

## ABSTRACT

FRANKLIN, MISTY ANN. Factors affecting seed production in natural populations of *Lysimachia asperulifolia* Poir. (Primulaceae), a rare, self-incompatible plant species. (Under the direction of Jon M. Stucky and Thomas R. Wentworth.)

*Lysimachia asperulifolia*, commonly known as Rough Leaved Loosestrife, is a federally endangered, rhizomatous herb restricted to ecotones between long-leaf pine savannas and pocosins in North and South Carolina. Botanists and land use managers across the species' range have observed low rates of seed production in natural populations. Low seed production may prevent establishment of new populations and production of new genotypes, thus limiting the ability of the species to survive diseases and changing environmental conditions. I conducted a study to determine causal mechanisms of the limited seed production at five natural populations in North Carolina. Using field observations and experiments, I examined pollen fertility, pollen compatibility, pollinator limitation, and population structure of *L. asperulifolia*. In this paper, I present background information on natural causes of low seed production, previously published information about *L. asperulifolia* species biology, and results of my own experiments.

**Keywords:** *Lysimachia*, pollinator limitation, pollen fertility, pollen compatibility, genetic compatibility, Stem Distribution, sweat bees, *Lasioglossum*, *Augochlorella*, *Macropis*, pollinator observations, pan trap sampling, self-incompatibility, t-square analysis.

**FACTORS AFFECTING SEED PRODUCTION IN NATURAL POPULATIONS  
OF *LYSIMACHIA ASPERULIFOLIA* POIR. (PRIMULACEAE), A RARE, SELF-  
INCOMPATIBLE PLANT SPECIES**

by

**Misty Ann Franklin**

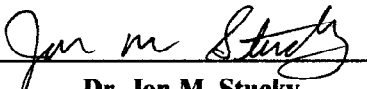
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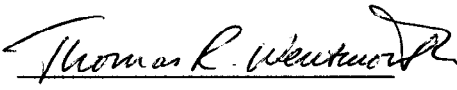
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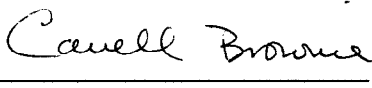
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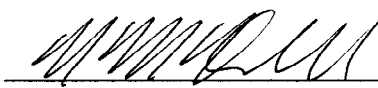
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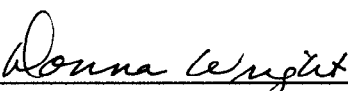
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## DEDICATION

This thesis is dedicated to the members of my family whose labor opened the doors of opportunity for me.

## BIOGRAPHY

I was born in an elevator on September 15, 1975 in Fort Bragg, NC. My mother says that's where I got my love of traveling, but I think that's really where I got my fear of falling. I lived with my parents and grandparents in Fayetteville, North Carolina until age 8, when my family moved to Marion, NC. It was in Marion that I discovered the great outdoors. My family and I spent countless weekend afternoons exploring hiking trails around the Blue Ridge Parkway and Linville Gorge, picking berries in abandoned fields, and playing in the creeks around our house.

The history of my education is marked by great teachers. I attended high school at North Carolina School of Science and Mathematics in Durham NC, where I graduated in 1993. I attended Smith College in Northampton, MA, where I graduated in 1997. Dr. John Burk was the first professor to introduce me to the fascinating world of plant ecology. While in college, I had the opportunity to participate in a Tropical Ecology program in Palau for one semester. Although I had known that I was interested in the environment, that experience cemented my plans to pursue the study of ecology. The tropical environment of Palau was enchanting (both marine and terrestrial), but I knew there was important work to be done closer to home, in my own corner of the world. In 1999, I learned about work being done by Dr. Jon Stucky and Ms. Donna Wright at North Carolina State University in Raleigh, NC. I was happy to get the opportunity to work with Dr. Stucky and Ms. Wright, as well as Dr. Thomas Wentworth on a topic that seems to combine some of the most popular aspects of ecology: pollination biology, endangered species, fire-frequency, and wetland ecology. I expect to receive my M.S. in Botany in December 2001. I hope to continue learning after I receive my degree. My interests include wetland ecosystems, plant-pollinator interactions, endangered species, and ecological restoration.

## ACKNOWLEDGEMENTS

This work has been the result of collaboration between scientists and government representatives from across North Carolina. Here, I take the opportunity to acknowledge a few of these collaborators, but wish to emphasize my appreciation to everyone who has contributed to making this research possible.

Dr. Jon Stucky has been the cornerstone of my involvement with *Lysimachia asperulifolia* research from the beginning. His patient mentorship, unflagging interest in this project, and availability and willingness to answer questions or discuss ideas at any time have contributed infinitely to both my research and my education at NCSU. Dr. Stucky has been an inspirational teacher and friend to me and numerous other students who have interacted with him during my time at NCSU. Dr. Thomas Wentworth has been instrumental in planning all phases of the research and analysis of this project. He has also been an influential role model as professor, researcher, and friend. Ms. Donna Wright has also been an important sounding board for ideas and seems to have inexhaustible reserves of interest and ideas for this and other research projects. Developing an effective experimental design and statistical analysis of data would not have been possible without the patient help of Dr. Cavell Brownie and Nick Haddad. T'ai Roulston played an important role in planning the phases of research related to pollinators.

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Last, but not least, I would like to thank my family and friends for their patience, kindness, and understanding during these last two years. I especially want to acknowledge my mother, Mrs. Joyce Gallion who has never hesitated to contribute funding, encouragement, interest, and love. Andrew

Buchanan and C. F. Goldsmith have truly gone above and beyond the call of friendship in providing understanding, encouragement, sympathy, field help, and daily words of wisdom that have guided me through my studies at NCSU.

## TABLE OF CONTENTS

	Page
LIST OF TABLES .....	vii
LIST OF FIGURES .....	viii
LITERATURE REVIEW .....	1
Life History of <i>Lysimachia asperulifolia</i> .....	1
Factors That Contribute To Low Seed Production .....	4
Literature Cited .....	9
<b>FACTORS AFFECTING SEED PRODUCTION IN NATURAL POPULATIONS OF <i>LYSIMACHIA ASPERULIFOLIA</i> POIR. (PRIMULACEAE), A RARE, SELF-INCOMPATIBLE PLANT SPECIES</b>	
Abstract .....	11
Introduction .....	12
Methods .....	14
Study Sites .....	15
Stem Distribution .....	15
Fluorescent Powder .....	16
Pollinator Observations .....	16
Pan Trap Sampling .....	17
Pollen Stainability .....	18
Verifying Self-Incompatibility .....	19
Genetic Compatibility .....	19
Pollinator Limitation .....	20
Results and Discussion .....	21
Stem Distribution .....	21
Distances between nearest flowering stems .....	21
T-Square analysis .....	22
Fluorescent Powder .....	22
Pollinator Observations .....	23
Pan Trap Sampling .....	24
Fruit Production .....	25
Pollen Stainability .....	26
Verifying Self-Incompatibility .....	26
Genetic Compatibility .....	26
Pollinator Limitation .....	27
Seed Germination .....	27
Genetic Compatibility Study .....	27
Pollinator Limitation Study .....	27
Conclusions .....	28
Acknowledgements .....	29
Literature Cited .....	30
Appendices .....	51

## LIST OF TABLES

1. Distances between study sites .....	32
2. Treatment classes for genetic compatibility study.....	34
3. Treatment classes for pollinator limitation Study.....	36
4. Vegetative and flowering stem counts before and after prescribed burns .....	37
5. Powder movement at 3 populations of <i>L. asperulifolia</i> during June 2000 .....	39
6. Results of Two-way ANOVA of square-root transformed frequency of visits to inflorescences by all visits, xenogamous visits, and geitonogamous visits .....	41
7. Pollinators trapped while visiting flowers and caught in pan traps .....	42
8. Percent of flowering stems producing capsules at each study site .....	44
9. Results of two-way ANOVA performed on percent pollen stainability .....	45
10. Mean percent pollen stainability, standard deviations, and Tukey groupings for 2000 (pre-burn) and 2001 (post-burn) .....	46
11. Results of three-way ANOVA performed on square-root transformed seed and fruit production data .....	47
12. Production and germination trends for seeds produced by genetic compatibility treatments in 2000 and 2001 .....	49
13. Production and germination trends for seeds produced by pollinator limitation treatments in 2000 .....	50

## LIST OF FIGURES

## LITERATURE REVIEW

1.1. Schematic diagram of interactions that contribute to low seed production.....	8
<b>FACTORS AFFECTING SEED PRODUCTION IN NATURAL POPULATIONS OF <i>LYSIMACHIA ASPERULIFOLIA</i> POIR. (PRIMULACEAE), A RARE, SELF-INCOMPATIBLE PLANT SPECIES</b>	
2.1. Map of Eastern North Carolina counties with research sites.....	33
2.2. Schematic diagram of treatment classes for pollen compatibility study .....	35
2.3. Box plot of the distances (in centimeters) between flowering stems for each year at each site .....	38
2.4. Frequency histogram of intervals containing geitonogamous, xenogamous, and visit types.....	40
2.5. Number of solitary bees in pan traps compared with total insects caught in traps of each color .....	43
2.6. Mean number of seeds produced by each treatment category .....	48

## LITERATURE REVIEW

*Lysimachia asperulifolia* Poir., commonly known as Rough Leaved Loosestrife, is a federally endangered, rhizomatous herb restricted to ecotones between long-leaf pine savannas and pocosins in North and South Carolina. Botanists and land use managers across the species' range have observed low seed production in natural populations. Low seed production may prevent establishment of new populations and production of new genotypes, thus limiting the ability of the species to survive diseases and changing environmental conditions. In this chapter, I present background information on the life history of *Lysimachia asperulifolia*, then explore some ecological factors that cause low seed production in other species, and discuss how factors contributing to low seed production in other species might also be acting in populations of *L. asperulifolia*.

