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Floral Biology and Breeding System of the Crenulate Leadplant, *Amorpha herbacea* var. *crenulata*, an Endangered South Florida Pine Rockland Endemic

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ABSTRACT: The federally endangered crenulate leadplant, *Amorpha herbacea* Walter var. *crenulata* (Rydb.) Isely, is an endemic shrub of the globally imperiled pine rocklands of southern Florida. Crenulate leadplant is near extinction in the wild due to heavy habitat loss, fire suppression, altered hydrology, and invasion by non-native species. This study examined the floral biology and breeding system of the leadplant and factors that may help explain its decline and provide direction for conservation. Protogynous flowers and a high pollen/ovule ratio suggest a reproductive strategy of outcrossing, while a binucleate pollen grain indicates possible gametophytic self-incompatibility. Hand pollinations show that while the leadplant is capable of some self-fertilization, it is significantly more successful in setting fruit when cross-pollinated, and produces a greater percentage of seed when outcrossed. This predominantly self-incompatible species may, therefore, suffer decreased reproductive fitness in its few remnant localities.

Index terms: *Amorpha herbacea* var. *crenulata*, bees, breeding system, endangered plant, Fabaceae, pine rockland, protogyny, self-incompatibility

INTRODUCTION

Amorpha herbacea Walter var. *crenulata* (Rydb.) Isely, the crenulate leadplant, is a federally endangered shrub endemic to the globally imperiled pine rocklands of extreme southern Florida (USFWS 2006). The crenulate leadplant (Fabaceae: Papilionoideae) has dwindled to near extinction in the wild, where it exists solely as remnant adult populations in highly altered sites. Its primary threats are habitat destruction and fragmentation, fire suppression, drainage, and invasion by non-native species (FDACS 2000).

The pine rockland habitat of the crenulate leadplant has been largely destroyed throughout urban and suburban Miami-Dade County (USFWS 2006). With a tree canopy composed solely of slash pine (*Pinus elliotii*), the pinelands support a number of endemic shrubs and herbs now listed as threatened or endangered. Pine rocklands outside of Everglades National Park once covered nearly 65,000 hectares, but now have been reduced to small fragments. In Miami-Dade County, the pine rocklands occur along the Miami Rock Ridge, an exposed portion of the Pleistocene Miami Limestone formation approximately 80 kilometers long and 6 to 14 kilometers wide (DERM 1993).

Crenulate leadplant had a restricted range even before habitat destruction took its toll. The shrub was constrained to an area approximately 19 km long and 8 km wide in Miami-Dade County (DERM 1993). This range is within the northern Biscayne

region of the Miami Rock Ridge, much of which historically was close to the transverse glades and eastern edge of the Everglades (Snyder et al. 1990). Crenulate leadplant is associated with the seasonally hydrated, pineland-marl prairie ecotone, which essentially no longer exists in Miami-Dade County (FDACS 2000).

The study of plant breeding systems is vital to species and habitat conservation (Richards 1997). If a plant is self-incompatible, it must have access to the pollen of a different genetic individual for successful reproduction. If pollinators are necessary, conservation requires habitat to support the pollinators and to sustain plant populations large enough to enable cross-pollination. Thus, knowledge of breeding systems is important in formulating integrated management strategies (Koptur 2006). It also has important practical applications in managing endangered plants to conserve their genetic variability (Kearns and Inouye 1993), since breeding systems play a crucial role in shaping population genetic structure (Hamrick 1989).

This study examined the floral and reproductive biology of crenulate leadplant, factors that may help explain its decline in the wild and assist in achieving the immediate federal objectives of preventing extinction and increasing populations. Modern conservation is strongly oriented toward habitat protection, but this does not remove the need to understand the autecology and requirements of individual species (Noss et al. 1997), especially those that have become so rare that they require individual listing

and recovery planning to avert extinction (Atwood and Noss 1994).

METHODS

Study Plants and Sites

Crenulate leadplant is a multi-stemmed shrub, readily resprouting from its base after periods of dry weather, mowing, or fire (Figure 1a). Its white inflorescences are tinged with red and its small flowers have bright orange pollen, making an attractive display for flower visitors (Figure 1b,c). We studied the crenulate leadplant in the wild and in a greenhouse. Study sites included two Miami-Dade County parks with naturally occurring leadplant populations. A third, privately owned site was monitored initially, but later abandoned as a study area after it was cleared.

Greenhouse study plants were obtained as seedlings from Fairchild Tropical Botanic Garden in Coral Gables, Florida. Seeds from two different sources produced the seedlings from which our study plants were grown. We potted seedlings individually in 4-inch containers in an alkaline potting mix consisting of equal parts fine-grain pumice, silica sand, peat moss, and pine bark soil conditioner. The plants were placed on two tables in the Florida International University greenhouse where they received regular watering and occasional treatment for ants (baits made of sugar, boric acid, and water) and scale insects (insecticidal soap spray). A half-strength mixture of liquid Miracle-Gro fertilizer (15-30-15, N-P-K) was applied every few months equally to all plants. The plants were stepped up to 1-gallon containers, and some to 3-gallon pots, over the course of several years. They were cut back when stems grew too long and in order to induce flowering.

