparts and then transfers self-pollen to the visitor (Darrault and Schlindwein 2005). Even though the complex pollination mechanism in-

Fig. 2 Fruit set of *Angadenia berteroi* ($n = 108$). Fruit set calculated from flowers and fruit over 4 mo from four pine rockland forest sites in Miami-Dade County (site 1 = Rockdale Preserve, site 2 = Nixon Smiley Park, site 3 = Larry and Penny Thompson Park, site 4 = Navy Wells Preserve) and two burn units in Everglades National Park (site 5 = unit I1 and site 6 = unit G).

Table 1

Results of Hand-Pollinations of *Angadenia berteroi*

Treatment	Seed weight (g; $n = 356$)	Fruit length (cm; $n = 55$)
Self-pollination	.79 ± .07[C]	6.70 ± 1.25[B]
Cross-pollination among siblings	1.05 ± .04[B]	8.78 ± .80[AB]
Cross-pollination	1.28 ± .03[A]	9.98 ± .48[A]

Note. Summary data showing means ± standard error of the mean for seed weight and fruit length. Superscript letters indicate significant differences at the 0.05 significance level.

creases pollination efficiency, avoids autogamy, and favors cross-pollination, it does not prevent geitonogamy (Darrault and Schlindwein 2005).

The yellow campanulate flowers of *A. berteroi* have nectar and are visited by bees and butterflies. The sugar concentration of the nectar (30%–67%) is within the range of values reported for flowers pollinated by bees (~40%; Proctor et al. 1996) but is higher than typical values for butterfly pollination (Baker and Baker 1983). This is also higher than the concentration reported for several other nonasclepioid species in the Apocynaceae, with sugar concentrations of ~30%: *Macrosiphonia petraea*, *Mandevilla laxa*, and *Mandevilla pentlandiana* (Galetto 1997); *Rauvolfia grandiflora* (Lopes and Machado 1999); and *Hancornia speciosa* (Darrault and Schlindwein 2005). In addition, the flowers of *A. berteroi* possess architectural attributes of oligophilic flowers. These flowers tend to have a specialized shape and to have very few species of visitors but a high chance of cross-pollination (Richards 1997). This agrees with our field observations, in that very few bees and butterflies visited the flowers of *A. berteroi*. The opening time of the flowers and our observations in the field suggest that *A. berteroi* may be pollinated by crepuscular or diurnal insects, and perhaps the flowers are visited most frequently early in the morning. More work is needed to verify this and to determine the array of visitors to flowers of this native species.

Hand-pollinations indicated that *A. berteroi* is a facultatively xenogamous species (sensu Cruden 1977: primarily outcrossing, self-incompatible, and requiring pollinators for sexual reproduction). Several authors have suggested that floral architecture in the Apocynaceae minimizes self-pollination (Lipow and Wyatt 1999; Darrault and Schlindwein 2005; Pinto et al. 2008), and in our experiments, unmanipulated flowers set virtually no fruits.

Some degree of self-compatibility has been reported previously in the Apocynaceae (Lin and Bernardello 1999; Torres and Galetto 1999). The majority of the seeds within a fruit may be full siblings (Lipow and Wyatt 1999) because the pollen in the Apocynaceae aggregates in tetrads or masses (with pollen grains either held together by an adhesive substance or in pollinia in Asclepioids). Pollen aggregation causes pollen grains to be removed and to be deposited collectively and simultaneously onto a single stigma, increasing the probability that the seeds within a fruit will be full siblings (Harder and Johnson 2008). In addition, a visitor can remove a large number of pollen grains in a single visit, enough to pollinate all the ovules of a flower (Darrault and Schlindwein 2005). The high genetic similarity between siblings, due to the possible existence of full siblings with 100% similarity produced by all seeds in a fruit sharing the same parents, may explain the relatively low fruit set of cross-pollination among siblings. In our experiments, crosses from unrelated individuals had the highest fruit set (26%); this result is in accordance with Lipow and Wyatt (1999), Lopes and Machado (1999), and Darrault and Schlindwein (2005), where highest fruit set resulted from crosses between unrelated individuals. A negative relationship between reproductive success (fruit production) and plant relatedness has been shown in many non-Apocynaceae species as well (Schlichting and Devlin 1992; Elam et al. 2007; Jakobsson et al. 2009; Pinto-Torres and Koptur 2009).

Fruits of *A. berteroi* take 2 mo to mature, and few fruits release viable seeds at the end of the maturation period. The total number of seeds produced was positively correlated with fruit length, as also reported by Torres and Galetto (1999). Seeds from self-pollinated fruits were black and weighed less, and because they failed to germinate, we conclude that they were inviable. In addition, the fruit length of self-pollinations was only 6.7 cm (vs. 9.98 cm in cross-pollinated fruits), evidence of low seed production after self-pollination. The high percentage of self-sterile individuals and the small number of seeds produced after self-pollination suggests a late-acting self-incompatibility mechanism in *A. berteroi*. Self-sterility in most individuals in a population, combined with the self-pollen tubes growing into the style, resulting in the abortion of those ovules and seeds, may imply postzygotic self-incompatibility (Lipow and Wyatt 1999, 2000). Ovule abortion from self-pollination or mating with close relatives probably contributes to the natural low levels of fruit set in *Angadenia*, as in other species in the Apocynaceae (Lipow and Wyatt 1999; Finer and Morgan 2003; Pinto et al. 2008), but more detailed observations are needed to determine this.