### **Life History of *Lysimachia asperulifolia***

*Lysimachia asperulifolia* (Primulaceae), commonly known as Rough Leaved Loosestrife, is a self-incompatible, insect-pollinated, rare species that often produces few seeds in natural populations, even when flower production is high (Frantz, 1984). It is a perennial wetland species endemic to the coastal plain and sand hills of North and South Carolina (Frantz, 1995). It is typically found growing in the ecotones between long-leaf pine savannas and pocosins, where periodic disturbance by fire and long hydroperiod provide habitat essential to its growth. The species was listed as Federally Endangered in 1987 due to the threatened nature of its habitat (caused by wetland draining, fire suppression, and land development) and the existence of only nine known population centers (Frantz, 1995). Many of the factors that have contributed to the destruction and fragmentation of southeastern long-leaf pine savannas have probably contributed to the rarity of this species.

I conducted a survey of 155 flowering stems in 2001 and found that flowers are borne in racemes of 1 – 31 flowers on typically unbranched stems, with an average of 9 flowers per flowering stem. Most flowers open before 9:00 am, remain open 4-7 days, and have receptive (shiny) stigmas throughout their 4-7 day life (Frantz, 1984). The average number of ovules per ovary is 16. Frantz (1984) showed that *L. asperulifolia* is self-incompatible. The flowering season usually lasts from the last week in May until the

third week in June, and, across its range, the species has been observed to produce far more flowers than fruits. Capsules become ripe during late September to mid-October. Individual fruiting stems produce 1-3 capsules. Fertile capsules contain 1 - 9 seeds, with an average of 4. Seeds have no apparent specialized dispersal mechanism and probably do not move far from the parent plant. The primary means of reproduction appears to be vegetative (by rhizome growth and fragmentation) (Frantz, 1984). Because of the species' highly restricted seed dispersal and clonal nature, genetic relatedness among near neighbors in natural populations is expected to be high. It is likely that stems in close proximity have a high probability of being ramets of individual clones.

The historically frequent occurrence of fire in the Southeastern United States has resulted in selection for fire-resistant traits in the regional vegetation (Lemon, 1949). Like other fire-resistant species, *Lysimachia asperulifolia* has features preventing destruction of vital regenerative tissues, specifically well-protected rhizomes. However, unlike other fire-resistant species, it does not have provision for abundant production and efficient dissemination of reproductive bodies (Lemon, 1949).

#### *Pollinators of L. asperulifolia*

Frantz (1984) identified six species of bees that visit flowers of *L. asperulifolia* at populations in the Green Swamp and the Croatan National Forest. The majority of flower visits were made by two species of halictid bees, *Lasioglossum sensu Danforth (1999) (=Dialictus)* and *L. coreopsis*. Other visitors included *L. rohweri*, *Augochlorella striata*, and two more unidentified individuals of the genus *Lasioglossum (= Dialictus)*. *Augochlorella* is a small, metallic bluish green eusocial bee that is common and represented by seven species in the USA (Michener et al., 1994). Individuals in the genus *Lasioglossum* are considered abundant, and generally the most common genus of bees in most north temperate localities (Michener et al., 1994). *Lasioglossum* sp. and *Augochlorella striata* are nearest-neighbor pollinators; individuals visit many inflorescences in close proximity and only occasionally bypass near-neighbor plants (Aspinwall and Christian, 1992, Waddington, 1979). These bees usually make very short flights and flight distances increase as flower density decreases (Waddington, 1979). Frantz noted that pollinators of *L. asperulifolia* frequently revisited flowers on individual stems before visiting other

stems so that geitonogamous and autogamous visits were common. Because *L. asperulifolia* is self-incompatible, effective pollination can only occur when pollen is transported between flowers with different self-incompatibility alleles. In *L. asperulifolia*, it is expected that under the nearest neighbor foraging strategy exhibited by *Lasioglossum* sp. and *Augochlorella striata*, the probability of effective pollination decreases as the size and/or density of the clone increases. This probability is lower within dense clusters of stems growing in close proximity than among the clusters or among populations, as is the case for populations of other species (Aspinwall and Christian, 1992, Campbell, 1989, Waddington, 1979).

#### *Potential Pollinators of L. asperulifolia*

Although Frantz did not observe bees of the genus *Macropis* at her two study sites, Simpson et al., (1983) predicted that *Macropis* spp. would visit *L. asperulifolia*. Like many other *Lysimachia* species that are pollinated by *Macropis* spp., *L. asperulifolia* does not produce nectar but does produce oils on capitate trichomes within the corolla. *Macropis* spp. collects floral oils for use as a food source in larval provisions and to waterproof larval brood chambers (Cane et al., 1983; Buchmann, 1987). Buchmann (1987) cited evidence that oil-producing *Lysimachia* species in the New World are dependent on *Macropis* for pollination.

The known range of *Macropis* is included within the distribution of the 78 oil-producing *Lysimachia* species (Buchmann, 1987, Cane et al., 1983). *Macropis* bees collect oil by clinging to filaments while curled around the anthers (Cane et al. 1983, Buchmann 1987). During this process, the bees become ventrally dusted with pollen, which they transfer to protruding stigmas of subsequent flowers. While the capitate trichomes are present in *L. asperulifolia* (reported by Simpson et al. and verified by authors), no studies have attempted to determine whether *Macropis* is associated with this species.

The four species of *Macropis* in Eastern and Central USA are uncommon (Michener et. al, 1994). All four eastern species of *Macropis* have been collected in North Carolina and deposited in the North Carolina Insect Collection at North Carolina State University, but none were collected east of Raleigh. No records of *Macropis* visits to any species of *Lysimachia* have been reported in North Carolina; however, we could locate no evidence that previous researchers have attempted to document such an association.

### **Factors Contributing To Low Seed Production**

In this section, I present some elements that may be interacting to suppress seed production in natural populations of *Lysimachia asperulifolia*. A schematic diagram of hypothetical interactions is presented in Figure 1.1. This diagram was designed to highlight the complex web of interactions between plants, populations, pollinators, and their environment.

#### *Habitat Fragmentation*

Compared to many other temperate habitats, the floras of regularly burned, unfragmented long-leaf pine savannas are species rich (Wells, 1928, 1931, Lemon, 1949, Walker and Peet, 1984, Taggart, 1990, Beckage and Stout, 2000). Widespread habitat degradation of long-leaf pine savannas in the Southeastern United States, where *Lysimachia asperulifolia* was historically found, has involved grazing by feral pigs, tree damage by the naval stores industry, altered fire regimes, timber extraction, and wetland draining, as well as fragmentation by agricultural, residential, and industrial development (Frost et al., 1986). When natural vegetation is fragmented into small patches, numerous ecological processes that involve the animal and plant inhabitants may be affected (Aizen and Feinsinger, 1994, Jennersten, 1988). Changes in the physical structure of the habitat, competition from invaders, intrinsic demographic changes that increase extinction probabilities for small populations, and scale dependent dispersal processes can all cause species in surviving fragments to decline or vanish entirely (Aizen and Feinsinger, 1994). If fragmentation and destruction affect certain animal or plant populations participating in mutualistic interactions, effects could cascade and affect other species as well (Aizen and Feinsinger, 1994). Scarcity of suitable foraging material for pollinators, competition with exotic species, and herbicide and pesticide use may reduce numbers of critical pollinators and limit pollinator activity. Plant populations in fragmented habitats have been found to receive fewer floral visits and host fewer pollinator species than populations in continuous vegetation, which in turn can decrease seed production by plant survivors (Aizen and Feinsinger, 1994, Jennersten, 1988). Pollinator limitation has been found to contribute to low seed sets in natural and agricultural populations (Kearns, et al., 1998). Low local pollinator populations could be

partly responsible for the observed low seed production in populations of *L. asperulifolia*, a species known to be entomophilous (Frantz, 1984).

#### *Population Structure and Pollen Dispersal*

Population sizes and densities of savanna herbs including *L. asperulifolia* fluctuate yearly in response to environmental stimuli, including fire (Lemon, 1949, Taggart, 1990, Beckage and Stout, 2000). These changes in plant density and availability of floral resources can influence pollinator flight patterns, as bee pollinators minimize flight costs by minimizing flight distances between successively visited flowers, thereby maximizing the foragers' net energy gain (Waser and Price, 1983, Ellstrand et al., 1978, Levin & Kerster 1969, Kearns, 1993). A positive relationship between flower density and visitation rate has been found for both solitary bees and bumblebees (Thompson, 1981). In plant populations where the relatedness of plants declines with distance, density-dependent pollinator behavior should bring about a higher frequency of mating between related plants in dense populations than in sparse populations, thus causing a negative correlation between plant density and outcrossing (Ellstrand et al., 1978, Levin and Kerster, 1969). Pollen dispersal distance can affect the fitness of a cross by influencing parental fecundity and lifetime fitness of offspring. For self-incompatible clonal species, where nearest neighbors are usually genetically identical, pollen transfer between nearest neighbors could result in incompatible crosses. Handel (1985) has demonstrated that seed production in self-incompatible insect-pollinated plants may be influenced by the interaction of highly restricted pollen flow distances and increasing size of the clone. I predict similar dynamics are at work within the *L. asperulifolia* communities.

