

EFFECTS OF FLORIVORY ON THE POLLINATION OF FLOWERS: AN EXPERIMENTAL FIELD STUDY WITH A PERENNIAL PLANT

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Plants experience damage to both their vegetative and their reproductive parts. Loss of leaf area can affect subsequent photosynthesis and resources available for growth and reproduction; damage to flowers can result in loss of ovules and seeds by consumption, but herbivory may also disfigure flowers, interfering with their functions of attracting and rewarding pollinators. We examined natural populations of the butterfly pea, *Centrosema virginianum*, in pine rockland habitat in Everglades National Park (intact habitat) and a pine rockland fragment in suburban Miami-Dade County to answer the following questions: (1) What is the breeding system of *C. virginianum*? (2) What are the pollinators of this species in southern Florida pine rocklands? And (3) how are flower herbivores affecting pollinator visitation and subsequent fruit set? Controlled hand-pollination experiments revealed this species to be self-compatible but requiring visitation/pollination for fruit set. Cross-pollinated flowers and open-pollinated flowers set substantially more seed per fruit than did self-pollinated flowers. Flowers are visited by a variety of bees (*Bombus pensylvanicus*, *Xylocopa micans*, *Megachile* spp., and *Melissodes* spp.), which serve as pollinators. Flowers were produced abundantly in areas that had experienced recent fires, and roughly half of the flowers were damaged by one of two florivore guilds (blister beetles that ate the flowers and petal-sucking flies in the family Agromyzidae). Damaged flowers were visited much less frequently by pollinators than were undamaged flowers, and, consequently, they set many fewer fruit and much less seed. We conclude that florivory is a major impediment to successful pollination and plant sexual reproduction of *C. virginianum* in areas where the species naturally occurs.

Keywords: florivory, pollination, bees, blister beetles, Agromyzidae, flies, breeding system.

Introduction

Folivory (flower herbivory) directly decreases the amount of photosynthetic tissue a plant has and, thus, it indirectly reduces the plant's growth, reproduction, and survival (Kulman 1971). Many indirect effects of defoliation caused by the interaction of multiple herbivore species have been described. Such effects include increased susceptibility to parasitic fungi (Wallin and Raffa 2001), modifications in the synthesis of secondary compounds (Crawley 1983; Armbruster et al. 1997; Adler 2000), and modifications of reproductive traits such as flower size (e.g., Lehtilae and Strauss 1999), flower shape (Mothershead and Marquis 2000), and flower color (Cardel 2004). These changes, especially in petal size and floral tube length, may have a detrimental effect on plant reproductive success as a consequence of changes in pollinator behavior (Armbruster and Mziray 1987; Karban and Strauss 1993; Strauss 1997; Krupnick et al. 1999; Mothershead and Marquis 2000).

Many plant species depend on strategies to enhance the effectiveness of pollinators, such as rewards (e.g., nectar, pollen), an attractive floral display (e.g., color, odors), and mechanisms that promote pollinator visitation (Faegri and van der Pijl 1979); these factors interact to increase reproductive fitness of animal-pollinated flowering plant species.

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Florivory is widespread (Breadmore and Kirk 1998), and the very features that evolve to attract pollinators can also attract herbivores (Adler and Bronstein 2004; Irwin et al. 2004; McCall and Irwin 2006). Florivory may affect pollination interactions directly, by altering aspects of the floral display and discouraging visitors as well as by destroying reproductive parts of the flower (Leavitt and Robertson 2006; Pohl et al. 2006; Sánchez-Lafuente 2007). It is essential to measure how herbivory, especially florivory, affects plant-pollinator interactions and, ultimately, plant reproductive success.

Potential plastic responses to herbivory include effects on both male and female reproductive traits (Delph et al. 1997; Lehtilae and Strauss 1999), changes in the quality and number of pollen grains produced because of nutrient stress, and changes in resource allocation by the plant (Young and Stanton 1990; Stephenson et al. 1992; Quesada et al. 1995) and, consequently, a reduction in pollen grain deposition on stigmas by pollinators (Faegri and van der Pijl 1979; Crawley 1986; Les 1988; Kawarasaki and Hori 1999; Krupnick et al. 1999; Lopez et al. 1999; Yashiro et al. 1999). The mating system of a particular plant population may be altered when the proportion of chasmogamous flowers is decreased (Steets and Ashman 2004; Steets et al. 2006), when the number of flowers and/or size of the floral display is diminished (Elle and Hare 2002; Penet et al. 2009), and when floral visitors avoid flowers that have been disfigured by florivores (Leavitt and Robertson 2006; Pohl et al. 2006; Sánchez-Lafuente

2007). In this study, we examine the effects of florivory in the reproductive biology of *Centrosema virginianum*, a large-flowered legume of the Everglades uplands. The large, showy flowers of this widespread species make it attractive to both pollinators and to florivores.