Table 2

Results from Planting Seeds from Hand-Pollinations of *Angadenia berteroi*

Treatment	Seedling emergence ($n = 49$; 15 d)	Seedlings present ($n = 49$) 35 d	Seedlings present ($n = 49$) 56 d	Height (cm; $n = 153$; 56 d)
Self-pollination	0	0	6.25 ± 6.25[C]	1.50 ± .50[B]
Cross-pollination among siblings	22.28 ± 4.54[A]	24.94 ± 4.76[B]	23.18 ± 4.89[B]	3.75 ± .22[A]
Cross-pollination	23.50 ± 3.77[A]	38.54 ± 3.9[A]	36.73 ± 3.84[A]	4.42 ± .33[A]

Note. Summary data showing means ± standard error of the mean seedling emergence, percentage of seedlings present at each interval, and seedling height for each group of seeds. Superscript letters indicate significant differences at the 0.05 significance level. Presence of selfed seedlings at day 56 is due to two small seedlings that germinated late (possibly experimental error).
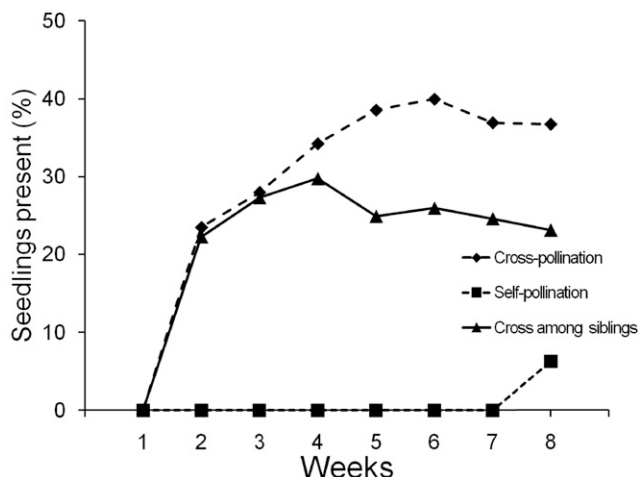
Fig. 4 Results of seed germination with *Angadenia berteroi*. Percent of total number of seeds that emerged from three hand-pollination treatments ($n = 49$).

Negative relationships among plant relatedness, seedling emergence, and progeny vigor were observed in this experiment. Seeds from crosses between unrelated individuals had higher seedling emergence and larger seedling size. These results contrast with the results of Torres and Galetto (1999), who found higher survival rates for seedlings from self-pollinations; they postulated that flowers that do not produce fruits contribute to pollen donation, increasing male fitness.

In addition, the relatively low fruit set after sibling crosses, combined with reduced seed weight and seedling vigor, suggests inbreeding depression (Richards 1997). The consequences of late-acting self-incompatibility, such as ovule and seed abortion, have been also assigned to inbreeding depression (Richards 1997). Ovules abort in some species of the Apocynaceae as a result of a late-acting self-incompatibility mechanism (Lipow and Wyatt 1999), which may be common in the family (Lipow and Wyatt 1999; Lopes and Machado 1999). Lipow and Wyatt (2000) found that the late-acting self-incompatibility system occasionally failed following self-pollinations and also after full-sibling cross-pollination. More research is needed in *A. berteroi* to determine the precise effects of self- or closely related pollen on fruit set and progeny vigor. Overall, the low percentage of self-fertile individuals, the low percentage of fruit set and seed set after self-pollination, plus the

occurrence of inviable seeds that do not germinate, suggest that *A. berteroi* has a late-acting self-incompatibility mechanism.

Natural fruit set in *A. berteroi* is fairly low. Several possible explanations for low fruit set are that resources needed to produce mature seeds may be limiting, insufficient numbers of compatible pollen grains may be reaching the stigma due to selfing and geitonogamy, and there may also be a late-acting self-incompatibility mechanism. Although we do not yet have data to test all these hypotheses and more experimental work in field and greenhouse is needed, our data suggest that flowers in the field are either not visited or not cross-pollinated and probably have a late-acting self-incompatibility mechanism. Perhaps low fruit set in nature can be attributed to crosses between closely related individuals and the negative effect of mixed pollen loads.

Angadenia berteroi is a showy but threatened species of the pine rocklands of south Florida. Its somewhat specialized pollination system (requiring long-tongued pollinators) and self-incompatible breeding system demonstrated in this study are not typical of all species in this habitat, and these may make it more likely than other species to experience negative effects of habitat fragmentation. If remnant populations in small fragments have little genetic diversity, the absence of suitable mates may limit sexual reproduction. This study can help guide conservation and restoration efforts for this and perhaps other pine rockland species.

Acknowledgments

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