#### *Pollen Compatibility*

As suitable habitat becomes increasingly fragmented, the increased distance between populations of self-incompatible species with low genetic diversity can limit seed production by reducing the probability of compatible crosses. Understanding compatibility at multiple spatial scales under natural conditions within a plant species range allows insight into the significance of genetic incompatibility,

especially in species where mating is restricted to local neighborhoods (Stacy, 2001, Waser and Price, 1983).

Genetic differentiation (which is influenced by pollen dispersal distance) within and among populations can produce a correlation between physical and genetic distance (Campbell, 1989) and genetic differentiation on a small spatial scale is possible (Waser and Price, 1983). In populations of self-incompatible species where vegetative reproduction is common, stems in close proximity have a high probability of being ramets of individual clones (Aspinwall and Christian, 1992). Levin and Kerster (1969) found physical distance between plants was positively correlated with genetic distance for *Liatris cylindracea* (Waser and Price, 1983). For self-incompatible species, only those pollinations involving stems of different rhizomes that are reproductively compatible would lead to seed production. Although sexual reproduction is not necessary for the continuation of the existing populations of rhizomatous perennials, low seed set may contribute to failure to disperse to new sites.

#### *Crossing Distance – Near Neighbor*

Seed production has been shown to be influenced by the distance between the pollen source and recipient (Kearns, 1993; Redmond, 1989; Stacy, 2001; Waser and Price, 1983). Seed set may be lower following crosses of neighboring plants than widely spaced plants, and seed and seedling mortality may be higher following self and near-neighbor crosses than following wide crosses (Levin, 1981; Redmond, 1989).

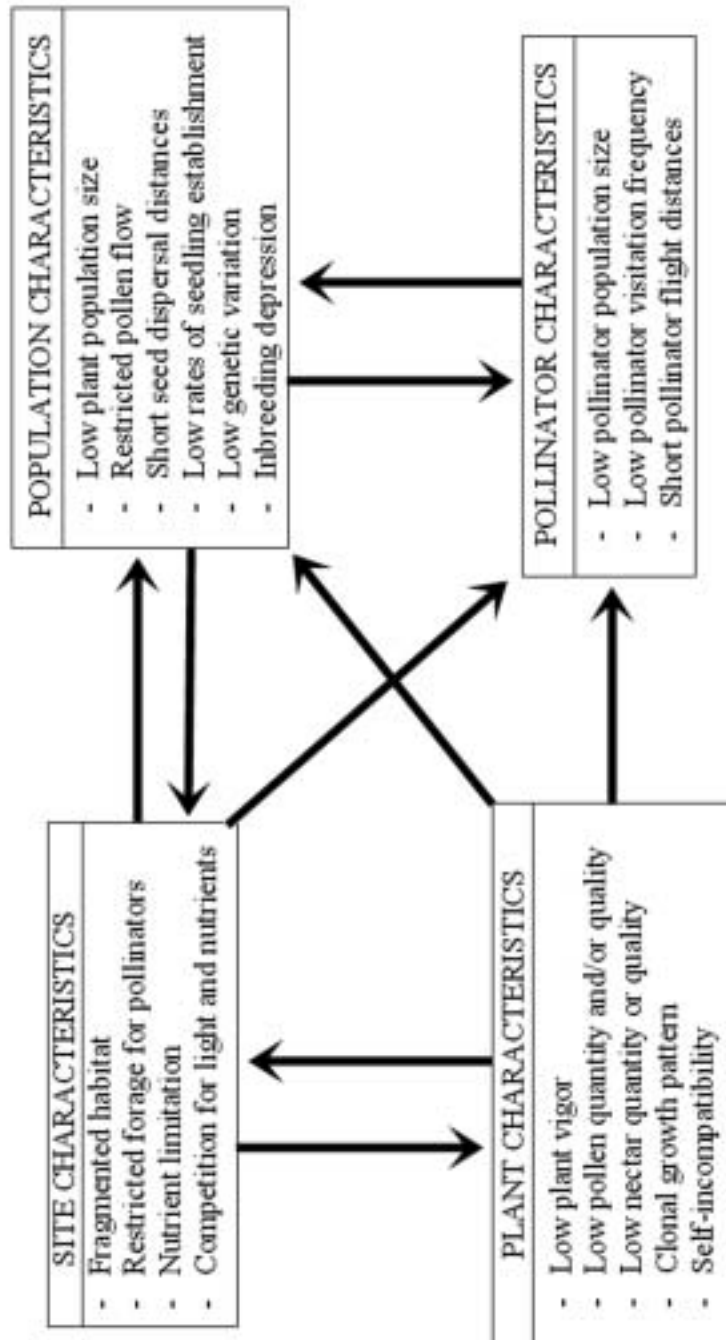
#### *Crossing Distance – Other Population*

Geographically separated populations may differ genetically due to founder effects, random genetic drift or selection (Stacy, 2001). For self-incompatible species, these genetic differences among populations could lead to increased chances of seed production from inter-population crosses when compared with crosses within populations, where there is a high probability of crosses between genetically closely related individuals.

### *Pollinator Limitation*

Low pollinator visitation rates and/or effectiveness can compound other factors that limit seed production by reducing pollen flow, especially if pollen dispersal is dependent on insects (Jennersten, 1988). If flowers are rarely visited by pollinators, the chances of effective pollen dispersal are decreased, thereby decreasing seed production (Willson and Schemke, 1980, Jennersten, 1988).

Given the evidence of human involvement in the decline of *Lysimachia asperulifolia* as a species, both directly through destruction of populations and indirectly through habitat destruction, we, as humans are ethically responsible to promote the reestablishment of healthy, breeding populations. With this goal in mind, I have undertaken to study some of the ecological factors currently limiting sexual reproduction in remnant populations. After we understand the natural barriers to seed production, we can begin to implement measures that may mitigate or remove these barriers. Until a thorough understanding of the reproductive ecology is available, however, attempts to augment seed production could be inefficient and speculative.



Ecological Interactions That Contribute To Low Seed Production

Figure 1.1. Schematic diagram of interactions that contribute to low seed production.

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FACTORS AFFECTING SEED PRODUCTION IN NATURAL POPULATIONS OF *LYSIMACHIA ASPERULIFOLIA* POIR. (PRIMULACEAE), A RARE, SELF-INCOMPATIBLE PLANT SPECIES

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*Lysimachia asperulifolia* Poir. (Rough Leaved Loosestrife), a perennial, rhizomatous herb, is restricted to the fire-influenced ecotones between long-leaf pine savannas and pocosins in the Coastal Plain of North and South Carolina. This species produces few seeds and is thought to be self-incompatible. Low seed production may prevent establishment of new populations and production of new genotypes, limiting the ability of the species to survive diseases and changing environmental conditions. We conducted a study in five natural populations to determine causal mechanisms of limited seed production and to determine if and how the individual causal mechanisms and the resultant level of seed production were affected by prescribed fire. Our study consisted of the following: 1) We measured distances between flowering and vegetative stems to determine patterns of Stem Distribution before and after prescribed burning. All populations grew in an aggregated pattern both years and distance between nearest vegetative stems did not change after burning, except at one site, where distance decreased. Average distances between nearest flowering stems decreased at all sites after burning, even when overall number of flowering stems remained constant or decreased. 2) We artificially pollinated flowers using pollen from varying distances within and between populations to determine if seed production is related to crossing distance. Between-population crosses consistently produced more seeds than within-population crosses. 3) We compared seed production from unmanipulated flowers with that from flowers which had received additions of pollen to determine if pollinator limitation is a factor limiting seed production. There was no difference in seed production between control flowers and those receiving additional pollen loads. 4) After burning, we observed insect flower visitation frequency and behavior and found slightly more xenogamous than geitonogamous visits and low visitation frequency. We used fluorescent powder to estimate powder movement distances, and

found powder movement infrequent and limited to within 7 meters of the source flowers. 5) We estimated the relative density of halictid bees in the pollinator community using pan trap sampling. The species of pollinators represented in the pan traps were the same as those observed visiting flowers. The rate of capture by pan traps was similar to that from other pan trap studies. Factors listed here strongly suggest that low levels of variability for compatibility alleles within populations, low pollinator visitation frequency, and limited pollen dispersal are the major factors limiting seed production.

### *INTRODUCTION*

*Lysimachia asperulifolia* (Primulaceae), commonly known as Rough Leaved Loosestrife, is a self-incompatible rare species that produces few seeds in natural populations, even when numerous flowers are produced. Sexual reproduction is not necessary for continuation of the existing populations of this rhizomatous perennial species. However, low seed set coupled with habitat destruction restrict the rate of establishing new populations. Additionally, low seed production represents a limitation to genetic recombination that can restrict the ability of populations to respond to diseases and long-term environmental change. We hypothesize that the observed low seed production across the species' range is the result of a suite of biological and ecological factors, including low pollen fertility, low genetic diversity within populations, low rates of pollinator visitation, and short pollen movement distances.

