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Reproductive biology of *Asimina parviflora* (Annonaceae)

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ABSTRACT

NORMAN, E. M., K. RICE AND S. COCHRAN (Department of Biology, Stetson University, DeLand, FL 32720). Reproductive biology of *Asimina parviflora* (Annonaceae). Bull. Torrey Bot. Club 119: 1–5. 1992.—Flowering and fruiting of the small flowered pawpaw, *Asimina parviflora* (Michx.) Dunal, was studied in central Florida to determine phenology, pollination, breeding system and germination. This species flowers from mid-February to the end of March. The protogynous flowers occasionally overlap in pistillate and staminate phases. Diptera are the main visitors to the flowers. Bagged flowers occasionally produce fruits (6.8% in 1984). In open-pollinated flowers 3.2% matured fruits in 1984, and 4.5% matured in 1987. Hand cross-pollinations resulted in 16% fruit-set in 1984 and 25% in 1987. Hand self-pollinations resulted in 10.4% fruit-set in 1987. Seed numbers per flower were significantly larger for cross pollinations. Of cross-pollinated seeds, 17.5% germinated; of open-pollinated, 10%; and of self-pollinated, 0%.

Key words: Annonaceae, *Asimina parviflora,* pawpaw, reproductive biology, fly pollination.

The Annonaceae, the largest of the primitive families of flowering plants, consists of approximately 130 genera and 2000 species (Cronquist 1981), most of which are tropical. We have been studying the only two extratropical genera, *Asimina* and *Deeringothamnus,* in order to understand their evolutionary history and compare their reproductive characteristics with those of tropical Annonaceae.

Asimina, with eight species (Kral 1960), can be divided into two groups based on morphology, ecology and geographical distribution. *Asimina parviflora* (Michx.) Dunal and *A. triloba* (L.) Dunal have small- to mid-sized purple flowers, are widespread and inhabit mesic woodlands. The other six species grow in more xeric habitats and are primarily limited to Florida. They possess mid- to large-sized flowers that are lighter in color.

Asimina parviflora is a deciduous shrub or small tree that occurs in or near the Coastal Plain from Virginia to Texas. It has received little attention, except for limited observations by McDaniel (1970) on its apparent self-compatibility. The flowers of *A. parviflora* are the smallest in the genus and are borne pendant on the previous year's wood. A typical shrub has 3–7 branches, each with 4–6 flowers. Maturation is basipetal. Petals are green in bud and turn maroon at anthesis. There were four plants that produced only yellow flowers at DeLand (DL). The six petals are in two series. The outer petals are thin, whereas the inner ones are thick, and basally saccate, forming a chamber around the reproductive organs with an apical opening approximately 3 mm in diameter. Our studies were designed to answer the following questions: 1) What is its phenology? 2) What are its floral visitors? 3) What is its breeding system?

Materials and Methods. In 1984 and 1987 data were collected for *A. parviflora* at Blue Springs State park (BS), Orange City, Volusia County, Florida, along both sides of the boardwalk where there are approximately 40 individuals. An additional population of 20 plants was studied in 1987 at a 5 hectare site in DeLand (DL), Volusia Co. Neither site showed any evidence of root cloning, as commonly occurs in the closely related *A. triloba.* Both sites are mesic hammocks, characterized by a canopy of *Quercus virginiana* Mill., *Q. nigra* L., *Q. hemisphaerica* Bartr., *Persea borbonia* (L.) Spreng., *Carya glabra* (Mill.) Sweet, and abundant vines such as *Gelsemium sempervirens* (L.) Aiton f., *Vitis rotundifolia* Michx., and *Smilax* spp.

Counts of reproductive structures were made in 1984 from 10 flowers from 10 different plants. These counts included: stamens, carpels and ovules per carpel, and pollen from 10 stamens

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Table 1. Reproductive characters of *Asimina parviflora* (mean ± SE and range).

Characters	*A. parviflora*	
Number of stamens/ flower	95 ± 26	(86–115)
Pollen/stamen	308 ± 84	(240–360)
Carpels/flower	3.3 ± 1.6	(2–5)
Ovules/carpel	7.2 ± 3.0	(5–10)
Pollen/ovule	1240 ± 996	(644–2200)

of each flower. Ratios of pollen to ovule were computed. Ten flowers were monitored during March 1984 and 1987 to determine duration of each sexual phase. In March 1984, 1987, and 1989, insects visiting flowers were collected and examined for pollen. Observations were made during a total of 10 daylight hours concentrated in the afternoon.