The goal of this study was to determine levels and types of florivory experienced by *C. virginianum* plants in their native habitat. To understand how florivory may directly affect the pollination and fruit set of this species, it was also essential to determine the breeding system of the plants to determine whether they must be visited, and what kind of pollen they require, in order to set fruit. This study examines the following questions: (1) What is the breeding system of *C. virginianum*? (2) what are the pollinators of this species in southern Florida pine rocklands? And (3) how are flower herbivores affecting pollinator visitation and subsequent fruit set?

Methods

Study Species

We studied natural populations of butterfly pea *Centrosema virginianum* Benth (Fabaceae: Papilionoideae) that were located in natural areas of southern Florida. *Centrosema virginianum* is an herbaceous perennial vine bearing large, showy flowers and pinnately trifoliate leaves. The leaves have a lateral pair of stipules and elongated petiolules. The plants may climb by means of the stem twining around other plants. Flowers of *C. virginianum* are highly specialized, complex flowers, as evidenced by their resupinate form, which is adapted for pollination by large insects, most commonly Hymenoptera (fig. 1; Faegri and van der Pijl 1979). The standard (or banner) has a conspicuous spur on the back, which separates this genus from the rest in the tribe Clitoriinae, that is, *Clitoria*, *Periandra*, and *Clitoriopsis* (Polhill and Raven 1978). The other petals (wings plus keel) form a structure that surrounds the anthers and pistil. This structure



Fig. 1 Sterno-tribic pollination of *Centrosema virginianum* by a medium-sized bee, *Megachile campanulae*, at Long Pine Key in Everglades National Park, Florida.

is pushed back by a visitor of the right size to expose the stigma and promote the transfer of pollen while the visitor takes the flower's nectar. Nine fused filaments, with the single stamen below (diadelphous stamens), surround the carpel, which is distinctive of the tribe Papilionoideae. This arrangement is characteristic of nototribic (dorsal) deposition and removal of pollen (Faegri and van der Pijl 1979), and this is the manner in which large bees are dusted with pollen; smaller bees often visit in the opposite orientation (sterno-tribic) and receive pollen on their ventral surface (fig. 1). If the flowers are successfully pollinated, the ovary grows to produce a mature fruit in 4–6 wk (Y. Cardel, personal observation).

Study Sites

The butterfly pea is distributed from the southern United States to the northern regions of South America (Isely 1990). In southern Florida, it occurs in pine rockland habitats (Snyder et al. 1990). The canopies of pine rockland habitats are dominated by *Pinus elliottii* var. *densa* growing on exposed limestone substrates (O'Brien 1998). The pine rockland understory is made up of shrubs, palms, grasses, and herbs, containing over 522 taxa; ~12% of these are pine rockland–endemic species (Gann et al. 2002). This ecosystem is globally endangered as a result of fragmentation, habitat destruction, and degradation (DERM 2004; Koptur 2006).

Pine rockland communities are fire-dependent ecosystems (Snyder et al. 1990). Fires are often induced by lightning during the wet season (May through September) and anthropogenic causes during the dry season (November through April; Geiger 2002). Many herbaceous species of this habitat, including *Jacquemontia curtisii* (Spier and Snyder 1998), *Ruellia succulenta* (Geiger 2002), *Chamaecrista keyensis* (Liu and Koptur 2003), and the subject of this study, *C. virginianum*, exhibit fire-stimulated reproduction.

For this study, we selected three locations where *C. virginianum* was abundant: Pine Shore Preserve (PS) and Rockdale Pineland (RD), both of which are pine rockland fragments under the management of Miami-Dade County, and Long Pine Key (LPK), in Everglades National Park (ENP). The PS site (25.650°N, –80.374°W) is 32 ha in area and includes ~202 plant taxa (Gann et al. 2002). Rockdale (25.635°N, –80.340°W) is 154 ha in area, with 210 plant taxa reported. Both preserves are located in residential neighborhoods in southwestern Miami-Dade County and are less than 10 mi from each other and more than 20 mi from LPK. LPK (25.313°N, –80.938°W), an area in the northeast section of ENP, is surrounded by other natural habitats (sawgrass prairie and hardwood hammock); there are 1036 plant taxa reported for all habitats combined (Gann et al. 2002).

Breeding System

To study the breeding system of *C. virginianum* populations in south Florida pine rocklands, we selected five plants that were producing abundant flowers and that were separated by at least 10 m from each other, at each of the two locations (PS and LPK). A number was assigned to each individual plant to identify different genotypes, for a total of 10 plants from two sites. Cuttings from each plant were col-

lected to reproduce each genotype in the Florida International University greenhouse, where hand pollinations were conducted in a controlled environment. Plants were fertilized to facilitate initial establishment and growth. We used the greenhouse both to facilitate pollinations and to avoid fruit abortion due to resource limitation. We were then able to compare the success of the different treatments under uniform conditions in a pollinator-free environment; the location also made it logistically possible to perform the crosses using plants collected from different sites, to assure plants were not related.