*L. asperulifolia* appears to reproduce primarily by rhizome growth and fragmentation (Frantz, 1984). The few seeds that are produced have no apparent specialized dispersal mechanism and probably do not move far from the parent plant. Because of the species' highly restricted seed dispersal and clonal nature, genetic relatedness among near neighbors is expected to be high. It is likely that stems in close proximity are ramets of individual clones. As suitable habitat becomes increasingly fragmented, the increased distance between populations combined with low genetic diversity within isolated populations can limit seed production by reducing the probability of compatible crosses. Understanding cross-fertility at multiple spatial scales under natural conditions within the species range allows insight into the importance of genetic incompatibility in suppressing seed production (Stacy, 2001; Waser and Price, 1983).

*Lysimachia asperulifolia* grows in a fire-adapted ecosystem and appears to benefit from periodic burning. Without regular prescribed fire, vegetative growth in the species appears to be suppressed due to competition with pocosin shrubs and savanna grasses. We hypothesize that burning increases vegetative growth and flower production, thereby increasing the chances of seed production. Positive effects of fire on seed production have been found in other species (Enright, 1999). However, if burning increases production of genetically incompatible flowers, seed production may not increase even in the presence of increased flowering.

Simpson et al. (1983) predicted that bees in the genus *Macropis* would be associated with *Lysimachia asperulifolia*. Like many other *Lysimachia* species that are pollinated by *Macropis* spp., *L. asperulifolia* does not produce nectar but does produce oils on capitate trichomes within the corolla. *Macropis* spp. collect and use floral oils as a food in larval provisions and for waterproofing larval brood chambers (Cane et al., 1983; Buchmann, 1987). *Macropis* spp. collect oil by clinging to filaments while curled around the anthers, during which process the bees become ventrally dusted with pollen which they transfer to protruding stigmas of subsequent flowers (Cane, et al. 1983; Buchmann, 1987).

In a previous study of the visitors to *L. asperulifolia* flowers, Frantz (1984) did not find *Macropis*; she observed only non oil-collecting bees of the genera *Lasioglossum* and *Augochlorella*. Individuals of these genera most commonly move between nearest neighbors when foraging at flowers (Aspinwall and Christian, 1992; Waddington, 1979). Bees at *L. asperulifolia* flowers have been observed to revisit flowers and stems so that geitonogamous and autogamous visits are common (Frantz, 1984). For self-incompatible clonal species, where nearest neighbors are usually genetically identical, pollen transfer between nearest neighbors would result in incompatible crosses. The probability of effective pollination decreases as the size of the clone increases (Aspinwall and Christian, 1992). Density-dependent pollinator behavior should bring about a higher frequency of mating between related plants in dense populations than in sparse populations, thus causing a negative correlation between plant density and outcrossing (Ellstrand et al., 1978; Levin and Kerster, 1969).

In addition to expected short pollen flow distances and low genetic compatibility, we hypothesize that low visitation frequency also limits sexual reproduction in populations of *Lysimachia asperulifolia*.

Anecdotal evidence from land managers across the species range has suggested that pollinator visitation frequency at loosestrife flowers is very low. Populations occur in areas of natural vegetation that has been fragmented into small patches. When fragmentation affects animal or plant populations participating in mutualistic interactions, these effects could cascade through their symbionts (Aizen and Feinsinger, 1994). Flower populations in fragmented habitats often have lower visitation frequency and lower pollinator species richness than flower populations in continuous vegetation. Low visitation rates can restrict pollen flow, which in turn can decrease seed production by plant survivors (Aizen and Feinsinger, 1994; Jennersten 1988). Small local pollinator populations could be responsible for our expected low pollinator visitation rates. Pollinator limitation has been found to limit fruit production in studies of other species (Willson and Schemke, 1980; Jennersten, 1988; Kearns et al., 1998).

Finally, low plant fertility caused by factors such as nutrient limitation, disease, or inbreeding depression could be limiting sexual reproduction in natural populations of *Lysimachia asperulifolia*. Low pollen fertility can directly influence seed production by preventing pollen germination and tube growth.

The components of pollination ecology -- flowering stem density, genetic compatibility, pollen flow distances, flower visitation, and pollen fertility -- are individual parts of a natural web of plant-pollinator interactions that can be acting independently or in concert to influence seed production. In an effort to understand the importance of these components in a natural setting, we addressed the following questions: 1) Across populations, how consistent is the flowering response to fire, as assessed by pollen fertility, number of flowering stems, distance between flowering stems, dispersion pattern of individual stems, and seed production? 2) Does the distance between the pollen source and the pollen recipient influence the number of seeds produced (i.e. Is seed production limited by pollen compatibility within populations)? 3) Does augmenting pollen transfer from the maximum range within a population enhance seed production (i.e. Is seed production limited by pollinator activity)? Our hypothesis is that self-incompatibility and low genetic diversity, coupled with pollinator limitation, work together to inhibit seed production in natural populations.

*METHODS* –

## Study Sites

We conducted research in North Carolina at five natural populations of *L. asperulifolia*: two populations in the Croatan National Forest (CNF; Carteret County), two populations in the Green Swamp (GS; Brunswick County) and one population at Camp Lejeune Marine Corps Base (C; Onslow County) (Table 1 and Figure 2.1). The two CNF study sites were designated Hibbs Road (H) and Millis Road (M). The H population grows in four groups labeled A – D with areas between the groups containing no stems. The two GS study sites were designated G1 and G2. All of the study sites are in areas of protected habitat that have long-term management plans which require prescribed burns every 3 – 5 years. Prior to the start of the study, CNF-M was burned in February 1999; CNF-H in November 1997; and C, during the dormant season of 1999. CNF and C sites were next burned during the spring of 2001. Data were collected from these sites during the growing seasons of 2000 and 2001 before and after the most recent burn. GS was included in the study in 2001; it had been burned during September 2000. The landscapes at all five sites were heterogeneous mosaics of long-leaf pine savanna and pocosin. All study populations were located in ecotones between pine savannas and pocosins (peat bogs dominated by dense thickets of evergreen shrubs). *L. asperulifolia* is typically found growing in this habitat where, apparently, periodic disturbance by fire and a long hydroperiod provide habitat essential to its growth. Detailed descriptions of vegetation characteristic of this habitat can be found in Wells (1932). A list of co-flowering species in the study sites is presented in Appendix A.

## Stem Distribution

In three of the populations, we censused or sampled vegetative and flowering stems and determined distances between neighboring stems during the growing seasons immediately before (2000) and immediately following (2001) the most recent prescribed burn to detect post-fire changes in stem number and distribution. Because the species can reproduce vegetatively by means of underground rhizome growth and fragmentation, determining whether two stems are ramets of the same or of different genets is difficult. For this reason, we did not attempt to establish genetic relatedness between neighboring stems.

At H and C, we censused inter-stem distances. Due to the large number of stems at M, we sampled, rather than censused, distances. We compared square-root transformed pre-burn and post-burn distances at each site using analysis of variance followed by contrasts. The analysis was carried out using SASPROC GLM with an LSMEANS statement (SAS 2000).

We determined the type of stem distribution pattern (random, uniform, or clustered) using the T-square analysis as described by Ludwig and Reynolds (1988). For a detailed description of T-square methods, see Appendix B.

### **Fluorescent Powder**

During the flowering season of 2000, we estimated the extent of insect-mediated pollen dispersal. We used bright powdered dyes as pollen surrogates to mimic pollen movement (Kearns and Inouye, 1993, Brown and Mitchell, 2001, Thompson, 1981, Waser and Price, 1983). During the second week of June, at M, H, and C, we applied Day-Glo brand Blaze Orange powder to the dehiscing anthers of two flowers of one stem near the center of each population. We applied Day-Glo brand Aurora Pink powder to two flowers on one stem near the edge of each population. Three days after powder application, we examined flowers in the surrounding area for dye particles (xenogamy). The intervening days were sunny, with no precipitation. We checked for powder movement to the stigmas of the flowers that originally received powder (autogamy) and other flowers on the same stem (geitonogamy). Because the effectiveness of dye powder as a pollen analogue has not been tested for *L. asperulifolia*, and because of the low number of replications, we used the presence/absence of powder on flowers only as a preliminary indicator of pollen movement.

### **Pollinator Observations**

We observed insects visiting flowers of *L. asperulifolia* at the study populations during the flowering season of 2001 to estimate pollinator visitation frequency and understand movement patterns. We watched patches of sunlit flowering stems for 10-minute intervals on sunny or partly cloudy days, and noted the number of xenogamous and geitonogamous visits. Here, a xenogamous visit was defined as one

involving an insect visiting a flower on a monitored stem after having arrived from some unspecified different stem. A geitonogamous visit was defined as one involving an insect visiting a flower on a monitored stem after having previously visited another flower on the same stem. Observations at each population included twelve 10-minute intervals before 1000 (Morning), twelve intervals between 1000 and 1700 (Midday), and twelve intervals between 1700 and 2015 (Evening). We conducted pollinator observations throughout the species' blooming period, from June 1 – 11 at C, June 4 – 6 at G1, June 6 – 11 at G2, May 31 – June 19 at H, and June 4 – June 18 at M. We collected and identified insect visitors that remained on the flowers after completion of each 10-minute observation interval and deposited them in the insect collection at North Carolina State University.