Floral Biology and Phenology

Crenulate leadplant has unusual flowers for its legume subfamily, the Papilionoideae, comprised of species known for their five-petaled, zygomorphic flowers. Crenulate leadplant flower corollas are reduced to a single standard petal; hence the generic

name *Amorpha*, meaning “without shape,” or “deformed” (Linnaeus 1753; Rydberg 1919; Isely 1990; McMahon 2002).

We observed leadplant inflorescences and flowers on plants in the field for growth patterns and for timing of male and female reproductive functions. To determine the growth of the leadplant’s spike-like terminal racemes, we measured 24 racemes on six plants daily until first flower opening. Inflorescence expansion ceased once flowers started to open.

Three racemes on two plants were monitored hourly for 30 hours following first flowering activity to determine the number of the protogynous flowers in female-phase and male-phase. We looked at six maturing racemes on four plants, and counted the number of female-phase and male-phase flowers at mid-inflorescence. To record the time an inflorescence lasted, from first flower opening to pollen dispersal, we monitored 11 racemes on four plants daily. To determine the average number of flowers per centimeter of inflorescence, we measured five racemes with a millimeter ruler and counted the number of flowers on each raceme.

Individual reproductive components were measured with a millimeter ruler using 30 flowers from at least six leadplants for each component. We measured calyx length, petal length, style protrusion beyond the standard petal, longest stamen exertion beyond the standard, flower length from base of the calyx to the tip of the longest exerted stamen, fruit length, and seed length.

Stigma Receptivity

We tested for stigmatic esterase activity, an enzymatic indicator of receptivity which also identifies the location of the receptive stigmatic surface (Kearns and Inouye 1993). Five styles were removed from leadplant flowers in each of three stages: just before flower opening, immediately following flower opening, and after anther dehiscence. The test solution was 2.5 mg of the substrate alpha-naphthyl acetate dissolved in three drops of acetone in a

test tube. Five ml of phosphate buffer (0.1 M, pH 7.0) were added to the dissolved alpha-naphthyl acetate and shaken well. Then, 12.5 mg of tetrazotized o-dianisidine blue dye were added to the solution and shaken again. A control solution was prepared that contained everything but the alpha-naphthyl acetate (Kearns and Inouye 1993). Styles were immersed in small quantities of the test and control solutions, and observed 10 minutes later for a strong reddish coloration that indicates enzymatic activity.

Pollen Nuclei

Pollen grains of crenulate leadplant were observed using two different DNA probes: (1) DAPI (4', 6-diamidino-2-phenylindole), a double-stranded DNA-specific dye that fluoresces blue in proportion to the amount of DNA in a nucleus after excitation with UV light; and (2) Hoechst 33258 (bisbenzimidazole derivative). We used fresh pollen from one greenhouse plant. Preserved pollen from three other individuals was fixed in a 1:3 acetic acid: ethanol solution for several hours, followed by 30 minutes in 50% ethanol, then stored in 70% ethanol (Kearns and Inouye 1993). We placed the fresh pollen in a drop of DAPI solution on a microscope slide under a Leitz dialux 20 microscope. We placed preserved pollen from three plants in a drop or two of Hoechst 33258 on three microscope slides and added cover slips. The slides were then rinsed with a phosphate buffer, observed under the microscope, and photographed.

Pollen Count

We estimated the number of pollen grains per leadplant flower by placing 10 dehiscing anthers from one flower in a micro-centrifuge tube and adding 0.1 ml of lactophenol-aniline blue (Kearns and Inouye 1993). We placed the tube in a vortex mixer for approximately 30 seconds to evenly suspend grains in the solution, and piped the solution into the wells of a hemacytometer. We observed the hemacytometer under a compound microscope and counted pollen grains within its grid. Pollen production per flower was estimated

Habit (a)



Branch (b)



Inflorescence (c)



Figure 1. Crenulate leadplant habit (a), branch (b), and inflorescence (c).

using the following formula:

$$0.1 \text{ ml} \times \frac{\text{mean \# pollen per sample}}{0.0009 \text{ ml}} \times \frac{1}{\# \text{ flowers}}$$

We used a total of three flowers from each of three plants, and had six replicate samples per flower.

Hand Pollinations

To determine the relative success of self versus outcross pollen, we conducted hand-pollination treatments over two flowering seasons on leadplants in the greenhouse. The greenhouse environment provided consistent light, temperature, and humidity for all plants, and prevented pollinators from visiting flowers. The success of different pollen sources in fertilizing ovules was measured by fruit and seed set (Kearns and Inouye 1993) (see below).

We performed hand pollinations using pollen from different inflorescences on the same plant to test for selfing (geitonogamy) and using pollen from flowers on different genetic individuals (genets) to test for outcrossing (xenogamy). Same-flower self-pollinations were not performed due to the separation in time of stigma receptivity and anther dehiscence. Many self-compatible species undergo no within-flower, or even within-ramet, self-pollination as a result of dichogamy (Richards 1997). Controls (no manipulation) were conducted to see if automatic self-pollination occurred. No pollination bags were used initially, since even the finest mesh bags can alter the environment of the flowers inside, and temperatures and humidity inside the greenhouse often were high. We bagged developing fruit to insure none of the fruit came off the plants before it was collected and counted. We did not test for seed production without sexual reproduction (agamospermy), as this test involves emasculating flowers by removing anthers before they dehisce (Kearns and Inouye 1993). It was not possible to remove the leadplant flower's 10 stamens without mutilating its tiny gynoecium.