Hand-pollination experiments were performed on the propagated clones using five flowers per plant per treatment. The four treatments were (1) apomixis (emasculation); (2) nonmanipulated flowers, or controls, which also tests for autogamy; (3) self-pollination, in which pollen from the same plant was applied to the stigma; and (4) hand-cross-pollination, where a mixture of pollen from at least three flowers of three different plants was applied to the stigma. Additionally, we monitored 50 flowers for the open-pollination treatment on the same plants in the field for comparison with other treatments. To apply the pollen, we used a fine paintbrush (number 00). All five treatments were performed on the same individual plant whenever possible. Mature fruits were collected when they appeared filled and black; fruit set, seed set, and germination rate were compared across treatments and sites.

Cuttings from a total of 35 plants from the three study sites were propagated in the greenhouse. To estimate the pollen : ovule ratio, a total of 150 flowers (two to six flowers per plant) were collected the day before opening. Each set of undehiscent anthers was kept in an Eppendorf tube and air-dried until the pollen was released. Pollen was stored in 70% ethyl alcohol and then dyed using the method in Cruden (1977) and counted using a hemacytometer. The ovaries of the same flowers were dissected, and the number of ovules were counted under a dissecting microscope. The pollen : ovule ratio was calculated using the estimated total number of pollen grains divided by the total number of ovules.

Pollinator Visitation

Flowers of *C. virginianum* are abundant after prescribed fires occur in the pine rocklands of southern Florida. Two of our three study populations of *C. virginianum* had been recently burned before the field work was performed, providing ample flowering for us to monitor flower visitors. The LPK site is part of the prescribed fire program managed by the United States National Park Service at ENP, and it was burned on June 8, 2001. The PS site was burned in January 2001 as a result of an arson fire, which had beneficial effects for flowering.

To determine which floral visitors transport pollen of *C. virginianum*, observations were conducted for 10-min periods every half hour during morning hours, twice monthly, from January through July. At least two specimens of each insect observed visiting *C. virginianum* flowers were captured with an insect net. We used chloroform to sedate the insects to collect pollen grains from their bodies, and we photographed them for later identification. Pollen was removed from insects by touching their bodies with small cubes of solidified agar. When insects woke up, they were released. Agar

cubes were stored in tagged glass tubes to transfer them from the field to the lab, where microscope slide preparations were made by melting the agar and dyeing it with acid fuchsin (Kearns and Inouye 1993). A compound microscope was used to verify the presence of *C. virginianum* pollen grains. Voucher specimens of each pollinator species were collected and mounted for taxonomic determination of the insects; these have been deposited in the collection at Everglades National Park.

Floral Herbivory

Flowers open before 0800 hours and are often damaged by 1000 hours, with floral herbivores feeding on flowers until early afternoon, when the flowers start to wither and close. We observed flowers midmorning and classified the damage, attributing the types of damage to the two most abundant floral herbivores. We collected specimens of each type of florivore during May 2001 for taxonomic determination (specimens deposited in ENP collection). When the herbivores were determined and we learned what kind of damage each herbivore caused, we haphazardly selected a group of flowers that showed each type of floral damage, as well as an equal number of intact flowers as controls, at the two sites, ultimately matching a total of ~20 flowers per day. Control flowers were in the same locations as damaged flowers, and both types were observed simultaneously. We chose flowers that were a random subset of florivore damage levels; all flowers were only partially damaged. We performed 20-min pollinator watches on both groups of flowers between 1000 and 1330 hours during 10 sunny days from late May through July. At the end of the study period each day (1330 hours), we bagged all the flowers observed that day with nylon bags in order to later collect fruit that might be set. Fruits were collected after they matured, and we also recorded flowers that did not set fruit. Seeds per flower per treatment were counted, scarified, hydrated for 12 h, and germinated in petri dishes with moist cotton balls.

The visitation rates of each insect to undamaged and damaged flowers were compared using one-way ANOVA (SPSS 2002). Normality and equal variances were checked before analysis using the Shapiro-Wilk test and Levene's test. Fruit sets between visited damaged and undamaged control flowers were compared using χ^2 tests (SPSS 2002), as were proportions of seed germinating from undamaged flowers and damaged flowers.

Results

Breeding System

No fruit was set in the emasculating treatment (table 1). A single control flower produced a fruit with five seeds; flowers that opened subsequently on that plant did not set fruit. We therefore considered that fruit to be an artifact. Emasculating and control treatments were not included in subsequent statistical analysis.

