

3-29-2004

Linking herbivory and pollination : costs and selection implications in *Centrosema virginianum* Bentham (Fabaceae: papilionoideae)

Yuria Cardel

Florida International University

DOI: 10.25148/etd.FI14052571

Follow this and additional works at: <https://digitalcommons.fiu.edu/etd>



Part of the [Biology Commons](#)

Recommended Citation

Cardel, Yuria, "Linking herbivory and pollination : costs and selection implications in *Centrosema virginianum* Bentham (Fabaceae: papilionoideae)" (2004). *FIU Electronic Theses and Dissertations*. 2042.
<https://digitalcommons.fiu.edu/etd/2042>

This work is brought to you for free and open access by the University Graduate School at FIU Digital Commons. It has been accepted for inclusion in FIU Electronic Theses and Dissertations by an authorized administrator of FIU Digital Commons. For more information, please contact dcc@fiu.edu.

FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

LINKING HERBIVORY AND POLLINATION: COSTS AND SELECTION
IMPLICATIONS IN *CENTROSEMA VIRGINIANUM* BENTHAM
(FABACEAE: PAPILIONOIDEAE)

A dissertation submitted in partial fulfillment of the
requirements for the degree of
DOCTOR OF PHILOSOPHY

in

BIOLOGY

by

Yuria Cardel

2004

To: Dean R. Bruce Dunlap
College of Arts and Sciences

This dissertation, written by Yuria Cardel, and entitled Linking herbivory and pollination: costs and selection implications in *Centrosema virginianum* Bentham (Fabaceae: Papilionoideae), having been approved in respect to style and intellectual content, is referred to you for judgment.

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

David Lee

Steven Oberbauer

Jorge Peña

Jennifer Richards

Suzanne Koptur, Major Professor

Date of Defense: March 29, 2004

The dissertation of Yuria Cardel is approved.

Dean R. Bruce Dunlap
College of Arts and Sciences

Dean Douglas Wartzok
University Graduate School

Florida International University, 2004

© Copyright 2004 by Yuria Cardel

All rights reserved.

ACKNOWLEDGMENTS

During my dissertation research I had the opportunity to have support of many people who I wish to extend my appreciation to and thank. I especially wish to thank Dr. Suzanne Koptur, my major professor for accepting me as her student and being always supportive, helpful, and very patient. I truthfully thank to my committee members Dr. Jennifer Richards, Dr. Steve Oberbauer, Dr. Jorge Peña and Dr. David Lee. Their valuable academic advice and guidance was essential in the success of my dissertation. I thank Dr. Paulette Johnson and Dr. Samuel Shapiro for their statistical advice. I appreciate the field assistance from Paul Sharp, John Geiger and Jose García-Franco. My labmates, John Geiger, Hong Liu, Jed Redwine, Heather Gamper, Philip Gonsiska, Lauren Linares and Elena Pinto-Torres provided support, friendship, and helped me along the way. I would like to thank John Geiger for helping me with my research in the greenhouse and ideas to improve it. I extend my special gratefulness to my husband, Paul Sharp for his unconditional support and love. I wish to thank my parents and sisters for being there for me always. Thanks to Byron and Beverly Sharp and to the rest of my family here in Miami for all their caring and support.

The CONACyT (Mexican national council of science and technology) provided financial help for tuition and additional expenses (Beca No. 119886). The Latin American and Caribbean Center through the Florida Mexico Institute afforded out-of state tuition wavers. This research also benefited from the F.I.U. Tropical Biology Research Awards.

ABSTRACT OF THE DISSERTATION

LINKING HERBIVORY AND POLLINATION: COSTS AND SELECTION

IMPLICATIONS IN *CENTROSEMA VIRGINIANUM* BENTHAM

(FABACEAE: PAPILIONOIDEAE)

by

Yuria Cardel

Florida International University, 2004

Miami, Florida

Professor Suzanne Koptur, Major Professor

This research first evaluated levels and type of herbivory experienced by *Centrosema virginianum* plants in their native habitat and how florivory affected the pollinator activity. I found that populations of *C. virginianum* in two pine rockland habitat fragments experienced higher herbivory levels (15% and 22%) compared with plants in the protected study site (8.6%). I found that bees (Hymenoptera) pollinated butterfly pea. Furthermore, I found that florivores had a negative effect in the pollinators visitation rates and therefore in the seed set of the population.

I then conducted a study using a greenhouse population of *C. virginianum*. I applied artificial herbivory treatments: control, mild herbivory and severe herbivory. Flower size, pollen produced, ovules produced and seeds produced were negatively affected by herbivory. I did not find difference in nectar volume and quality by flowers among treatments. Surprisingly, severely damaged plants produced flowers with larger pollen than those from mildly damaged and undamaged plants. Results showed that plants

tolerated mild and severe herbivory with 6% and 17% reduction of total fitness components, respectively. However, the investment of resources was not equisexual.

A comparison in the ability of siring seeds between large and small pollen was necessary to establish the biological consequence of size in pollen performance. I found that fruits produced an average of 18.7 ± 1.52 and 17.7 ± 1.50 from large and small pollen fertilization respectively. These findings supported a pollen number-size trade-off in plants under severe herbivory treatments. As far as I know, this result has not previously been reported.

Lastly, I tested how herbivory influenced seed abortion patterns in plants, examining how resources are allocated on different regions within fruits under artificial herbivory treatments. I found that self-fertilized fruits had greater seed abortion rates than cross-fertilized fruits. The proportion of seeds aborted was lower in the middle regions of the fruits in cross-fertilized fruits, producing more vigorous progeny. Self-fertilized fruits did not show patterns of seedling vigor. I also found that early abortion was higher closer to the peduncular end of the fruits. Position of seeds within fruits could be important in the seed dispersion mechanism characteristic of this species.

TABLE OF CONTENTS

CHAPTER	PAGE
Introduction.....	1
Chapter 1 – The breeding system and reproductive implications of herbivory in <i>Centrosema virginianum</i> Bentham (Fabaceae: papilionoideae) in southern Florida populations	7
References.....	24
Chapter 2 – Effects of herbivory on reproductive success in <i>Centrosema virginianum</i> (Fabaceae: Papilionideae) through female and male components of fitness	39
References.....	54
Chapter 3 – Evidence of intraspecific size-number trade-off In pollen production after severe herbivory episodes on <i>Centrosema virginianum</i> Bentham (Fabaceae: Papilionideae).....	66
References.....	72
Chapter 4 – Patterns of seed abortion in response to leaf herbivory in a perennial legume herb <i>Centrosema virginianum</i> Bentham (Fabaceae: Papilionoideae)	79
References.....	90
Conclusion	102
Vita.....	105

LIST OF TABLES

TABLE	PAGE
Chapter 1.	
TABLE 1. Herbivory classes and their corresponding percentages of foliar area consumed (FAC).	29
TABLE 2. Total fruit set and seed set of pollination treatments.	31
TABLE 3. Two-way ANOVA table for seed set of <i>Centrosema virginianum</i> under three pollination treatments.	31
TABLE 4. Flower visitors of <i>Centrosema virginianum</i>	33
TABLE 5. ANOVA results comparing pollinator visitation rates between undamaged and damaged flowers of <i>Centrosema virginianum</i>	35
Chapter 2.	
TABLE 1. Experimental approach: randomized complete block design with defoliation treatment as the fixed factor.....	57
TABLE 2. Analysis of variance results on male fitness components.	58
TABLE 3. Three-way analysis of variance results on female fitness components.....	59
TABLE 4. Tolerance to herbivory in five fitness components.	60
Chapter 3.	
TABLE 1. Results of two-way ANOVA on pollen produced and pollen size.	77
TABLE 2. Results of two-way ANOVA on seeds produced and seed mass.....	77
Chapter 4.	
TABLE 1. ANOVA for a split-split plot design for proportion of seed abortion testing the effects of four levels of herbivory, pollination treatment (self and cross) and region within the fruit.....	97

LIST OF FIGURES

FIGURE	PAGE
Introduction.	
Diagram 1. Conceptual model of research.....	6
Chapter 1.	
1. A bee, <i>Megachile campanulae</i> pollinating <i>Centrosema virginianum</i> at Long Pine Key in Everglades National Park	28
2. <i>Centrosema virginianum</i> carpel surrounded by nine fused filaments with the single stamen below, which is characteristic of the Papilionoideae tribe. ..	28
3. Exponential model used to calculate the foliar area consumed (FAC) of <i>Centrosema virginianum</i> from the Herbivory Index.....	29
4. Frequency (%) histogram of herbivory on <i>Centrosema virginianum</i> at the three study sites.	30
5. Mean and standard deviations of seed set of <i>Centrosema virginianum</i> after three pollination treatments on plants from Long Pine Key (LPK) and Pine Shore preserve (PS).	32
6. <i>Epicauta strigosa</i> (blister beetle) eating a flower of <i>C. virginianum</i> taken at Pine Shore site.	33
7. Mean number of visitors per watch per day of <i>Centrosema virginianum</i> at (a and b) LPK and (c and d) PS during ten days.	34
8. Pooled visitation rates of <i>Centrosema virginianum</i> pollinators at Long Pine Key (LPK) and Pine Shore preserve (PS).	36
9. Fruit set and seed set produced by damaged flowers of <i>Centrosema virginianum</i> pollinated at Long Pine Key (LPK) and Pine Shore (PS)	37
10. Percent germination of <i>Centrosema virginianum</i> seeds.	38
Chapter 2.	
1. The relationship between mean genotype female fitness (seed set) between damaged with mild herbivory, severe herbivory and undamaged plants.	61
2. The relationship between mean male fitness per genotype between damaged plants with mild herbivory, severe herbivory and undamaged plants.	62
3. The relationship between mean pollen size per genotype between damaged with mild herbivory, severe herbivory and undamaged plants.....	63
4. Association between female and male components for undamaged plants.	64
5. Relationship between female and male reproductive success for severe damaged plants.	65

Chapter 3.

1. Experimental design of hand pollinations to compare siring ability between small and big pollen grains.	74
2. Relationship between female and male reproductive success for severe damaged plants.	75
3. Mean (± 1 SD) pollen number per flower produced by undamaged and damaged plants.	76
4. Mean (± 1 SE) pollen size (area projected, μm^2) per flower produced by undamaged and damaged plants.	76
5. Mean (± 1 SD) number of seeds produced per cross by undamaged and damaged plants.	78

Chapter 4.

1. Proportion of seeds aborted of <i>Centrosema virginianum</i> fruits after four levels of herbivory.	93
2. Mean ± 1 SE of seed mass and seed number of X-fruits from control maternal plants and plants under ~33% of herbivory.	94
3. Mean ± 1 SE of seed mass and seed number of X-fruits from plants under severe herbivory.	95
4. Mean ± 1 SE of seed mass and seed number of S-fruits from control plants and under severe herbivory.	96
5. Mean ± 1 SE of the proportion of seeds aborted in each fruit region among four herbivory treatments.	98
6. Frequency histogram of early abortion of seeds among four herbivory treatments of X-fruits and S-fruits.	99
7. Mean ± 1 SE of the proportion of seeds aborted in each fruit region among four herbivory treatments, excluding peduncular region D.	100
8. Mean ± 1 SE seedling dry mass among herbivory treatments within fruits.	101

INTRODUCTION

Herbivory is defined as the consumption of plant tissues or fluids by a consumer, called a herbivore, and usually it does not have lethal effects (Crawley 1983). Commonly, herbivory is considered a positive interaction for the herbivore and negative for the host plant (Cantu et al. 1999). It is well known that the reduction of a plant's photosynthetic tissue decreases nutrient provision that could affect the number and quality of its offspring (fitness) (Crawley 1983, Hendrix 1988, Abrahamson 1989, Karban and Strauss 1993, Koptur et al. 1996, Mutikainen 1996).

Related studies have emphasized the importance of the effects of herbivory on the allocation of nutrients to male and female reproductive traits on plants (Hendrix 1988, Strauss 1991, Karban and Strauss 1993, Mutikainen 1996, Krupnick 1999, Lehtilae and Strauss 1999). Female traits affected by reduction of foliar area have been the focus of many studies where plant seed number and seed mass are the female fitness components used to estimate the negative effect of herbivory (Crawley 1983, Hendrix 1988, Karban and Strauss 1993, Koptur et al. 1996, Niesenbaum 1996, Parra-Tabla and Bullock 1998, Irwin and Brody 1999). Undoubtedly, the consequences of herbivory on female fitness components are critical to understand part of the possible natural selection effects of herbivory in plant communities (Strauss 1991). However, the counterpart male fitness components have been understudied (Strauss 1991, Karban and Strauss 1993, Strauss 1997, Agrawal et al. 1999, Irwin and Brody 1999, Lehtilae and Strauss 1999, Strauss 1999).

Male fitness is defined as the number of seeds sired by pollen (Crawley 1997, Strauss 1997). Pollen contributes half of the genes to the next generation of sexually reproducing plants (Crawley 1997). If herbivory has a significant effect on male reproductive traits, cumulative effects on both female and male reproductive traits may affect the fitness of a plant species. Consequently, it is important to investigate how reproductive traits are affected by herbivory and how much such changes contribute to the status of plant communities.

Recently, a few studies have investigated indirect pathways through which herbivory may affect male and female plant fitness (Strauss 1991, Lowenberg 1994, Lohman et al. 1996a, Lohman et al. 1996b, Mutikainen 1996, Niesenbaum 1996, Strauss 1997, Krupnick 1999, Lehtilae and Strauss 1999, Strauss 1999). Such indirect effects always include those related to the behavior of pollinators, such as changes in aspect and size of flowers or amount and quality of rewards, which all may affect the time pollinators spend per flower. Few of these studies have analyzed the individual attributes of flowers affected by herbivory. According to Strauss (1997), there is little information about indirect effects of herbivory. Because pollination affects both female and male plant fitness, it is important to include indirect effects of herbivory as a fundamental component in any analysis of the effects of herbivory on fitness.

The purpose of my dissertation is to describe how herbivory may play a selective force affecting the fitness of *Centrosema virginianum*, considering both direct impacts of herbivory on plant populations as well as indirect effects on pollinators as a result of changes in plant reproductive traits. Diagram 1 shows a conceptual model that

summarizes the goals of my research. This model shows many possible ways in which herbivory may directly affect floral traits, and also indirect effects on components of fitness through changes in pollinator behavior, studied in this dissertation.

All chapters were written in the format of journal articles. Chapter I includes an evaluation and understanding of local natural populations of *Centrosema virginianum* (Fabaceae), the study system. This chapter contains a description of the breeding system of the plant studied. Also, I summarized the amount of leaf tissue consumed in different localities, including the type of herbivory and the identity of the herbivores. In addition, I have evaluated the amount of floral herbivory in the field, its effects in the pollinator visitation and a brief investigation of how fruit and seed set is affected by florivores.

Using the information contained in Chapter I as a precedent, I elaborated three studies involving greenhouse experiments. Each of these investigated several ways in which foliar herbivory could affect total plant fitness. Chapter II summarized the effects of herbivory on male and female fitness components, including flower size, nectar quality, pollen produced, pollen size, ovules produced and seeds produced. Chapter III describes a study of the effects of herbivory in pollen grain size and pollen number, and their ability to sire seeds. Finally, Chapter IV describes the selectivity of seed abortion of the study plant and how parental plants allocate resources to reproduction under different herbivory levels. This research project and all the specimen collections were done under permits provided by the Everglades National Park through the National Park Service, United States Department of the Interior (permits no. 003, 032, and 068) and the Miami-Dade County, Park and Recreation Department (permit no. 0021).

REFERENCES

- Abrahamson, W. G. 1989. Plant-Animal Interactions: An Overview. Pages 481 in W. G. Abrahamson, editor. Plant-Animal Interactions. McGraw-Hill Book Company, New York.
- Agrawal, A. A., S. Y. Strauss, and M. J. Stout. 1999. Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. *Evolution* **53**:1093-1104.
- Cantu, L., G. Castro, J. Cazares, M. Hidalgo, S. Magaña, and V. Vazquez. 1999. Patrones de herbivoría en especies leñosas de la selva mediana de la Mancha, Veracruz. Pages 137-146 in R. Dirzo, editor. Memorias del curso de campo 1999, Posgrado en ecología y manejo de recursos naturales. Instituto de Ecología, A.C., Xalapa, Veracruz.
- Crawley, M. J. 1983. Herbivory. The Dynamics of Animal -Plant Interactions. University of California Press, Berkeley.
- Crawley, M. J. 1997. Plant Ecology, 2nd edition. Blackwell Scientific Publications, London.
- Hendrix, S. D. 1988. Herbivory and its impact on plant reproduction. Pages 246-263 in J. a. L. L. D. Lovett Doust, editor. Plant reproductive ecology. Oxford University Press, New York.
- Irwin, R. E., and A. K. Brody. 1999. Nectar-robbing bumble bees reduce the fitness of *Ipomopsis aggregata* (Polemoniaceae). *Ecology* **80**:1703-1712.
- Karban, R., and S. Y. Strauss. 1993. Effects of herbivores on growth and reproduction of their perennial host, *Erigeron glaucus*. *Ecology* **74**:39-46.
- Koptur, S., C. L. Smith, and J. H. Lawton. 1996. Effects of artificial defoliation on reproductive allocation in the common vetch, *Vicia sativa* (Fabaceae: Papilionoideae). *American Journal of Botany* **83**:886-889.
- Krupnick, G. A. W., A. E. 1999. The effect of floral herbivory on male and female reproductive success in *Isomeris arborea*. *Ecology* **80**:135-149.
- Lehtilae, K., and S. Y. Strauss. 1999. Effects of foliar herbivory on male and female reproductive traits of wild radish, *Raphanus raphanistrum*. *Ecology* **80**:116-124.

- Lohman, D. J., A. R. Zangerl, and M. R. Berenbaum. 1996b. Impact of floral herbivory by parsnip webworm (Oecophoridae: *Depressaria pastinacella* Duponchel) on pollination and fitness of wild parsnip (Apiaceae: *Pastinaca sativa* L.). American Midland Naturalist **136**:407-412.
- Lowenberg, G. J. 1994. Effects of floral herbivory on maternal reproduction in *Sanicula arctopoides* (Apiaceae). Ecology **75**:359-369.
- Mutikainen, P. a. D. L. F. 1996. Effects of herbivory on male reproductive success in plants. Oikos **75**:353-358.
- Niesenbaum, R. A. 1996. Linking herbivory and pollination: Defoliation and selective fruit abortion in *Lindera benzoin*. Ecology **77**:2324-2331.
- Parra-Tabla, V., and S. H. Bullock. 1998. Factors limiting fecundity of the tropical tree *Ipomoea wolcottiana* (Convolvulaceae) in a Mexican tropical dry forest. Journal of Tropical Ecology **14**:615-627.
- Strauss, S. Y. 1991. Direct, indirect, and cumulative effects of three native herbivores on a shared host plant. Ecology **72**:543-558.
- Strauss, S. Y. 1997. Floral characters link herbivores, pollinators, and plant fitness. Ecology **78**:1640-1645.
- Strauss, S. Y., D. H. Siemens, M. B. Decher and T. Mitchell-Olds. 1999. Ecological costs of plants resistance to herbivores in the currency of pollination. Evolution **53**:1105-1113.

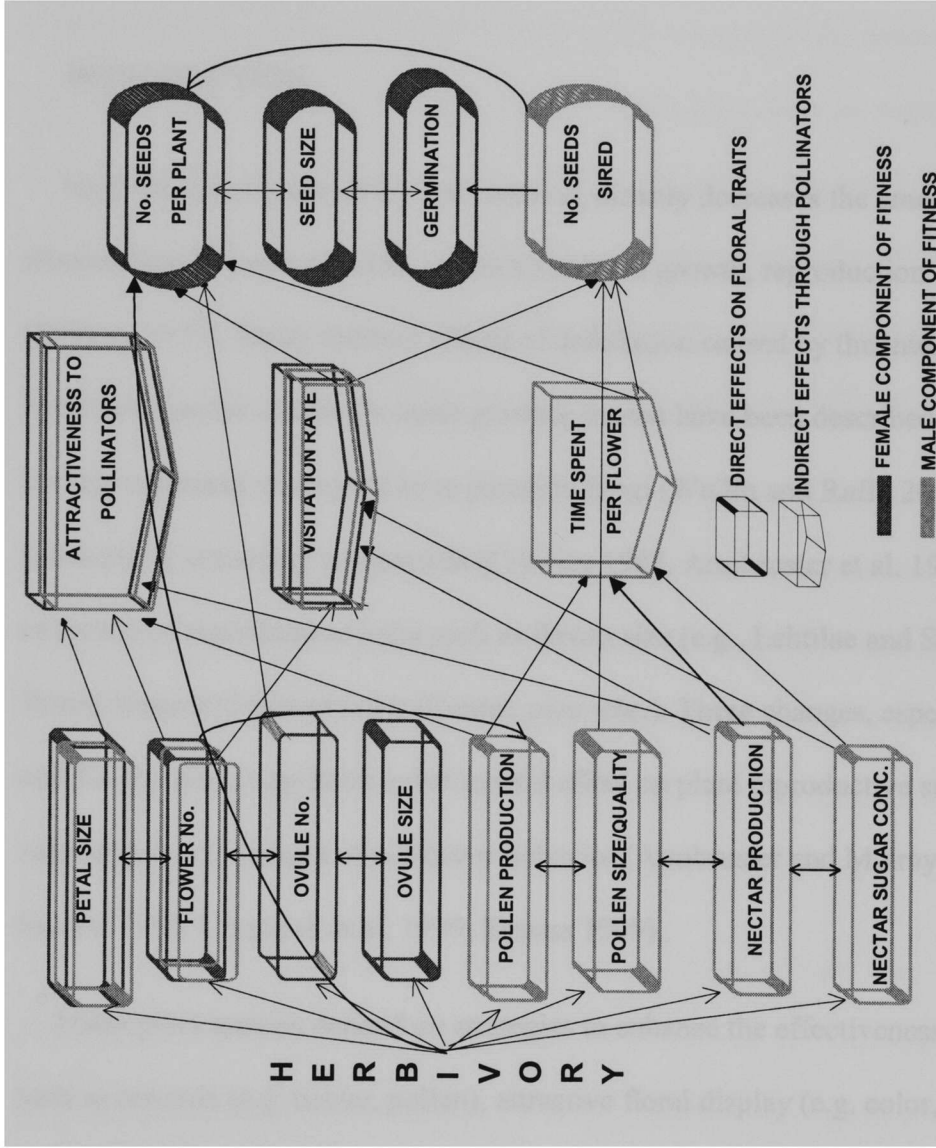


Diagram 1. Conceptual model of research. This model shows many possible ways in which herbivory may directly affect floral traits, and also indirect effects on components of fitness through changes in pollinator behavior (adapted from Strauss 1997).

CHAPTER I

THE BREEDING SYSTEM AND REPRODUCTIVE IMPLICATIONS OF HERBIVORY IN *CENTROSEMA VIRGINIANUM* BENTHAM (FABACEAE: PAPILIONOIDEAE) IN SOUTHERN FLORIDA POPULATIONS.

INTRODUCTION

Herbivory, as measured by leaf removal, directly decreases the amount of photosynthetic tissue and thus indirectly reduces growth, reproduction and survival (Kulman 1971). Many indirect effects of defoliation caused by the interaction of multiple herbivore species within the same growing season have been described. Such effects include increased susceptibility to parasitic fungi (Wallin and Raffa 2001), increase in the synthesis of secondary compounds (Crawley 1983, Armbruster et al. 1997, Adler 2000), reduction of reproductive traits such as flower size (e.g., Lehtilae and Strauss 1999), flower shape and flower color (Cardel, *pers. obs.*). These changes, especially in petal size and floral aspect, 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, Krupnick et al. 1999, Strauss 1999).

Many plant species depend on strategies to enhance the effectiveness of pollinators such as rewards (e.g. nectar, pollen), attractive floral display (e.g. color, odors) and other mechanisms that promote pollinator visitation (Faegri and van der Pijl 1979). All these factors interact to increase reproductive fitness of animal-pollinated flowering plant species. Herbivory, and especially floral herbivory, can also affect pollination interactions, by directly decreasing floral display. To understand how herbivory and

especially florivory affect plant-pollinator interactions it is essential to measure their effects on plant reproductive success.

Potential plastic responses to herbivory include changes in the quality and number of pollen grains produced because of nutrient stress, changes in the resource allocation by the plant, and indirectly a reduction in pollen grain deposition on stigmas by pollinators (Faegri and van der Pijl 1979, Crawley 1986, Les 1988, Kawarasaki and Hori 1999, Lopez et al. 1999, Yashiro et al. 1999). The breeding system of a particular plant population may be altered when the pollen:ovule ratio changes in response to herbivore damage (Crawley 1986), or when floral visitors avoid flowers disfigured by florivores.