Greenhouse plants that produced at least three inflorescences were used as experi-

mental plants. All treatments (self-pollination, cross-pollination, and no-pollination control) were done on each individual, and replicated if there were enough racemes available. Inflorescences were pollinated only once. All male-phase (earlier-opened) flowers were removed prior to hand pollinations. Removing flowers with exerted stamens prevented their pollen from coming into contact with hand-pollinated flowers. All receptive flowers on a single raceme received the same hand-pollination treatment. We used an OptiVISOR to magnify the flowers for precision pollen application.

Pollen for hand pollinations was obtained from field and greenhouse plants. It was taken in the form of whole or partial inflorescences broken off the paternal plants by forceps, placed in glassine envelopes, and used within several hours of harvesting the flowers. The pollen-donor raceme was held at its base by forceps and rubbed, brush-like, over all protruding styles on the maternal inflorescence until pollen was generously distributed and visible on the stigmatic surfaces. The bright, yellow-orange leadplant pollen is easily seen on stigmas. We used ample pollen supply since, in some plants, more than one pollen grain per ovule is required to initiate seed production (Kearns and Inouye 1993).

Racemes were marked with jewelers' tags to indicate self- or hand-pollination treatments, or control. Fruits were collected when brown (mature) or when they easily came off inflorescences. Fruits were stored in paper envelopes and air-dried. Fruits and seeds were later weighed to 0.1 mg on an American Scientific Products electronic analytical balance.

Pollination and Insect Visitors

Formal insect pollination studies of crenulate leadplant were not conducted; however, some observations of insect visitors were made in the field. We photographed and / or collected specimens whenever possible for identification. We reared one caterpillar successfully to eclosion on a diet of crenulate leadplant. The adult butterfly was determined by an entomological taxonomist, and then released.

Statistical Analysis

Data are presented as means \pm 1 S.D. As the data were not normally distributed, we applied square root transformations for 2001 and 2002 fruit count data. We used a one-way repeated measures analysis of variance (ANOVA) on the number of fruit produced by hand pollinations and controls, and a Bonferroni test at a 5% level for post hoc comparisons. Between-year differences were tested using a two-way, repeated-measures ANOVA. For 2002 percent fruit set data, we used a one-way ANOVA with arcsine square root transformations and Fisher's LSD at a 5% level for post hoc comparisons. Two-way, repeated measures ANOVA of treatment and year was carried out on raw percent seed set. We used a paired-sample t-test to compare fruit and seed weights for cross- and self-pollination treatments.

RESULTS

Floral Biology and Phenology

The flower of crenulate leadplant is dichogamous, with a difference in timing between stigma and pollen presentation. The protogynous flower presents a single receptive style before 10 monadelphous stamens are exerted and dehisce. Stamens eventually extend past the style, but are adjacent to it at one point during their exertion (Figure 2). The anthers we observed did not dehisce until after stamens were exerted beyond still-receptive stigmas. The inflorescence is a spike-like raceme that ranges from only a few centimeters in length to more than 30 cm (Figure 3). Flowers mature from the bottom of the raceme toward the apex in an orderly succession, although some buds open out of sequence. Flowers are arranged in a spiral around the inflorescence axis, becoming more crowded toward the tip. We observed plants to bloom in the field as early as March and as late as mid-November. Individual plants may flower off and on throughout the season, with flowering usually accompanying flushes of new growth.

Inflorescence Characteristics

Inflorescences grew an average of 1.2 ± 0.8 cm per day while expanding ($n = 57$ daily measurements) with a range of 0.0 to 3.3 cm per day. Growth stopped just before or when the first flower(s) opened. By the middle of the second day after flowers began to open, inflorescences had an average of 16 ± 4.6 flowers in either female or male phase. The number of flowers becoming active within that initial period ranged from 11 to 20 ($n = 3$ racemes), with the

females outnumbering the males. By the time inflorescences were midway through their bloom, male-phase flowers predominated. Leadplant racemes expanded over an average of one week ($n = 11$ racemes, mean = 7.0 ± 3.0 days), from time of first flower opening until pollen dispersal. The shortest inflorescence (3.1 cm) expanded over three days, and the longest inflorescence (22.5 cm) opened flowers over 11 days. There were approximately 10 flowers per centimeter of leadplant inflorescence ($n = 77.5$ cm, mean = 9.8 ± 1.2 flowers).

Total flower counts ranged from 107 on the shortest raceme (10.5 cm) to 208 on the longest (20.5 cm) ($n = 5$ racemes, mean = 150.6 ± 37.2 total flowers).

Flower Opening and Floral Characters

Leadplant flowers opened during day and nighttime hours. The flower style first uncurls from a tightly closed bud. As the style straightens, the bud widens to reveal a portion of the single white banner petal enclosing bright yellow-orange anthers. Within hours of style emergence, the stamens begin to exert. They are of varying lengths during this process, in which the banner petal either extends and fans open slightly or extends and remains slightly folded around the style. Stamens do not exert simultaneously; nor do anthers dehisce simultaneously. The style begins to wilt or retract as stamens reach full length, and the style eventually is entirely enclosed by the banner petal. The male flower phase generally last two to three times as long as the female phase. In nature, flowers usually are spent, with all pollen dispersed, within 48 hours.