All flowers that were hand pollinated (self and cross, 100%) set fruit, and open-pollination flowers set close to 80% fruit (table 1). We used the mean number of ovules produced to calculate the percentage of seeds produced. Two-way ANOVA results on square-root-transformed seed set

Table 1

Total Fruit Set and Seed Set of Pollination Treatments				
Pollination treatment	N	% fruit set	No. seeds per fruit \pm SD (range)	% seed set
Open ^a	37	81.08	15.8 \pm 3.9 (3–18)	83.24
Emasculation ^b	48	0	n/a	0
Self ^b	50	100	8.0 \pm 3.2 (0–18)	37.87
Cross ^b	50	100	16.9 \pm 2.2 (11–21)	84.37
Control (autogamy) ^c	50	.02	n/a	.03

Note. Open-pollination treatment was monitored in the field at two sites (combined here, as they were not substantially different) using the same genotypes studied in the greenhouse (10 genotypes, five each from two sites). Number of flowers (N) differed due to flower availability. Seed set was calculated using the mean number of ovules per flower. n/a = not applicable.

^a Performed in the field.

^b Performed in the greenhouse.

^c One fruit with five seeds.

(Levene's equal variance test passed $P = 0.490$) showed no significant interaction between site and treatment ($F_{2,27} = 1.76$, $P = 0.18$), and seed sets between the two sites (PS and LPK) were not significantly different ($F_{2,27} = 0.017$, $P = 0.897$). Seed set of selfed flowers was significantly lower than that of crossed and open-pollinated flowers ($F_{2,27} = 113.14$, $P < 0.001$; fig. 2).

The mean number (\pm SE) of pollen grains produced by 150 flowers from 35 undamaged plants of *Centrosema virginianum* was 3700.5 ± 372.7 per flower. The mean number (\pm SE) of ovules produced by the same flowers was 19.7 ± 1.2 . The pollen : ovule ratio (\pm SE) using the mean of the 150 samples for pollen grains and ovules was 187 ± 19 , which is precisely within the range that Cruden (1977) gives for facultative autogamy (168.5 ± 22.1).

Pollinator Visitation

Both pollinators and florivores were observed to visit flowers of *C. virginianum*. We considered all of the bees to be pollinators of *C. virginianum* because they carried *C. virginianum* pollen grains on their bodies; the other insects were florivores (table 2). We counted a maximum of 100 grains on all bees that were captured after a flower visit. All the bee species observed were seen at both sites where detailed observations were made (PS and LPK), except for *Bombus pennsylvanicus*, which was only observed at LPK during this study. During the pollinator watches, we pooled all *Megachile* species and *Colletes distinctus* because it was difficult to be certain about their identity without capturing them. *Xylocopa micans* (the carpenter bee) was the most active pollinator at LPK, followed by *B. pennsylvanicus*, *Melissodes communis*, *Megachile campanulae wilmingtoni*, *Megachile polycaris*, and *C. distinctus* (table 3; fig. 4a). In contrast, the pollinator guild at PS was dominated by the group containing *Megachile* spp. and *C. distinctus* (table 3; fig. 4c).

Floral Herbivory

The most abundant florivores observed at the two sites were the blister beetles (Coleoptera: Meloidae) *Epicauta strigosa* (Gyllenhal) and *Lytta aenea* (Say). These beetles usually ate flowers starting with the nectar, and then they continued

on, in sequence, to the petals, pollen, and sometimes part of the style and filaments (fig. 3). The majority of the flowers attacked by this type of insect were partially damaged; only a few were totally eaten. Other authors have documented *E. strigosa* blister beetles as predators on another Florida plant, the endemic legume *Chapmannia floridana* (Gunn et al. 1980). The secondmost abundant type of floral damage was caused by a fly from the family Agromyzidae (species not identified). These flies have been reported to be seed predators of other species in the South Florida pine rocklands (Geiger 2002; Huey et al. 2007), but these agromyzid flies were observed sucking petal tissue, which resulted in petal discoloration. The corollas of damaged flowers changed color from pink-purple to black-gray in spots (from 4 to 8 mm in diameter), dramatically altering the appearance of the flowers to the human eye and, presumably, to that of pollinators as well (see below). Florivory by the echo moth *Seirarctia echo*, was observed at LPK during late summer of 2001. Caterpillars of this moth consumed more than the 50% of the flower, including the reproductive parts; these caterpillars are generalist feeders and have been reared eating leaves and flowers of many other plants, including rough-leaved velvetseed *Guet-*

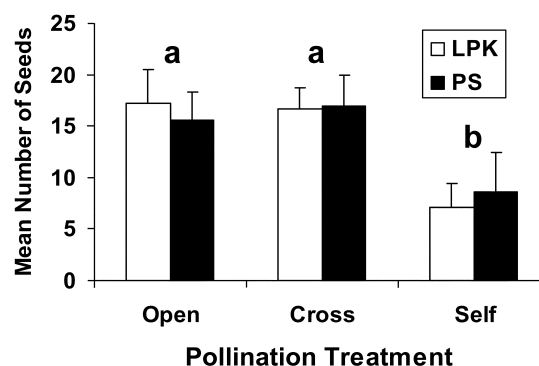


Fig. 2 Mean and standard deviations of seeds produced by flowers of *Centrosema virginianum* after three pollination treatments on plants from Long Pine Key (LPK) and from Pine Shore preserve (PS). Open-pollination data were collected from plants growing in the field; "cross" and "self" data were obtained from hand pollinations conducted on the same genotypes (clones) in the greenhouse. Different letters indicate significant difference determined by ANOVA ($P < 0.05$).