In March 1984, we assigned 242 flowers on 30 plants into three groups: 1) buds were bagged before the stigmas became receptive to test for autogamy; 2) to determine possible potential fruit production, buds were bagged, emasculated when stigmas became receptive and cross-pollinated with pollen from donors located at least 6 m away; 3) to determine average fruit production, buds were tagged but not bagged. The bags were made of fine-mesh stocking material with drawstrings at the base. Pollen was transferred to stigmas with a needle and its presence was confirmed with a hand lens. In March 1987, 225 buds on 60 plants were used to conduct the same three tests. We used only flowers on stems >2 mm in diameter, as previous observations revealed that no fruits were produced on smaller stems. Approximately 25% of the flowers are borne on stems <2 mm in diameter. Another experimental group of 48 buds was added to determine self-compatibility. In this treatment, buds were bagged. When stigmas became receptive, they were self-pollinated either with pollen from the same flower or, more commonly, with pollen from another flower on the same plant. Abscission of fruits was also monitored biweekly from the beginning of April to the end of June 1987. Fruits and seeds were collected and counted in mid-July 1984 and at the end of June 1987. In 1987, soon after fruit collection, 40 seeds from each of the three treatments were planted in groups of 20 in 20-cm × 26-cm clay pots containing a 1:1:1 mixture of soil, peat and vermiculite. The pots were placed outside under partial shade and were watered as necessary.

Results. FLORAL MORPHOLOGY. The flowers of *A. parviflora* are similar to those of *A. triloba* except for the former's smaller size and smaller chamber opening. The petals have a thicker texture and the inner whorl lacks the corrugated tissue at the base of the adaxial surfaces that is typically found in other pawpaws. The reproductive characteristics are summarized in Table 1.

PHENOLOGY AND FLORAL LONGEVITY. The plants were in flower from mid-February until the end of March in 1984 and 1987. In 1989 flowering began a month earlier and lasted approximately two months. In more northern states the blooming period is from April to May (Radford *et al.* 1968). The odor of maturing flowers is slight and yeasty. The flowers are protogynous. In the flowers that we monitored the stigmas became shiny and remained so for 4–9 days ($\bar{x}$

Table 2. Results of breeding experiments in *Asimina parviflora*.

Treatment	Site	Year	Sample size	% flowers setting mature fruit	Mean number of berries per flower ± SD and range	Mean number of seeds per berry ± SD and range
Direct autogamy	BS	1984	29	6.9	2.5 ± 0.7 (2–3)	3.8 ± 2.1 (1–8)
	BS	1987	27	0	0 ± 0 (0)	0 ± 0 (0)
	DL	1987	23	0	0 ± 0 (0)	0 ± 0 (0)
Open-pollination	BS	1984	188	3.2	1.8 ± 1.2 (1–4)	4.4 ± 2.1 (1–9)
	BS	1987	59	1.7	1 ± 0 (1)	1 ± 0 (1)
	DL	1987	68	7.4	1.8 ± 0.8 (1–3)	6.3 ± 2.7 (2–9)
Hand-pollination (cross)	BS	1984	25	16	2.5 ± 1.3 (1–4)	6.6 ± 2.8 (3–11)
	BS	1987	24	20.8	2.6 ± 0.9 (1–3)	6.1 ± 1.9 (4–10)
	DL	1987	24	29.2	3 ± 1 (2–4)	6 ± 2 (3–10)
Hand-pollination (self)	BS	1987	24	8.3	1 ± 0 (1)	5 ± 0 (5)
	DL	1987	24	12.5	2 ± 1.1 (1–3)	5.6 ± 1.4 (4–8)

= 6.5 ± 3.58 SD). Pollen was available for 2–3 days ($\bar{x}$ = 2.7 ± 0.67 SD). In half the flowers observed, there was an overlap of one day between the two sexual phases. In the other half, pollen was released when the stigmas turned brown.

FLORAL VISITORS. Insect visitors to flowers were few, with the greatest activity in the late afternoon. The most common floral visitors were a group of small Diptera: *Drosophila melanogaster* Meigen or *D. simulans* Sturtevant, milichiid flies, clusiid flies and frit flies. These insects hovered around the flowers, sometimes entering them, and seemed to forage around the glistening stigmas. We did not observe any pollen on approximately 25 small flies that we examined with a dissecting microscope. However, pollen could easily have been lost in handling. The only two kinds of insects captured with pollen were *Lucilia* sp. (Calliphoridae) (N = 6), the greenbottle fly, and small nitidulid beetles (N = 3). Both had pollen around their mouthparts. On several occasions, we saw *Lucilia* visiting at least two flowers on one plant.