The leadplant's five-lobed, persistent flower calyx averaged 3.2 ± 0.3 mm in length, and the obcordate standard petal (banner) averaged 5.6 ± 0.9 mm in length from base to tip. The base of the banner begins within the calyx. On average, leadplant styles protruded 1.7 ± 0.3 mm beyond the standard. There was much greater variability in the longest stamen's exertion beyond the banner, with an average length of 5.1 ± 1.5 mm. Overall, the leadplant flower, including the longest exerted stamen, averaged 9.7 ± 1.6 mm in length. Like all legumes, the ovary of the leadplant is superior and unicarpellate, and placentation is marginal. The ovary is approximately 1 mm long, compressed, and contains two ovules.

Fruit and Seed Characteristics

The leadplant fruit is a glandular-dotted, indehiscent legume, maturing from green to brown. Fruit measured approximately 5.6 ± 0.4 mm in length. Seeds were compressed,

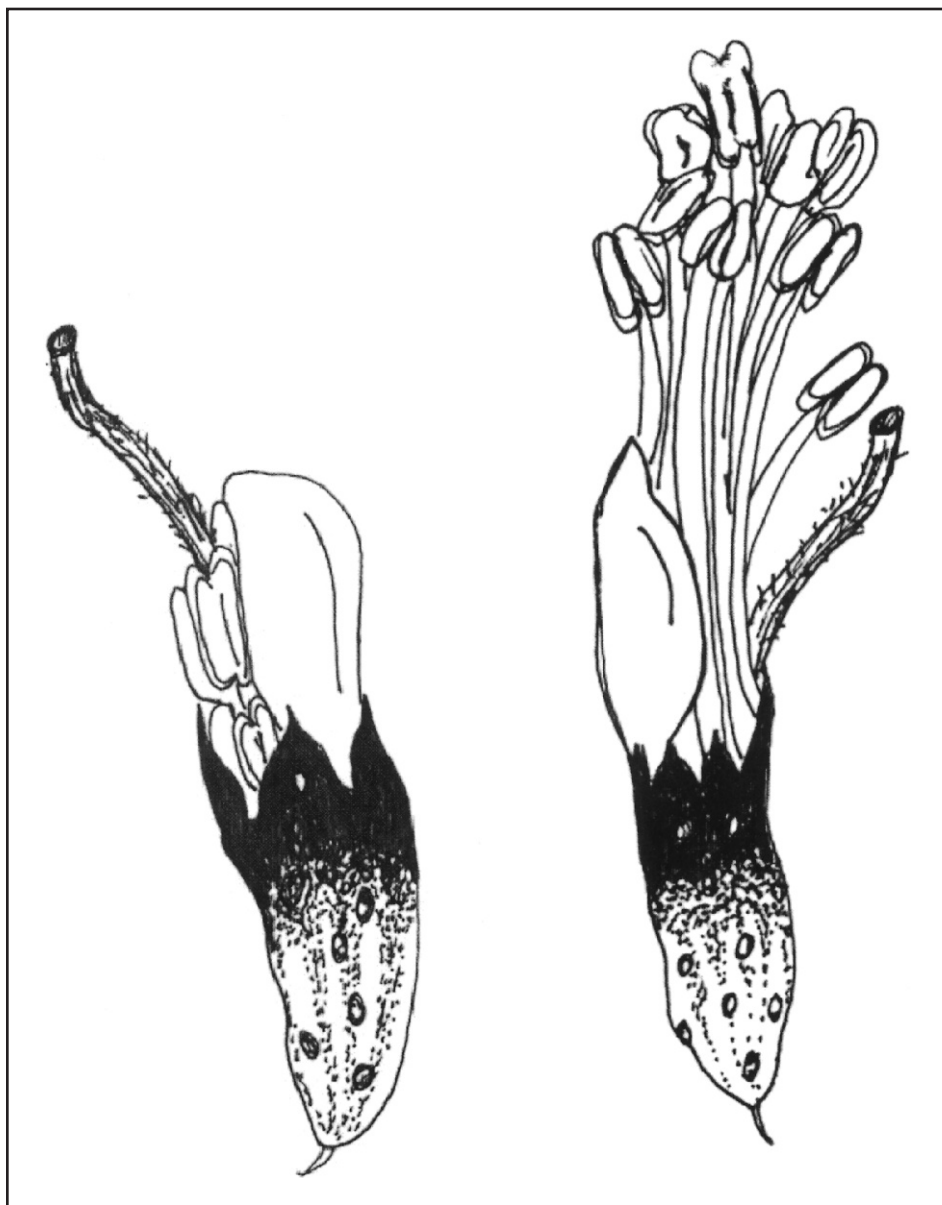


Figure 2 . Line drawing of flowers of crenulate leadplant. Female phase (left) – style exerted and receptive; anthers beginning to show. Male phase (right) – all ten stamens exerted, full display with all anthers dehiscent.

varying in color from olive green to milk chocolate brown, curved at one end, and pitted. The average seed length was 3.1 ± 0.3 mm. Generally only one of two seeds matures per fruit (Long and Lakela 1971), with single healthy seeds filling the entire seed compartment. Two-seeded fruit have been found on some robust, *ex situ* field and container specimens (Fellows 2002).