We tested the hypotheses that visitation frequencies were the same at all sites and during all times of day. We analyzed number of visits per 10-minute interval per inflorescence using a two factor ANOVA with fixed effects of site and time of day. Square root transformed data were used for this analysis. Attempts to analyze the data using a program that accounted for Poisson-error distributions (GENMOD) resulted in non-convergence, so comparisons using ANOVA are presented. These comparisons should be treated as approximations.

We also used linear regression to analyze the effects of number of open flowers in the patch and number of inflorescences in the patch on visitation rates, and to correlate visitation rates with seed production in populations.

### **Pan Trap Sampling**

We used pan traps to sample insect populations at the study sites to estimate the relative abundance of halictid bees in the *L. asperulifolia* populations. Pan traps have been used as flower models to monitor insect populations in habitat fragments and managed/restored habitats (Leong and Thorp, 1999; Aizen and Feinsinger, 1994). Bowl color has been shown to influence the kinds and numbers of insects caught in pan traps (Leong and Thorp, 1999; Williams et al., 2001). Because bees demonstrate strong responses to colors associated with floral rewards such as pollen and nectar, they are good candidates for pan trap sampling (Leong and Thorp, 1999). We set out pan traps consisting of small plastic bowls (Solo™

party bowls, Urbana, Illinois, USA). We chose white, blue, and yellow bowls because these were the predominant floral colors that occurred at the study site during the time of sampling. We filled each bowl with water, to which we added a few drops of a surfactant (unscented aqueous dishwashing detergent). We placed the traps within dense floral patches of *L. asperulifolia* at flower height. During this study, *L. asperulifolia* was the dominant yellow flowering species in the study sites, although *Rhexia lutea*, *Hypericum reductum*, and *H. galioides* were also present.

In each population, we arranged one pan trap of each color in random order at the center of a patch of flowering stems. We took samples in 2001 during the peak blooming period of *L. asperulifolia*. We sampled on June 1 and June 11 at C, June 6 and June 11 at GS, on June 6 and 11 at H, and on June 5 and 11 at M. We left pan traps out each day from 0800 until 1700. Weather conditions were clear, warm, and calm on all sampling days. We emptied traps at the end of each sampling day and stored specimens in 70% ethanol until they were identified. T.R. identified bee specimens to genus and, when possible, species. We did not further examine non-bee insects and other arthropods present in the pan trap samples. We deposited bee specimens in the North Carolina Insect Collection at North Carolina State University in Raleigh, North Carolina.

### **Natural Fruit Production**

We counted number of stems naturally producing one or more fruits and calculated percent of inflorescences bearing fruit in September during 2000 and 2001, to assess whether seed production was higher in the populations after the fire.

### **Pollen Stainability**

We tested pollen stainability using Cotton Blue stain in Lactophenol, as described by Kearns and Inouye (1993). This dye lactophenol stains nonabortive pollen but not abortive pollen (Kearns and Inouye, 1993). Pollen stainability is an indicator of male fertility that can be used both as an estimate of population fecundity and as a test to ensure the use of live pollen in artificial pollination experiments so as not to confound compatibility tests (Kearns and Inouye, 1993). We removed three anthers from one flower of

each stem to be used in the pollen compatibility and pollinator limitation studies (described below). We transferred pollen from the collected anthers to slides and stained pollen with cotton blue in lactophenol. We used a systematic method of viewing the slides under a microscope, beginning at the edge of the cover slip and scrolling from left to right across the stage. Pollen grains were observed, making sure to include grains along the edge of the cover slip as well as those in the middle of the slip. We scored the first 100 pollen grains encountered for the presence/absence of stain.

We used percent pollen stainability as a measure of pollen fertility and compared fertility among sites and years. We hypothesized that different sites would have similar levels of pollen fertility and that pollen fertility would increase after the prescribed fire. We analyzed the percent stainability data using a two-way ANOVA to test for effects due to site, year, and interaction between site and year.

We also used percent pollen stainability as a screen when choosing flowering stems to be included in the genetic compatibility, self-incompatibility, and pollinator limitation phases of this study. We used only those stems with flowers producing >70% stainable pollen in the compatibility study.

### **Verifying Self-Incompatibility**

During 2001, we bagged buds and then self-pollinated flowers on 14 stems to verify the previous reports of loosestrife self-incompatibility (Frantz, 1984).

### **Genetic Compatibility**

During the flowering seasons of 2000 and 2001, we artificially cross-pollinated flowers with pollen from varying distances within and among populations to examine the effect of outcrossing distance on seed production. At the beginning of each growing season, we enclosed buds in nylon hosiery to exclude pollinators from flowers as they opened. We designated male pollen donors and female pollen recipients by using pollen stainability as described above.

We used pollen from each donor stem for all treatment categories at least once. We assigned each pollen recipient stem to one of the following treatments: Unbagged/Unpollinated, Bagged, Near Neighbor Cross, Distant Neighbor Cross, Other Population Cross (Table 2 and Figure 2.2). Three emasculated

flowers per stem received pollen from the same pollen donor. We removed stigmas from all non-pollinated flowers on each pollen recipient stem to avoid variation due to resource allocation to differing numbers of developing seeds on different pollinated stems. The number of flowers in each population limited the number of replicates for each treatment.

We analyzed square-root transformed seed and fruit counts in SAS (2000) using PROC GLM with the stem as the unit of replication and seed or fruit production averaged across all three treatment flowers on the stem. We tested for effects due to site, treatment, and pollen donor.

At the end of each growing season, we collected capsules from treatment stems and counted seeds in each capsule. We moist-stratified the seeds in a refrigerator at 40 degrees for 30 days, and then sowed them in a greenhouse according to methods used by the North Carolina Botanical Garden (Robert Gardner, personal communication). We tested non-germinating seeds for viability using tetrazolium according to the methods described in Kearns (1993).

### **Pollinator Limitation**

During the 2001 growing season, we conducted a study to determine if seed production was pollinator-limited. We used the pollen donors from the “Pollen Compatibility” study. As recipients, we used only stems producing >70% stainable pollen. We assigned each flowering stem to one of the following treatment categories: Unbagged/Artificially Pollinated (We applied pollen to all receptive flowers on the stem and left anthers intact.); Unbagged/Unmanipulated (Flowers were completely unmanipulated.) (see Table 3). We used within-population pollen for the Unbagged/Artificially Pollinated treatment because within-population pollen movement most closely resembles the likely natural pollination system of these populations, which are widely separated and rarely receive pollen from other populations.

We analyzed square-root transformed number of seeds and capsules produced with SAS PROC GLM with the stem as the unit of replication and seed or fruit production averaged across all three treatment flowers on the stem. We tested for effects due to site, treatment, and interaction between site and treatment.

## **RESULTS AND DISCUSSION**

### **Stem Distribution**

In all cases except C, the total number of stems increased after prescribed burning (Table 4). At C, the total number of stems decreased. The number of vegetative stems increased after the prescribed burn at all sites except H- Group A, where the number of vegetative stems remained constant and H – Group C, where the number of vegetative stems decreased. The populations generally showed an increase in number of vegetative stems after the fire (Table 4).

We observed an inconsistent flowering response to fire among populations. C and H groups A and C showed an increase in flowering, while M and H groups B and D had fewer flowers after the burn. The different responses to fire could be the result of natural burn patterns in a landscape marked by plowed fire lines remaining from previous years. H groups B and D are both on the pocosin side of fire lines that separate the savanna from the pocosin. The microhabitat could have caused the fire to burn these groups with less intensity than the groups of stems not growing in the fire lines.

Because all populations have a consistent history of prescribed burning, the populations may have already been under optimal growth conditions and therefore showed a limited increase after burning. More dramatic increases in flowering and vegetative stems would be expected if the prescribed burns had been introduced after a history of fire suppression.

### **Distances Between Nearest Flowering Stems**

Nearest neighbor distances for flowering stems decreased after the prescribed burn at all sites except C and H-D (Figure 2.3). At C, the distance between flowering stems increased, even though the actual number of flowering stems also increased. This suggests that fire caused an expansion in the size of the flowering population at C. At M, even though there were fewer flowering stems after burning, the stems were closer together. This suggests an opposite kind of response as that at C.

The decreased distance between flowering stems following fire noted at two populations could actually decrease pollen flow distances and apparent outcrossing, even though the denser post-burn patches may be more attractive to pollinators. In dense populations where the relatedness of plants declines with

distance, density-dependent pollinator behavior is expected to bring about a higher frequency of mating between related plants than in sparse populations, thus causing a negative correlation between plant density and outcrossing (Ellstrand et al., 1978; Levin and Kerster, 1969). For self-incompatible clonal species, where nearest neighbors are usually genetically identical, pollen transfer between nearest neighbors could result in incompatible crosses. Handel (1985) has demonstrated that seed production in self-incompatible insect-pollinated plants may be influenced by the interaction of highly restricted pollen flow distances and increasing size of the clone. This effect could confound seed production increases that might otherwise be caused by increased numbers of flowers and increased pollinator visitation frequency.