Table 2
Flower Visitors of *Centrosema virginianum*

Species	Type of visitor	Site observed
<i>Bombus pensylvanicus</i> (Degeer)	Pollinator	LPK
<i>Colletes distinctus</i> Cresson*	Pollinator	LPK/PS
<i>Megachile campanulae wilmingtoni</i> Mitchell*	Pollinator	LPK/PS
<i>Megachile polycaris</i> Say*	Pollinator	LPK/PS
<i>Melissodes communis</i> Cresson	Pollinator	LPK/PS
<i>Xylocopa micans</i> Lepeletier	Pollinator	LPK/PS
<i>Epicauta strigosa</i> (Gyllenhal)	Florivore	LPK/PS
<i>Lytta aenea</i> (Say)	Florivore	LPK/PS
Agromyzidae fly	Florivore	LPK/PS
<i>Seiractia echo</i> (Smith)	Florivore/foflivore	LPK
Grasshopper nymph (not identified) ^a	Florivore	PS

Note. All species listed as pollinators were caught at flowers and carried pollen on their bodies. Species indicated by an asterisk were easily confused, and for this reason they were pooled in visitation data. LPK = Long Pine Key; PS = Pine Shore.

^a Observed only once.

tarda scabra (S. Koptur, unpublished data), flowers of sabal palm, saw palmetto, and silver palm (S. Koptur and R. Khor-sand, unpublished data), and the native cycad, coontie *Zamia pumila* (Negron-Ortiz and Gorchoy 2000). We observed no pollinator visits to flowers damaged by this species.

Each major bee pollinator species (or species group) visited undamaged flowers more frequently than damaged flowers, especially during the morning hours until midday (fig. 4). ANOVA showed that all pollinators visited substantially fewer damaged flowers than undamaged flowers (table 3; fig. 5a). Flowers damaged by blister beetles and agromyzid flies did not set any fruit at the PS site (fig. 5b), whereas control flowers at PS produced a total of 15 fruits and 138 seeds; the difference in fruit set between damaged and undamaged flowers visited by pollinators was dramatic (no statistical test performed on these data). Only two fruits (~3%) were produced from blister beetle-damaged flowers at LPK, with 25 seeds total, and six fruits (~12%) were produced from flowers damaged by agromyzid flies, with 55 seeds total. Control flowers at LPK set 29 fruits with 327 seeds total, which was also a highly significant difference and more than 10 times greater than the reproductive success of flowers damaged by florivores. (Because flowers were not bagged before observation of florivory and subsequent visitation, there is also a chance they may have been visited before our observations). The average number of seeds produced in those fruits from flowers damaged by the flies (9.2 seeds) was lower than the number of those from flowers damaged by blister beetles (12.5 seeds), perhaps because the fruit from fly-damaged flowers showed evidence of seed predation.

Seed germination percentages per treatment did not differ between blister beetle-damaged flowers and control flowers (fig. 5c), but seeds from flowers with agromyzid fly damage showed a lower germination percentage, perhaps as a result of seed predation (and perhaps by the same agent). We were not able to see or rear the insects causing this type of damage, so this speculation warrants further study.

Discussion

Breeding System

This study revealed that *Centrosema virginianum* is not capable of apomixis, as none of the emasculated flowers set fruit. Control treatments confirmed that *C. virginianum* requires the services of a visitor to be pollinated. All self-pollinated flowers set fruit, but they produced fewer seeds than did cross-pollinated flowers. The pollen : ovule ratio indicates facultative inbreeding (Cruden 1977). Therefore, we conclude that *C. virginianum* is fully self-compatible and has a mixed-mating system in these populations. The fact that open-pollinated controls in the field set somewhat less fruit than did hand-pollinated greenhouse plants (80% vs. 100% fruit set) suggests that some flowers may go unvisited and that there may be either some pollinator limitation or resource limitation keeping plants from setting fruit. Furthermore, seed set of open pollinated fruit at levels equal to flowers that were cross-pollinated by hand in the greenhouse suggests that those open-pollinated flowers were probably cross-pollinated.