Stigma Receptivity

The leadplant stigma, a tiny area at the tip of the style, first becomes receptive while in the bud. Flowers in two early stages had stigmas staining dark purple-brown when tested for esterase, indicating enzymatic activity. Stigmas lose receptivity some time after stamens exert. We observed stigmas that still appeared receptive after stamens began to extend. When the style wilted or retracted within the banner petal, it was impossible to observe the stigma for receptivity, and further pollination appeared unlikely.

Pollen Characteristics and Pollen/Ovule Ratio

The leadplant has a binucleate pollen grain (Figure 4). Pollen from four leadplant individuals clearly showed the binucleate nature of the grain, meaning it is shed while containing two cells: a vegetative cell and a generative cell.

The leadplant flower's 10 stamens together produced an estimated mean pollen production of 4654 ± 2350 grains. Dividing pollen number by uniform ovule number of two gave a ratio of $2327 \pm 1175:1$. Individual plants varied in their pollen / ovule ratio: of three leadplant flowers sampled, the lowest estimated ratio was 1370:1, and the highest was 3639:1.

Hand Pollinations

Cross-pollinations of crenulate leadplant in 2001 and 2002 produced significantly more fruit than self-pollinations and controls ($F = 76.67$, $df = 2, 18$, $P < 0.0001$, combined years). In 2001, greenhouse cross-pollinations of five individuals (six

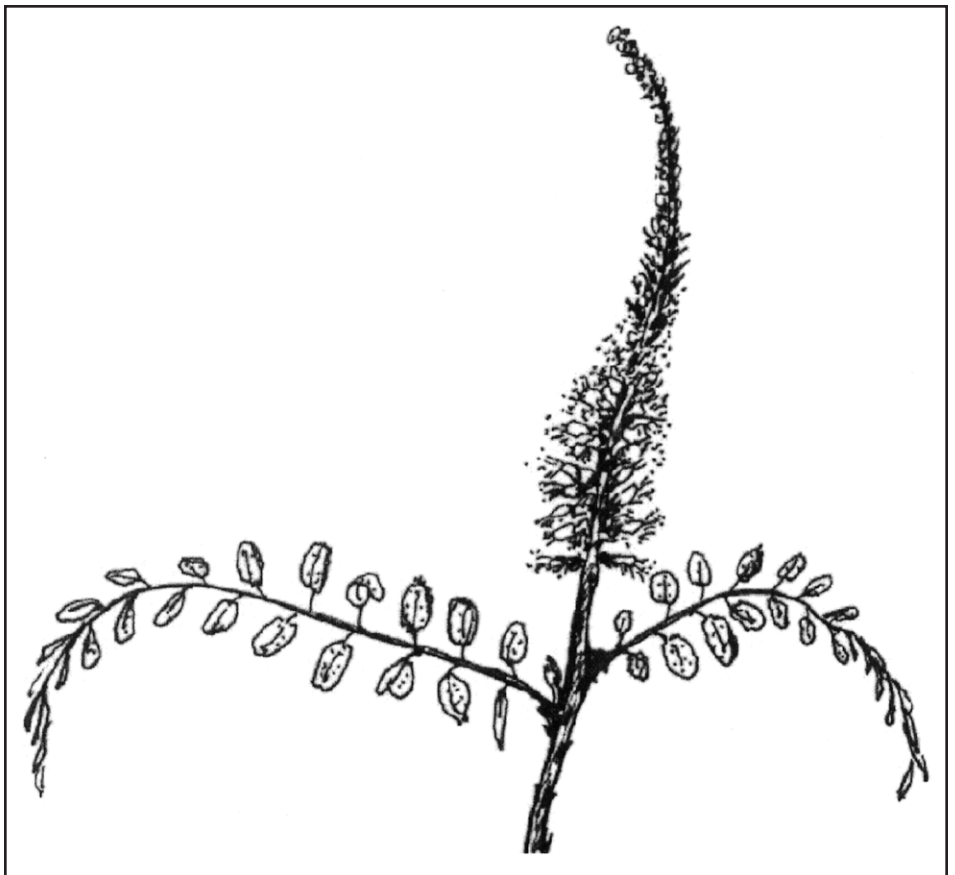


Figure 3. Line drawing of crenulate leadplant inflorescence with oldest flowers at the bottom, buds at the top.

racemes, including one replicate) produced 239 fruit, a mean fruit set of 41.6 ± 16.3 per raceme. Self-pollinations of the same five plants (five racemes) produced 50 fruit, a mean fruit set of 10 ± 8.5 per raceme (Figure 5). Controls produced a mean fruit set of 5.2 ± 5.5 per raceme. One-way repeated measures ANOVA and post-hoc comparisons showed a significant difference between cross-pollinated fruit set and that of self-pollinated racemes and controls ($F = 41.33$, $df = 2, 8$, $P < 0.001$).