### **T-Square Analysis**

T-Square calculations revealed that vegetative stems in all populations sampled were aggregated for both years. The aggregated pattern was highly significant for both 2000 (preburn) and 2001 (postburn). The *C*-value derived from T-square calculations is an index of dispersion, with values significantly above .50 indicating aggregation. *C*-values were as follows: .8484 (2000) and .7387 (2001) at C; .6769 (2000) and .6819 (2001) at M; and .9047 (2000) and .8799 (2001) at H – C. The *z*-value is an indication of significance (numbers greater in absolute magnitude than 1.96 are significant at  $P=0.05$ ). *Z*-values were as follows: 8.53 (2000) and 5.23 (2001) at C; 4.74 (2000) and 3.99 (2001) at M, and 16.109 (2000) and 6.96 (2001) at H – C. Clumping of flowers make them more desirable for bumblebee visitation (Real, 1982).

### **Fluorescent Powder**

Of the 428 flowers sampled for fluorescent powder deposition, powder was moved to the petals, stamen, or styles of only 17 flowers. The stigmas of only 2 flowers received powder so that reproductively effective pollen dispersal was extremely limited (Table 5). Of the 19 instances when powder did move, it was deposited a maximum of 5.5 meters from the source flower. These observations indicated that pollinators probably do not frequently disperse pollen to stigmas widely in these populations and that flower visitors may be so small that they do not contact stigmas when they visit flowers to collect pollen, and these results served as the basis for planning the research we did in 2001 to further explore pollinator

activities. When powder movement distances are considered along with the low visitation frequency and the known foraging patterns of *Lasioglossum* and *Augochlorella*, the evidence presents a good case for short actual pollen flow distances in these populations of aggregated inflorescences.

### **Pollinator Observations**

Overall flower visitation rates were very low. Of the 185 10-minute observation intervals, 159 intervals had zero pollinator visits (Figure 2.4). The overall mean flower visit frequency per inflorescence per 10-minute observation interval was .178.

When we analyzed frequency of total visits per inflorescence per 10-minute interval using square-root transformed data, we found differences due to time of day and site (Table 6). The highest total visitation frequency and the highest xenogamous visitation frequency were in the morning. The site differences were due to the higher visitation frequency at M than G2. Surprisingly, M and G2 have the highest number of flowering stems, but M has the most frequent visits while G2 has the least frequent visits. This observation is inconsistent with studies conducted by Thompson (2001), who found that the number of open flowers in the surrounding patch had a significant positive effect on the mean number of insect visits. Regression using square-root transformed data indicated no significant relationship of number of flower visits per inflorescence per 10-minute interval with number of open flowers or inflorescences in the patch.

Pollinators were most active in morning and evening, and least active during mid-day. Because *L. asperulifolia* flowers do not produce nectar and *Lasioglossum* spp. and *Augochlorella* spp. do not collect oil, the only important floral resource for pollinators was pollen, which is most abundant in the morning (Frantz, 1984).

We observed no statistically significant differences in visit type among sites or times, although all sites had slightly more xenogamous visits overall ( $p = .20$ ).

Our presence did not visibly disturb visiting insects and we easily captured pollinators with the aid of killing jars. Of the pollinators trapped while visiting flowers, individuals in the genus *Lasioglossum* were most abundant (Table 7). We did not collect any specimens of *Macropis*, the oil-collecting pollinator

of other *Lysimachia* species. All collected pollinators were small halictid bees in the genera *Lasioglossum* and *Augochlorella* (Table 7).

### **Pan Trap Sampling**

We used pan traps to sample halictid bees in the area. The bees collected in pan traps were in the same genera as the pollinators we collected from flowers (Table 7). During the combined total of 300 hours during which traps were set, traps caught only 12 pollinators. Traps caught no potential pollinators at GS. While each trap caught a total of 4 sweat bees each at C, H, and M, traps at each site caught an average of 36 other types of insects.

Because of the low numbers of potential pollinators caught, we were not able to perform statistical analysis for pan trap data. Yellow traps caught the most non-halictid insects (Figure 2.5). The low frequency of trapping sweat bee pollinators combined with the low flower visitation frequency presents a strong case for low pollinator populations at all study sites.

We used the pan traps for sampling to determine if there were other pollinator species present in the area that were not visiting the flowers. The traps did not indicate a large presence of pollinators in the area and did not reveal any species other than those caught on *L. asperulifolia* flowers. The rate of capture by pan traps was 0.20 pollinators caught per trap-hour across all populations except GS. Our rate of capture for bee species is very similar to that from two other pan trap studies: Cane et al. (2000) caught 0.233 bees per trap-hour in desert vegetation dominated by creosote bush, while Leong and Thorp (1999) caught 0.331 bees per trap-hour in one year and 1.8 bees per trap-hour in the second year at vernal pool sites in California (calculations based on authors data and text).

Because plant-pollinator systems inherently possess wide stochastic variation from year-to-year (Williams et al. 2001), additional data would be necessary before conclusions could be made about the long-term bee abundance at these populations. Species composition and abundance within bee communities can vary greatly within and among sites (Roubik, 2001). Intense sampling across years and sites will be required to document natural dynamics of populations and communities (Williams et al. 2001).

Neither pollinator observations nor pan trap samples yielded evidence of association between *Lysimachia asperulifolia* and *Macropis* bees. Species of *Lysimachia* and *Macropis* seem to be unequally coadapted, as *Macropis* is limited to loosestrife for larval provisions, but *Lysimachia* often reproduces by vegetative propagation and/or nonspecialist pollen vectors (Cane et al., 1983). In North America, oil-producing *Lysimachia* are abundant and widespread, but the *Macropis* bees are much more rare than their floral hosts (Buchmann, 1987).

### **Fruit Production**

The percent of inflorescences producing one or more fruits was lower after the prescribed burn than before the burn at all sites except M (Table 8). The total number of stems producing one or more fruits was lower after the burn at all sites except C, where the number of fruiting stems produced increased from 4 to 6. Theoretically, this trend in fruit production could result from several factors, including pollinator limitation (in the presence of increased numbers of inflorescences and no change in pollinator numbers, individual stems might receive fewer visits), decreased outcrossing resulting from increased aggregation of genetically incompatible flowering stems after the burn or other environmental factors.

Year 2001 percent fruit production is positively correlated with pollinator visitation frequency ( $r^2 = 0.95$ ). Because we did not have pollinator visitation data for 2000, we were unable to correlate visit frequency with seed production across years. When we regressed other variables (year, flower number, distance between flowering stems, and percent pollen stainability) from both years on fruit production, we found no strong correlations. The strongest relationship we found between these other variables and fruit production was for year ( $r^2 = 0.33$ ). The low number of sites (replicates) and low number of fruits limited efforts to uncover correlations between ecological parameters and natural fruit production.

Energy or nutrient availability can limit fruiting and seed production (Willson and Schemske, 1980). The poor seed production observed after augmenting natural stigmatic pollen loads via artificial pollination may be due to low resource availability. Combinations of inadequate pollinator service and limited physical resources have been suggested for other species (Campbell, 1985; Willson and Schemske, 1980). We did not attempt to determine whether these factors are important for our study populations.

### **Pollen Stainability**

We found significant differences in pollen stainability among sites, but no differences in percent pollen stainability due to year or interaction between year and site (Table 9). Each site had significantly different pollen stainability when compared with the other two sites (Table 10). These differences in pollen stainability could be caused by environmental factors or inbreeding depression. We observed no consistent relationship between pollen stainability and seed production.

### **Verifying Self-incompatibility**

The 14 bagged, artificially self-pollinated stems produced zero seeds and zero fruits. These results are consistent with previous reports of self-incompatibility in *L. asperulifolia*.

### **Genetic Compatibility**

Statistical analysis indicated significant differences in seed and fruit production due to site, treatment, and donor (Table 11 and Figure 2.6). Interactions between site and donor were imbedded in the analysis in the form of treatment category (Other Population treatment – a factor composed of both site and donor -- was expected to produce more seeds than within population treatments). H was the only site to produce statistically >0 seeds and fruits per stem. OP was the only treatment to produce statistically >0 seeds and fruits per stem. H was the only site that produced seeds from any within-population crosses (including the naturally pollinated unbagged treatment). This could indicate higher genetic diversity within the H population than within the other two study sites. This would not be surprising, given the differences in population structure (H is composed of 4 distinct groups of stems, whereas M and C are each composed of one large group of stems).

Isozyme data have recently increased in use to permit detailed examination of the population structure of clonal plant species (Aspinwall and Christian, 1992). Using genetic analysis to directly sample the population structure of populations would be a logical next step for understanding clonal distribution in natural populations. Our experimental evidence suggests that genetic similarity between mates is important

to plant fitness and that pollinators appear to transfer pollen over short distances rather than apparently optimal distances (between populations).

### **Pollinator Limitation**

We were unable to detect evidence of pollinator limitation affecting seed and fruit production. Although each of the unbagged treatments did produce seeds and fruits, there was statistically no difference between the treatments (seeds:  $p=0.08$ , fruits:  $p=0.09$ ), and neither of the treatments produced average production of statistically greater than zero seeds or fruits per stem. Again, we detected a difference due to site ( $p=0.03$  for seeds and  $p=0.0622$  for fruits), with H producing the most seeds and fruits and neither M nor C producing an average of statistically  $>0$  seeds or fruits per stem. Our failure to demonstrate pollinator limitation may not indicate lack of pollinator limitation in nature, but instead could be the result of within-population genetic incompatibility repressing seed production from all crosses and confounding our results. Pollinator limitation could extend to the observed failure of pollinators to move pollen between populations and produce effective crosses. Other studies have found pollen-limitation to be a factor in limiting fruit production (Willson and Schemske, 1980).