A number of recent studies have investigated interactions between pollination and herbivory (Agrawal et al. 1999, Krupnick et al. 1999, Krupnick 1999, Lehtilae and Strauss 1999, Strauss 1999, Strauss and Agrawal 1999, Timmerman-Erskine and Boyd 1999, Adler 2000, Groom 2001, Leege and Wolfe 2002). Few of these, however, have studied populations of native species in their natural habitats (Brewer and Platt 1994, Frazee and Marquis 1994, Krupnick et al. 1999, Krupnick 1999).

In this study my goal was to determine levels and type of herbivory experienced by *Centrosema virginianum* plants in their native habitat and to document how these kinds of herbivory affect the pollination and breeding system of this species. My research examines the questions (1) what are the natural levels of herbivory on foliage and flowers? (2) What is the breeding system of *C. virginianum*? (3) What are the pollinators of this species in southern Florida pine rocklands? (4) How are flower herbivores affecting pollinator visitation and subsequent fruit set?

STUDY SYSTEM

Species

I studied natural populations of butterfly pea, *Centrosema virginianum* Bentham (Fabaceae: Papilionoideae), located in southern Florida. *Centrosema virginianum* is an herbaceous perennial vine bearing pinnately trifoliate leaves with elongated petiolules and a pair of stipules lateral to each leaf.

Flowers of *Centrosema virginianum* are highly specialized, as evidenced by their resupinate form. This form is adapted for pollination by large insects, most commonly Hymenoptera (Fig. 1) (Faegri and van der Pijl 1979). The standard has a conspicuous spur on the back; this character separates this genus from the rest in the subtribe Clitoriinae, i.e., *Clitoria*, *Periandra*, and *Clitoriopsis* (Polhill and Raven 1978). The other petals form a structure that surrounds the anthers and pistil. This structure 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 surround the carpel, which is distinctive of the tribe Papilionoideae (Fig. 2). This arrangement is characteristic of nototribic deposition and removal of pollen (Faegri and van der Pijl 1979). If the flowers are successfully pollinated, the ovary grows to produce a mature fruit four to six weeks later (Cardel, *unpublished data*).

Study Sites

Centrosema virginianum 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. The canopies of pine rockland habitats are dominated by *Pinus elliottii* var.

densa growing on exposed limestone substrates (O'Brien 1998). The pine rocklands understory is made up of shrubs, palms, grasses and herbs containing over 522 taxa, and about 12% of these are pine rockland endemic species (Gann 2001). These ecosystems are globally endangered due to fragmentation, habitat destruction and degradation (DERM 2003).

Pine rockland communities are fire-dependent ecosystems. Fires are often induced by lightning during the wet season (May through September) and by anthropogenic causes during the dry season (November to April) (Geiger 2002). Several herbaceous species of this habitat exhibit fire-stimulated reproduction, including *Jacquemontia curtisii* (Spier and Snyder 1998), *Ruellia succulenta* (Geiger 2002), and *Chamaecrista keyensis* (Liu and Koptur 2003). *Centrosema virginianum* similarly shows enhanced flowering following fires (Cardel, *unpublished data*).

For this study, I selected three locations where *Centrosema virginianum* was abundant. Pine Shore Preserve (PS) and Rockdale Pineland (RD), both pine rockland fragments under the management of Miami-Dade County, and Long Pine Key (LPK) in Everglades National Park (ENP). The Pine Shore site (25.650°N, -80.374°W) was 3 ha and included about 202 plant taxa (Gann 2001). Rockdale (25.635°N, -80.340°W) was 15 ha with 210 plant taxa reported. Both preserves were located in residential neighborhoods in southwestern Miami Dade County. Long Pine Key (25.313°N, -80.938°W), an area in the NE section of Everglades National Park, was surrounded by other natural habitats (sawgrass prairie and hardwood hammock) with 1036 plant taxa reported for all habitats combined (Gann 2001).

METHODS

Evaluation of foliar herbivory

To assess the amount of foliar herbivory, a total of 75 *Centrosema virginianum* plants were marked and mapped at each study site during the dry season of 1999-2000. To select individual plants, I haphazardly located and marked plants that had at least four nodes. Each plant selected was at least a meter apart from another *C. virginianum* individual. All the leaves of those plants were collected, cutting the leaf off from the base of the petiole (to include petiolules whose leaflets were entirely consumed).

In the laboratory, I randomly selected nine leaflets per plant and assigned each leaflet to a damage class according to the total percentage of herbivory shown (Table 1). I used the modified method of Ake-Castillo et al. (2000) to calculate the herbivory index (HI) for each population of *C. virginianum*, using equation (1).

$$HI = \left(\sum_{i=0}^{i=5} \text{leaflet}_i * i \right) / (\text{no. of leaflets collected}); \text{ where } i = \text{class of damage.} \quad (1)$$

I calculated the percentage of foliar area consumed in each population using the exponential model shown in Figure 3. Frequency histograms were generated to determine the distribution of levels of damage in the three populations.

Breeding system

To study the breeding system of *Centrosema virginianum* populations in south Florida pine rocklands, I selected five plants producing abundant flowers separated by at least 10 m from each other at each of two locations (PS and LPK). A number was haphazardly assigned to each plant to identify it as a different genotype for a total of 10 plants from

two sites. Cuttings from each plant were collected to reproduce each genotype in the Florida International University greenhouse, where I conducted hand-pollinations in a controlled environment to facilitate pollinations and to avoid fruit abortion due to resource limitation.

I performed hand-pollination experiments in the greenhouse on the propagated clones using five flowers per plant per treatment. The four treatments were: (1) apomixis (emasculature); (2) non-manipulated flowers or controls that also test for autogamy; (3) self-pollination, in which pollen from the same plant was applied to the stigma; (4) cross-pollination, where a mixture of pollen from at least three flowers of three different plants was applied to the stigma. Additionally, for an open-pollination treatment I monitored 50 flowers on the same plants in the field for comparison with other treatments. I chose to perform hand-pollinations in the greenhouse in order 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. To apply the pollen we used a fine paintbrush (number 00). Pollen from flowers on the same plant was used to perform self-pollinations. All five treatments were performed on the same individual plant whenever possible. Mature fruits were collected when filled and black; fruit set, seed set and germination rate were compared across treatments and sites using two-way ANOVA over square-root transformed data. Multiple comparisons were done with the Bonferroni procedure test at $\alpha=0.05$.

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 prior to opening. Each set of undehisced 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 of Cruden (1977) in which the pollen grains are stained with lactophenol-aniline blue (0.1ml). Pollen grains were placed in a vortex mixer. Each sample was shaken for 30 seconds. A subsample of the mixture was placed into the cells of a hemocytometer using a pipette. Pollen grains were counted in the large square on both sides of the hemocytometer, and 3 slides were made per sample.

Once the average number of pollen grains was calculated from each sample, I used equation (2) to calculate the total number of pollen grains per flower.

$$\text{No. Pollen grains} = [(\text{average number per quadrant}) / 0.0009 \text{ml}] (\text{dilution}) \quad (2)$$

The ovaries of the same flowers were dissected and the number of ovules 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 *Centrosema virginianum* and several other species are extremely abundant and attract flower visitors in profusion after prescribed fires in pine rocklands of southern Florida. Only two of the three study populations of *C. virginianum* had been recently burned to monitor flower visitors (LPK and PS), so I used these to study pollinator visitation. The LPK site is part of the prescribed fire program managed by the United States National Park Service at ENP and was burned in June 8th on 2001. The PS site was

burned during early January of 2001. After these fires, *C. virginianum* produced an abundance of flowers for observation.

To determine what floral visitors transport pollen of *Centrosema virginianum*, at least two specimens of each insect observed visiting *C. virginianum* flowers were captured with an insect net. I used chloroform to sedate the insects to collect pollen grains from their bodies, and photographed them for later identification. Pollen was removed from insects by touching their bodies repeatedly until pollen was removed with ~5x5x5 mm cubes of solidified agar. Once insects woke up they were released. Agar cubes were stored in tagged glass tubes to transfer from the field to the lab, until microscope slides preparations were made by melting the agar and dyeing them with acid fuchsin (Kearns and Inouye 1993). A compound microscope was used to verify presence of *Centrosema virginianum* pollen grains. Voucher specimens of each pollinator species were collected and mounted for taxonomic determination of insects.

Floral herbivory

To evaluate floral herbivory, I collected at least one specimen of each type of florivore for taxonomic identification during May 2001. Flowers open before 8am and are often damaged by 10am, with floral herbivores feeding on flowers until early afternoon when the flowers start to whither and close. I observed flowers mid-morning and classified the damage, attributing the types of damage to the two most abundant floral herbivores. Once the herbivores were identified, I haphazardly selected a group of flowers that showed each type of floral damage, with an equal number of intact flowers as controls at the two sites, matching approximately a total of twenty flowers per day. I performed 20 min.

pollinator watches on both groups of flowers, between 1000 and 1330 during ten sunny days between late May through July. I bagged all the flowers observed every day with nylon bags at the end of the study period (1330) to later collect fruit that might be set. I collected all the fruits after they matured and also recorded flowers that did not set fruit. Seeds per flower per treatment were counted, scarified, hydrated for 12 hours and germinated in petri dishes with moist cotton balls.

Visitation rate of each insect between undamaged flowers and damaged was compared using one-way ANOVA (SPSS 2002). Normality and equal variances were checked with Shapiro-Wilks and Levene's test before analysis. Proportions of seed germination between undamaged flowers and damaged flowers were compared using chi-square (SPSS 2002).

RESULTS

Evaluation of foliar herbivory

The sites differed strongly in foliar herbivory damage. At PS and RD, damage category 5 (50%-100%) was the most common damage category. The lowest amount of damage for PS was category 2 (6-12% of leaf damage) and for RD was category 0 (Fig. 4). In contrast, the population at LPK showed the highest frequency of damage in category 2 (6-12% of leaf damage) and the lowest in category 5 (50%-100% of leaf damage) (Fig. 4).

The Herbivory Index (HI) for the PS population was calculated to be 3.3. Using the exponential model to calculate the Foliar Area Consumed (FAC), this HI corresponds to 22% FAC. The RD population had a HI of 2.80, while that of LPK 2.06, corresponding to

FAC of 15% and 8%, respectively. The smallest habitat fragment therefore had the highest average percentage of foliar damage, and the “pristine” LPK site had the lowest damage index. This suggests that populations of *Centrosema virginianum* in fragmented habitats may be more susceptible to foliar herbivores.

Breeding System

No fruit was set in the emasculation treatment (Table 2). A single control flower produced a fruit with five seeds; flowers that opened subsequently on that plant did not set fruit. Therefore, I considered this fruit to be an artifact. Emasculation and control treatments were not included in subsequent statistical analysis.

All flowers that were hand pollinated (self and cross) set fruit, and open pollination flowers set close to 80% fruit (Table 2). Table 3 shows the two-way ANOVA results on square root transformed seed set (Levene’s equal variance test $P=0.490$). I used the mean number of ovules produced to calculate the percentage of seeds produced (see below). Seed set between the two sites (PS and LPK) was not significantly different ($F=0.017$, $P=0.897$; Table 3). Seed set of selfed flowers was significantly lower than that of crossed and open pollinated flowers (Fig. 5).

The mean number of pollen grains produced by 150 flowers from 35 plants of *Centrosema virginianum* was 3700.5 ± 372.7 (SE) pollen grains per flower. The mean number of ovules produced by the same flowers was 19.7 ± 1.2 (SE). The pollen:ovule ratio using the mean of the 150 samples for pollen grains and ovules was 187 ± 19 .

Pollinator visitation

Both pollinators and florivores were observed to visit flowers of *Centrosema virginianum*. All the bees were considered pollinators of *C. virginianum* because they carried *C. virginianum* pollen grains on their bodies; the other insects were florivores (Table 4). I counted a maximum of 100 *C. virginianum* grains per bee captured after a flower visit. All bee species observed were noted at both sites (PS and LPK) except *Bombus pennsylvanicus* (Hymenoptera: Apidae), which was only seen at LPK during this study. During analysis of the pollinator watches I pooled all bees in the genus *Megachile* (Hymenoptera: Megachilidae) and the species *Colletes distinctus* (Hymenoptera: Colletidae) because it was difficult to be certain about their identity without capturing them. *Xylocopa micans* (Hymenoptera: Anthophoridae) was the most active pollinator at LPK followed by *Bombus pennsylvanicus*, *Melissodes comunis*, *Megachile campanulae wilmingtongi*, *Megachile polcaris* and *Colletes distinctus* (Table 4, Fig. 7a). In contrast, the pollinator guild at PS was dominated by the bees *Megachile* spp. and *Colletes distinctus* (Table 4, Fig. 7c).

Floral herbivory

The most abundant florivores observed at the two sites were the blister beetles, *Epicauta strigosa* Gyllenhal and *Lytta aenea* Say (Coleoptera: Meloidae). These beetles ate flowers starting with the nectar, and then consuming in sequence petals, pollen, and sometimes part of the style and filaments (Fig. 6). The majority of the flowers attacked by blister beetles of insect were partially damaged; only a few were totally eaten. The second most abundant type of floral damaged was caused by a fly from the family

Agromyzidae (species not identified). Species of this family have been reported to be seed predators for other species in the South Florida pine rocklands (Geiger 2002), but these agromyzid flies were observed extracting petal fluids, resulting in petal discoloration. The corollas of damaged flowers changed color from pink-purple to black-gray in spots from 4 to 8mm in diameter, dramatically altering the appearance of the flowers to the human eye and presumably to pollinators as well (see below). Herbivory by the echo moth, *Seirarctia echo* (Lepidoptera: Arctiidae), was observed during late summer of 2001 at LPK. Caterpillars of this moth usually consumed more than the 50% of the flower, including the reproductive parts. I observed no pollinator visits to flowers damaged by this species.

Analysis of variance showed that pollinators visited damaged flowers less than undamaged flowers (Table 5, Fig.7). Also, results from comparison between the different types of damage and control flowers showed significant differences (Fig. 8).

Flowers damaged by blister beetles and agromyzid flies did not set fruit at the PS site (Fig. 9a). Control flowers at PS produced a total of 15 fruits and 138 seeds. 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, more than ten times the reproductive success of flowers damaged by florivores. The average number of seeds produced fruits from flowers damaged by the flies was lower than those from flowers damaged by blister beetles (Fig. 9b). Those fruits showed evidence of seed predation.

Seed germination percentages per treatment did not differ between blister beetle-damaged flowers and control flowers (Fig. 10), but seeds from flowers with agromyzid fly damage showed a lower germination percentage, perhaps as a result of the previously mentioned seed predation. I was not able to see or rear the insects causing this type of damage.

DISCUSSION

Evaluation of foliar herbivory

Centrosema virginianum plants in fragments had greater herbivory than plants in pristine habitat in Everglades National Park (15% and 22% vs. 9%). These results suggest that plants in fragmented habitats are damaged more by herbivory than those in pristine habitats. A possible explanation may be that there is lower species diversity in fragments, with a few more abundant species confined to smaller areas (e.g. theory of resource concentration, Root 1973). Also, plants in fragments may be isolated from predators and parasitoids of herbivores which may also be less abundant in fragments, and thus herbivore populations are released from these top-down controls (Kruess and Tschardtke 2000, Steffan-Dewenter and Tschardtke 2002). Another possible explanation may be the occurrence of fewer herbivores species and therefore lower interspecific competition in the fragments (Crawley 1983), resulting in larger herbivore populations and more damage.

Little is known about the biological consequences of herbivory in native plant communities and the dynamics of other ecological processes indirectly involved (e.g. pollination, competition). There are no studies to my knowledge assessing the effects of

fragmentation of pine rockland habitats on plant- herbivore interactions. Therefore, my study indicated that further investigations are necessary to understand how size of pine rockland fragment may play an important role in herbivory levels. Studies that include quantification of herbivory should be a top priority of monitoring programs of endangered habitats.

Breeding system

My study showed that *Centrosema virginianum* is not capable of apomixis because 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 cross-pollinated flowers. Therefore, I conclude that *Centrosema virginianum* is partially self-compatible and probably has a mixed mating system in these populations (Cruden 1977). Additionally, the fact that open-pollinated controls in the field set fruit at levels equal to hand cross-pollinated flowers in the greenhouse indicates there was no pollinator limitation at the two sites studied in the growing season following fire.

Strikingly different results were found in a study of butterfly pea by Spears (1987) comparing pollination in mainland and island populations on the western coast of Florida. In these populations, self-pollinated flowers produced between 6.7 to 23.3% fruit set and cross-pollinated flowers between 30 to 48.4% fruit set. I do not attribute the differences to greenhouse effects, because seed set between open treatments were done in the field and did not differ from cross pollination treatments done in the greenhouse; but it may be that those populations were under light and nutrient limitation.

Pollinator visitation

Large bees were observed to be frequent visitors of *Centrosema virginianum* flowers. *Bombus pennsylvanicus* and *Xylocopa micans* have been reported to effectively pollinate butterfly pea (Faegri and van der Pijl 1979, Spears 1987). Based on the results of this study, *Melissodes communis*, *Megachile campanulae wilmingtongi*, *Megachile policularis* and *Colletes distinctus* are also effective pollinators. Smaller bees (e.g., *Megachile* and *Colletes*) were more active in the PS fragment site compared with LPK (intact Everglades site). Studies of other pine rockland species have shown pollinator guilds to differ between fragmented habitats and intact habitats (Geiger 2002, Liu and Koptur 2003). Spears (1987) reported differences in pollinator guilds of *C. virginianum* among a far island, a near island and mainland populations. When studying pollination in *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 on pine rockland endemic, *Chamaecrista keyensis*, showed that *Xylocopa micans* was the dominant pollinator in urban-edge habitats, while *Megachile* spp. predominated in forest sites (Liu and Koptur 2003).

Floral herbivory

My results resemble other studies that have demonstrated that changes in pollinator behavior have detrimental effects in the reproductive output of plant species (Pellmyr and Thompson 1996, Corff et al. 1998, Ohashi and Yahara 1998, Parra-Tabla and Bullock 1998, Bigger 1999, Krupnick et al. 1999, Strauss 1999, Strickler and Freitas

1999, Cunningham 2000). Although I did not quantify the proportion of damaged flowers in the populations studied, I observed that roughly 50% of the flowers of *C. virginianum* were damaged by flower eaters. The most abundant florivores found were blister beetles from the family Meloidae and a species of agromyzid fly, neither of which specialized exclusively on *C. virginianum*, being observed on a variety of flowers. These species 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. Only 12% of flowers damaged by flies produced six fruits with 55 seeds. Interestingly, seeds from these fruits showed evidence of seed predation, which ultimately may reduce the mean number of seeds produced. I cannot prove that the seed predator responsible was the same fly species that damage 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 *Centrosema virginianum*, its effects on seed production are doubly negative. Directly, flies may be ovipositing in ovaries and their larvae destroying seeds in their development; indirectly, adult flies sucking petals disfigure flowers and reduce pollinator visitation, and pollination is required for fruit and seed set.

CONCLUSION

The reproductive success of populations of *Centrosema virginianum* in pine rocklands of southern Florida is affected by herbivores feeding in different ways, especially in fragmented habitats. Foliar herbivores damage more photosynthetic tissue in habitat fragments than in intact habitat, and at all sites the amount of damage is substantial. Floral herbivores reduce pollinator visitation to flowers, as damaged flowers

look different. This translates into substantially reduced fruit set for damaged flowers in this species that requires flower visits for pollination. It would also be ecologically interesting to see if plants vary in their resistance and tolerance to herbivory and their relationship with pollinators (Strauss 1997), since damage on flowers and leaves has profound direct and indirect effect on reproductive fitness.

The data presented here showed (1) differences in rates of herbivory in the different sites studied and (2) major effects from herbivory on plant reproduction, even in pristine habitats. These data indicate both the importance of plant-animal interactions for conservation and that anthropogenic activities can alter this interaction. Data presented here are from a single year of study. Nevertheless, this study contributes to understanding the unique dynamics of herbivores, pollinators and their host plants that result from microenvironmental characteristics of each habitat and its site-specific biotic structure.

REFERENCES

- Adler, L. S. 2000. Alkaloid uptake increases fitness in a hemiparasitic plant via reduced herbivory and increased pollination. *American Naturalist* **156**:92-99.
- Agrawal, A. A., S. Y. Strauss, and M. J. Stout. 1999. Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. *Evolution* **53**:1093-1104.
- Armbruster, W. S., J. J. Howard, T. P. Clausen, E. M. Debevec, J. C. Loquvam, M. Matsuki, B. Cerendolo, and F. Andel. 1997. Do biochemical exaptations link evolution of plant defense and pollination systems? Historical hypotheses and experimental tests with *Dalechampia* vines. *American Naturalist* **149**:461-484.
- Armbruster, W. S., and W. R. Mziray. 1987. Pollination and herbivore ecology of an African *Dalechampia* (Euphorbiaceae): Comparisons with New World species. *Biotropica* **19**:64-73.
- Bigger, D. S. 1999. Consequences of patch size and isolation for a rare plant: Pollen limitation and seed predation. *Natural Areas Journal* **19**:239-244.
- Brewer, J. S., and W. J. Platt. 1994. Effects of fire season and herbivory on reproductive success in a clonal forb, *Pityopsis graminifolia*. *Journal of Ecology* **82**:664-675.
- Corff, J. L., J. Agren, and D. W. Schemske. 1998. Floral display, pollinator discrimination, and female reproductive success in two monoecious *Begonia* species. *Ecology* **79**:1610-1619.
- Crawley, M. J. 1983. *Herbivory. The Dynamics of Animal -Plant Interactions*. University of California Press, Berkeley.
- Crawley, M. J. 1986. *Plant Ecology*. Blackwell Scientific Publications, London.
- Cruden, R. W. 1977. Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution* **31**:32-46.
- Cunningham, S. A. 2000. Effects of habitat fragmentation on the reproductive ecology of four plant species in mallee woodland. *Conservation Biology* **14**:758-768.
- DERM. 2003. Department of Environmental Resources Management. Miami-Dade, Florida.

- Faegri, K., and L. van der Pijl. 1979. The principles of pollination ecology. Pergamon Press, Oxford.
- Frazee, J. E., and R. J. Marquis. 1994. Environmental contribution on floral trait variation in *Chamaecrista fasciculata* (Fabaceae: Caesalpinoideae). American Journal of Botany **81**:206-215.
- Gann, G. D. 2001. Institute for Regional Conservation in The Institute for Regional Conservation Web Site.
- Geiger, J. 2002. The reproductive biology of *Ruellia succulenta* (Acanthaceae) and the effects of habitat fragmentation. Master of Science. Florida International University, Miami.
- Groom, M. J. 2001. Consequences of subpopulation isolation for pollination, herbivory, and population growth in *Clarkia concinna concinna* (Onagraceae). Biological Conservation **100**:55-63.
- Isely, D. 1990. Vascular flora of the Southeastern United States: Leguminosae (Fabaceae). The University of North Carolina Press, North Carolina.
- Karban, R., and S. Y. Strauss. 1993. Effects of herbivores on growth and reproduction of their perennial host, *Erigeron glaucus*. Ecology **74**:39-46.
- Kawarasaki, S., and Y. Hori. 1999. Effect of flower number on the pollinator attractiveness and the threshold plant size for flowering in *Pertya triloba* (Asteraceae). Plant Species Biology **14**:69-74.
- Kearns, C. A., and D. W. Inouye. 1993. Techniques for pollination biologists. University Press of Colorado, Niwot, Colorado.
- Kruess, A., and T. Tschardt. 2000. Species richness and parasitism in a fragmented landscape: experiments and field studies with insects on *Vicia sepium*. Oecologia **122**:129-137.
- Krupnick, G. A., A. E. Weis, and D. R. Campbell. 1999. The consequences of floral herbivory for pollinator service to *Isomeris arborea*. Ecology **80**:125-134.
- Krupnick, G. A. W., A. E. 1999. The effect of floral herbivory on male and female reproductive success in *Isomeris arborea*. Ecology **80**:135-149.

- Kulman, H. M. 1971. Effects of insect defoliation on growth and mortality of trees. *Annual Review of Entomology* **16**:289-324.
- Leege, L. M., and L. M. Wolfe. 2002. Do floral herbivores respond to variation in flower characteristics in *Gelsemium sempervirens* (Loganiaceae), a distylous vine? *American Journal of Botany* **89**:1270-1274.
- Lehtilae, K., and S. Y. Strauss. 1999. Effects of foliar herbivory on male and female reproductive traits of wild radish, *Raphanus raphanistrum*. *Ecology* **80**:116-124.
- Les, D. H. 1988. Breeding systems, population structure, and evolution in Hydrophilous Angiosperms. *Annals of the Missouri Botanical Garden* **75**:819-835.
- Liu, H., and S. Koptur. 2003. Breeding system and pollination of narrowly endemic herb of the lower Florida Keys: impacts of the urban-wildland interface. *American Journal of Botany* **90**:1180-1187.
- Lopez, J., T. Rodriguez-Riano, A. Ortega-Olivencia, J. A. Devesa, and T. Ruia. 1999. Pollination mechanisms and pollen-ovule ratios in some Genisteae (Fabaceae) from Southwestern Europe. *Plant Systematics and Evolution* **216**:23-47.
- O'Brien, J. J. 1998. The distribution and habitat preferences of rare *Galactia* species (Fabaceae) and *Chamaesyce deltopidea* subspecies (Euphorbiaceae) native to Southern Florida pine rockland. *Natural Areas Journal* **18**:208-222.
- Ohashi, K., and T. Yahara. 1998. Effects of variation in flower number on pollinator visits in *Cirsium purpuratum* (Asteraceae). *American Journal of Botany* **85**:219-224.
- Parra-Tabla, V., and S. H. Bullock. 1998. Factors limiting fecundity of the tropical tree *Ipomoea wolcottiana* (Convolvulaceae) in a Mexican tropical dry forest. *Journal of Tropical Ecology* **14**:615-627.
- Pellmyr, O., and J. N. Thompson. 1996. Sources of variation in pollinator contribution within a guild: The effects of plant and pollinator factors. *Oecologia* **107**:595-604.
- Polhill, R. M., and P. H. Raven, editors. 1978. *Advances in legume systematics Part 1*. Royal Botanical Gardens, Kew.
- Root, R. B. 1973. Organization of plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecological Monographs* **43**:95-124.