In 2002, greenhouse cross-pollinations of five individuals (six racemes, including one replicate) produced 165 fruit, a mean fruit set of 27.0 ± 16.2 per raceme (Figure 5). Self-pollinations of the same five plants (six racemes, including one replicate) produced 22 fruit, a mean fruit set of 2.8 ± 3.8 per raceme. Controls produced a mean fruit set of 1.1 ± 1.2 per raceme. Again, the difference between cross-pollinations and the other two treatments was highly

significant ($F = 31.04$, $df = 2, 8$, $P < 0.001$). There was no significant difference in treatments between years ($F = 4.53$, $df = 1, 8$, $P < 0.66$) and no interaction between year and treatment ($F = 0.40$, $df = 2, 16$, $P < 0.614$).

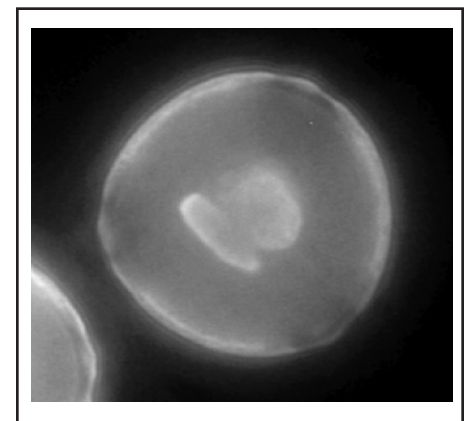


Figure 4. Pollen grain of *Amorpha crenulata* showing its two-nucleate status upon release.

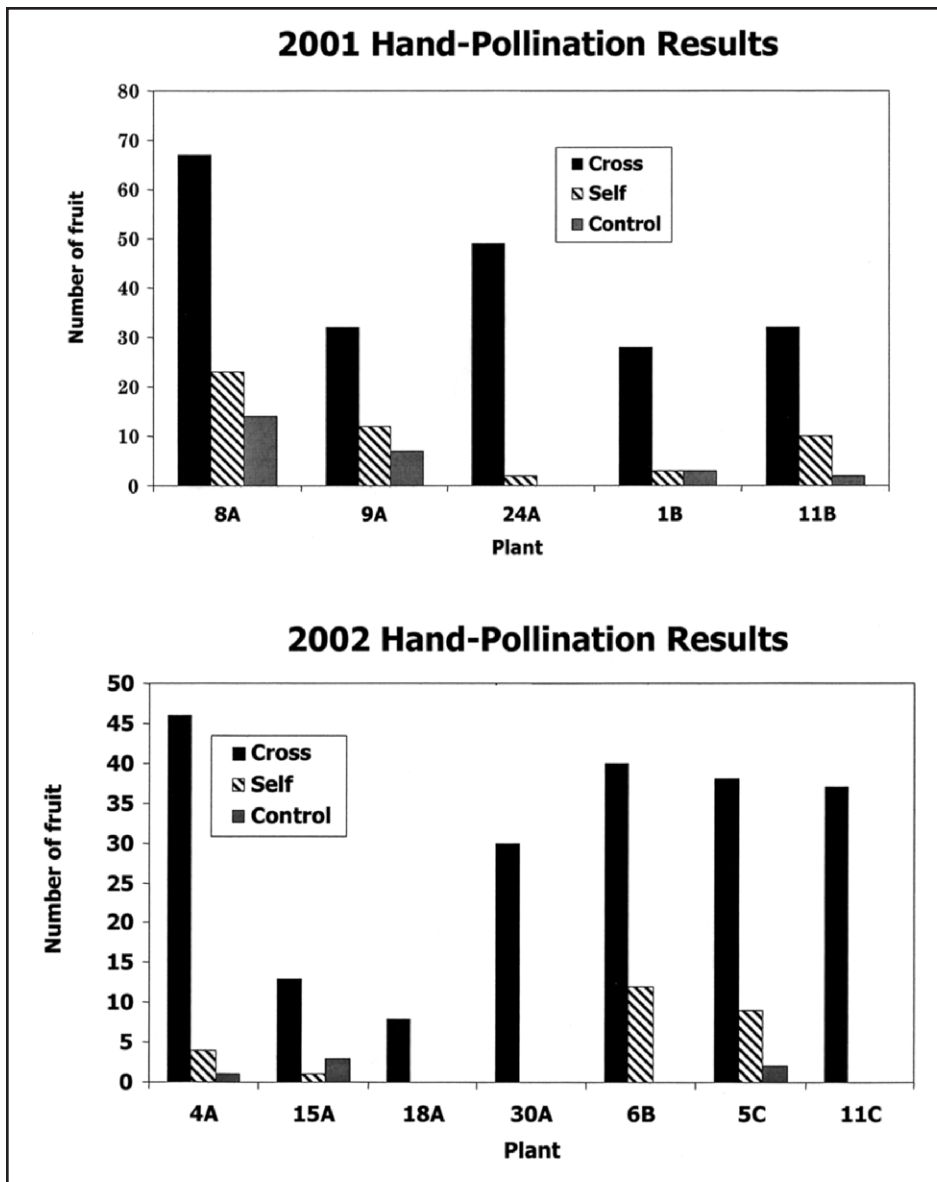


Figure 5. Fruit set from *Amorpha crenulata* hand-pollination treatments for 2001 (above) and 2002 (below). The number of fruit set with cross-pollination was greater than all other treatments on all plants.