Strikingly different results were found in an earlier study of the same species, in which pollination in mainland and island populations on the western coast of Florida were compared (Spears 1987). In those populations, self-pollinated flowers produced between 6.7% and 23.3% fruit set and cross-pollinated flowers produced between 30% and 48.4% fruit set. We attribute the differences to greenhouse effects, as plants growing in natural conditions may well be resource limited. In our study, we might have obtained different results by performing hand pollinations in the field, where plants might experience lower fruit production with nutrient limitation. Or, perhaps, the populations of *C. virginianum* on the west coast of Florida that were studied by Spears (1987) may have been more self-incompatible, and they are likely to be genetically different from populations in this study. Further investigation is indicated, particularly taking into ac-

Table 3

ANOVA results comparing pollinator visitation rates (total mean \pm SE per watch per day) between undamaged and damaged flowers of *Centrosema virginianum*

Pollinator, site	Undamaged	Damaged	$F_{1, 18}$	P
<i>Bombus pensylvanicus</i> :				
LPK	.016 \pm .005	.003 \pm .001	5.26	.045
PS	n/a	n/a	n/a	n/a
<i>Xylocopa micans</i> :				
LPK	.043 \pm .010	.007 \pm .002	8.99	.01
PS	.009 \pm .002	.0001 \pm .0001	15.19	.003
<i>Melissodes</i> spp.:				
LPK	.026 \pm .003	.0033 \pm .001	37.47	.001
PS	.036 \pm .010	.0009 \pm .0005	9.28	.012
<i>Megachile</i> spp.:				
LPK	.014 \pm .004	.002 \pm .001	5.45	.042
PS	.066 \pm .010	.007 \pm .003	20.79	.001

Note. All tests were significant at $\alpha = 0.05$. Degrees of freedom for each test are 1 among groups (damaged vs. undamaged) and 18 within groups (for each visitor per watch per day for 10 d). LPK = Long Pine Key; PS = Pine Shore. n/a = not applicable.



Fig. 3 Florivores on a flower of *Centrosema virginianum* at the Pine Shore site. *Top left*, flower with mild damage around corolla edges; *top right*, Agromyzidae fly on flower. *Bottom left*, blister beetle *Epicauta strigosa* before leaving flower; *bottom right*, flower with extensive blister beetle damage.

count plant size and measures of folivory at the different locations and comparing results of hand pollinations with plants under different nutrient regimes.

Pollinator Visitation

Large bees were observed to be frequent visitors of *C. virginianum* flowers. *Bombus pensylvanicus* and *Xylocopa micans* have been previously reported to effectively pollinate the butterfly pea (Faegri and van der Pijl 1979; Spears 1987).

On the basis of the results of our study, *Melissodes communis*, *Megachile campanulae wilmingtoni*, *Megachile polycaris*, and *Colletes distinctus* are also effective pollinators. Smaller bees of these genera were more frequent visitors in the PS fragment site compared with in LPK (intact Everglades site). Spears (1987) reported differences in pollinator guilds of *C. virginianum* among far-island, near-island, and mainland populations. Differences in pollinator guilds have also been reported in other pine rockland species when comparing disturbed and intact habitats. When studying pollination in