- Spears, E. E. J. 1987. Island and mainland pollination ecology of *Centrosema virginianum* and *Opuntia stricta*. *Journal of Ecology* **75**:351-362.
- Spier, L. P., and J. R. Snyder. 1998. Effects of wet- and dry-season fires on *Jacquemontia curtisii*, a south Florida pine forest endemic. *Natural Areas Ecology* **18**:350-357.
- SPSS. 2002. SPSS for Windows version 11.5. LEAD technologies, Inc.
- Steffan-Dewenter, I., and T. Tschamntke. 2002. Insect communities and biotic interactions on fragmented calcareous grasslands-a mini review. *Biological Conservation* **104**:275-284.
- Strauss, S. Y. 1997. Floral characters link herbivores, pollinators, and plant fitness. *Ecology* **78**:1640-1645.
- Strauss, S. Y., and A. A. Agrawal. 1999. Ecology and evolution of plant tolerance to herbivory. *TRENDS in Ecology and Evolution* **14**:179-185.
- Strauss, S. Y., D. H. Siemens, M. B. Decher and T. Mitchell-Olds. 1999. Ecological costs of plants resistance to herbivores in the currency of pollination. *Evolution* **53**:1105-1113.
- Strickler, K., and S. Freitas. 1999. Interactions between floral resources and bees (Hymenoptera: Megachilidae) in commercial alfalfa seed fields. *Environmental Entomology* **28**:178-187.
- Timmerman-Erskine, M., and R. S. Boyd. 1999. Reproductive biology of the endangered plant *Clematis socialis* (Ranunculaceae). *Journal of the Torrey Botanical Society* **126**:107-116.
- Wallin, K. F., and K. F. Raffa. 2001. Effects of folivory on subcortical plant defenses: Can defense theories predict interguild processes? *Ecology* **82**:1387-1400.
- Yashiro, K., Y. Sakai, and H. Namai. 1999. Relationships between pollen-ovule ratio and autofertility, self-compatibility, automatic self-pollination ability in heterogeneous incomplete autogamous plants, Thai mustard. *Breeding Science* **49**:39-42.



Fig. 1. A bee, *Megachile campanulae* pollinating *Centrosema virginianum* at Long Pine Key in Everglades National Park.

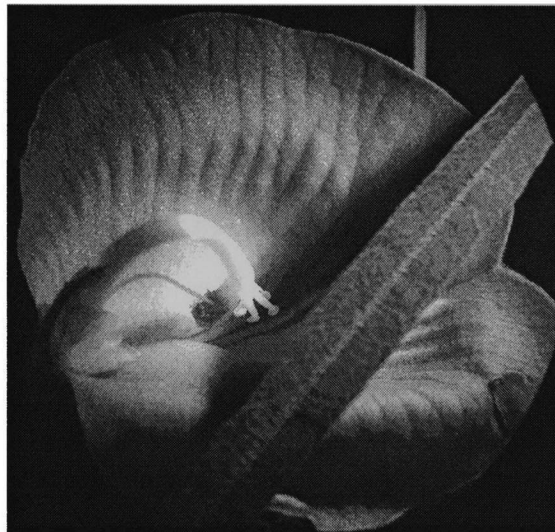
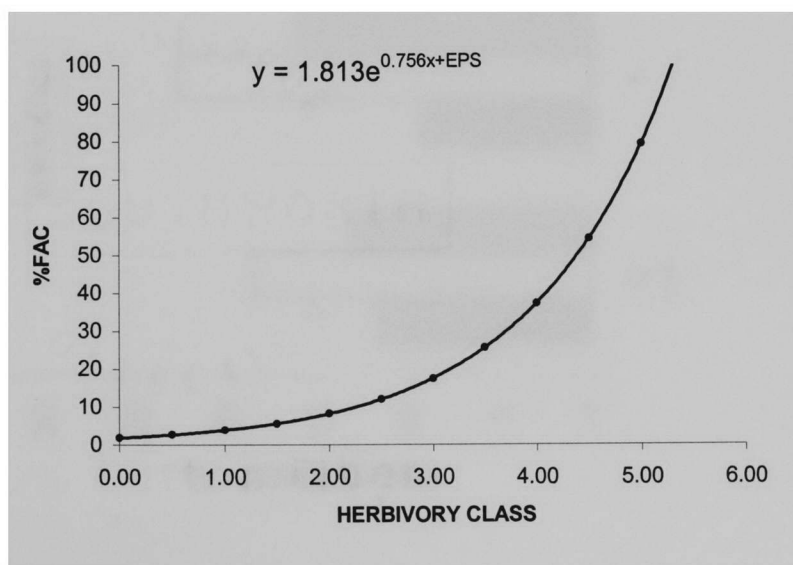


Fig. 2: *Centrosema virginianum* carpel surrounded by nine fused filaments with the single stamen below, which is characteristic of the Papilionoideae tribe.

TABLE 1. Herbivory classes and their corresponding percentages of foliar area consumed (FAC).

Herbivory Class	% of Foliar Area Consumed (FAC)
0	0
1	1-6
2	6-12
3	12-25
4	25-50
5	50-100

Fig. 3. Exponential model used to calculate the foliar area consumed (FAC) of *Centrosema virginianum* from the Herbivory Index.



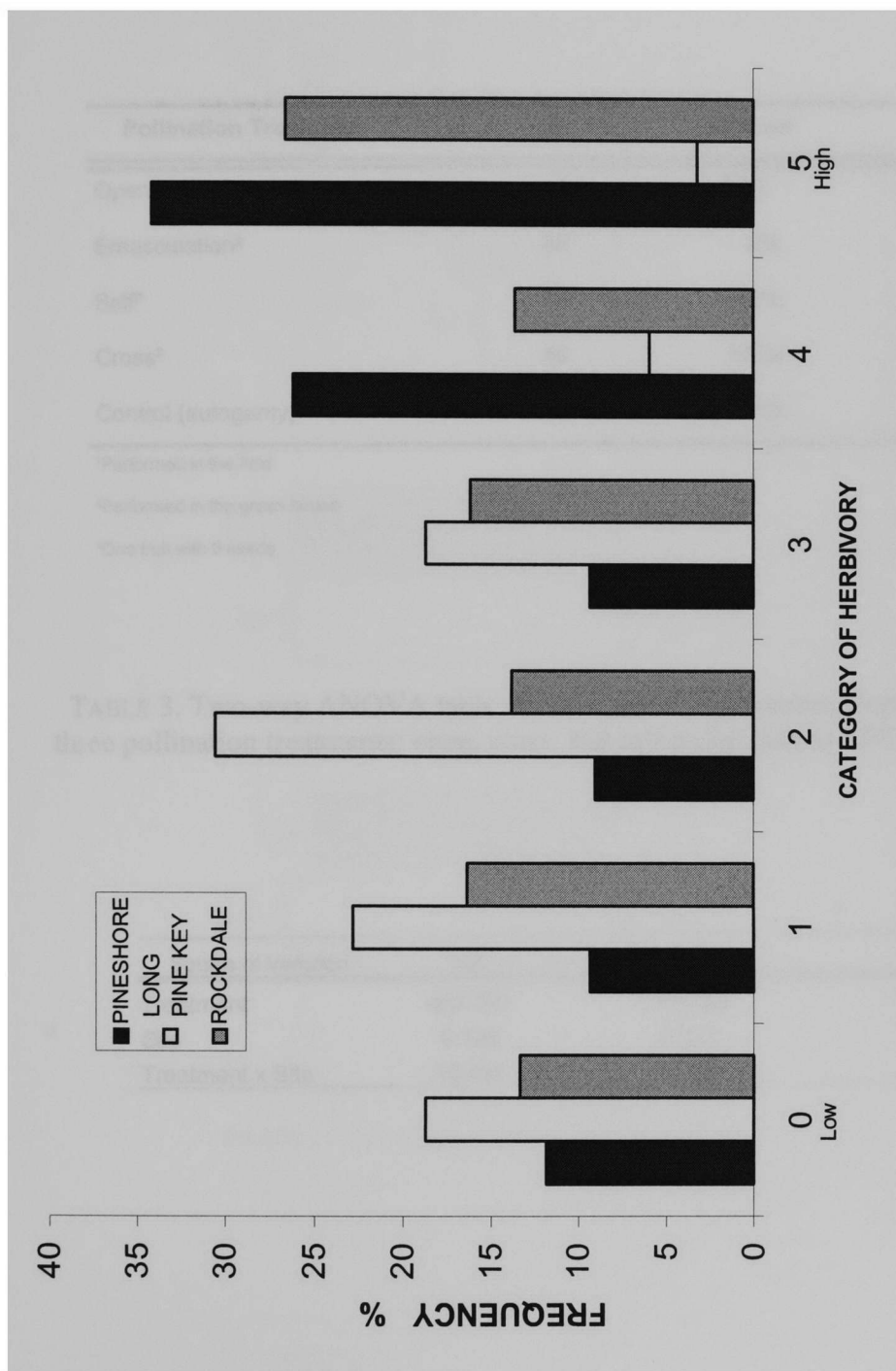


Fig 4. Frequency (%) histogram of herbivory on *Centrosema virginianum* at the three study sites.

TABLE 2. Total fruit set and seed set in pollination treatments. Open pollination treatment was performed in the field using the same genotypes as in the greenhouse. Number of flowers (N) differed due to flower availability. Seed set was calculated using the mean of ovules per flower.

Pollination Treatment	N	fruit set	seed set
Open ¹	37	81%	83%
Emasculation ²	48	0%	0%
Self ²	50	100%	38%
Cross ²	50	100%	84%
Control (autogamy) ^{2,3}	50	0%	0%

¹Performed in the field

²Performed in the green house

³One fruit with 5 seeds

TABLE 3. Two-way ANOVA table for seed set of *Centrosema virginianum* under three pollination treatments: open, cross, and self pollination at LPK and PS.

Source of Variation	MS	F	P
Treatment	867.791	113.140	<0.001
Site	0.128	0.017	0.8970
Treatment x Site	13.461	1.755	0.1790

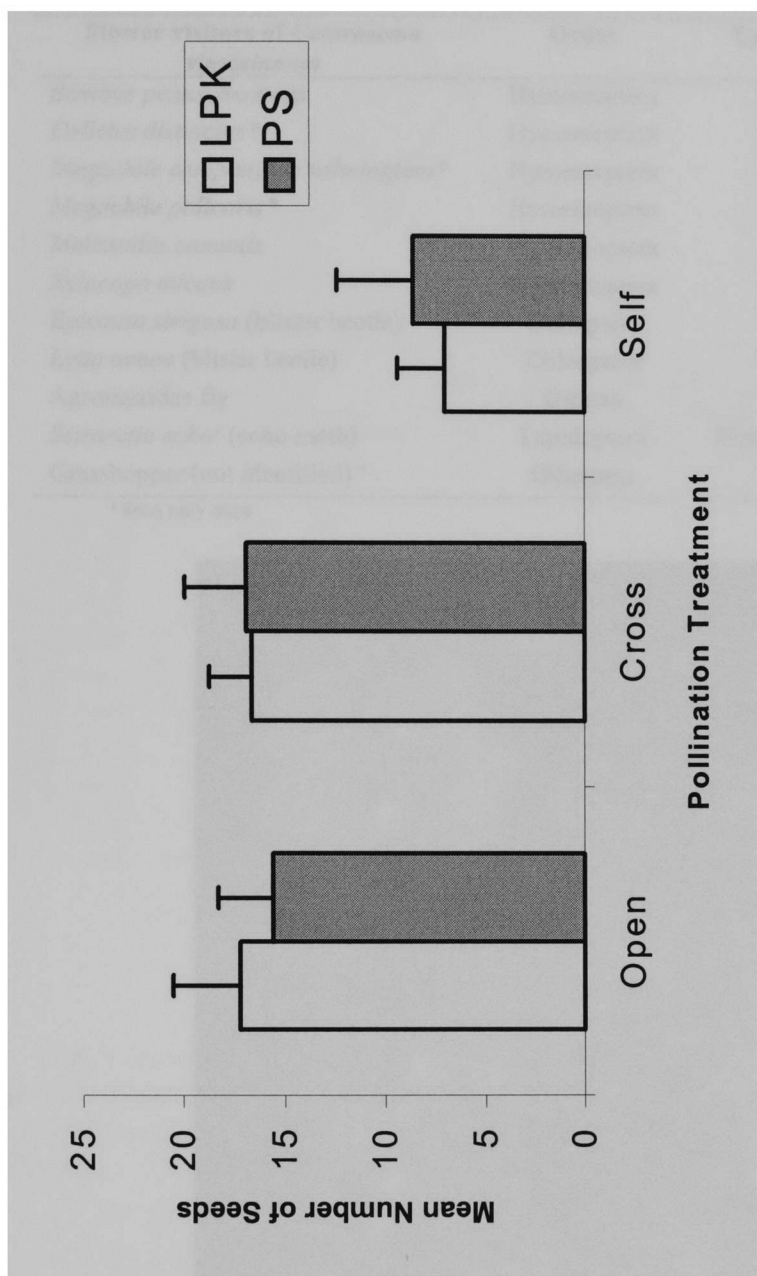


Fig. 5. Mean and standard deviations of seed set of *Centrosema virginianum* after three pollination treatments on plants from Long Pine Key (LPK) and Pine Shore preserve (PS). Open-pollination data were collected from plants growing in the field; "cross" and "self" data were obtained from hand-pollinations conducted on the same genotypes (clones) in the greenhouse. Different letters indicate significant difference at $\alpha=0.05$.

TABLE 4. Flower visitors of *Centrosema virginianum*. All species listed as pollinators were caught at flowers and carried pollen on their bodies. Species with a star were easily confused and for this reason were pooled in visitation data.

Flower visitors of <i>Centrosema virginianum</i>	Order	Type of visitor	Site Observed
<i>Bombus pennsylvanicus</i>	Hymenoptera	Pollinator	LPK
<i>Colletes distinctus</i> *	Hymenoptera	Pollinator	LPK/PS
<i>Megachile campanulae wilmingtongi</i> *	Hymenoptera	Pollinator	LPK/PS
<i>Megachile polcaris</i> *	Hymenoptera	Pollinator	LPK/PS
<i>Melissodes comunis</i>	Hymenoptera	Pollinator	LPK/PS
<i>Xylocopa micans</i>	Hymenoptera	Pollinator	LPK/PS
<i>Epicauta strigosa</i> (blister beetle)	Coleoptera	Florivore	LPK/PS
<i>Lytta aenea</i> (blister beetle)	Coleoptera	Florivore	LPK/PS
Agromyzidae fly	Diptera	Florivore	LPK/PS
<i>Seirarctia echo</i> ¹ (echo moth)	Lepidoptera	Florivore/Folivore	LPK
Grasshopper (not identified) ¹	Othoptera	Florivore	PS

¹ Seen only once



Fig. 6. *Epicauta strigosa* (blister beetle) eating a flower of *C. virginianum* taken at Pine Shore site. Top from left to right, undamaged flower; blister beetle and Agromyzidae fly. Bottom from left to right, blister beetle before leaving flower; flower with blister beetle damage.

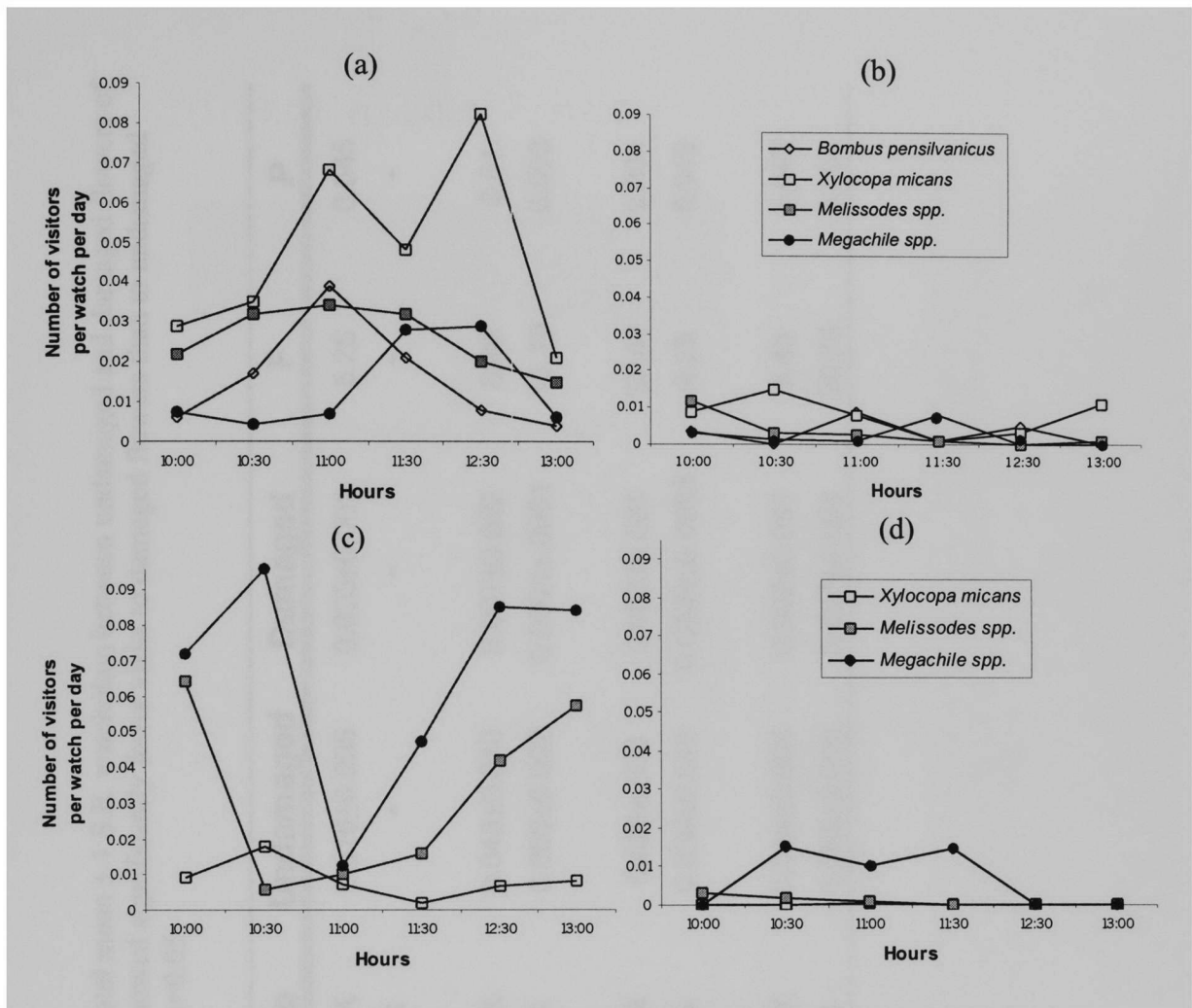


Fig. 7. Mean number of visitors per watch per day of *Centrosema virginianum* at LPK (a and b) and PS (c and d) during ten days. Panels to the left (a and c) are visitation rates for undamaged flowers. Panels to the right (b and d) are visitation rates for damaged flowers.

TABLE 5. Pollinator visitation rates (total mean $\pm$ 1 S.E. watch/day) between undamaged and damaged flowers of *Centrosema virginianum*. ANOVA showed significantly fewer visits to damaged flowers than to undamaged flowers. All tests were significant at $\alpha=0.05$.

Pollinator	Site	Undamaged	Damaged	F	P
<i>Bombus pennsylvanicus</i>	LPK	0.016 $\pm$ 0.005	0.003 $\pm$ 0.001	5.26	0.045
	PS	-	-	-	-
<i>Xylocopa micans</i>	LPK	0.043 $\pm$ 0.010	0.007 $\pm$ 0.002	8.99	0.01
	PS	0.009 $\pm$ 0.002	0.0001 $\pm$ 0.0001	15.19	0.003
<i>Melissodes</i> spp.	LPK	0.026 $\pm$ 0.003	0.0033 $\pm$ 0.001	37.47	0.001
	PS	0.036 $\pm$ 0.010	0.0009 $\pm$ 0.0005	9.28	0.012
<i>Megachile</i> spp.	LPK	0.014 $\pm$ 0.004	0.002 $\pm$ 0.001	5.45	0.042
	PS	0.066 $\pm$ 0.010	0.007 $\pm$ 0.003	20.79	0.001

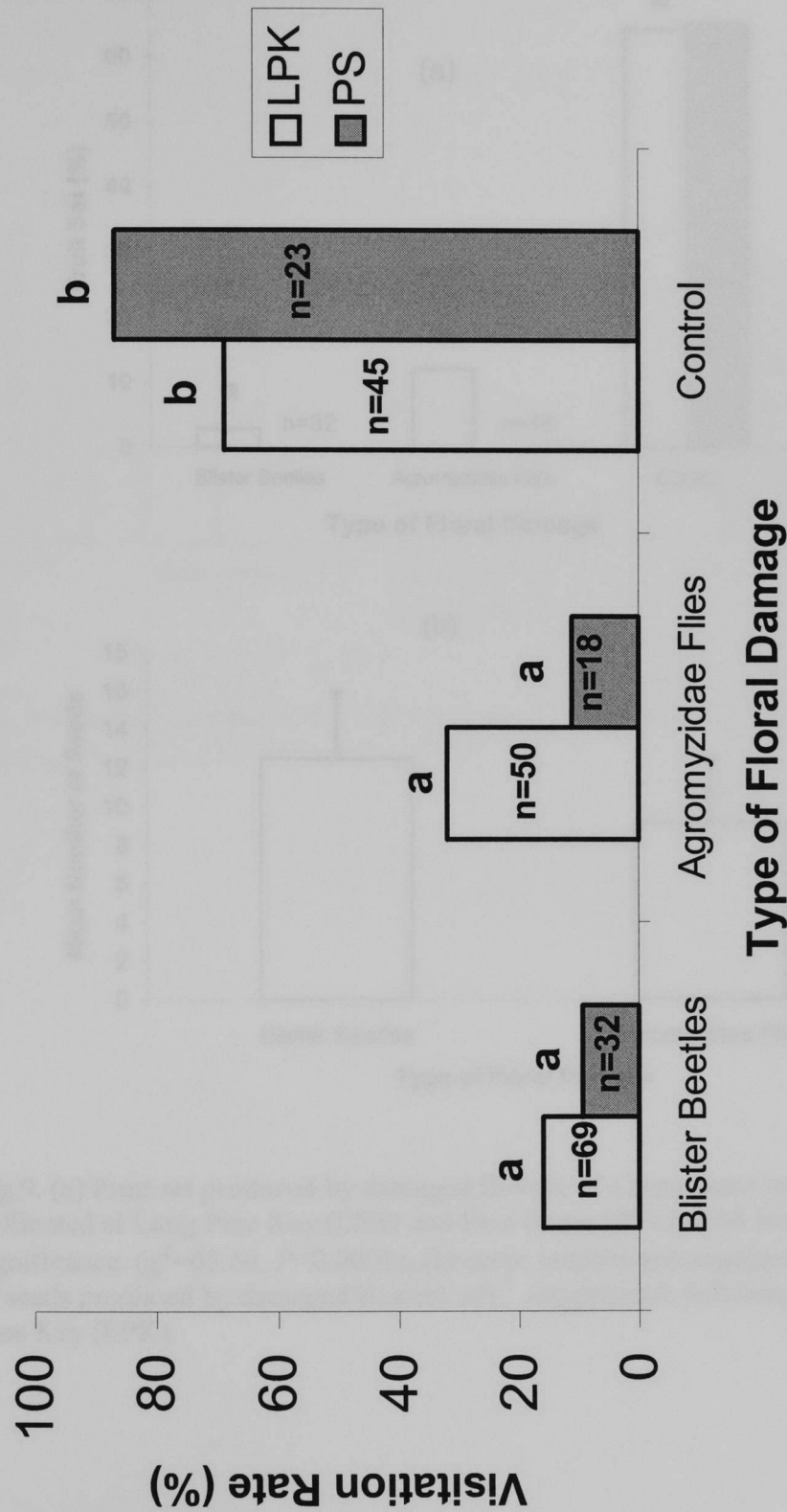


Fig. 8. Pooled visitation rates of *Centrosema virginianum* pollinators at Long Pine Key (LPK) and Pine Shore preserve (PS). Treatments with different letters (a and b) indicate significant difference between type of floral damage ($\chi^2=66.1$, $df=2$, $P<0.0001$ & $\chi^2=41.45$, $df=2$, $P<0.0001$).