### **Seed Germination**

#### ***Genetic Compatibility Study***

A total of 70 seeds were produced from all genetic compatibility treatment categories in 2000. Only 7 of the seeds germinated. All the seeds that germinated were in the Other Population treatment category (Table 12). A total of 10 seeds and 4 fruits were produced from all treatment categories in 2001. All of these seeds were in the Other Population treatment category. Three of these seeds germinated.

#### ***Pollinator Limitation Study***

A total of 30 seeds and 12 fruits were produced from all pollinator limitation treatment categories in 2001. Six seeds were produced in the Unbagged Artificially Pollinated category, one of those germinated, and two of the non-germinating seeds stained positive using tetrazolium (Table 13). Twenty-four seeds were produced in the Unbagged, Unmanipulated category, five of those germinated, and six of

the non-germinating seeds stained positive using tetrazolium (Table 13). The difference in number of seeds produced was not statistically significant, given the uneven number of stems included in each category and the high variation.

## CONCLUSIONS

Our research uncovered the following factors that limit seed production in *Lysimachia asperulifolia*:

- infrequent movement of simulated pollen (fluorescent powder)
- infrequent pollinator visitation
- prevalent genetic incompatibility within populations
- ineffective pollen transfer between compatible stems.

The pollinators observed in the area appear to be inadequate in transferring pollen between genetically compatible stems. This appears to be true, even though the pollinator inadequacy is overshadowed by the strong effect of low genetic variation within populations. No population of effective pollinator species was captured, so that even if genetic incompatibility were not a problem, seed production would still be low due to infrequent movement of pollen to stigmas.

The studies of self-incompatibility, pollinator visitation frequency, and population structure can be integrated to provide a plausible explanation for the paucity of seed set in *L. asperulifolia* at five natural populations. The low seed set within populations supports the conclusion that the populations consist of one or just a few large clones that have undergone a sequence of expansion and fragmentation. In populations that produced extremely low seed set, population expansion may be due almost entirely to clonal expansion. One would expect that even a small amount of sexual reproduction over time would generate substantial genetic heterogeneity within populations. However, genetic heterogeneity does not appear to be expressed through compatible cross-pollinations. Even when seeds or seedlings are produced, competition from dense grasses and shrubs in the pocosin-savanna ecotones may out-compete them. Other studies of clonal plant species have uncovered similar problems (Aspinwall and Christian, 1992).

The determination of causal mechanisms for the poor seed set of *L. asperulifolia* in natural populations has important implications for the conservation of this rare and endangered species. If restoration of extirpated populations is to be attempted through transplantation of rhizomes from a single population, care should be exercised in choosing material from different clones. If available, the use of seeds would promote a genetically diverse population and consequently a population, which could reproduce sexually. A more practical alternative would be to transplant rhizomes from two or more populations into individual sites, to ensure genetic diversity among founder plants.

It is clear that the species *Lysimachia asperulifolia* has numerous obstacles working against it. The low pollinator visitation rates, low genetic compatibility within populations, high distance between compatible flowers, and yearly fluctuations in number of flowering stems, all interact to suppress seed production. Without seed production, the species is just “hanging on” in the scattered localities where it persists, and has no realistic chance of colonizing new areas or increasing the genetic diversity of the species. Human intervention may be necessary to optimize conditions that would be favorable for seed production. Such activities might include planting new populations with plant material from diverse genotypes, introducing populations of natural pollinators into existing *L. asperulifolia* populations, and continuing research to develop a better understanding of the nutrient requirements of the species. With the ample evidence for human involvement in the destruction and degradation of populations of the species, it would seem appropriate that humans be involved in bringing the species back from the brink of an evolutionary dead end.

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Table 1. Distances between study sites (in kilometers).

Site	CNF-H	CNF-M	C	G1
CNF-M	16.5			
C	41.4	25.2		
G1	149	133	108	
G2	149	133	108	0.33

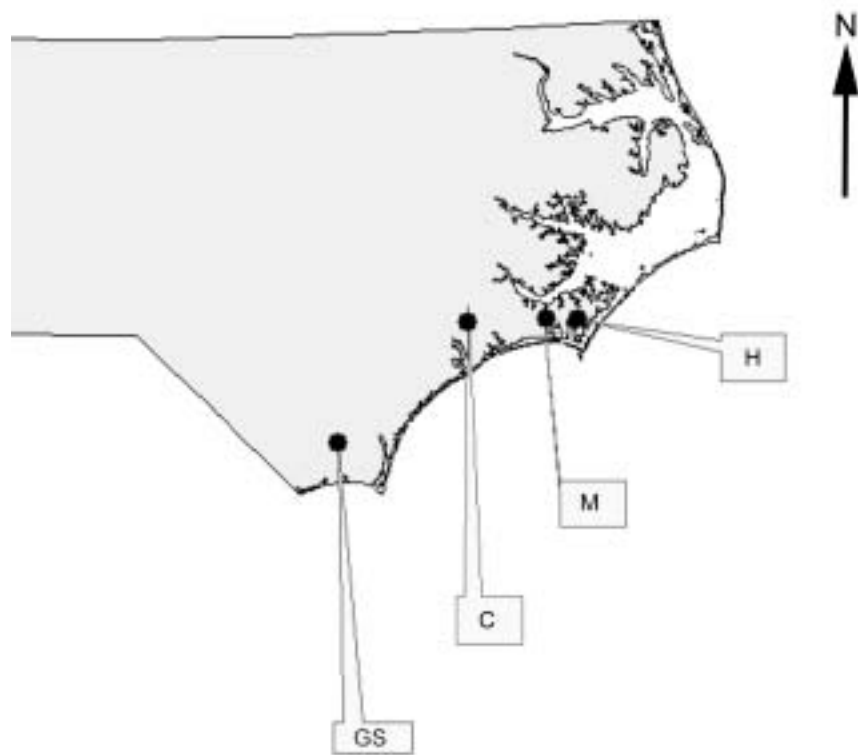


Figure 2.1 Map of Eastern North Carolina with research sites.

Table 2. Treatment classes for genetic compatibility study. n represents number of stems receiving treatment.

Treatment	Tested for:	2000 n	2001 n
Unbagged Unpollinated	Control	12	15
Bagged	Self Pollination and Self- incompatibility	10	12
Near Neighbor -- We bagged and pollinated flowers using pollen from an average distance of .55 m.	Xenogamy - optimum cross distance	13	13
Distant Neighbor -- We bagged and pollinated flowers using pollen from an average distance of 52.70 m.	Xenogamy - optimum cross distance	9	14
Other Population – We bagged and pollinated flowers, moving pollen among each population (see Table 1).	Xenogamy - optimum cross distance	11	20

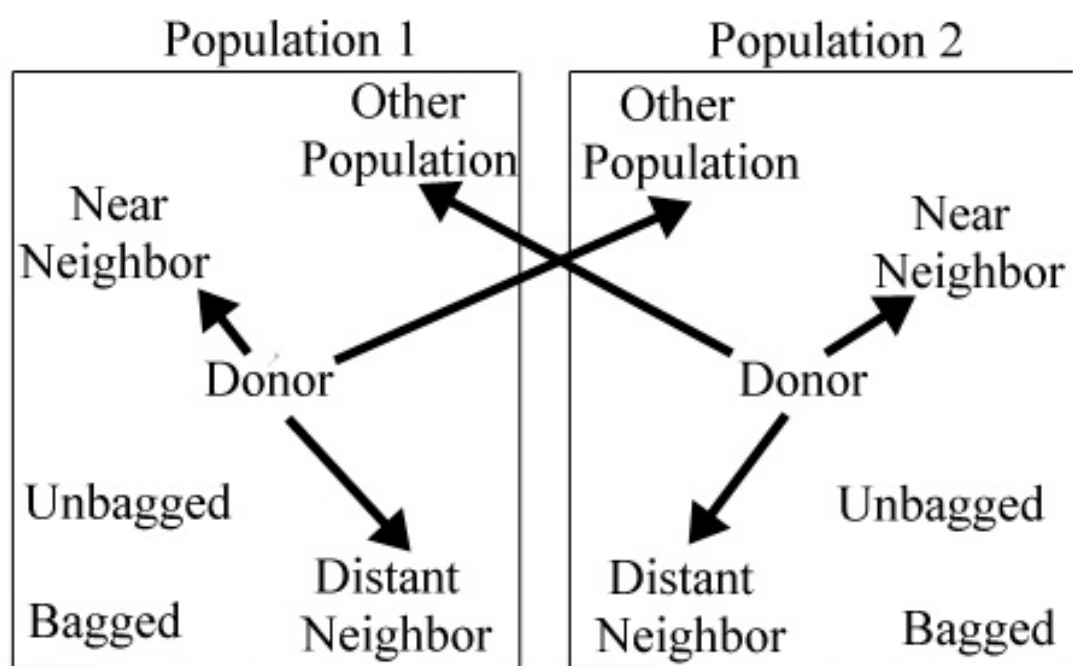


Figure 2.2. Schematic diagram of treatment Classes for Pollen Compatibility Study.