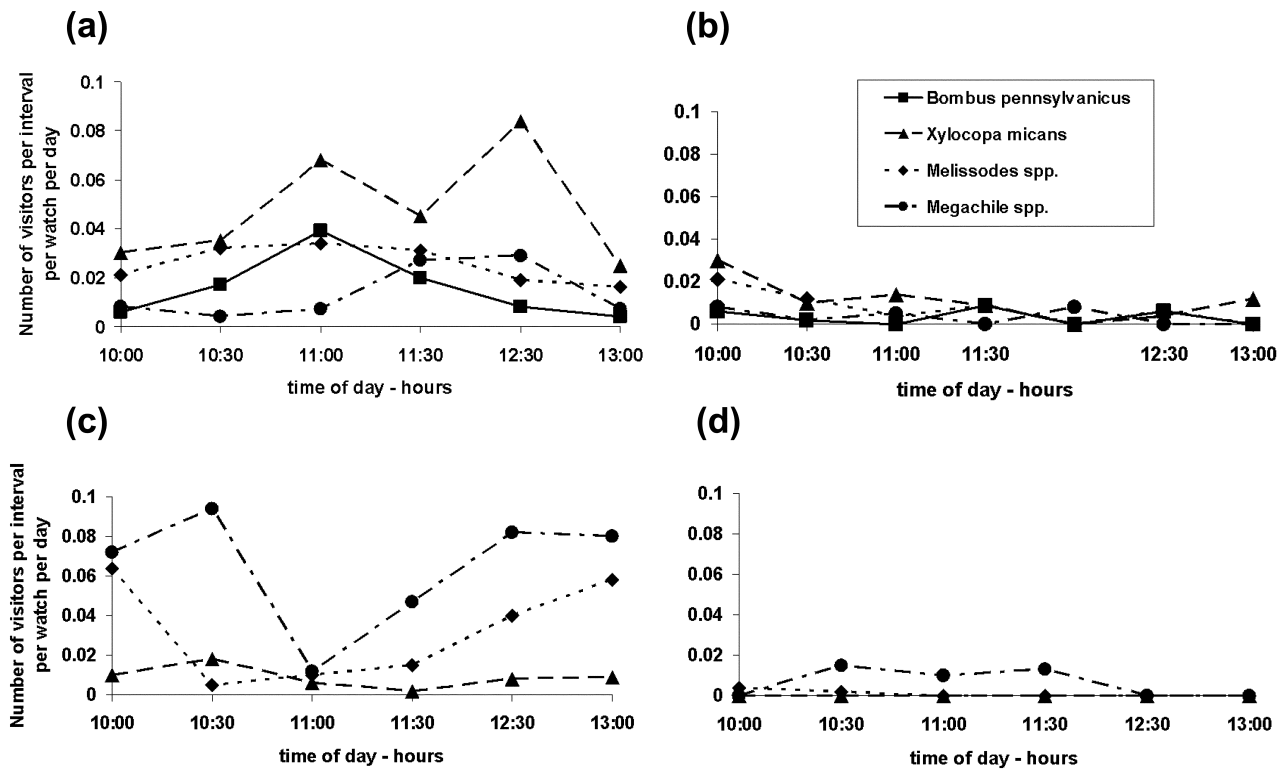


Fig. 4 Mean number of *Centrosema virginianum* visitors per watch per day at Long Pine Key (LPK; *a, b*) and at Pine Shore preserve (PS; *c, d*) over 10 observation days. Left panels (*a, c*) represent visitation rates for undamaged flowers. Right panels (*b, d*) represent visitation rates for damaged flowers.

Ruellia succulenta, Geiger (2002) found that bees predominated in the intact and large size class of pine rockland fragments, whereas Lepidoptera were predominant in the small and medium size classes of fragments. Studies of the pine rockland endemic *Chamaecrista keyensis* found *X. micans* to be the dominant pollinator in urban-edge habitats, while *Megachile* spp. predominated in forest sites (Liu and Koptur 2003).

Floral Herbivory

Although we did not quantify the proportion of damaged flowers in the populations studied, we observed that a substantial number (~50%) of the flowers of *C. virginianum* were damaged by flower eaters in both intact and fragment habitats. The most abundant florivores we encountered were blister beetles from the family Meloidae and a species of agromyzid fly, neither of which specialized exclusively on *C. virginianum* but instead were observed on a variety of flowers. These species nonetheless affected the seed set of *C. virginianum* in both populations studied, regardless of the amount of floral tissue consumed. Only two fruits were set from 69 flowers damaged by blister beetles, and only 12% of flowers damaged by flies set fruit, producing at total of six fruits with 55 seeds. Interestingly, seeds from the fruits of the fly-damaged flowers showed evidence of seed predation, which ultimately may reduce the mean number of seeds produced. We do not know whether the responsible seed preda-

tor was the same fly species that damaged the petals, but agromyzid flies have been reported ovipositing in and destroying seeds of other pine rockland species (Geiger 2002). If this fly is a seed predator of *C. virginianum*, its effects on seed production are doubly negative. Directly, flies may be ovipositing in ovaries, with their larvae destroying seeds in their development; indirectly, adult flies that suck petals disfigure flowers and reduce pollinator visitation, and pollination is required for fruit and seed set. However, if adult flies are ovipositing in ovaries and require seed set for larval survival, then feeding on the corollas of the same plants would be highly counterproductive (since those damaged plants will not get pollinated). Perhaps this scenario can be maintained if the flies are not specialized on only one plant species; this clearly warrants further study.

Our results concur with other studies that have demonstrated that changes in pollinator behavior can have marked effects on the reproductive output of plant species (Pellmyr and Thompson 1996; Le Corff et al. 1998; Ohashi and Yahara 1998; Parra-Tabla and Bullock 1998; Bigger 1999; Strauss and Agrawal 1999; Strickler and Freitas 1999; Cunningham 2000). Sometimes these changes in pollinator behavior are a result of florivory (Lohman et al. 1996; Krupnick et al. 1999). In a study of *Mimulus luteus*, seed set was halved in damaged flowers when hummingbird pollinators were present (Pohl et al. 2006). Flowers of *Lepidium* (Brassicaceae) with petals that had holes chewed in them produced half the amount of fruit and seed that undamaged flowers made