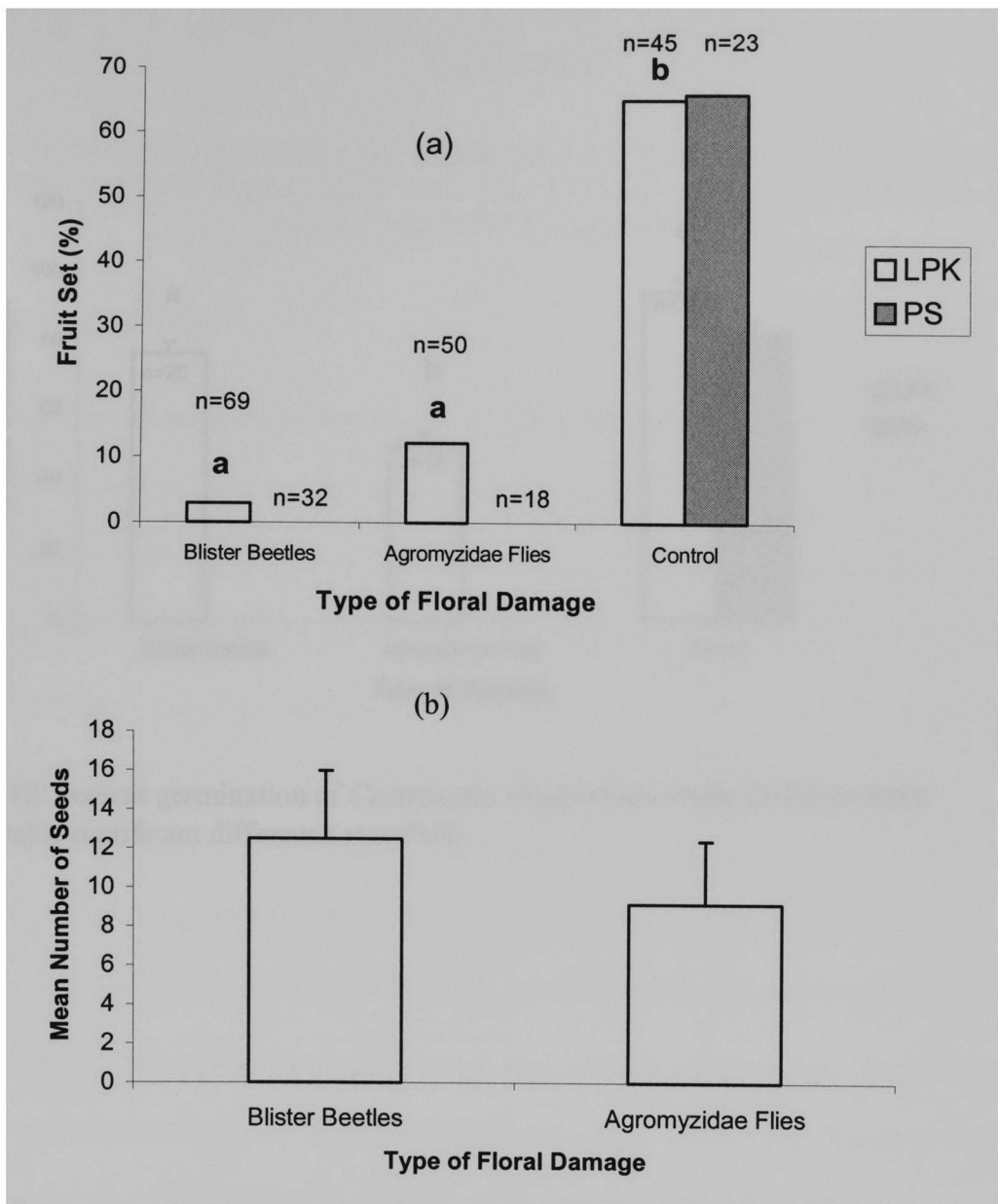


Fig.9. (a) Fruit set produced by damaged flowers of *Centrosema virginianum* pollinated at Long Pine Key (LPK) and Pine Shore (PS) at 0.05 level of significance. ($\chi^2=63.64$, $P<0.0001$); (b) mean number and standard deviation of seeds produced by damaged flowers of *C. virginianum* pollinated at Long Pine Key (LPK).

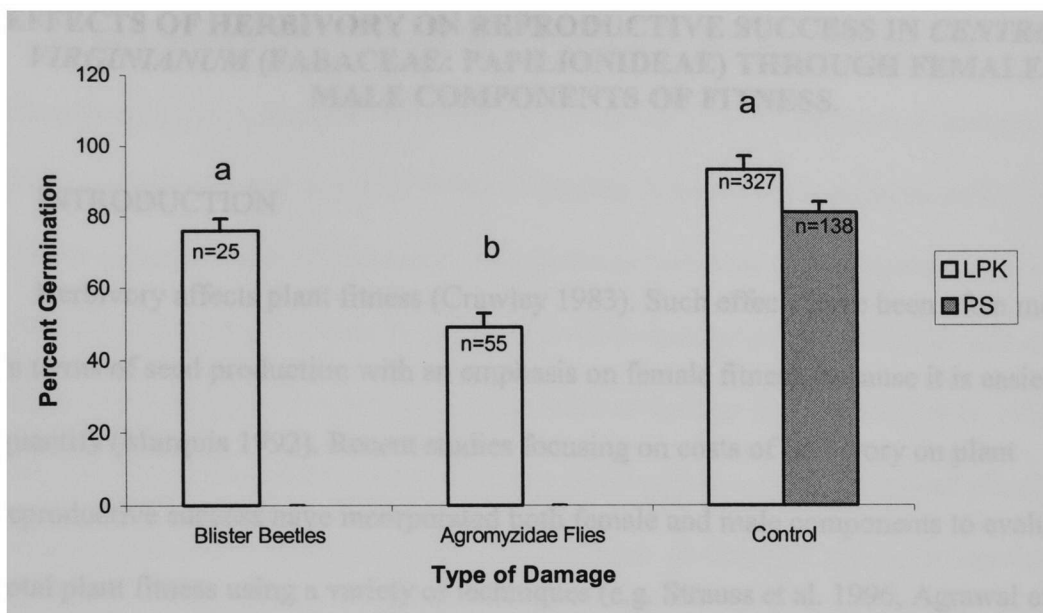


Fig. 10. Percent germination of *Centrosema virginianum* seeds. Different letter indicates significant difference at $\alpha=0.05$.

CHAPTER II

EFFECTS OF HERBIVORY ON REPRODUCTIVE SUCCESS IN *CENTROSEMA VIRGINIANUM* (FABACEAE: PAPILIONIDEAE) THROUGH FEMALE AND MALE COMPONENTS OF FITNESS.

INTRODUCTION

Herbivory affects plant fitness (Crawley 1983). Such effects have been often measured in terms of seed production with an emphasis on female fitness, because it is easier to quantify (Marquis 1992). Recent studies focusing on costs of herbivory on plant reproductive success have incorporated both female and male components to evaluate total plant fitness using a variety of techniques (e.g. Strauss et al. 1996, Agrawal et al. 1999, Lehtilae and Strauss 1999, Avila-Sakar et al. 2001). Some plants can shift allocation of resources from female to male components of fitness (Horovitz 1978, Charlesworth and Charlesworth 1981, Crawley 1997, Delph et al. 1997, Cobb et al. 2002). Because both components contribute to the subsequent generations, understanding the effects of herbivory on both female and male function, and how both functions relate to each other, have been the focus of several studies (e.g. Bertin 1982, Strauss et al. 1996, Agrawal et al. 1999, Lehtilae and Strauss 1999, Leege and Wolfe 2002).

Little is known about how hermaphroditic plants allocate resources to female and male components of fitness after sustaining leaf damage (Lehtilae and Strauss 1999). Less is known about this process in self-compatible species. Since 80% of the angiosperms have perfect flowers (Proctor et al. 1996, Niklas 1997) and most flowering plants are self-compatible (Crawley 1997), studies of reproductive success on species with self-

compatible hermaphroditic flowers are particularly important to understand the effects of leaf herbivory in components of fitness.

A way to relate female and male components of fitness, and to detect shifts in resource allocation on plants to favor one component over the other, is estimating tolerance to herbivory (Strauss and Agrawal 1999). Tolerance to herbivory is referred as the ability to regrow and reproduce after damage in response to herbivore attack (Strauss and Agrawal 1999). In this study, tolerance to herbivory will be used as the proportional reduction in a fitness component of the plant for a given level of damage (see Agrawal et al. 1999, Strauss 1999, Galen 2000), and can only be measured for a group of related plants, because tolerance is measured as the ratio of fitness of damaged and undamaged plants (Rausher et al. 1993). A genotype that appears severely damaged by herbivory may not be highly susceptible to herbivory, but rather, highly tolerant of herbivory, and the fitness of genotypes may be reduced by the cost of tolerance in environments with relatively low levels of herbivory (Fineblum 1995).

Typically, cost of tolerance could be measured by two methods: examining the relationship between genotype fitness means for damaged and undamaged plants, where a negative correlation demonstrates a cost of tolerance (Strauss and Agrawal 1999, Galen 2000). The alternative method consists of analyzing the relationship between tolerance and mean genotype fitness. I have chosen to examine both methods to estimate the cost of tolerance in *Centrosema virginianum*.

Pollen number and size have been reported to decrease as a consequence of herbivory (Bertin 1982, Schlichting and Devlin 1989, Strauss et al. 1996, Strauss 1997, Agrawal et al. 1999, Irwin and Brody 1999, Lehtilae and Strauss 1999). If pollen number and pollen

size are potential indicators of shifts in resource allocation between male and female components, it is appropriate to examine them to determine how costly herbivory may be in terms of male fitness. For facultative self-compatible species, which depend in part on the pollen load deposited on stigmas to produce seeds, pollen availability will be directly related to seed set (Crawley 1997). Additionally, smaller pollen grains may lose in the race for fertilization competing with normal size grains (Delph et al. 1997). Similarly, I used seeds produced and ovules produced to measure cost of tolerance to herbivory in terms of female fitness.

I conducted a study using experimental defoliation in the greenhouse to evaluate male and female fitness components directly affected by herbivory in a perennial, partially self-compatible hermaphroditic plant. My goal was to describe allocation shifts between both functions, and to determine which reproductive features, if any, were substantially affected by foliar damage. I measured how two levels of foliar herbivory affected flower size, nectar volume and nectar concentration, pollen production, pollen grain size, number of ovules produced, and number of seeds produced. My main questions were: (1) how does herbivory affect male and female fitness components? and (2) What is the relative cost of tolerance to leaf area loss to both components?

MATERIAL AND METHODS

Species

Centrosema virginianum Bentham (Fabaceae: Papilionideae) is a partially self-compatible perennial vine that bears pinnately trifoliate leaves. *C. virginianum* occurs in

pine rockland habitats in southern Florida and is widely distributed from the southern United States to the northern regions of South America (Isely 1990).

The flowers are entomophilous, with an unusual resupinate orientation adapted for pollination by large insects, most commonly bees (Faegri and van der Pijl 1979).

Although partially self-compatible, flowers require an insect for pollination. Neither apomixis nor autogamy occurs (Chapter 1). Each flower lasts only one day. If a flower is successfully pollinated, the superior ovary grows to produce a mature fruit, approximately four to six weeks later (Chapter 1).

In natural populations on southern Florida pine rocklands, herbivores remove between 30 to 70% of leaf tissue from more than 30% of plants (Chapter 1). A wide variety of herbivores, which include caterpillars, grasshoppers, beetles and scale insects (Orders Lepidoptera, Orthoptera, Coleoptera and Homoptera) are responsible for the damage.

Experimental Approach

Pine rockland habitats are fragmented and dotted with relatively small *Centrosema virginianum* populations. Ten source plants were selected from three different sites. To insure source plants were different individuals, source plants were separated by at least 10m from other plants, which would allow distinction of maternal effects. All plants were propagated by cuttings to establish three ramets per genotype. All plants were placed in 1500-ml pots with identical amounts of all-purpose fertilizer 15:30:15 (Miraclegrow^{TM}).

I randomly assigned one of three treatments per clone to all genotypes after the fifth trifoliate leaf was produced: control (undamaged), mild herbivory (one third of each

leaflet removed), and severe herbivory (two thirds of each leaflet removed) (Table 1). I used scissors to remove leaf tissue to the designated level. After the initial defoliation, all new leaves produced were manipulated in the same way on a weekly basis.

Female and male components

I measured petal size based on the longest axis of the standard of the flower on 15 flowers per plant per treatment. To measure nectar concentration, I used a hand-held refractometer for small volume that measures sugar concentration on a wt/wt basis (Bellingham & Stanley, Tunbridge Wells, UK). I used 1 μ l micropipettes to collect nectar from 15 flowers per plant per treatment, calculating the volume to the nearest .1 μ l for each flower.

A total of 15 flower buds per plant per treatment were collected the night prior to flower opening. All anthers per flower were placed in Eppendorf tubes until anthers dehisced, releasing all their pollen grains. I then added Carnoy's fixative (3:1 absolute ethanol: glacial acetic acid) to all tubes. After an hour, the tubes were centrifuged (mini centrifuge Costar, USA) to sediment out the pollen grains; the Carnoy's solution was removed and replaced with 70% ethanol to store samples until processing was done. To process the samples, all ethanol was replaced with 0.1 ml of lactophenol-aniline blue solution (Kearns and Inouye 1993). Each tube was shaken in a vibrator (FisherVortex Genie-2; Fisher Scientific, Pittsburgh, PA) for 15 seconds. Using a Pasteur pipette I placed a portion of the liquid in a hemacytometer, filling both wells of three hemacytometers with enough liquid to cover the entire grid, having a total of 6 replicates per sample. All the pollen grains on the four large squares of the grid were counted using

a light microscope. I calculated the total number of pollen grains as described in Kearns and Inouye (1993).

In order to measure pollen size, an identical procedure to fix pollen grains was performed on another set of 15 flowers per genotype per treatment. Once the Eppendorf tubes were ready to process, each sample was placed in the Vortex for 15 seconds. A hemacytometer was prepared as mentioned previously. A digital picture of each large center square was taken for the two wells. The remaining sample in the tube was placed again on the Vortex for 15 seconds and another two digital pictures were taken. The last step was repeated one more time to have a total of six pictures per flower. The area of a total of twenty five randomly selected pollen grains in the six pictures was calculated using ArcView GIS Image Analysis Extension (ArcViewGIS 1999).

I performed cross and self hand pollinations on 10 flowers per genotype per defoliation treatment per cross. Cross pollen was a mix of at least three flowers from healthy plants not involved in the herbivory experiment (collected from different localities). Once the fruit fully matured, I counted the number of ovules per fruit and the number of seeds produced. I compared ovule number and seeds produced between pollination treatments, defoliation treatments and genotype.

Analysis

I had a randomized complete block experimental design with 15 replicates (flowers). I used the experimental design procedure from StatGraphics software (Statistical Graphics Corp. 2001). Flower size, nectar concentration, pollen number and pollen size were response variables in each analysis. The defoliation treatment, with three levels, was

the fixed factor. The ten genotypes (maternal plants) were treated as blocks for all comparisons, with a total of 450 runs and 438 error degrees of freedom. Seed set was analyzed with a mixed model three-way ANOVA using herbivory treatment and type of cross as fixed factors and genotype as a random factor. For all data sets, homoscedasticity was tested with Cochran's C test and normality of residuals with the Shapiro-Wilk test (Analise-it Software Ltd. 2000, Statistical Graphics Corp. 2001). Ln-transformation was used to normalize the data for nectar concentration, flower size and pollen size. Square root transformation was used to normalize counts of seeds produced and number of ovules and pollen number (Krebs 1989). The chi-square test was used to compare treatments for nectar volume, since data could not be normalized by transformation.

The cost of tolerance and the relationship between female and male success

Similar to the study by Agrawal et al. (1999), I analyzed the female and male fitness components on plants under herbivory damage. I plotted the average values of seed produced per genotype of damaged plants against undamaged plants. Since *Centrosema virginianum* may have a mixed mating system (Chapter 1), I calculated the average of seeds produced per plant by self and cross hand pollinations for control and damaged plants.

I plotted ln-transformed average of pollen number and pollen size for damaged plants against pollen produced by undamaged plants. A negative correlation of fitness components of damaged plants versus undamaged plants indicates a cost of tolerance in reproduction after herbivory for male fitness components (Agrawal et al. 1999).

I then compared the relationship between male and female components of fitness using pollen produced and seeds produced using Pearson's product-moment correlation test to explore any association between them for undamaged and damaged plants. Data used for this test were square root transformed (seeds) and ln-transformed (pollen) to meet analysis assumptions.

Tolerance to herbivory for female and male reproductive success was calculated to determine the cost of tolerance to herbivory and to detect shifts of resource allocation among all female and male components of fitness measured. Similar to Agrawal et al. (1999), I divided the means of seeds produced and pollen sizes from damaged plants by undamaged plants. Total performance of the components of fitness measured was compared among treatments.

RESULTS

Female and male components

Damaged plants produced smaller flowers for both levels of herbivory compared with control plants ($F=221.07$, $P<0.001$, Table 2). The flower size decreased with increasing levels of herbivory (Table 2). A significant treatment-genotype interaction indicated that genotypes responded differently to foliar herbivory damage suggesting that flower size is also the result of the individual's genetics (Table 2).

Sugar concentration of nectar did not differ among treatments ($F=0.47$, $P=0.63$) Nectar volume was highly variable and no difference was detected in the chi-square analysis ($\chi^2=8.40$, $P=0.078$). Nectar volume ranged from 0.04 μ l to 1.81 μ l for undamaged

plants and 0.33 μ l to 1.86 μ l and 0.05 μ l to 1.87 μ l for plants that suffered mild and severe herbivory, respectively. Similar results have been reported for *Raphanus raphanistrum*, in which damaged plants produced only marginally less nectar than undamaged plants (Strauss et al. 1996, Lehtilae and Strauss 1999).

Damaged plants produced less pollen than undamaged plants for both mild and severe herbivory levels ($F=18.49$, $P<0.001$, Table 2). No differences within genotype were detected ($F=1.09$, $P<0.374$, Table 2) and the two levels of herbivory did not differ from each other, suggesting that pollen number is a conservative trait within the species.

I obtained surprising results from the measurement of pollen size. The results showed that after severe leaf damage, flowers produced larger pollen grains. Control plants and plants damaged with mild herbivory did not significantly differ in pollen size, but these two groups produced flowers with smaller pollen grains than plants sustaining severe herbivory (Bonferroni multiple comparisons, $\alpha=0.05$, $P<0.001$). This pattern suggests a possible trade-off between pollen size and pollen number.

Total seed set decreased concordantly with the level of damage compared with control plants (Table 2) and, type of pollination (i.e. self or cross) contributed to the number of seeds produced. Maternal effects were not significant and there was not any detectable interaction with them.

Fewer seeds were produced by self-pollination than by cross-pollination (Table 3). Seeds from fruits produced by cross-pollinations did not show difference under mild herbivory treatments compared with undamaged plants, but plants under severe damage produced significantly less seeds than plants from the mild damage and control treatments (Table 3). Genotype was not a significant factor ($F=1.25$, $P=0.328$), but the

interaction between treatment and genotype was significant ($F= 2.96$, $P<0.001$), evidence of the influence of genotype on the number seeds produced from cross-pollinated flowers.

The total number of seeds produced was significantly lower in plants under severe damage compared with mild damage and undamaged plants ($F=77.02$, $P<0.001$).

The cost of tolerance to herbivory and the relationship between female and male components of fitness

Female success was measured as the average number of the total square root transformed seeds per genotype. Number of seeds produced by plants under the mild herbivory treatment was correlated with seeds produced by the same genotype when undamaged ($r=0.57$, $P=0.08$, Fig. 1a). I found a significant positive correlation between number of seeds from undamaged plants against number of seeds from plants with severe damage ($r=0.67$, $P=0.03$, Fig. 1b). This suggests that there is a tendency for genotypes that produced the most seeds under herbivory stress also produced the most seeds when they were undamaged. In other words, *Centrosema virginianum* apparently has strategies to tolerate severe herbivory with little cost in seed production.

Pollen number in plants with herbivory was also positively correlated with pollen number in undamaged plants ($r=0.67$, $P=0.03$; and $r=0.53$, $P=0.11$ Fig.2). This means that *Centrosema virginianum* presumably tolerates herbivory without cost in pollen production. Pollen size showed a similar pattern for severe herbivory but not for mild herbivory (Fig. 3). Means of pollen size between undamaged plants and plants with mild damage were not statistically different but means between pollen produced were

marginally different (Table 1). This may be a strong indicator of a trade-off between pollen size and pollen number when plants were under severe resource stress.

There was no association between female and male components of fitness for undamaged plants ($r=0.1$, $P=0.78$, Fig. 4). This concurs with the theory that female success is related to resource availability and male success is determined by genetics (Bertin 1982). If there is no resource differential (e.g. in uniform greenhouse conditions), I expect no association between both functions. For damaged plants there was a tendency towards a negative association between female and male success but this was not statistically significant ($r=-0.38$, $P=0.28$, Fig.5). This may indicate that independent of genotype, at the most severe herbivory treatment plants favored the male fitness component over the female fitness component.

Table 4 summarizes the tolerance to herbivory calculated for each individual fitness component. These data support the possible trade-off between pollen size and pollen number and indicate that *C. virginianum* has a negative female-male component relationship.

DISCUSSION

Female and male components

In this study, flower size decreased with increasing levels of herbivory. Many studies have demonstrated that pollinators choose larger flowers over smaller flowers (for references see Galen 2000), which evidently has an indirect effect in fitness.

Floral nectar works also as a vector to attract pollinators. In this study, differences of nectar concentration and volume between damaged and undamaged plants were not detected. It has been demonstrated that flower display and nectar can be controlled within-plant to improve intraspecific (male-male) and interspecific competition for pollinators (Crawley 1997). Different pollinators tend to respond to different signals to choose between flowers (Strauss et al. 1996). If nectar is cheap to produce in terms of resources, it is understandable that different genotypes might be able to equally allocate enough carbon to maintain quality and quantity of nectar.

It is common that flower size is categorized as a male fitness component in self-incompatible species because it promotes pollen carry over (Lehtilae and Strauss 1999). Flower size and rewards (e.g. nectar, pollen) in partially self-compatible species, such as *Centrosema virginianum*, may be associated with both male and female fitness because both potentially could affect visitation of pollinators and fruit set due to pollen limitation. Initially, I treated both flower size and nectar as important components for female and male functions. Theoretically, hermaphroditic self-compatible plant species may not be equisexual due to genotype or environmental factors (Horovitz 1978). My results concur with this statement because my data show that genotype played an important role in the response of flower size to foliar herbivory. Some undamaged plants, presumably with higher fitness, produced bigger flowers maintaining this advantage when they were damaged. This suggests that larger flowered individuals of *Centrosema virginianum* could better tolerate the effects of herbivory on floral display. In addition, flowers that were cross pollinated produced close to 50% more seeds than self pollinated, suggesting that if attractiveness to pollinators is negatively affected, the male function of the flowers

will be severely affected. Therefore, petal size and rewards directly affect the male function.

Damaged plants compared with undamaged plants produced less pollen. Although statistically significant, little is known about the biological consequences of producing between 9% (mild herbivory) to 20% (severe herbivory) less pollen than undamaged pollen for *Centrosema virginianum*. Studies in other species showed that pollen loads have a positive correlation with pollen tube growth which is also correlated with the genetic quality of the offspring (Niesenbaum 1996, 1999, Winsor et al. 2000). Deposition of large pollen loads in *Cucurbita pepo* promotes intense pollen competition and results in progeny with greater reproductive output (Stephenson et al. 1988). Therefore, reduction in pollen production could potentially result in lower quality offspring in *C. virginianum*.

Although the relationship between pollen size and pollen quality has been controversial, there is still the possibility that some limiting resources contained in pollen are critical to pollen tubes to successfully reach the ovary (Cruzan 1990). *Centrosema virginianum* pollen responded to herbivory in an exceptional manner. My data suggest that plants of *C. virginianum* overcompensated pollen size under severe herbivory levels. My results also suggest that *C. virginianum* experienced a trade-off between pollen number and pollen size under high levels of herbivory. Interspecific trade-offs have been reported between pollen number and pollen size in species of papilionaceous legumes (Vonhof and Harder 1995). Pollen production trade-offs among individuals of the same species as a compensatory strategy have not yet been reported.

Total seed set was strongly affected by severe herbivory. In contrast, ovule number differences among treatments may not be biologically meaningful. Interestingly, the proportions of seeds produced by cross pollinations and those from self pollinations were preserved, suggesting that *Centrosema virginianum* is allocating resources to mature double the amount of crossed seeds, even under resource stress.