In 2002, we also estimated the number of receptive female flowers for hand pollinations and controls to obtain the percent of fruit by each treatment. There was a significant difference among the three treatments ($F = 14.36$, $df = 2, 8$, $P < 0.11$), and post-hoc tests showed cross-pollinations yielded a significantly higher mean percent fruit set than did self-pollinations and controls. Fruit weights in 2002 were not significantly different for cross- and self-pollination treatments ($t = 0.62$, $P < 0.29$).

The leadplant ovary contains two ovules,

but usually only one seed matures (Long and Lakela 1971). No fruit with two seeds was produced during greenhouse pollinations in 2001 and 2002, and a number of fruit in both self- and cross-pollinations produced no seeds. Undeveloped seeds appeared black and dust-like. Cross-pollinated fruit produced a significantly higher percentage of seed per mature fruit than self-pollinated fruit ($F = 12.67$, $df = 1, 8$, $P < 0.007$, based on one seed per fruit), as well as a higher overall mean number of seed. There was no significant difference in treatments between years ($F = 3.91$, $df = 1, 8$, $P < 0.083$) or in interaction between

year and treatment ($F = 1.57$, $df = 1, 8$, $P < 0.245$).

In 2001, 87 percent of the cross-pollinated fruit produced seed ($n = 215$ fruits). The mean number of seed was 37.4 ± 9.4 per outcross treatment inflorescence. Fifty-eight percent of the self-pollinated fruit produced seed ($n = 50$ fruits). The mean number of seed was 5.8 ± 6.1 per self-pollinated inflorescence.

In 2002, 88.5 percent of the cross-pollinated fruit produced seed ($n = 165$ fruits). The mean number of seed was 24.3 ± 21.8 per outcross treatment inflorescence. Fifty-four percent of the self-pollinated fruit produced seed ($n = 34$ fruits). The mean number of seed was 2 ± 2.4 per self-pollinated inflorescence. Though the numbers of fruit with seed clearly differed, there was no significant difference in the weights of seed produced by cross-pollination compared to seeds produced by self-pollination ($t = 1.61$, $P < 0.103$).

Pollination and Insect Visitors

The crenulate leadplant is a larval food source for the Cassius Blue butterfly, *Leptotes cassius* (Cramer) (Lepidoptera: Lycaenidae). The Cassius Blue lays its eggs singly on host plant buds, and caterpillars eat flowers and seedpods (Opler et al. 2006). We found several of the green, slug-like larvae on leadplant flower racemes feeding at nighttime. We observed potential pollinators on the leadplant including *Apis mellifera* L., a non-native honeybee from the Mediterranean (Apidae); *Agapostemon splendens* Lepeletier, a conspicuous, metallic green sweat bee (Halictidae); and *Dianthidium floridiense* Schwarz, a native, leaf-cutting solitary bee (Megachilidae).

Several species of ants were observed on crenulate leadplants, including the non-native crazy ant, *Paratrechina longicornis* (Latreille) (Hymenoptera: Formicidae). Other insect visitors included two weevils, *Artipus floridanus* Horn and *Pachnaeus litus* (Germar) (Coleoptera: Curculionidae) that were probable herbivores. An unidentified hairstreak butterfly (Lepidoptera: Lycaenidae) was observed with its proboscis

inserted into a crenulate leadplant calyx, perhaps looking for nectar, and probably pollinating the flowers.

DISCUSSION

The crenulate leadplant has several characteristics suggesting a system of facultative outbreeding and gametophytic self-incompatibility. Dichogamy in the leadplant is recognized as an outcrossing mechanism, and is intrafloral and incomplete. A leadplant flower presents its receptive stigma before stamens are exerted, but there is a point when one or more of its 10 stamens are adjacent to the stigma, and self-mating conceivably could occur. The flowers we observed resolved this positional interference by delaying pollen release until stamens were exerted past still-receptive stigmas.

Incomplete protogyny provides an opportunity for cross-pollination before self-fertilization is possible (Lloyd and Webb 1986). If the selective force for a plant's dichogamy is avoidance of self-mating, incomplete protogyny is expected to evolve (Lloyd and Webb 1986). However, if a plant is strongly self-incompatible and dichogamous, it is unlikely that dichogamy was selected to reduce selfing (Lloyd and Webb 1986).

In addition to possible within-flower pollination (autogamy), the leadplant has an opportunity to self-mate via different flowers on the same plant (geitonogamy). A leadplant raceme can bear female- and male-phase flower blossoms at the same time (asynchronous), and usually there is more than one same-phase flower on the raceme (synchronous). Because not all female flower phase are completed before males release their pollen, there is a chance for a pollinator to carry pollen from one flower to another flower with a receptive stigma on the same raceme. In addition, there may be two or more inflorescences on a plant bearing flowers in and out of synchrony with other flowers, enabling geitonogamy.

Our observations suggest that dichogamy in the crenulate leadplant serves as more than a backup for outcrossing. It allows

prolonged pollen presentation: the male-phase leadplant flower can last three times longer than the female phase. The stamens fan out and anthers disperse pollen over an extended period, while the banner petal folds over the wilting style, protecting it from further exposure to its own pollen. Dichogamy is associated with longer-lived flowers relative to homogamy (Schoen and Ashman 1995). In addition, in accordance with Lloyd and Webb's predictions (1986), leadplant stamens and anthers offer signals and rewards to floral visitors. While a single crenulate leadplant flower is tiny and unremarkable, a number of synchronous male-phase flowers on a raceme are eye-catching, with bright yellow-orange pollen and white banner petals contrasting with dark purple flower calyxes and raceme rachis. The stamens present a generous reward of pollen.