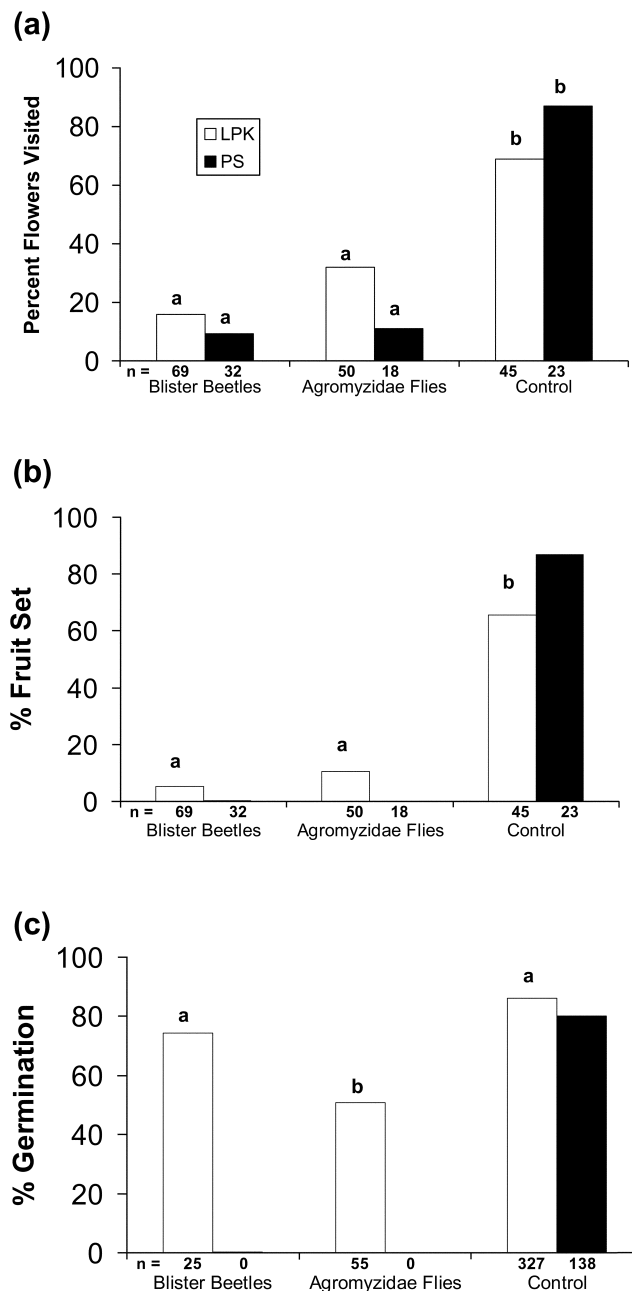


Fig. 5 Visitation, fruit set, and seed germination comparisons for damaged and undamaged flowers of *Centrosema virginianum*. *a*, Visitation rates of pollinators at Long Pine Key (LPK; white bars) and at Pine Shore preserve (PS; black bars). Bars indicate the proportion of the total number of flowers (damaged by type or undamaged control) that were visited (sample sizes of each group at each site indicated below the bar). Treatments with different letters indicate significant difference between type of floral damage ($\chi^2 = 66.1$, $df = 2$, $P < 0.0001$ and $\chi^2 = 41.45$, $df = 2$, $P < 0.0001$). *b*, Fruit set by damaged flowers of *C. virginianum* pollinated at the LPK and PS sites; ($\chi^2 = 63.64$, $P < 0.0001$), as no fruit was set by damaged flowers at PS. *c*, Percent germination of *C. virginianum* seeds. Different letter indicates significant difference of χ^2 at $\alpha = 0.05$. No seeds were set in damaged flowers at PS.

(Leavitt and Robertson 2006). In a clever experiment (Sánchez-Lafuente 2007), complex flowers of *Linaria lilacina* (Scrophulariaceae) were manipulated to remove upper, lower, or both lips of the corolla. This study revealed that pollinators were less likely to visit and pollinate flowers with missing parts (especially the lower lip), resulting in lower fruit and seed sets. Both naturally and artificially damaged flowers of *Nemophila menziesii* receive fewer visits from pollinators and set less fruit and seed than do undamaged flowers (McCall 2008).

In self-compatible species that can set fruit without pollinator visits, florivory can increase selfing (Penet et al. 2009). However, in species like *C. virginianum* that require visitation for fertilization and fruit set, the most important direct effect of florivory is to inhibit visitation and therefore preclude fruit set. Plants with larger floral displays may attract more visitors (Sánchez-Lafuente 2007) and set more fruit (Salomão et al. 2006), but they are also more likely to experience greater herbivory (Sánchez-Lafuente 2007), and these effects may carry over to the following year in perennial plants (Ehrlén 2002). Clearly, the butterfly pea is one of the “pollinator-dependent plants suffering intense herbivory on flowers” in which we would expect to see “correlated evolution of mutualism- and antagonism-related traits” (Herrera et al. 2002, p. 16824). Beetle florivores may benefit some plants as pollinators (Gottsberger 1977), and they certainly do so in Annonaceae such as *Asimina* spp. (Cox 1998) and *Duguetia* spp. (H. P. Neto, personal communication). It seems unlikely, however, that beetle florivory provides pollination in the specialized flowers of this papilionoid legume *C. virginianum*.

The reproductive success of populations of *C. virginianum* in the pine rocklands of southern Florida is affected by florivores feeding in different ways, and floral herbivores reduce pollinator visitation to flowers because damaged flowers look different and are visited less by pollinators. This translates into substantially reduced fruit set for damaged flowers, as this species requires flower visitation for pollination. The data presented here show major effects of florivory on plant reproduction, in both pristine and fragmented habitats. This study contributes to the understanding of the direct effects of herbivores on the interaction between pollinators and plants; indirect effects of florivory on this interaction will be examined in subsequent studies.

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