The cost of tolerance to herbivory and the relationship between female and male components of fitness

This study showed that *Centrosema virginianum* is capable of tolerating mild levels of herbivory with no cost for the total fitness components measured. In general, damaged plants were able to tolerate 33% continuous leaf removal with only a 6% reduction in total fitness components. With mild defoliation, male components were marginally more affected than female components, but still no clear patterns were detected.

Plants experiencing severe herbivory (~66% leaf removal) tolerated damage quite well, experiencing only approximately 17% reduction in the total components of fitness measured during my experiment. However, male components of fitness were favored over female components of fitness. The average of total male fitness components was reduced by 6.1%, while the average of total female fitness components decreased 32.3%.

Female fitness components, in the currency of seed set, dropped to two-thirds the amount produced by undamaged plants. However, the correlation did not show cost in the tolerance of plants to severe damage for seed set. Strauss and Agrawal (1999) demonstrated that correlations between means of family fitness of damaged and undamaged plants may be obscured when there is across-family variance. Since genotype

was a significant factor in the seed production of self-seeds, variation in genotype responses may be masking costs of tolerance in the currency of seed set in this method of assessment.

Total male fitness components did not change among treatments. *Centrosema virginianum* was able to compensate for a decrease in pollen number with an increase in pollen size. *C. virginianum*'s strategy to tolerate severe herbivory exemplifies a trade-off between pollen number and size. Tolerance to herbivory data support this finding. Nevertheless, a true trade-off in male components of fitness on *C. virginianum* individuals after severe herbivory requires further study comparing the capacity of small and large pollen grains to sire seeds.

Although correlation analysis was not significant, I found a negative relationship between female and male reproductive success when plants were damaged. This strongly implies that gender specialization is related to factors beyond genetic characteristics. Both female and male components of fitness could vary yearly in natural populations of hermaphroditic plant species and this variation could be balanced by natural selection (Bertin 1982). Different levels of herbivory may then be affecting reproductive success, promoting changes in resource allocation, and sporadic events of extreme herbivory may direct adaptive responses of *C. virginianum* to pollinators through floral traits and rewards.

REFERENCES

- Agrawal, A. A., S. Y. Strauss, and M. J. Stout. 1999. Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. *Evolution* **53**:1093-1104.
- Analise-it Software Ltd. 2000. Analise-it, version 1.71. Analise-it Software, Ltd. England, UK.
- ArcViewGIS. 1999. Image Analysis Extension. ArcView GIS 3.2, ESRI. USA.
- Avila-Sakar, G., G. A. Krupnick, and A. G. Stephenson. 2001. Growth and resource allocation in *Cucurbita pepo* ssp. *texana*: Effects of fruit removal. *International Journal of Plant Sciences* **162**:1089-1095.
- Bertin, R. I. 1982. Paternity and fruit production in trumpet creeper (*Campsis radicans*). *The American Naturalist* **119**:694-709.
- Charlesworth, D., and B. Charlesworth. 1981. Allocation of resources to male and female functions in hermaphrodites. *Biology Journal of the Linnean Society* **15**:57-74.
- Cobb, N. S., R. T. Trotter Iii, and T. G. Whitham. 2002. Long-term sexual allocation in herbivore resistant and susceptible pinyon pine (*Pinus edulis*). *Oecologia* **130**:78-87.
- Crawley, M. J. 1983. *Herbivory. The Dynamics of Animal -Plant Interactions*. University of California Press, Berkeley.
- Crawley, M. J. 1997. *Plant Ecology*, 2nd edition. Blackwell Scientific Publications, London.
- Cruzan, M. B. 1990. Variation in pollen size, fertilization ability, and postpollination siting ability in *Erythronium grandiflorum*. *Evolution* **44**:843-856.
- Delph, L. F., M. H. Johannsson, and A. G. Stephenson. 1997. How environmental factors affect pollen performance: ecological and evolutionary perspectives. *Ecology* **78**:1632-1639.
- Faegri, K., and L. van der Pijl. 1979. *The principles of pollination ecology*. Pergamon Press, Oxford.

- Fineblum, W. L. a. M. D. R. 1995. Tradeoff between resistance and tolerance to herbivore damage in a morning glory. *Nature* **377**:517-520.
- Galen, C. 2000. High and dry: Drought stress, sex-allocation trade-offs, and selection on flower size in the alpine wildflower *Polemonium viscosum* (Polemoniaceae). *The American Naturalist* **156**:72-83.
- Horovitz, A. 1978. Is the hermaphrodite flowering plant equisexual? *American Journal of Botany* **65**:485-486.
- Irwin, R. E., and A. K. Brody. 1999. Nectar-robbing bumble bees reduce the fitness of *Ipomopsis aggregata* (Polemoniaceae). *Ecology* **80**:1703-1712.
- Isely, D. 1990. Vascular Flora of the Southeastern United States: Leguminosae (Fabaceae). The University of North Carolina Press, North Carolina.
- Kearns, C. A., and D. W. Inouye. 1993. Techniques for pollination biologists. University Press of Colorado, Niwot, Colorado.
- Krebs, C. J. 1989. *Ecological Methodology*. Harper and Row, Publishers, New York.
- Leege, L. M., and L. M. Wolfe. 2002. Do floral herbivores respond to variation in flower characteristics in *Gelsemium sempervirens* (Loganiaceae), a distylous vine? *American Journal of Botany* **89**:1270-1274.
- Lehtilae, K., and S. Y. Strauss. 1999. Effects of foliar herbivory on male and female reproductive traits of wild radish, *Raphanus raphanistrum*. *Ecology* **80**:116-124.
- Marquis, R. J. 1992. The selective impact of herbivores. University of Chicago Press, Chicago Illinois.
- Niesenbaum, R. A. 1996. Linking herbivory and pollination: Defoliation and selective fruit abortion in *Lindera benzoin*. *Ecology* **77**:2324-2331.
- Niesenbaum, R. A. 1999. The effects of pollen load size and donor diversity on pollen performance, selective abortion, and progeny vigor in *Mirabilis jalapa* (Nyctaginaceae). *American Journal of Botany* **86**:261-268.
- Niklas, K. J. 1997. The evolutionary biology of plants. The University of Chicago Press, Chicago and London.

- Proctor, M., P. Yeo, and A. Lack. 1996. The natural history of pollination. Timber Press, Portlan, Oregon.
- Rausher, M. D., K. Iwao, E. L. Simms, N. Ohsaki, and D. Hall. 1993. Induced resistance in *Ipomoea purpurea*. *Ecology* **74**:20-29.
- Schlichting, C. D., and B. Devlin. 1989. Male and female reproductive success in the hermaphroditic plant *Phlox drummondii*. *The American Naturalist* **133**:212-227.
- Statistical Graphics Corp. 2001. Statgraphics Plus version 5.1. Manugistics, Inc. Rockville, MD.
- Stephenson, A. G., J. A. Winsor, and C. D. Schlichting. 1988. Evidence of non-random fertilization in the common zucchini, *Cucurbita pepo*. Pages 502 pp. in M. Cresti, P. Gori, and E. Pacini, editors. Sexual Reproduction in Higher Plants. Springer-Verlag, New York, U.S.
- Strauss, S. Y. 1997. Floral characters link herbivores, pollinators, and plant fitness. *Ecology* **78**:1640-1645.
- Strauss, S. Y., and A. A. Agrawal. 1999. Ecology and evolution of plant tolerance to herbivory. *TRENDS in Ecology and Evolution* **14**:179-185.
- Strauss, S. Y., J. K. Conner, and S. L. Rush. 1996. Foliar herbivory affects floral characters and plant attractiveness to pollinators: implications for male and female plant fitness. *The American Naturalist* **147**:1098-1107.
- Strauss, S. Y., D. H. Siemens, M. B. Decher and T. Mitchell-Olds. 1999. Ecological costs of plants resistance to herbivores in the currency of pollination. *Evolution* **53**:1105-1113.
- Vonhof, M. J., and L. D. Harder. 1995. Size-number trade-off and pollen production by Papilionaceous legumes. *American Journal of Botany* **82**:230-238.
- Winsor, J. A., S. Peretz, and A. G. Stephenson. 2000. Pollen competition in a natural population of *Cucurbita foetidissima* (Cucurbitaceae). *American Journal of Botany* **87**:527-532.

Genotype	Herbivory Treatment		
	CLONE 1	CLONE 2	CLONE 3
1	U	SH	MH
2	SH	MH	U
3	SH	MH	U
4	U	MH	SH
5	MH	SH	U
6	SH	U	MH
7	U	SH	MH
8	MH	U	SH
9	MH	SH	U
10	SH	U	SH

TABLE 1. Experimental approach: randomized complete block design with defoliation treatment as the fixed factor. U=undamaged; MH=one third or mild damage; SH=two thirds or severe damage.

Fitness component	Undamaged	Mild Herbivory	Severe Herbivory	Source	df	F	P
Flower Size (cm) N=450	3.75±0.027 ^a	3.29 ±0.029 ^b	3.05 ±0.024 ^c	Treatment Genotype (block) Treatment*Genotype	2 9 18	221.07 18.56 19.926	<0.001 <0.001 <0.001
Nectar (sugar concentration) N=450	39.89 ±1.089	40.72 ±1.09	39.83 ±1.09	Treatment Genotype (block) Treatment*Genotype	2 9 18	0.47 1.13 0.85	0.628 0.341 0.634
Pollen Number N=450	3723.46 ± 82.78 ^a	3355.16 ± 82.78 ^b	3011.74 ± 82.78 ^b	Treatment Genotype (block) Treatment*Genotype	2 9 18	18.49 1.09 0.74	<0.001 0.374 0.773
Pollen Size (µm ²) N=450	1984.41±25.84 ^a	1961.09 ±25.82 ^a	2371.78 ±26.05 ^b	Treatment Genotype (block) Treatment*Genotype	2 9 18	65.95 1.27 0.81	<0.001 0.252 0.692

TABLE 2. Analysis of variance results of male fitness components. Because my experiment was a randomized complete block design, the variation between blocks is removed from the experimental error to increase the precision of the experiment (Krebs, 1989). ANOVA was performed on ln-transformed data, except for pollen number. Data given are actual means ± 1SE per treatment across genotype.

Fitness component	Undamaged	Mild Herbivory	Severe Herbivory	Source	df	F	P
Seeds produced (self+cross) N=600	13.22 ± 4.70 ^a	12.46 ± 5.02 ^a	4.85 ± 2.78 ^b	Treatment	2	296.95	<0.001
				Pollination	1	131.14	<0.001
				Genotype	9	2.19	0.167
				Treatment*Pollination	2	5.99	0.010
				Treatment*Genotype	18	.82	0.663
				T*G*P	18	3.42	<0.001
Seeds produced (cross) N=300	17.10 ± 2.19 ^a	16.61 ± 2.70 ^a	6.63 ± 2.33 ^b	Treatment	2	237.14	<0.001
				Genotype	9	1.25	0.328
				Treatment*Genotype	18	2.96	<0.001
Seeds produced (self) N=300	9.33 ± 3.02 ^a	8.31 ± 2.94 ^b	3.34 ± 2.34 ^c	Treatment	2	77.02	<0.001
				Genotype	9	5.12	0.002
				Treatment*Genotype	18	3.20	<0.001
Ovules N=600	20.03 ± 1.36 ^a	19.81 ± 1.37 ^b	19.80 ± 1.28 ^b	Treatment	2	6.42	0.008
				Genotype	9	1.36	0.275
				Treatment*Genotype	18	1.61	0.053

TABLE 3. Three-way analysis of variance results of female fitness components. ANOVA was performed on square root-transformed data. Data given are actual means ± 1SE per treatment/pollination across genotype. I included two-way ANOVA results for seeds produced from self and cross-pollination separately.

Fitness Components	% Tolerance to mild herbivory (one third of leaf removal)	% Tolerance to severe herbivory (two thirds of leaf removal)
Flower Size	87.7	81.3
Pollen Number	90.1	80.8
Pollen Size	98.8	119.5
Seeds Produced (cross+self)	94.2	36.6
Ovule Number	98.9	98.8

TABLE 4. Tolerance to herbivory in five fitness components. Tolerance for each trait was calculated by dividing the mean of the trait that resulted from damage treatments by the mean of the trait that resulted of undamaged plants.

(a)

(b)

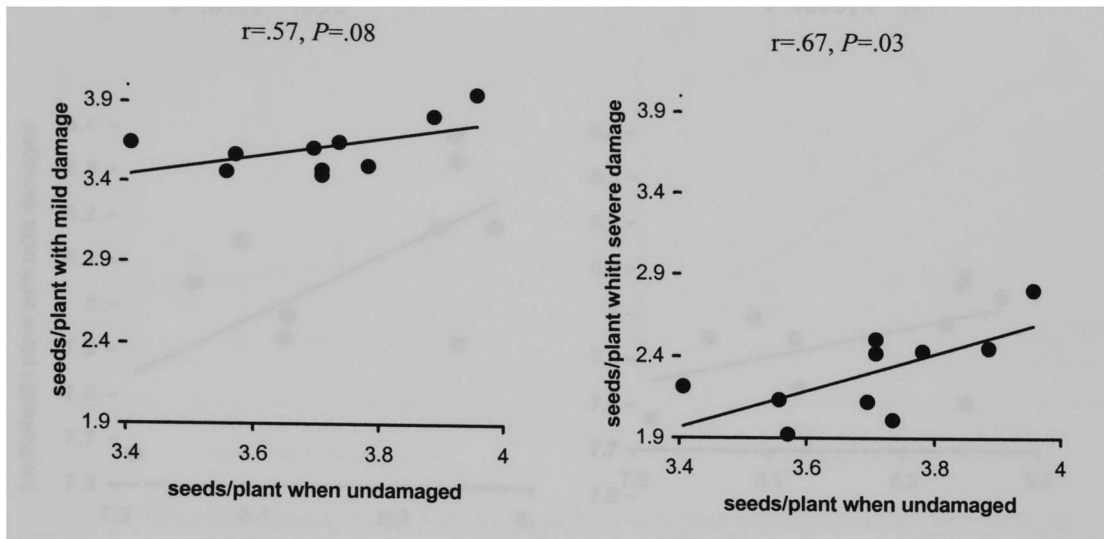


Fig. 1. The relationship between mean genotype female fitness (seed set) between damaged with mild herbivory (a), severe herbivory (b) and undamaged plants. Each point represents the average of seeds produced by genotype. Pearson's correlation was done using square-root of seeds + 0.5-transformed. Negative association indicates a cost of tolerance (Agrawal et al., 1999). The regression line is provided only to show the nature of the relationship, with no causation implied.

(a)

(b)

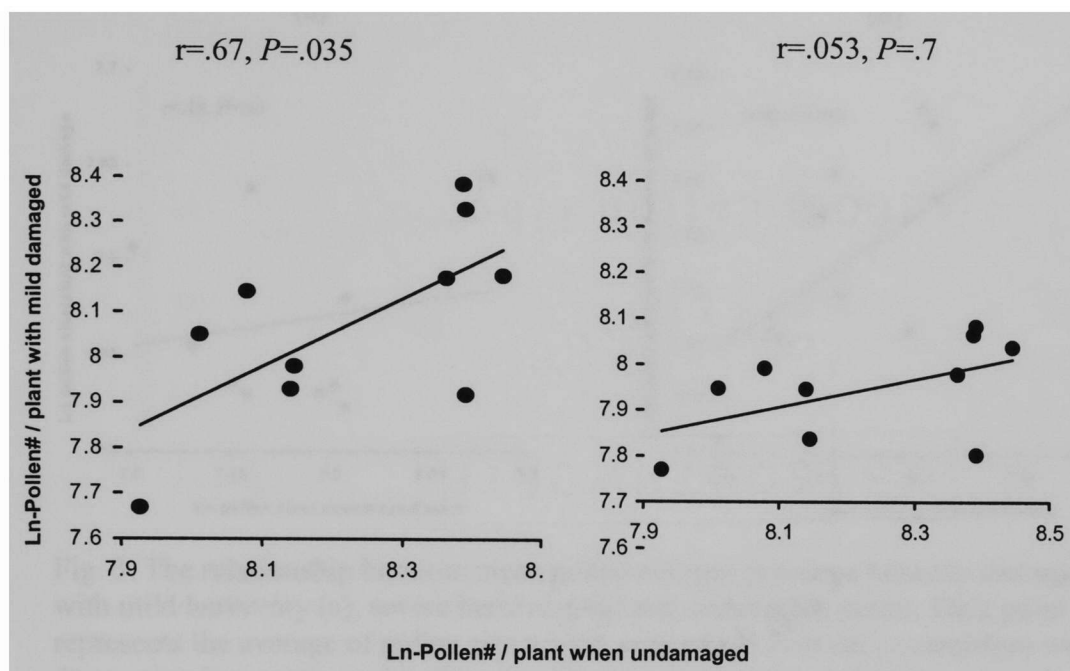


Fig. 2. The relationship between mean male fitness per genotype (pollen produced) between damaged with mild herbivory (a), severe herbivory (b) and undamaged plants. Each point represents the average of pollen produced within a genotype. Pearson's correlation was done using ln-transformed pollen number. Negative association indicates a cost of tolerance (Agrawal et al., 1999). The regression line is provided only to show the nature of the relationship, with no causation implied.

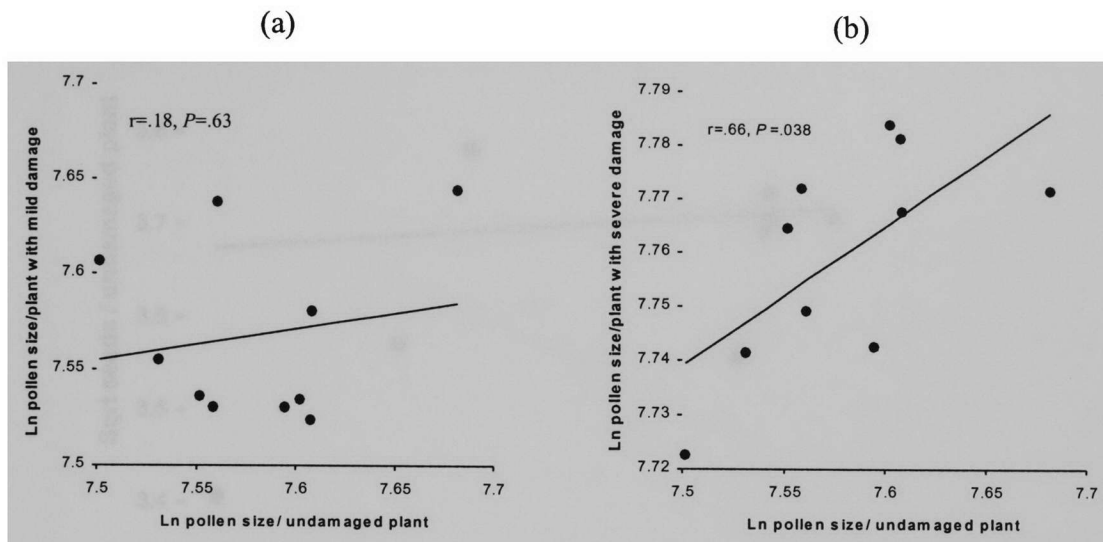


Fig. 3. The relationship between mean pollen size per genotype between damaged with mild herbivory (a), severe herbivory (b) and undamaged plants. Each point represents the average of pollen size within a genotype. Pearson's correlation was done using ln-transformed pollen size. Negative association indicates a cost of tolerance (Agrawal et al., 1999). The regression line is provided only to show the nature of the relationship, with no causation implied.

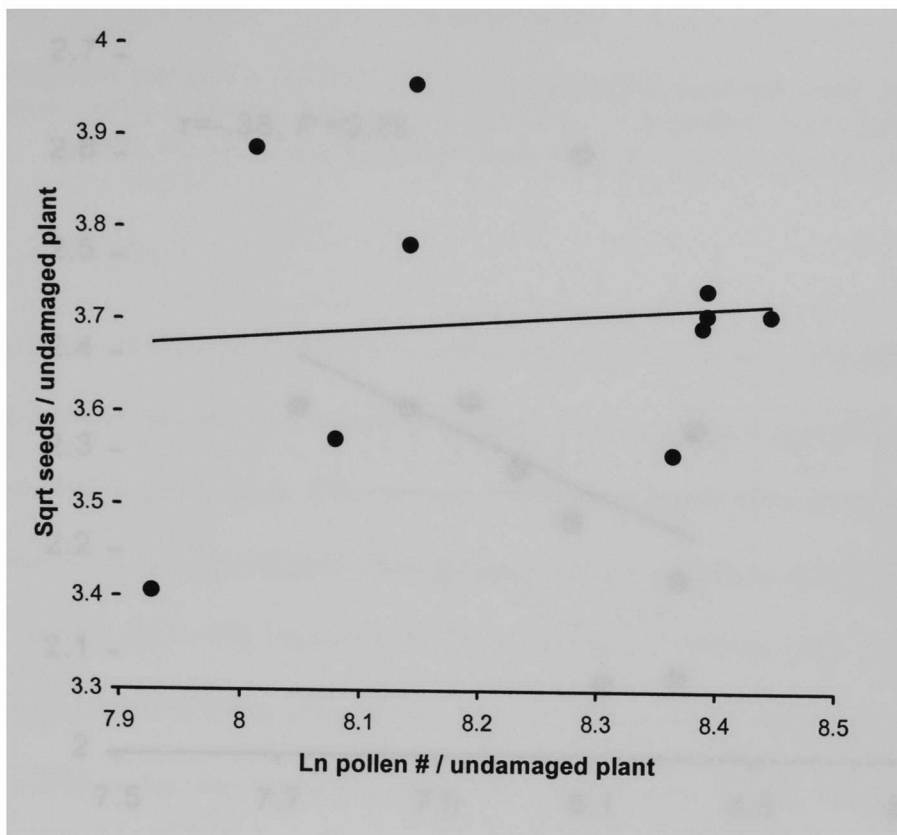


Fig. 4. There is not apparent association between these female and male components for undamaged plants. Pearson's correlation was done using ln-transformed pollen number and seeds produced square root-transformed. The regression line is provided only to show the nature of the relationship.

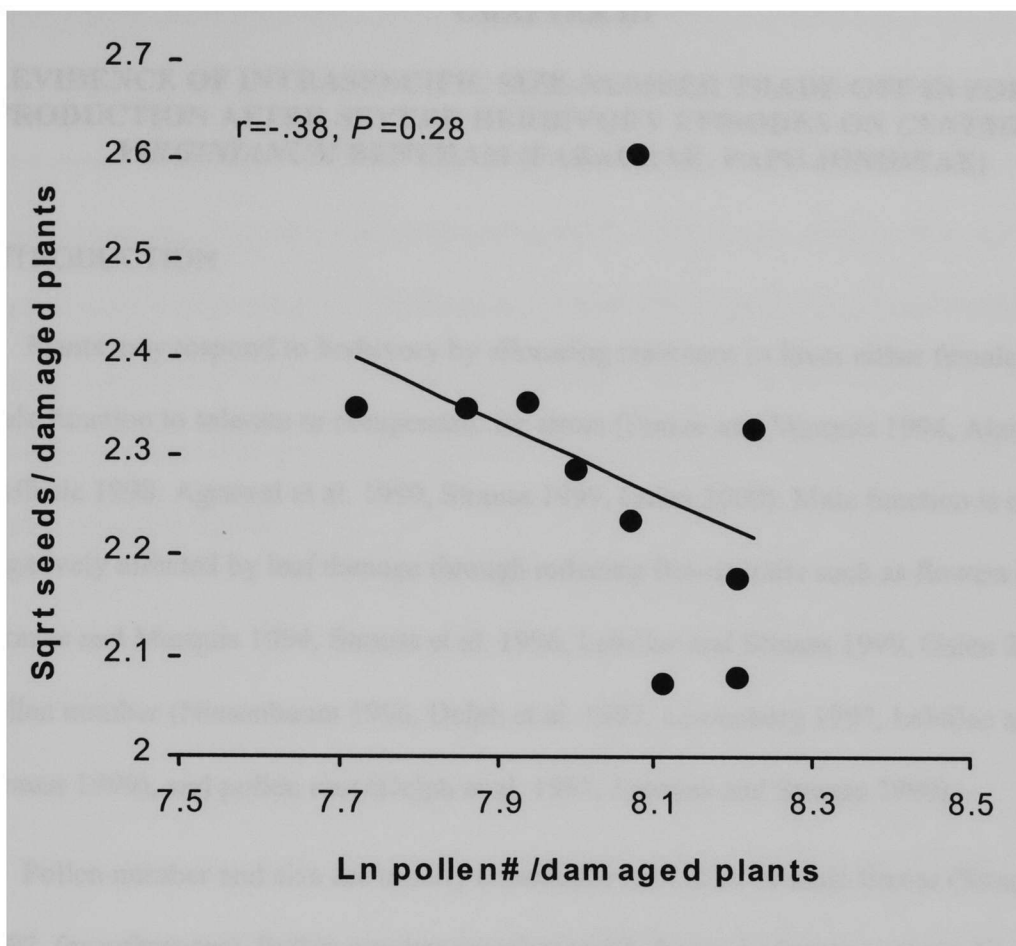


Fig. 5. Relationship between female and male reproductive success for severe by damaged plants. Pearson's correlation was done using ln-transformed pollen number and seeds produced square root-transformed. The regression line is provided only to show the nature of the relationship.