BREEDING SYSTEM. Table 2 summarizes the results of the breeding experiments. Direct autogamy occurred only in 1984. In both 1984 and 1987, open-pollinated samples set fewer fruits and seeds than hand-pollinated ones. The especially low fruit- and seed-set at BS in 1987 in the former treatment (Table 2) may have been due to an infestation of larvae of the leafroller, *Choristoneura parallela* Robinson (Tortricidae) in some flowers. In both locations a greater percentage of mature fruits and more seeds were produced by cross-pollination than by self-pollination. Seed yield per cross-pollinated flower ($\bar{x}$ = 4.2 ± 1.2 SE) was significantly higher ($P <$ 0.01 Tukey-Kramer Test) from that in self-pollinated flowers ($\bar{x}$ = 0.90 ± 0.46 SE) and open-pollinated flowers ($\bar{x}$ = 0.46 ± 0.29 SE). Differences in seed production between the latter two treatments were not significant.

Under all four pollination treatments, fruit abscission was concentrated in the earlier phase of development (Fig. 1). Both hand-pollinated groups showed a much higher initial level of fruit-set in comparison with the open-pollinated treatment. This is undoubtedly due to the larger numbers of pollen tetrads transferred by hand rather than naturally. Approximately 50% of fruits initiated from cross-pollinations matured. In the self- and open-pollinated groups only 25% and 38% matured, respectively. The cross-pollinated flowers also retained a significantly higher proportion of berries per flower ($P <$ 0.01 Tukey-Kramer Test) than did the other two treatments (Fig. 1).

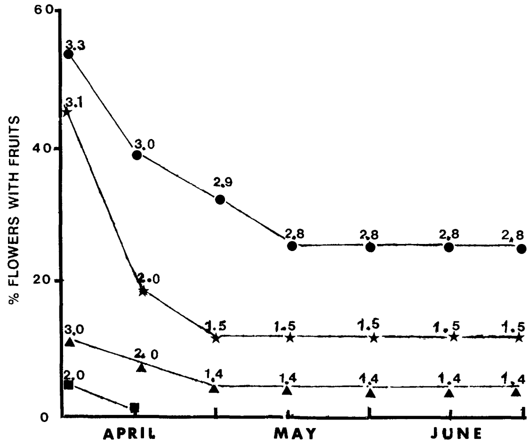

Fig. 1. Time course of fruit abortions in four treatments in 1987: ● = cross-pollination N = 48; ★ = self-pollination N = 48; ▲ = open-pollination N = 127; ■ = direct autogamy N = 50. Numbers above the lines indicate the mean number of berries per fruit.

SEED GERMINATION. Fruits of *A. parviflora* take 3–4 months to mature; those harvested in 1987 were still green, but the seeds appeared to be mature based on their dark brown coloration. Pawpaw seeds have a large amount of endosperm and a minute embryo. This embryo develops slowly, taking 3–10 months for the upper part of the hypocotyl or, more commonly, the epicotyl to appear above ground. Seven seeds (17%) of the cross-pollinated group and four seeds (10%) of the open-pollinated sample germinated. None of the self-pollinated seeds germinated.

Discussion. *Asimina parviflora* flowers are characterized by fewer stamens and lower pollen/ovule ratio than its congeners (Norman and Clayton 1986). This ratio would place *A. parviflora* in the facultatively xenogamous category (Cruden 1977). Although *A. parviflora* is protogynous, half of the sampled flowers showed an overlap of at least one day in the female and male phases. This is similar to the situation in *A. triloba* (Willson and Schemske 1980). There is less overlap in *A. obovata* and *A. pygmaea* (Norman and Clayton 1986).