The results of controlled greenhouse pollinations strongly indicate that the crenulate leadplant is mostly self-incompatible. Data for cross-pollinations, self-pollinations, and controls for individual plants showed a highly significant difference in fruit set. All greenhouse cross-pollinations resulted in fruit set, while only a fraction of self-pollinations set fruit. The difference in seed set percentages of mature fruit for cross- and self-pollinations also was substantial.

Historically, partial self-incompatibility and consequent outcrossing may have facilitated the greatest possible levels of genetic diversity in crenulate leadplant populations, given the species' limited range. Outcrossing also could have mitigated problems of limited seed dispersal. Seed dispersal affects the overall distribution of genes within a population (Proctor et al. 1996). We observed naturally occurring leadplant seedlings that appeared to germinate directly under maternal plants, placing relatives in close proximity. In the past, water most likely played a role as a seed dispersal agent, since the leadplant historically occurred in transverse glades and seasonally inundated habitats (G. Gann, Director, Institute for Regional Conservation, pers. comm.). Water no longer appears to be a factor in seed dispersal due to habitat drainage, and seed

dispersal is likely to be more restricted in the last century.

Characteristics suggesting a system of outbreeding include a high pollen/ovule ratio (Cruden 1977). Our data show a ratio for crenulate leadplant within Cruden's described range for xenogamy. A high pollen/ovule ratio is associated with plants bearing highly localized stigmatic areas, such as the tiny receptive "wet" stigma at the tip of the crenulate leadplant style (Cruden and Miller-Ward 1981). The wet stigma and crenulate leadplant's binucleate pollen grain are strongly correlated with gametophytic self-incompatibility (GSI) (Richards 1997). GSI is a chemical form of self-recognition, differing from dichogamy in that it occurs after pollen is deposited on a stigma. The incompatibility reaction is mediated by the binucleate pollen grain, and involves inhibition of the pollen tube within the style. The reaction is expressed after the second pollen grain mitosis in the pollen tube (Richards 1997), although Lewis (1949) and Pandey (1959, 1970) report other times of inhibition.

Reproductive Strategy

Results of hand pollinations of crenulate leadplant indicate a system in which self-fertilization is possible, but outcrossing is probably the common mode of fruit and seed production. Such a combined reproductive strategy is not unusual in plants (Richards 1997), since self-mating as well as cross-mating can confer fitness benefits onto offspring (Holsinger 1992). Self-fertilization may serve as a backup in case outcrossing fails (Proctor et al. 1996). The balance between the two systems varies widely, depending on the life history and ecology of the species (Proctor et al. 1996). Even if selfing is possible, most plants favor cross-fertilization (Proctor et al. 1996) because outcrossing confers more genetic diversity (Richards 1997). Genetic diversity refers to the amount of genetic variability among individuals of the same species, and is directly related to a species' ability to survive environmental change (Mazzotti 1990). Reduced genetic variation may increase a species' risk in the face of long-term biotic or abiotic environmental

change (Frankel and Soulé 1980; Soulé 1980). In the case of crenulate leadplant, such a decrease may have made it difficult for populations to adjust to habitat changes in hydrology and light.

CONCLUSIONS

Not only is crenulate leadplant extremely rare due to the limited number of individuals in the wild, its pine rockland habitat is classified as globally imperiled (FNAI 2004). This leaves the crenulate leadplant vulnerable to problems inherent in small populations, such as inbreeding depression and genetic drift, as well as those correlated with habitat fragmentation. The leadplant's need for outcrossing further compounds the dangers of small populations by reducing the availability of suitable mates (Kearns et al. 1998). Additional drawbacks are invasion by non-native species, suppression of natural fire regimes, and altered hydrology (Koptur 2006).

Optimum management and species restoration plans include consideration of breeding systems and a familiarity with the plant's natural history. Given the right combination of soil, light, water, and open habitat for pollinators to find the plant, crenulate leadplant thrives and sets fruit, and its seeds germinate easily, although seedling survival appears spotty (L. Linares, pers. observation; Koptur 2006).

Fairchild Tropical Garden biologists introduced leadplants at a Restored Translocation Site (RTS), an endemic pine rockland community owned by Florida Department of Transportation about 42 km south of Miami (Miami-Dade County, Florida) (Maschinski et al. 2006; Wendelberger et al. 2008). Four types of propagules were used in the introduction: seedlings, rescued whole plants, cuttings, and one to seven-year-old nursery plants. Larger plants had the best survival regardless of their origin. Biologists concluded that with more than 100 native species establishing at the RTS, it was likely that the leadplant would also, forming a self-sustaining population (Wendelberger et al. 2008). The array of threatened plants endemic to pine rocklands, and the increasing knowledge and restoration

work associated with this habitat, hold promise for further protection and benefits (Koptur 2006). As described above, successful reintroductions and outplantings also may assist the crenulate leadplant and its imperiled pineland cohorts.

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