CHAPTER III

EVIDENCE OF INTRASPECIFIC SIZE-NUMBER TRADE-OFF IN POLLEN PRODUCTION AFTER SEVERE HERBIVORY EPISODES ON *CENTROSEMA VIRGINIANUM* BENTHAM (FABACEAE: PAPILIONIDAE)

INTRODUCTION

Plants may respond to herbivory by allocating resources to favor either female or male function to tolerate or compensate for stress (Frazee and Marquis 1994, Aizen and Raffaele 1998, Agrawal et al. 1999, Strauss 1999, Galen 2000). Male function is often negatively affected by leaf damage through reducing flower traits such as flowers size (Frazee and Marquis 1994, Strauss et al. 1996, Lehtilae and Strauss 1999, Galen 2000), pollen number (Niesenbaum 1996, Delph et al. 1997, Lowenberg 1997, Lehtilae and Strauss 1999), and pollen size (Delph et al. 1997, Lehtilae and Strauss 1999).

Pollen number and size are usually considered indicators of male fitness (Strauss 1997, for references). Pollen number correlates with the load of grains potentially deposited on stigmas. Progeny produced from large pollen loads have greater vigor and/or have higher reproductive output as adults than those from small pollen loads (Windsor 1987, Stephenson et al. 1988, Winsor et al. 2000). On the other hand, studies have suggest that higher nutrient content (resources) in pollen grains could result in a greater ability to sire seeds, suggesting that bigger grains are associated with better post-pollination performance (Aizen and Raffaele 1998, for references). All of these studies have shown that both the number and size of pollen decreases after herbivory episodes.

Results from previous experiments (Chapter 2 of this dissertation) showed a difference in pollen size between damaged and undamaged *Centrosema virginianum*

plants. Plants under 66% leaf removal treatments produced less pollen but bigger pollen grains than undamaged plants. In this study, my goal was to verify whether the *Centrosema virginianum* response to severe foliar damage was consistent with previous experiments, and if so, to compare the siring ability of large and small pollen. My experimental design compared differences in the ability of pollen produced by severely damaged plants with control or undamaged plants to sire seeds. Ultimately, I hoped to provide evidence of a number-size trade-off for pollen produced by *C. virginianum* under severe herbivory stress.

METHODS

Three plants (genotypes) remained from an earlier experiment investigating the effects of defoliation on floral characters (Cardel, unpublished data). To compare siring ability of pollen grains from undamaged plants (smaller grains) and damaged plants (bigger grains), and test any possible trade-off between pollen produced and pollen size, I propagated the study plants from the remaining genotypes by cuttings. Once two ramets per genet were fully established independently in eleven-liters pots, identical amounts of all-purpose fertilizer 15:30:15 (Miraclegrow^{TM}) were applied. I clipped two thirds of every leaf from one ramet of each of the three genets, leaving the other three ramets as controls (no manipulation, Fig.1).

To verify that pollen from damaged plants was larger, I collected undehisced anthers from three flowers from each plant per treatment. Each set of anthers (i.e. a flower) was collected every third day and from different heights within a plant.

Each set of anthers per flower was placed in Eppendorf tubes until anthers dehisced releasing the pollen grains. I then added Carnoy's fixative (3:1 absolute ethanol: glacial acetic acid) to all tubes. After an hour, the tubes were centrifuged (mini centrifuge Costar, USA) to sediment out the pollen grains; the Carnoy's solution was removed and replaced with 70% ethanol to store samples until processing was done. To process the samples, all ethanol was replaced with 0.1 ml of lactophenol-aniline blue solution (Kearns and Inouye 1993). Each tube was shaken in a vibrator (FisherVortex Genie-2; Fisher Scientific, Pittsburgh, PA) for 15 seconds. Using a Pasteur pipette I placed a portion of the liquid in a hemacytometer, filling both wells of three hemacytometers with enough liquid to cover the entire grid, having a total of 6 replicates per sample. All the pollen grains on the four large squares of the grid were counted using a light microscope. I calculated the total number of pollen grains as described in Kearns and Inouye (1993). (Fig. 2). The area of a total of twenty five randomly selected pollen grains in the six pictures was calculated using ArcView GIS Image Analysis Extension (ArcViewGIS 1999). The grid of the hemacytometer was used as a reference to calculate the real area of the fixed pollen. Two SEM pictures of pollen from undamaged plants were used to verify if measurements provided by this method were representative of the pollen size (projected area).

Measurements of pollen number and size were verified for equality of error variances with Levene's test using the SPSS statistical package (SPSS Inc. 2000). Normality was checked with Shapiro-Wilks test (Analyse-it Software Ltd. 2000). Two-way ANOVA was used to compare pollen produced and grain size. Treatment was used as the fixed factor and genotype was treated as a random factor (SPSS Inc. 2000). I square-root

transformed the pollen grain counts with equation 1 to stabilize the variance and to meet ANOVA assumptions (Krebs 1989).

$$\text{pollen grains}' = \sqrt{(\text{pollen grains}) + (3/8)} \quad (1)$$

Three different genotypes (unmanipulated plants), grown in identical conditions (pots and fertilizer), were used as pollen recipients (maternal plants). I performed three hand cross-pollinations per plant per treatment using as pollen donors (paternal plants) each of the experimental plants mentioned above. Each possible combination between pollen donor plants and pollen recipient plants will be referred as a cross (Fig. 1). Flowers were emasculated prior to pollination. All the pollen grains from two to three flowers from donor plants were placed on the receptive part of the stigma of recipient plants until the stigma surface was completely covered. Each pollination per plant was spaced at least two days apart to minimize any abortion due to selectivity of resource allocation from the recipient plant (Niesenbaum 1996). A total of nine crosses with three replicates were performed.

Fruits were collected as soon as they reached maturity. I compared the number of seeds produced and average seed weight per plant per treatment with two replicates using two-way ANOVA. Treatment was used as the fixed factor and cross was treated as a random factor (SPSS Inc. 2000). I square root transformed the seed counts to stabilize the variance and to meet ANOVA assumptions (Krebs 1989).

RESULTS

Flowers from damaged plants produced fewer and larger pollen grains compared with undamaged plants (Fig. 3, Fig.4). ANOVA results showed significant differences between treatments but differences among genotypes were not significant (Table 1). These results concurred with results presented in Chapter 2, suggesting consistency in the response of *Centrosema virginianum* to herbivory.

Results from the hand pollination experiment showed that larger pollen grains yielded fruit with significantly more seeds than smaller pollen grains from undamaged plants (18.7 ± 1.52 vs. 17.7 ± 1.50 ; $F=10.49$, $P=0.01$; Fig. 5). Although the statistical analysis was significant, the biological impact of producing a single seed more on average may not be that substantial. Nevertheless, for perennial plants this difference may have a long-term positive effect.

The average weight of seeds produced by damaged and undamaged plants did not differ (Table 2). Type of cross was statistically significant for both seeds set and seed mass (Table 2). This may be due to maternal effects related to compatibility among genotypes and/or male-male competition in the ovary.

DISCUSSION

Studies have shown that environmental factors may affect pollen size, which in turn is related to pollen performance (Lau 1995, Quesada 1995, Mutikainen 1996, Delph et al. 1997, Aizen and Raffaele 1998, Kelly et al. 2002). If that is true for *Centrosema virginianum*, my study provides some of the first data showing a trade-off between pollen number and pollen size. My data showed evidence of a change in resource allocation,

illustrated by the consistency of the responses of pollen characteristics to herbivory by most genotypes (number vs. size). This suggests a trade-off between pollen number and size under extreme conditions of resource limitation.

Presently, the importance of pollen size to pollen performance after pollination is controversial (Aizen and Raffaele 1998, for review). Siring ability is ultimately one of the best indicators of pollen performance. My results indicated that resource limitation by herbivory directly affected pollen grain size in *C. virginianum* (Chapter 2; this study), which suggests that pollen size reflects resource content. Therefore, resource allocation in pollen grains was important for determining their siring success.

Both environmental and genetic influences on pollen contribute to the role of herbivory on the male fitness component of a species. My results confirmed that growing conditions of parental plants affect pollen performance. However, they did not reveal how much variation is genetic. Crossing pairs showed variation in seed set. This may be an indicator of such genetic influence but I was unable to evaluate it.

In conclusion, my findings support the idea that there is a trade-off in the number of pollen produced and the grain size under severe herbivory episodes, a finding not previously reported to my knowledge. It is possible that pollen tube growth may be mediated by the style and pollen competition with different donors pollinating simultaneously (Cruzan 1990). However, these results indicated that donors might have influence on their fertilization success and that influence may be attributed to pollen grain content.

REFERENCES

- Agrawal, A. A., S. Y. Strauss, and M. J. Stout. 1999. Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. *Evolution* **53**:1093-1104.
- Aizen, M. A., and E. Raffaele. 1998. Flowering-shoot defoliation affects pollen grain size and postpollination pollen performance in *Alstroemeria aurea*. *Ecology* **79**:2133-2142.
- Analyse-it Software Ltd. 2000. Analyse-it, version 1.71. Ltd. England, UK.
- ArcViewGIS. 1999. Image Analysis Extension. ArcView GIS 3.2. ESRI, USA.
- Cruzan, M. B. 1990. Variation in pollen size, fertilization ability, and postpollination siting ability in *Erythronium grandiflorum*. *Evolution* **44**:843-856.
- Delph, L. F., M. H. Johannsson, and A. G. Stephenson. 1997. How Enviromental Factors Affect Pollen Performance: Ecological and Evolutionary Perspectives. *Ecology* **78**:1632-1639.
- Frazee, J. E., and R. J. Marquis. 1994. Environmental contribution to floral trait variation in *Chamaecrista fasciculata* (Fabaceae: Caesalpinoideae). *American Journal of Botany* **81**:206-215.
- Galen, C. 2000. High and dry: Drought stress, sex-allocation trade-offs, and selection on flower size in the alpine wildflower *Polemonium viscosum* (Polemoniaceae). *The American Naturalist* **156**:72-83.
- Kearns, C. A., and D. W. Inouye. 1993. Techniques for pollination biologists. University Press of Colorado, Niwot, Colorado.
- Kelly, J. K., A. Rasch, and S. Kalisz. 2002. A Method to Estimate Pollen Viability from Pollen Size Variation. *American Journal of Botany* **89**:1021-1023.
- Krebs, C. J. 1989. *Ecological Methodology*. Harper and Row, Publishers, New York.
- Lau, T. C., X. Lu, R. T. Koide and A. G. Stephenson. 1995. Effects of soil fertility and mycorrhizal infection on pollen production and pollen grain size of *Cucurbita pepo* (Cucurbitaceae). *Plant Cell and Environment* **18**:169-177.

- Lehtilae, K., and S. Y. Strauss. 1999. Effects of foliar herbivory on male and female reproductive traits of wild radish, *Raphanus raphanistrum*. *Ecology* **80**:116-124.
- Lowenberg, G. J. 1997. Effects of floral herbivory, limited pollination, and intrinsic plant characteristics on phenotypic gender in *Sanicula arctopoides*. *Oecologia* **109**:279-285.
- Mutikainen, P. a. D. L. F. 1996. Effects of herbivory on male reproductive success in plants. *Oikos* **75**:353-358.
- Niesenbaum, R. A. 1996. Linking herbivory and pollination: Defoliation and selective fruit abortion in *Lindera benzoin*. *Ecology* **77**:2324-2331.
- Quesada, M., K. Bollman and A. G. Stephenson. 1995. Leaf damage decreases pollen production and hinders pollen performance in *Cucurbita texana*. *Ecology* **76**:437-443.
- SPSS Inc. 2000. SPSS for Windows. Version 11.5. U.S.
- Stephenson, A. G., J. A. Winsor, and C. D. Schlichting. 1988. Evidence of non-random fertilization in the common zucchini, *Cucurbita pepo*. Pages 502 pp. in M. Cresti, P. Gori, and E. Pacini, editors. Sexual Reproduction in Higher Plants. Springer-Verlag, New York, U.S.
- Strauss, S. Y. 1997. Floral characters link herbivores, pollinators, and plant fitness. *Ecology* **78**:1640-1645.
- Strauss, S. Y., J. K. Conner, and S. L. Rush. 1996. Foliar herbivory affects floral characters and plant attractiveness to pollinators: implications for male and female plant fitness. *The American Naturalist* **147**:1098-1107.
- Strauss, S. Y., D. H. Siemens, M. B. Decher and T. Mitchell-Olds. 1999. Ecological costs of plants resistance to herbivores in the currency of pollination. *Evolution* **53**:1105-1113.
- Windsor, J. A., L. E. Davis and A. G. Stephenson. 1987. The relationship between pollen load and fruit maturation and the effect of pollen load on offspring vigor in *Cucurbita pepo*. *American Naturalist* **129**:643-656.
- Winsor, J. A., S. Peretz, and A. G. Stephenson. 2000. Pollen competition in a natural population of *Cucurbita foetidissima* (Cucurbitaceae). *American Journal of Botany* **87**:527-532.

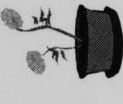
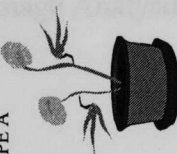
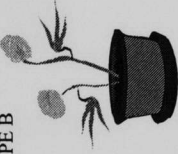
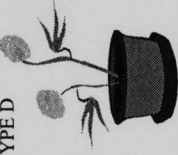
Paternal plant or pollen donor	Maternal plant or pollen recipient		
GENOTYPE 1 	GENOTYPE 1 UNDAMAGED-DAMAGED 	GENOTYPE 2 UNDAMAGED-DAMAGED 	GENOTYPE 3 UNDAMAGED-DAMAGED 
GENOTYPE A 	D1A 3 POLLINATIONS U1A 3 POLLINATIONS	D2A 3 POLLINATIONS U2A 3 POLLINATIONS	D3A 3 POLLINATIONS U3A 3 POLLINATIONS
GENOTYPE B 	D1B 3 POLLINATIONS U1B 3 POLLINATIONS	D2B 3 POLLINATIONS U2B 3 POLLINATIONS	D3B 3 POLLINATIONS U3B 3 POLLINATIONS
GENOTYPE D 	D1C 3 POLLINATIONS U1C 3 POLLINATIONS	D2C 3 POLLINATIONS U2C 3 POLLINATIONS	D3C 3 POLLINATIONS U3C 3 POLLINATIONS

Fig. 1. Experimental design of hand pollinations to compare siring ability between small and big pollen grains. Three genotypes (1, 2, 3) were used as pollen donors with two treatments: undamaged and damaged (two thirds of each leaf removed). Recipients are different genotypes (A, B, C). Three hand pollinations per plant per treatment were performed (referred in text as cross).

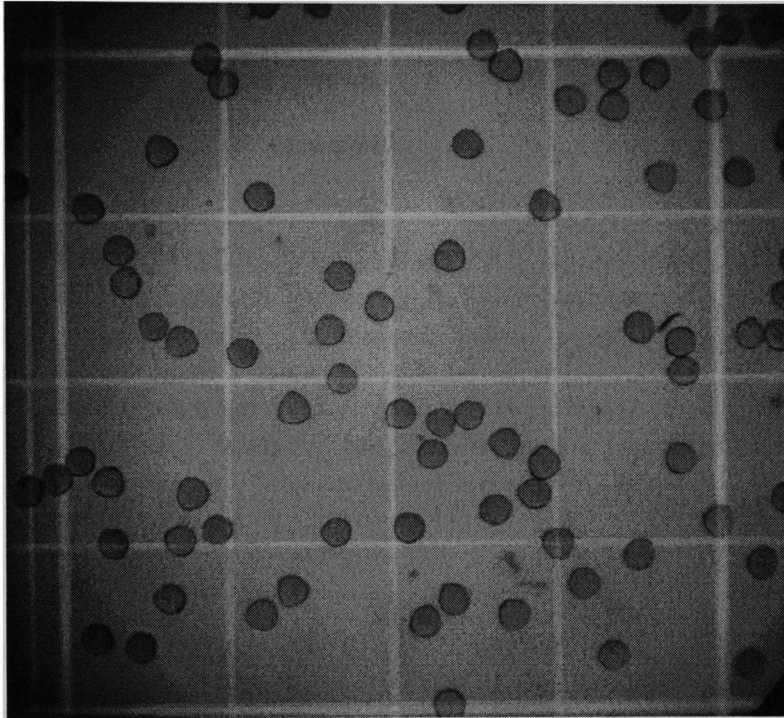


Fig. 2. Digital picture of *Centrosema virginianum* pollen used to measure pollen size with ArcView, Image Analysis.

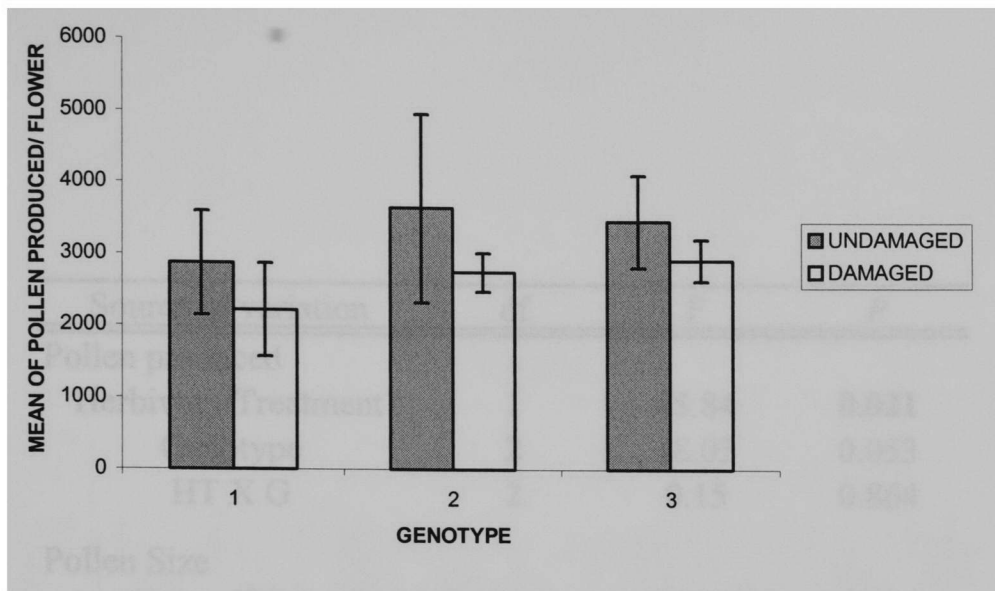


Fig. 3. Mean ($\pm 1SD$) pollen number per flower produced by undamaged and damaged plants. The number assigned to each genotype was arbitrary. Undamaged plants produced more pollen grains than damaged plants ($F=45.8$, $P=0.021$). Differences between genotypes were not significant ($F=18.0$, $P=0.053$). Statistical analysis was performed over transformed data (see text).

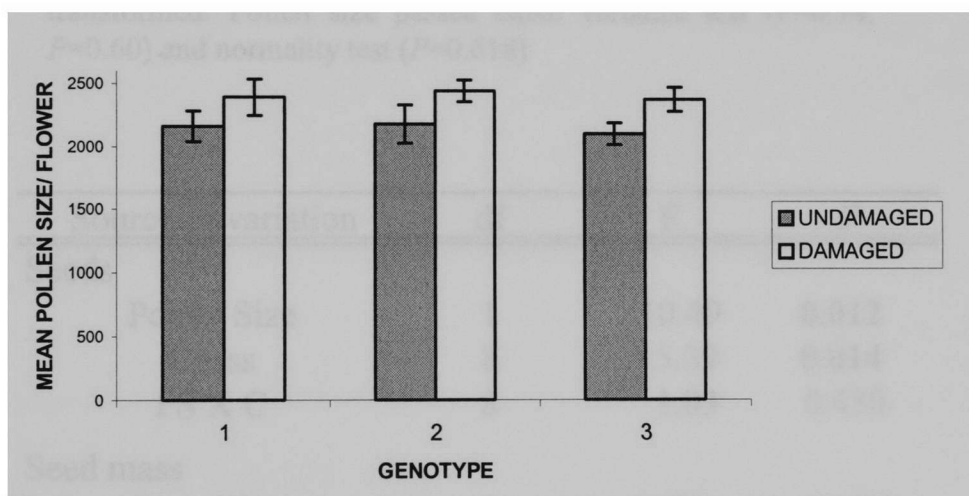


Fig. 4. Mean ($\pm 1SE$) pollen size (area projected, μm^2) per flower produced by undamaged and damaged plants. The number assigned to each genotype was arbitrary. Pollen grains produced by damaged plants were bigger than pollen grains produced by undamaged plants ($F=377.8$, $P=0.003$). Differences between genotypes were not significant ($F=9.6$, $P=0.9$).

Source of variation	df	F	P
Pollen produced			
Herbivory Treatment	1	45.84	0.021
Genotype	2	18.03	0.053
HT X G	2	0.15	0.864
Pollen Size			
Herbivory Treatment	1	377.83	0.003
Genotype	2	9.63	0.094
HT X G	2	0.09	0.912

TABLE 1. Results of two-way ANOVA on pollen produced and pollen size. Data from pollen produced were square-root transformed. Pollen size passed equal variance test ($F=0.74$, $P=0.60$) and normality test ($P=0.618$)

Source of variation	df	F	P
Seeds			
Pollen Size	1	10.49	0.012
Cross	8	5.39	0.014
PS X C	8	1.03	0.430
Seed mass			
Pollen Size	1	0.522	0.491
Cross	8	106.28	<0.001
PS X C	8	0.255	0.976

TABLE 2. Results of two-way ANOVA on seeds produced and seed mass. Data from seeds produced were square root ($x+3/8$)-transformed and seed mass data was ln-transformed to meet ANOVA assumptions.

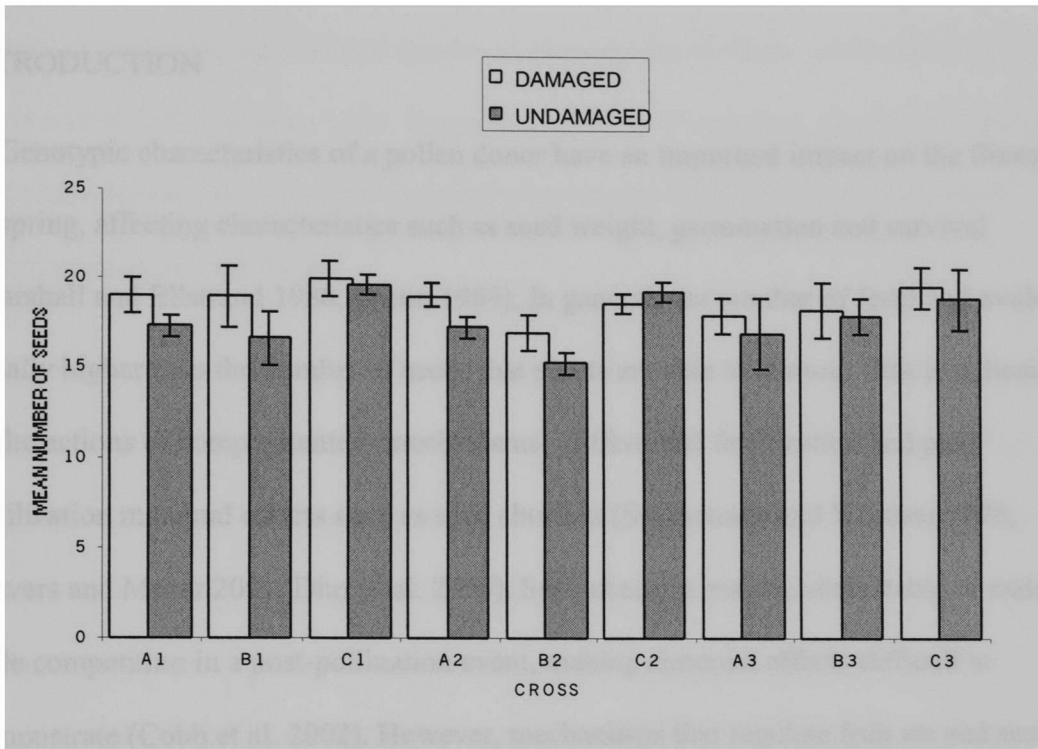


Fig. 5. Mean (± 1 SD) number of seeds produced per cross by undamaged and damaged plants. Statistical analysis was performed on transformed data (see text).