The Annonaceae are primarily beetle pollinated (Gottsberger 1970, 1977; Schatz 1985; Rogstad 1986; Bernhardt and Thien 1987). Fly pollination is much rarer (Gottsberger 1970, 1988). Thien (1980) notes, however, that flies act as pollen vectors in other primitive woody dicot families. Flies were the most common visitors to flowers of *A. parviflora.* The purple pigmentation, smell, and glistening stigmas may be attractive to Diptera (Percival 1965). Nitidulid beetles also visited flowers of *A. parviflora* and are commonly found in other genera of Annonaceae (Gottsberger 1970; Gazit *et al.* 1982; Murray and Johnson 1987) and other members of the Magnoliales (Thien 1974; Armstrong and Irvine 1989). Flies are also the chief visitors of *A. triloba* (Robertson 1929; Willson and Schemske 1980). Nitidulids have also been observed in the flowers of that species (Willson; Johnson personal communication). Thus, *Asimina* has two contrasting, but overlapping, pollination syndromes. The forest inhabitants, *A. parviflora* and *A. triloba,* are primarily myophilous, whereas the remainder of the genus is cantharophilous, pollinated especially by large scarabs (Kral 1960; Winnett-Murray 1980; Norman and Clayton 1986; Austin personal communication). We have also occasionally observed nitidulids in flowers of *A. obovata* and *A. reticulata.* They have also been noted in *A. angustifolia* (Winnett-Murray 1980). On the other hand, flies sometimes visit the flowers of *A. pygmaea* (Uphof 1933 and personal observation).

Members of the Annonaceae are primarily self-compatible (Gottsberger 1970; Bawa *et al.* 1985; Rogstad 1986; Bernhardt and Thien 1987). Although *A. parviflora* is self-compatible, several factors appear to lower fruit and seed yield when selfing does take place. These factors may be polygenic postzygotic incompatibility systems and/or homozygosity of lethal or defective genes acting in the embryo and endosperm (Stephenson and Bertin 1983). McDaniel (1970), looking for pawpaws that could be bred to *A. triloba* to induce self-compatibility, suggested that *A. parviflora* might be a good candidate. He selfed several flowers of one plant and obtained one mature fruit with two seeds. The breeding system of *A. triloba* appears to be similar to that found in *A. parviflora.* Some clones or cultivars are self-compatible, despite low fruit production, while others require cross-pollination to ensure fruit production (Zimmerman 1941; McDaniel 1970). The percent fruit-set produced naturally in *A. triloba* in Illinois ($\bar{x}$ = 0.45%, Willson and Schemske 1980) is considerably lower than that in *A. parviflora* ($\bar{x}$ = 3.8%). This difference is probably due to a lower level of pollen transfer in *A. triloba.* Willson and Schemske increased fruit-set to levels comparable to ours when cross-pollination was effected. On the other hand, the higher percentage of flowers producing fruits ($\bar{x}$ = 8%) in *A. pygmaea* and *A. obovata* (Norman and Clayton 1986) may be attributed to more abundant pollen transfer in these species. Scarabs are often covered with pollen. Winnett-Murray (1980) counted 850 pollen tetrads per insect (N = 3) in flowers of *A. angustifolia.*

Our results show that fruit and seed production in *A. parviflora* is influenced by maternal resources and by the amount and type of pollen available. It was observed that stems <2 mm in diameter never set mature fruits and that stems in general bore a maximum of three fruits. Pollinators were infrequent and carried small pollen loads. The timing of fruit abortion in *A. parviflora* in the early developmental phase is typical for most species studied (Stephenson 1981). The higher level of fruit retention in cross- vs. self-pollinated fruits is also expected (Stephenson 1981). In contrast, Willson and Schemske (1980) found that *A. triloba* aborted <1% of both self- and cross-pollinated fruits.

The number of seeds that germinated was low. Because the populations of *A. parviflora* at DL and BS were small, it is likely that the pollen parent will be closely related to recipient flowers and that the progeny will show inbreeding depression (Baker 1985). Other factors such as immature embryo, or insufficient moisture, also might have contributed to poor germination. The complete lack of germination in "selfed" seeds is puzzling and demands further examination.

Gottsberger (1988) believes that there is an evolutionary tendency in the Annonaceae from small unspecialized flowers, pollinated by small nitidulid beetles, to larger flowers, with feeding tissue and strong fragrances, that attract larger beetles, primarily members of the Scarabaeidae. Under this scenario, *A. parviflora* would be basic in the genus *Asimina.* The closely related and more wide-ranging species, *A. triloba,* possesses several derived characters: 1) larger flowers with corrugated tissue on the inner petals; 2) ring-porous wood, an advanced condition (Mauseth 1988) unusual in the Annonaceae (Metcalf 1987); and 3) vegetative reproduction from root sprouts.

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