CHAPTER IV

PATTERNS OF SEED ABORTION IN RESPONSE TO LEAF HERBIVORY IN A PERENNIAL LEGUME HERB *CENTROSEMA VIRGINIANUM* BENTHAM (FABACEAE: PAPILIONOIDEAE)

INTRODUCTION

Genotypic characteristics of a pollen donor have an important impact on the fitness of offspring, affecting characteristics such as seed weight, germination and survival (Marshall and Ellstrand 1988, Bertin 1989). In general, the number of fertilized ovules is usually higher than the number of seeds that plants are able to mature. This is indicative of the actions of complementary mechanisms: differential fertilization and post-fertilization maternal effects such as seed abortion (Stephenson and Windsor 1986, Travers and Mazer 2001, Diaz et al. 2003). Seed abortion may be attributable to male-male competition in a post-pollination event, making maternal effects difficult to demonstrate (Cobb et al. 2002). However, mechanisms that regulate fruit set and seed mass are clearly under maternal influence as well (Nakamura 1968, Stephenson and Bertin 1983, Lee and Bazzaz 1986).

Under resource limitation, selective mating by maternal plants may be represented by abortion patterns of fruits and seeds. If this selective abortion occurs, plants may be capable of increasing progeny fitness by adjusting reproductive allocation (Koptur et al. 1996, Marshall and Oliveras 2001, Shaner and Marshall 2003) and sex allocation (Avila-Sakar et al. 2001, Kudo et al. 2001).

Herbivory decreases resources available to plants by reducing photosynthetic tissue and presumably promoting shifts in resource allocation (Crawley 1983). A recent study

demonstrated that herbivory in maternal plants of wild radish grown in a greenhouse negatively affected components of progeny fitness, especially increasing seed abortion and seed characteristics (Agrawal 2001). Other studies of leaf herbivory effects on legume reproduction showed that species of *Vicia* produced fewer seeds after defoliation (Brown et al. 1987, Nielsen 1990, Koptur et al. 1996). If herbivory promotes abortion, it may also influence the degree of maternal plant selectivity aborting certain seeds to optimize resources available.

It is well known that fertilization is nonrandom for some species (Stephenson and Windsor 1986, Stephenson et al. 1988, Winsor et al. 2000, Avila-Sakar et al. 2001). Preferred regions of fertilization within fruit are related to higher vigor of seedlings, which in turn is related to higher fitness (Stephenson et al. 1988). If similar patterns apply to the mechanisms of selective seed abortion, detailed knowledge of both (1) the frequency of abortion in different regions of the fruit and (2) the seed characteristics of those regions will be required to prove that plants chose the quality of their progeny to optimize the use of resources. I suggest that if fertilization is position-dependent, position-dependent seed abortion within fruits strongly indicates a choice by maternal plants to favor certain offspring genotypes.

I conducted an experiment to test how herbivory influenced seed abortion between self-pollinated and cross-pollinated plants. I analyzed post-fertilization patterns of seed abortion when maternal plants had different amounts of leaf area removed. I compared how resource allocation in different regions within fruits changed under these artificial herbivory treatments. A change in the ability of the same pollen donor to sire seeds on

damaged maternal plants compared with undamaged plants indicates that higher selectivity of abortion occurs under resource limitation.

METHODS

The Plant

Centrosema virginianum Benth (Fabaceae: Papilionideae) is a perennial vine that bears trifoliate leaves. Pods produce an average of 19 seeds when flowers are cross-pollinated and 13 seeds when self-pollination occurs (Chapter 1). Seeds are ballistically projected from pods by sudden separation of the legume valves along both sutures. *C. virginianum* occurs in pine rockland habitats in southern Florida and is widely distributed from the southern United States to the northern regions of South America (Isely 1990). Natural levels of herbivory range from 1% to more than 60% herbivory in individual plants (chapter 1). *C. virginianum* provides several advantages for this study: 1) it is self-compatible, which allowed comparisons of fertilization success between crossed and self-pollinated fruits; 2) flowers are not capable of automatic self-pollination, which facilitated controlled hand-pollination treatments; 3) it produces flowers year-round in the greenhouse and it is easy to propagate from cuttings; 4) it produces pods in which is easy to distinguish early aborted seeds (or unfertilized ovules) and late aborted seeds.

Main Experiment

I selected 15 genotypes maternal plants. A genotype number was assigned to each plant. Genets were propagated by cuttings to have four replicates of each genotype. Once plants were fully established in the Florida International University (FIU) greenhouse, I randomly applied four herbivory treatments by partially clipping leaf tissue using scissors

to the assigned level. The treatments were: zero herbivory (control); ~33% herbivory (one third of each leaflet removed); 50% (half of each leaflet removed); ~66% (two thirds of each leaflet removed). Clipping treatments were applied throughout the experiment to every leaf produced after it fully expanded.

Five unrelated plants were grown in the FIU greenhouse and used as pollen donors for xenogamous cross-pollinations. Pollen from at least three flowers from plant donors was mixed and used to perform cross-pollinations. Self- pollinations were done using at least three flowers from the same maternal plant. The first 15 fruits produced were collected from each plant per cross per treatment as soon as they reached maturity. At least one of each self and cross hand pollination was done every other day. Henceforth, fruits and seeds produced by self-pollination and cross-pollination will be referred as S-fruits or S-seeds and X-fruits or X-seeds, respectively.

Each fruit collected was divided in four sections. Each section contained ~25% of the total number of ovules, with “A” the stylar end and “D” the peduncular end (similar to Stephenson et al. 1988). For each ovule position I recorded whether or not a seed was present, and if not, whether it was aborted early (or not fertilized) or late (aborted after seed was initiated). I weighed all seeds collected and then planted them in individual trays with 5 by 10 cells, tracking the identity of each seed. Before planting, seeds were scarified with 90% sulfuric acid for 5 min. If seeds did not germinate, I manually removed a small section of the seed coat with a scalpel. Subsequently these seeds germinated successfully. I did not compare germination rates among treatments because germination procedures were not uniform. After 30 days from emergence, I harvested a

subsample of seedlings from seeds that germinated successfully. Seedlings were dried in an oven for 72 hrs at 80° C, and I obtained dry mass.

Data analysis

Seed abortion was compared among herbivory treatments and pollination treatments with both considered as fixed factors. I used a randomized complete-block design ANOVA to compare proportion of seeds aborted (Statgraphics 2001). I arcsine – transformed the proportions of seeds aborted to meet ANOVA assumptions.

In order to test for compensation between the number of seeds produced and seed weight I performed linear regression analysis over each of the herbivory treatments for both types of pollination. I square root-transformed the number of seeds and seed weight to meet normality assumptions of statistical analysis. A negative slope would confirm a trade-off between the variables, making selective allocation of resources difficult to differentiate. No association between seed number and seed weight would allow me to detect resource allocation to specific regions of the fruits.

To evaluate the effects of herbivory on the proportion of seeds aborted by region in fruits produced by cross and self-pollinations, I used the split-split plot design (S. Shapiro, FIU, personal communication). Herbivory treatment was considered as a fixed factor. The blocks were split by pollination treatment (self and cross) and split again by fruit region (A, B, C, D). Genotypes were used as replicates by plot (random factor) and interactions with them were considered to evaluate genotype effect in the variance components. ANOVA was run on SPSS software (SPSS Inc. 2002) using the proportion

of arcsine-transformed seeds aborted to meet analysis assumptions. I used the linear mixed model procedure with Type III sums of squares and the restricted maximum likelihood method.

Chi-square analysis was used to compare dry mass of the seedlings harvested and early abortion rates between treatments. Early abortion rates also included unfertilized ovules because I could not tell the difference between unfertilized ovules and early aborted seeds.

RESULTS

Seed abortion

S-fruits had higher abortion rates than X-fruits throughout all herbivory treatments ($F=576.73$, $P<0.001$; Fig.1). Severely defoliated plants showed higher rates of abortion for both S-fruits and X-fruits compared with little (~33%) or no leaf removal ($F=292.36$, $P<0.001$; Fig.1).

Seed number vs. seed mass

Linear regression analysis of seed mass and seeds produced by control plants and plants with low leaf damage showed negative slopes for X-fruits. Figure 2 shows the relationship between mean of seed mass vs. seeds produced. I eliminated three fruits from control plants and five from 33% herbivory treatment that were outliers. In contrast, maternal plants that experienced higher levels of herbivory showed no significant association (Fig. 3). S-fruits showed consistency across all treatments with trends to

compensate for number and mass ($r^2=0.08$, $F=26.35$, $P=0.013$ and $r^2=0.13$, $F=31.52$, $P=0.001$). Control and ~66% herbivory are shown in Figure 4.

Herbivory effects in selective seed abortion

Proportion of seeds aborted differed within fruits with pollination and herbivory treatments (Table 1). These results and the consistency of patterns among herbivory treatments suggested that abortion was not randomly located. Control plants and plants with low levels of leaf damage did not differ on the proportion of X-seed abortion along the fruit regions but severely damaged plants did (Fig. 5). In these plants, abortion was lower in the fruit regions B and C (Fig. 5). For the ~33% treatment, region D showed the highest proportion of seed abortion. On the other hand, S-seed abortion differed significantly only for region D of the fruit for all treatments (Fig. 5).

As I was able to distinguish early aborted and unfertilized ovules from late aborted seeds, I constructed a histogram of frequencies of early aborted seeds. Early abortion was higher in the peduncular regions of both X-fruit and S-fruit ($\chi^2=21.31$, $P=0.01$; $\chi^2=144.49$, $P<0.001$; Fig. 6). I was not able to distinguish whether early abortions were unfertilized ovules or aborted fertilized ovules. If mechanisms involved in late abortion of seeds are different from those causing early seed abortion, comparison of abortion within fruits excluding region D or peduncular end should provide a better understanding of the results. In this comparison the only significant difference was among X-fruits, which aborted more seeds in the proximate region for severely damaged plants (Fig. 7). I did not detect any abortion patterns in S-fruits after excluding region D of the fruits (Fig. 7).

Progeny and their position along the fruit

I found a contrast in the performance of progeny from regions B, C and D compared with A ($\chi^2=35.43$, $P<0.01$, Fig. 8) in terms of seedling dry mass at 30 days. This suggests that position-dependent resource availability and abortion are interacting with pollen-donor success in X-fruits. I did not find a pattern for S-fruits, which seems to have a random distribution of seedling dry mass along the fruit position ($\chi^2=15.81$, $P=0.07$, Fig. 8).

DISCUSSION

I have demonstrated that herbivory strongly increases abortion of seeds in *Centrosema virginianum* plants grown in the greenhouse. The data suggest that abortion of seeds also happens on undamaged plants and is higher in fruits that were self-pollinated. This indicates that selective abortion occurs because plants allocate more resources to cross-pollinated fruits. Plants under extreme herbivory also maintained this pattern.

The difference in seeds produced may be the result of both the reduction of resource availability and unfertilized ovules, which in this study were lumped with early abortion. Abortion of unfertilized ovules was not attributed to pollen limitation because large loads of pollen were deposited on the receptive surface of the stigma. Because abortion rates behaved similarly among all control plants (see next section), I attribute the variation in seed abortion to maternal effects. Because both types of hand pollination were done at the same time, possible preference of X-seeds over S-seed may explain the higher levels of S-seeds aborted.

Because changes in seed number per fruit were not compensated by changes in individual seed mass of X-fruits, seed number and seed mass were considered indicators of resource allocation per fruit in maternal plants under extreme resource limitation. For S-fruits, neither variable is a complete indicator of resource allocation and both should be considered jointly as total seed weight per fruit (Marshall and Ellstrand 1988).

Centrosema virginianum exhibited shifts in resource allocation in response to herbivory.

Seed number was compensated by seed weight on undamaged plants or with low herbivory damage in fruits resulting from cross-pollination. Similar patterns were also found among fruits resulting from self-pollination on herbivory treatment plants. In contrast, X-fruits from plants under severe damage maintained seed mass regardless of seed number. Interestingly, these results are different from findings in annual legumes.

Vicia sativa, an annual legume with autogamous fruit production, showed that after increasing levels of herbivory plants produced fewer and smaller seeds (Koptur et al. 1996). It is likely that perennial plants like *C. virginianum* invest more resources in high quality progeny sacrificing number of seeds when resources are scarce, as they have opportunity for future reproduction, while annuals have only one opportunity to reproduce.

Differential donor success can occur at any stage in the reproductive cycle (Stephenson and Bertin 1983). In my experiment, maternal effects showed a great influence on both post-pollination and post-fertilization processes. First, a high frequency of early abortion in specific regions of the fruit indicates a strong influence of pollen competition in the ovary. According to theory, when resources are scarce plants should abort some fruits and seeds, and selectively mature only those of high quality (Janzen

1977), resulting in offspring with greater vigor (Niesenbaum 1996). Second, strong patterns of abortion with treatments in X-fruits compared with similar abortion rates within S-fruits demonstrate that maternal effects also direct offspring quality.

My progeny-vigor results concurred with studies of nonrandom fertilization within fruits of zucchini that showed that more seeds were sired in specific regions of the zucchini and high vigor progeny resulted from those seeds (Stephenson et al. 1988). Seeds of *C. virginianum* produced in middle regions of X-fruits from severe herbivory treatments produced more vigorous seedlings. Although S-seedlings in the A region weighed less than seedling from other fruit regions, I could not find patterns for the seedlings in the other fruit regions, suggesting a more random allocation of resources with S-pollination. This less selective process may have been a position-dependent resource effect rather than a selection for better genotypes.

I found that early abortion was the main contributor to abortion of seeds closer to the base of pods. I hypothesize that basal regions in the ovary have a spatial and temporal nutritional advantage (Stephenson et al. 1988). Furthermore, a recent study of ballistic seed projection in *Vicia sativa* and *Croton capitatus* demonstrated that more seeds were projected from the upper rather than the lower half of the plants and number of fruits and seed produced were position dependent (Garrison et al. 2000). I did not test for difference in seed abortion at different heights of the plant. However, if position along the plant is important for seed dispersal, position within fruits could be also important in the active dehiscence of the fruits. It is reasonable to predict that seeds at the base of a dehiscing legume will not move as far from the plant as those in the middle and seeds at the tip will

likely be flying the furthest, if they are not stuck in the edges. This phenomenon should be further investigated.

Overall, I analyzed selectivity of abortion through a variety of factors present in a hermaphroditic self-compatible species. I demonstrated that resource limitation increases discrimination of seeds between crosses and among donor genotypes. Early processes of reproduction, such as fertilization, especially interactions with the maternal plants, need to be further studied to understand in which stages maternal plants affected by herbivory modify resources allocation strategies.

REFERENCES

- Agrawal, A. A. 2001. Transgenerational consequences of plant responses to herbivory: An adaptive maternal effect? *The American Naturalist* **157**:555-569.
- Avila-Sakar, G., G. A. Krupnick, and A. G. Stephenson. 2001. Growth and resource allocation in *Cucurbita pepo* ssp. *texana*: Effects of fruit removal. *International Journal of Plant Sciences* **162**:1089-1095.
- Bertin, R. I. 1989. Pollination Biology. Pages 480 in W. G. Abrahamson, editor. Plant-animal interactions. McGraw Hill, New York.
- Brown, V. K., A. C. Gange, I. M. Evans, and A. L. Storr. 1987. The effect of insect herbivory on the growth and reproduction of two annual *Vicia* species at different stages in plant succession. *Journal of Ecology* **75**:1173-1190.
- Cobb, N. S., R. T. Trotter III, and T. G. Whitham. 2002. Long-term sexual allocation in herbivore resistant and susceptible pinyon pine (*Pinus edulis*). *Oecologia* **130**:78-87.
- Crawley, M. J. 1983. Herbivory. The Dynamics of Animal -Plant Interactions. University of California Press, Berkeley.
- Diaz, M., A. P. Moller, and F. J. Pulido. 2003. Fruit abortion, developmental selection and developmental stability in *Quercus ilex*. *Oecologia* **135**:378-385.
- Garrison, W. J., G. L. Miller, and R. Raspet. 2000. Ballistic seed projection in two herbaceous species. *American Journal of Botany* **87**:1257-1264.
- Isely, D. 1990. Vascular Flora of the Southeastern United States: Leguminosae (Fabaceae). The University of North Carolina Press, North Carolina.
- Janzen, D. H. 1977. A note on optimal mate selection by plants. *American Naturalist* **111**:365-371.
- Koptur, S., C. L. Smith, and J. H. Lawton. 1996. Effects of artificial defoliation on reproductive allocation in the common vetch, *Vicia sativa* (Fabaceae: Papilionoideae). *American Journal of Botany* **83**:886-889.
- Kudo, G., T. Maeda, and K. Narita. 2001. Variation in floral sex allocation and reproductive success within inflorescences of *Corydalis ambigua* (Fumariaceae): Pollination efficiency or resource limitation? *Journal of Ecology* **89**:48-56.

- Lee, D. A., and F. A. Bazzaz. 1986. Maternal regulation of fecundity: nonrandom ovule abortion in *Cassia fasciculata* Michx. *Oecologia* **68**:459-465.
- Marshall, D. L., and N. C. Ellstrand. 1988. Effective mate choice in wild radish: evidence for selective seed abortion and its mechanisms. *The American Naturalist* **131**:739-756.
- Marshall, D. L., and D. M. Oliveras. 2001. Does differential seed siring success change over time or with pollination history in wild radish, *Raphanus sativus* (Brassicaceae)? *American Journal of Botany* **88**:2232-2242.
- Nakamura, R. R. 1968. Maternal investment and fruit abortion in *Phaseolus vulgaris*. *American Journal of Botany* **73**:1049-1057.
- Nielsen, B. S. 1990. Yield responses of *Vicia faba* in relation to infestation levels of *Sitona lineatus* L. (Coleoptera: Curculionidae). *Journal of Applied Entomology* **110**:389-407.
- Niesenbaum, R. A. 1996. Linking herbivory and pollination: Defoliation and selective fruit abortion in *Lindera benzoin*. *Ecology* **77**:2324-2331.
- Shaner, M. G. M., and D. L. Marshall. 2003. Under how wide a set of conditions will nonrandom mating occur in *Raphanus sativus* (Brassicaceae)? *American Journal of Botany* **90**:1604-1611.
- SPSS Inc. 2002. SPSS for Windows. Version 11.5. U.S.
- Statgraphics. 2001. Statistical Graphics Corp., version 5.1. Manugistics, Inc. Rockville, MD.
- Stephenson, A. G., and R. I. Bertin. 1983. Male competition, female choice, and sexual selection in plants. Pages 109-149 in L. Real, editor. *Pollination Biology*. Academic Press, Orlando, Florida.
- Stephenson, A. G., and J. A. Windsor. 1986. *Lotus corniculatus* regulates offspring quality through selective fruit abortion. *Evolution* **40**:453-458.
- Stephenson, A. G., J. A. Winsor, and C. D. Schlichting. 1988. Evidence of non-random fertilization in the common zucchini, *Cucurbita pepo*. Pages 502 pp. in M. Cresti, P. Gori, and E. Pacini, editors. *Sexual Reproduction in Higher Plants*. Springer-Verlag, New York, U.S.

- Travers, S. E., and S. J. Mazer. 2001. Trade-offs between male and female reproduction associated with allozyme variation in phosphoglucosomerase in an annual plant (*Clarkia unguiculata*: Onagraceae). *Evolution* **55**:2421-2428.
- Winsor, J. A., S. Peretz, and A. G. Stephenson. 2000. Pollen competition in a natural population of *Cucurbita foetidissima* (Cucurbitaceae). *American Journal of Botany* **87**:527-532.

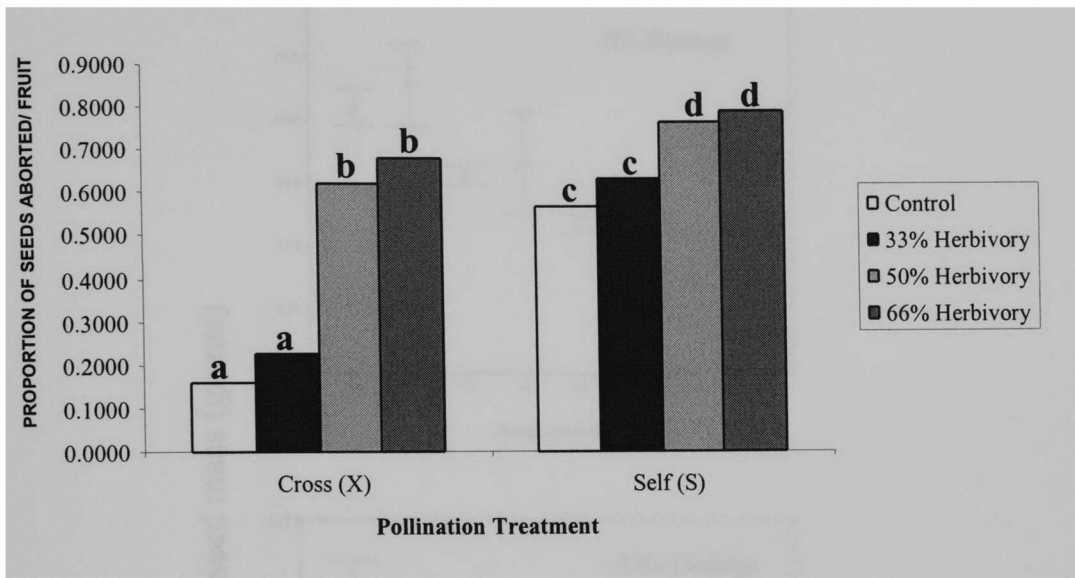


Fig. 1. Proportion of seeds aborted of *Centrosema virginianum* fruits after four levels of herbivory. X-fruits showed lower proportion of seeds aborted than S-fruits ($F=576.73$, $P<0.001$). Maternal plants with high levels of herbivory aborted more seeds than control or low levels of herbivory ($F=292.36$, $P<0.001$). Different letters mean statistical difference at $\alpha=0.05$. Statistical analysis was done on arcsine-transformed proportions ($N=120$; $n=15$).

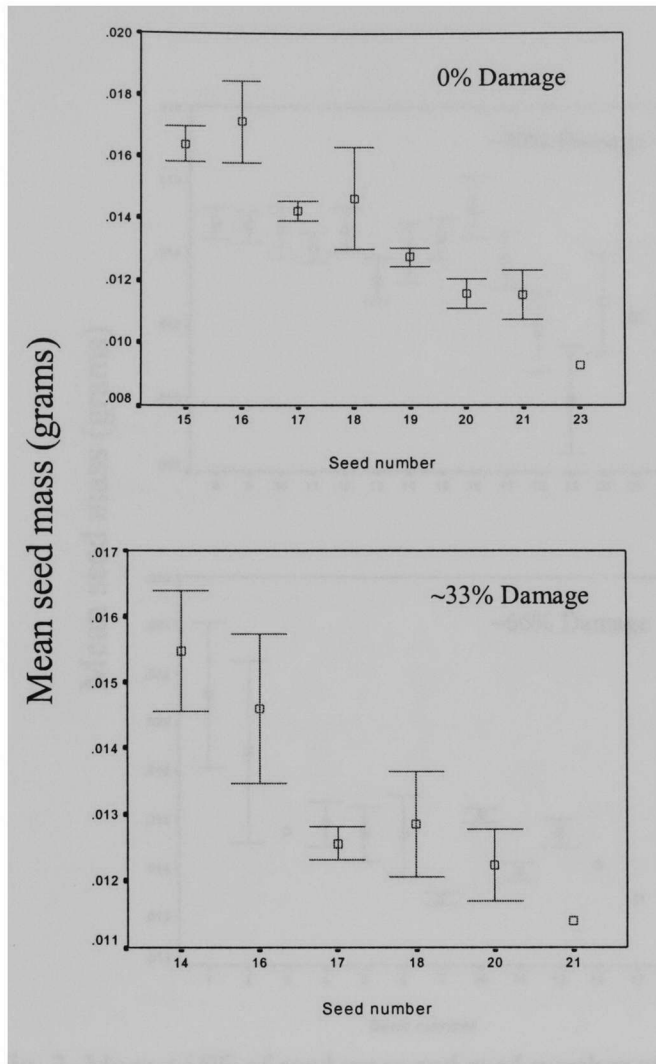


Fig. 2. Mean ± 1 SE of seed mass and seed number of X-fruits from control maternal plants (top) and plants under $\sim 33\%$ of herbivory (bottom). Square root-transformed seed number and seed weight were used in linear regression analysis ($r^2=0.48$, $F=206.31$, $P<0.001$ and $r^2=0.51$, $F=258.25$, $P<0.001$).

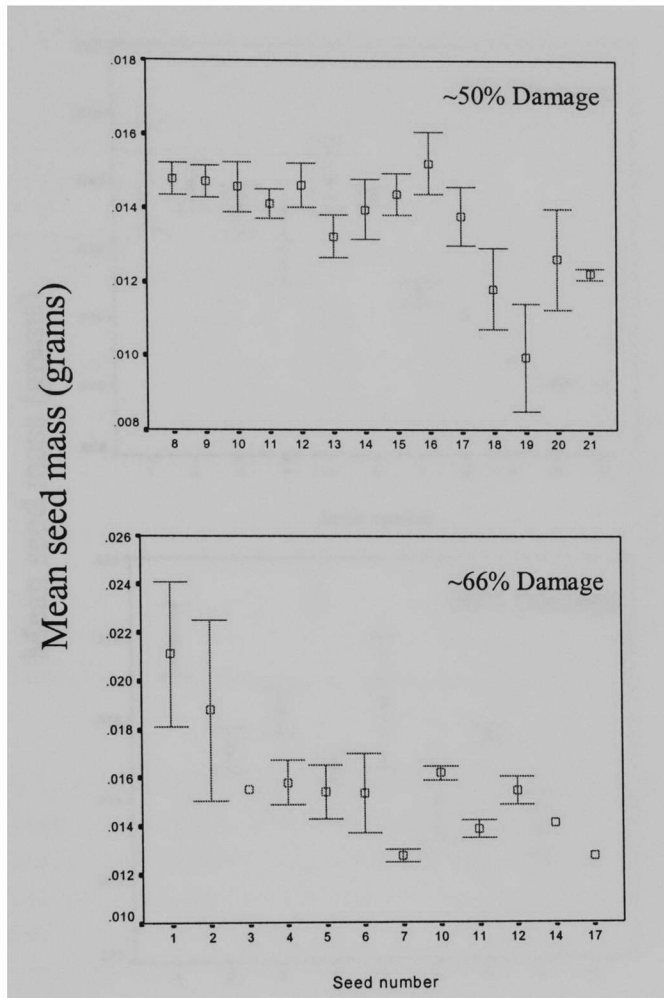


Fig. 3. Mean ± 1 SE of seed mass and seed number of X-fruits from plants under severe herbivory; 50% leaf removal (top) and plants under ~66% of herbivory (bottom). Square-root transformed seed number and seed mass were used in linear regression analysis ($r^2=0.01$, $F=29.14$, $P=0.160$ and $r^2=0.06$, $F=35.42$, $P=0.311$).

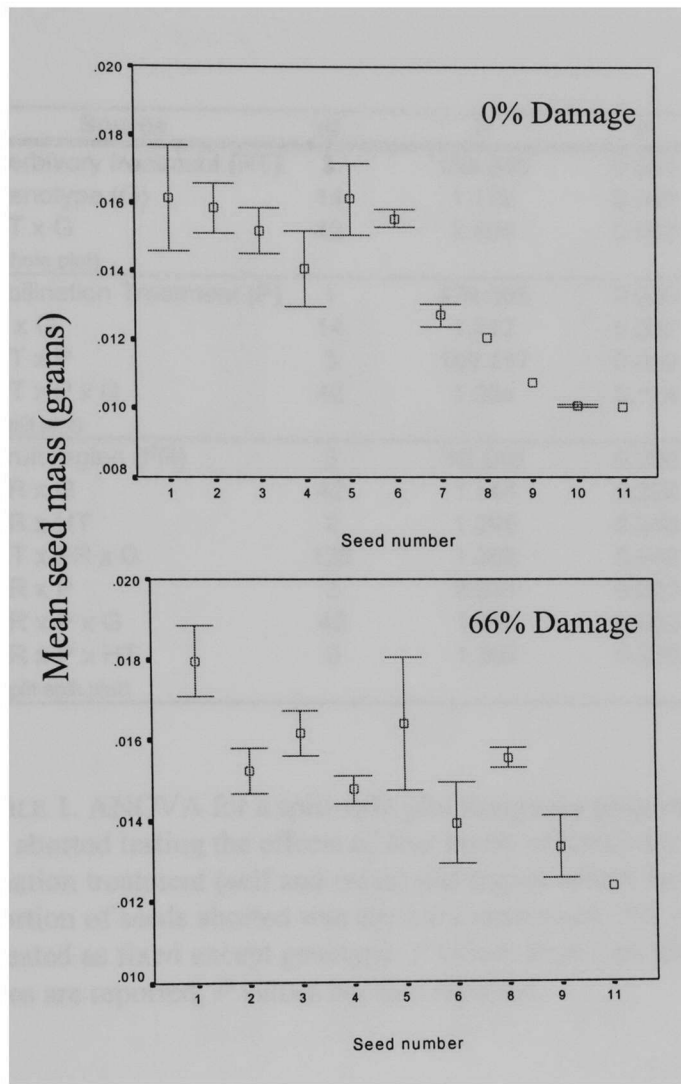


Fig. 4. Mean ± 1 SE of seed mass and seed number of S-fruits from control plants and under severe herbivory; 0% leaf removal (top) and plants under $\sim 66\%$ of herbivory (bottom). Square-root transformed seed number and seed mass were used in linear regression analysis ($r^2=0.08$, $F=26.35$, $P=0.013$ and $r^2=0.13$, $F=31.52$, $P=0.001$).

Source	df	F	P
Herbivory treatment (HT)	3	159.345	0.000
Genotype (G)	14	1.172	0.348
HT x G	42	2.505	0.002
(whole plot)			
Pollination Treatment (P)	1	476.365	0.000
P x G	14	1.212	0.307
HT x P	3	149.417	0.000
HT x P x G	42	1.334	0.114
(split plot)			
Fruit region (FR)	3	18.540	0.000
FR x G	42	1.144	0.326
FR x HT	9	1.295	0.246
HT x FR x G	126	1.362	0.042
FR x P	3	8.351	0.000
FR x P x G	42	1.978	0.002
FR x P x HT	9	1.367	0.210
(split-split plot)			

TABLE 1. ANOVA for a split-split plot design for proportion of seeds aborted testing the effects of four levels of herbivory, pollination treatment (self and cross) and region within the fruit. Proportion of seeds aborted was arcsine transformed. All variables are treated as fixed except genotype. *F* values from type III sums of squares are reported, *P* values are also reported.

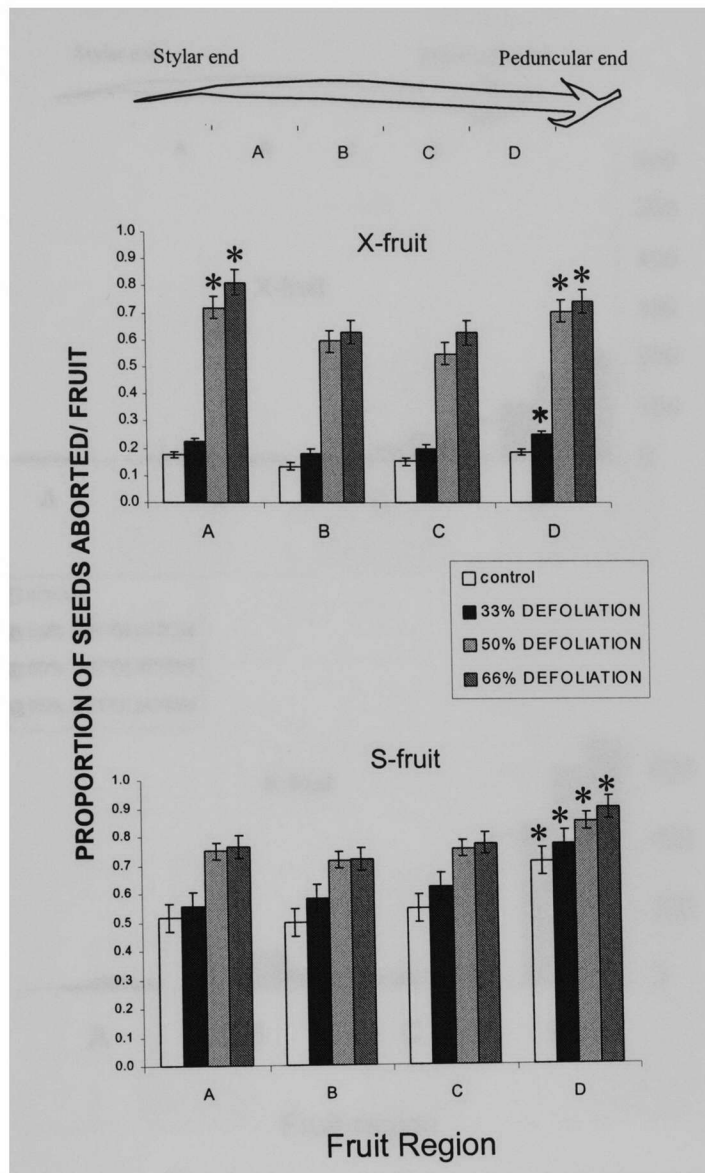


Fig. 5. Mean ± 1 SE of the proportion of seeds aborted in each fruit region among four herbivory treatments. Bars with asterisk were significantly different within herbivory treatment with respect to bars with no asterisk. X-fruits are represented on the top graph and S-fruits on the bottom graph. ($\alpha=0.05$).

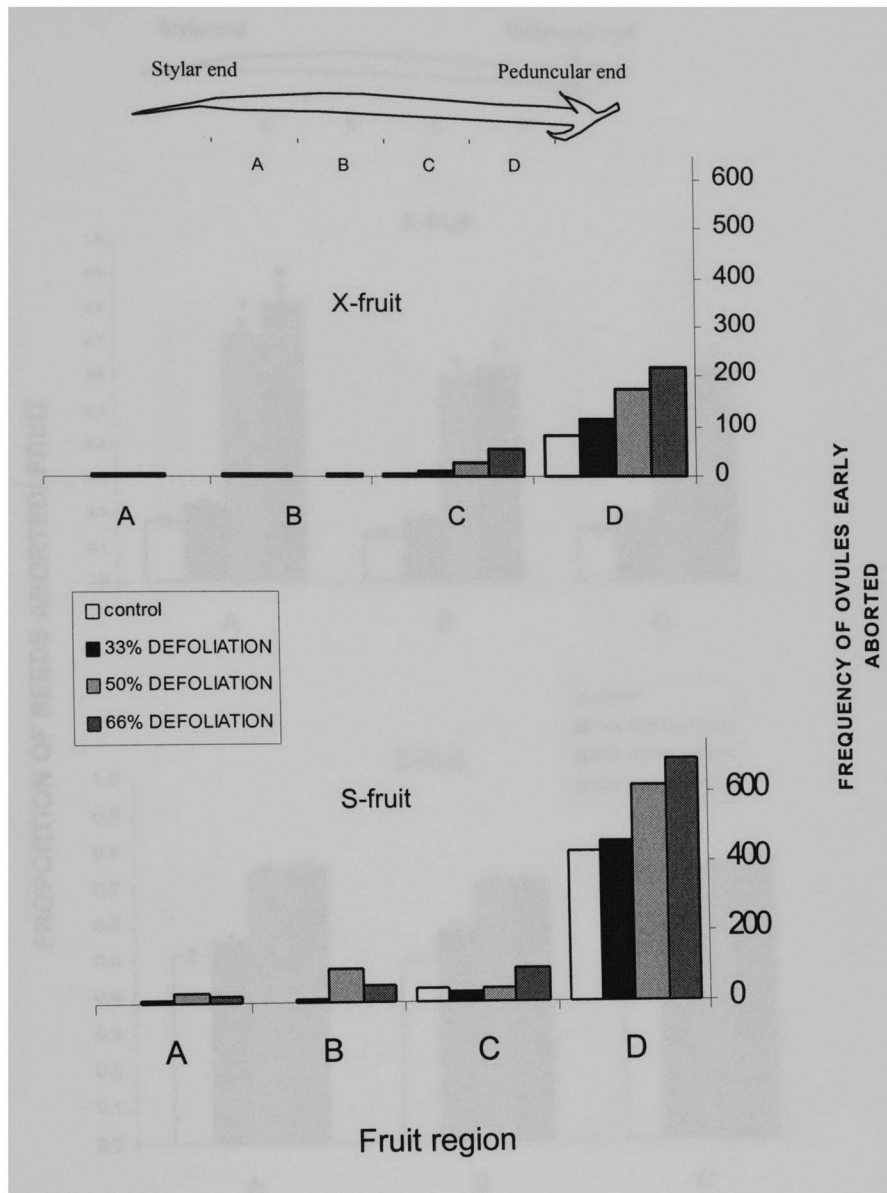


Fig. 6. Frequency histogram of early abortion of seeds among four herbivory treatments of X-fruits, top; and S-fruits, bottom ($\chi^2=21.31$, $P=0.01$; $\chi^2=144.49$, $P<0.001$).

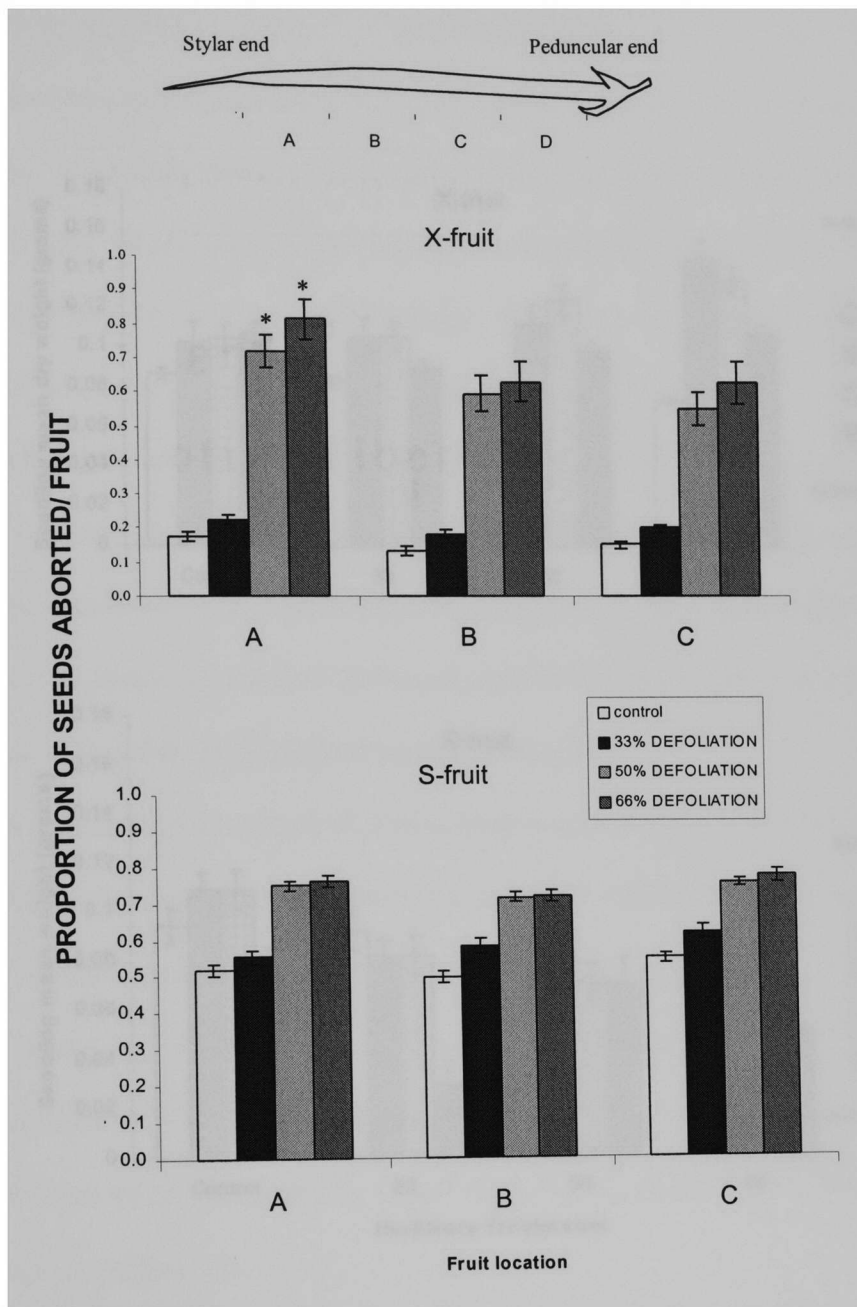


Fig. 7. Mean ± 1 SE of the proportion of seeds aborted in each fruit region among four herbivory treatments, excluding peduncular region D. Bars with asterisk were significantly different within herbivory treatment with respect to bars without asterisk. X-fruits are represented on the top graph and S-fruits on the bottom graph. ($\alpha=0.05$).

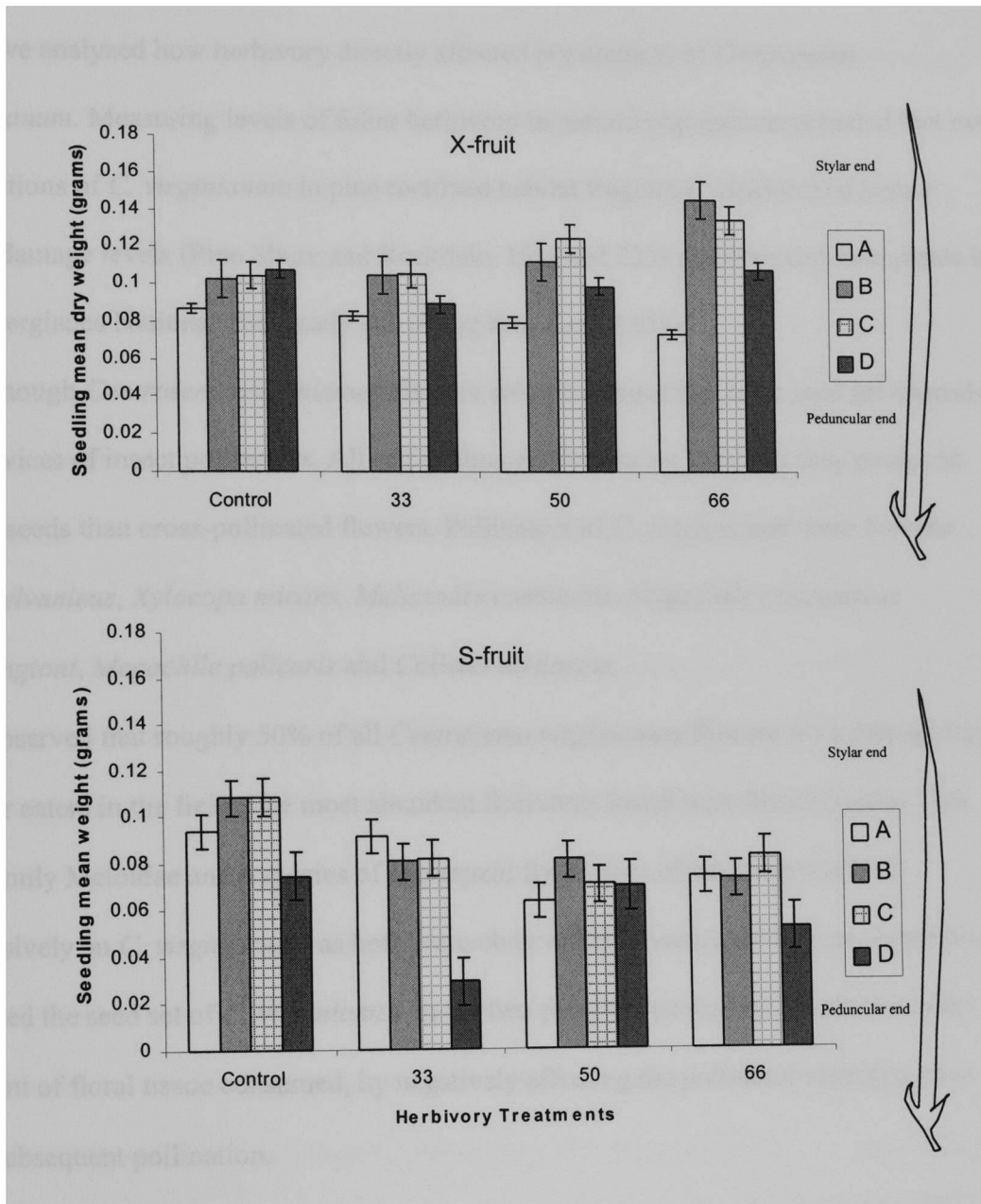


Fig. 8. Mean ± 1 SE seedling dry weight among herbivory treatments in each fruit region. X-fruits are represented on the top graph ($\chi^2=35.43$, $P=0.02$) and S-fruits on the bottom graph. ($\chi^2=15.81$, $P=0.07$).

CONCLUSION

I have analyzed how herbivory directly affected populations of *Centrosema virginianum*. Measuring levels of foliar herbivory in natural populations revealed that two populations of *C. virginianum* in pine rockland habitat fragments experienced higher foliar damage levels (Pine Shore and Rockdale, 15% and 22% respectively) than plants in the Everglades National Park study site (Long Pine Key, 8.6%).

Although *Centrosema virginianum* flowers are self-compatible, their seed set depends on services of insect pollinators. All self-pollinated flowers set fruit, but they produced fewer seeds than cross-pollinated flowers. Pollinators of *C. virginianum* were *Bombus pennsylvanicus*, *Xylocopa micans*, *Melissodes communis*, *Megachile campanulae wilmingtoni*, *Megachile policularis* and *Colletes distinctus*.

I observed that roughly 50% of all *Centrosema virginianum* flowers were damaged by flower eaters in the field. The most abundant florivores found were blister beetles from the family Meloidae and a species of agromyzid fly, neither of which specialized exclusively on *C. virginianum*, as both were observed on a variety of flowers. Florivores affected the seed set of *C. virginianum* in the two populations studied, regardless of the amount of floral tissue consumed, by negatively affecting the pollinator visitation rates and subsequent pollination.

Flower size, pollen produced, and seeds produced were negatively affected by foliar herbivory. I did not find significant differences in nectar produced, nectar quality (sugar concentration), and ovules produced between damaged and undamaged plants. Surprisingly, severely damaged plants produced flowers with bigger pollen than flowers produced by both mildly damaged and undamaged plants.

Results showed that damaged plants tolerated herbivory with 6% (mild herbivory) and 17% (severe herbivory) reduction of total fitness. However, the investment of resources was not equisexual, favoring male components under extreme resource stress.

Centrosema virginianum was able to compensate a decrease in pollen number with an increase in pollen size.

The species strategy to tolerate severe herbivory showed a trade-off between pollen number and size. The ability to sire seeds differed between larger pollen versus smaller pollen, but only by one seed per fruit on average. Although the difference was statistically significant, I did not examine the biological consequences of these findings. Moreover, seed weight sired by both pollen types did not vary. These results are indicative of a true trade-off between pollen number and pollen size by severely damaged plants, never reported to my knowledge. These results confirmed that growing conditions of parental plants affected pollen performance.

I found a negative relationship between female and male reproductive success when plants were damaged. This strongly implies that gender specialization is related to factors beyond genetic characteristics. Different levels of herbivory may be affecting reproductive success, promoting changes in resource allocation. Sporadic events of extreme herbivory may direct adaptive responses of *C. virginianum* to pollinators through floral traits and rewards.

This project demonstrated that resource limitation increases maternal discrimination, increasing seed abortion in fruits from cross-pollinations and differing among donor genotypes. These findings suggest that maternal plants direct the selection of offspring quality. Experiments showed that resource stress promotes selectivity in abortion within

fruits, favoring regions towards the center of the pods in cross-fertilized fruits.

Furthermore, seedlings that were the products of seeds within favored regions were more vigorous. In contrast, self-fertilized fruits showed more random abortion patterns. Also, fruits aborted early a higher number of seeds (or ovules) in basal regions of the pods.

This may be due in part to interactions with pollen competition in the ovary. If position along the plant is important for active dehiscence (demonstrated for *Vicia sativa* with similar fruit dehiscence), the position of seeds within individual fruits is also likely to be important for seed dispersal. If seeds in distal parts of the fruit are dispersed further from the maternal plants, these positions may be more favorable for seed development, and ovules or developing seeds in those positions are more likely to be retained.

This research has raised a number of interesting questions in a plant that has many good features for further experimentation. If greenhouse grown plants and their flowers show dramatic differences in response to herbivory based on the type of pollination they receive, it is reasonable to expect that plants in natural habitats may have even more dramatic responses under field conditions with fluctuating nutrients, light, and water. Plants in small habitat fragments may be pollen limited and experience less cross pollination and more mating with close relatives or little pollination.

My research highlights the importance of considering understudied ways in which total plant fitness may be affected by herbivory, contributing to determine how herbivores and pollinators together act as selective agents in the evolution of plant species.

VITA

YURIA CARDEL

January 29, 1972	Born, Xalapa, Veracruz, Mexico
December, 1995	B.S. Biology (Minor in Ecology) Universidad de las Americas, Puebla, Mexico
1995-1996	AFRICAM Safari Zoo, Clinic responsible Puebla, Mexico
1996-1999	INAOE (National Institute of Astronomy, Optics and Electronics) Large Millimetric Telescope Project Site selection Team
1999-2004	PhD Candidate in Biology, Florida International University

HONORS, FELLOWSHIPS, AND AWARDS:

Awarded (2001) Tropical Biology Research Grant- \$1,500.00

Awarded (2000) Tropical Biology Research Grant- \$2,000.00

Awarded (1999) Graduate Student Fellowship CONACyT
(National Council of Sciences and Technology, Mexico) -
\$40,000.00

Universidad de las Americas

(1995) Undergraduate Thesis Honors
Undergraduate Honors in Biology
College Honors

(1994) Jaguar Award
(Best biology undergraduate student)

(1990) Jenkins Science Fellowship (90%tuition)