Table 3. Treatment classes for Pollinator Limitation Study.

Treatment	n
Unbagged Artificially Pollinated – We moved pollen an average distance of 35 m.	12
Unbagged Unmanipulated – We left all flowers unmanipulated.	21

Table 4. Vegetative and flowering stem counts before and after prescribed burns. Year 2000 data are pre-burn and year 2001 data are post-burn.

Population	Population Area m <sup>2</sup>	Total Number of Stems		Number of Vegetative Stems		Number of Flowering Stems	
		2000	2001	2000	2001	2000	2001
H Group A	340	457	471	456	456	1	15
H Group B	11	102	269	89	268	13	1
H Group C	111	150	185	132	159	18	26
H Group D	10	95	139	61	129	34	10
C	175	547	233	510	126	59	107
M	321	Unknown	1216	Unknown	928	368	288

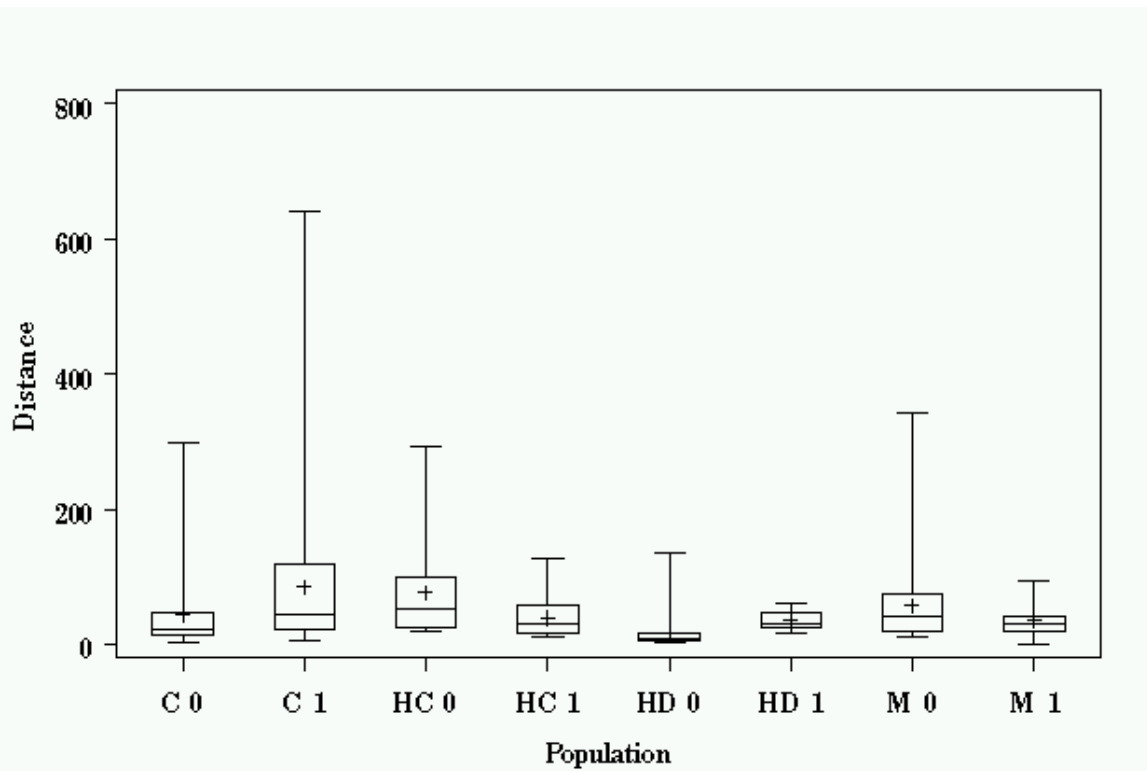


Figure 2.3. A box plot of the distances (in centimeters) between flowering stems for each year at each site. 0 indicates year 2000 and 1 indicates year 2001. Inter-stem distances were censused at all sites except M. + represents mean distance and the cross bar represents median distance. Whiskers extend to extreme observations.

Table 5 – Powder movement at 3 populations of *L. asperulifolia* during June 2000.

Study Population	Position Of Source Flower In Study Population	Total Number Of Flowers Sampled In Study Population	Number Of Flowers That Received Powder via Pollinators	Number Of Flowers With Powder Deposited On Stigma	Maximum Powder Dispersal Distance (m)	Maximum Possible Dispersal Range in Population (m)
H	Edge	129	3	0	5	60
H	Center	129	1	0	5	60
M	Edge	229	3	1 (Autogamy)	5.5	40
M	Center	229	10	1 (Xenogamy)	5.5	40
C	Edge	70	0	0	None	30
C	Center	70	0	0	None	30

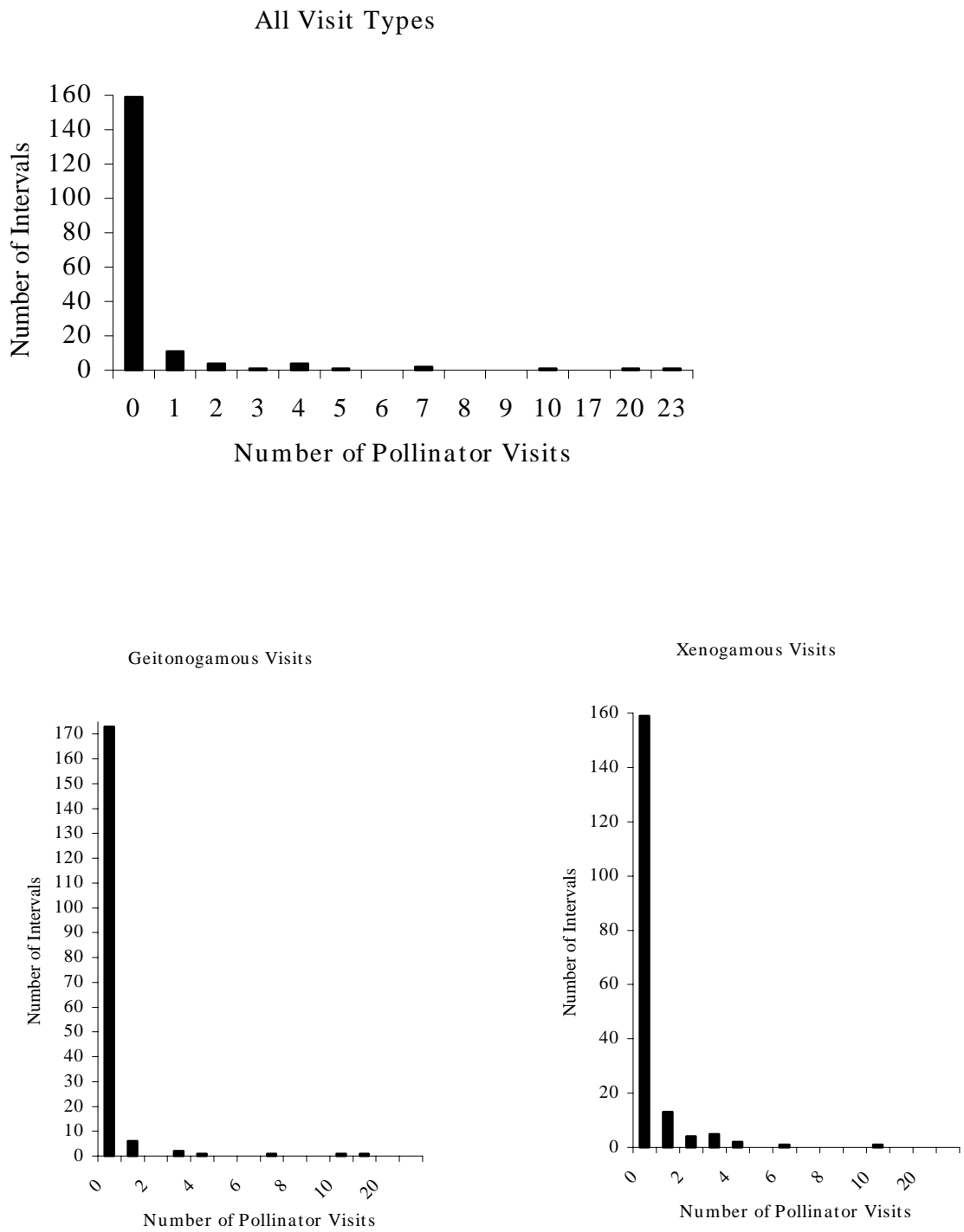


Figure 2.4. Frequency histogram of intervals containing geitonogamous, xenogamous, and all visit types.

Table 6 – Results of Two-way ANOVA performed on square-root transformed frequency of visits to inflorescences by All Visits, Xenogamous Visits, and Geitonogamous Visits. Site = Study site; Time categories are Morning (before 1000), Midday (1000-1700), and Evening (after 1700).

		Total Visits			Xenogamous			Geitonogamous		
Source	df	SS	F	PR>F	SS	F	PR>F	SS	F	PR>F
Time	2	0.952	3.32	0.039	0.679	4.15	0.017	0.187	1.41	0.246
Site	4	1.651	2.88	0.025	0.929	2.84	0.026	0.769	2.91	0.023
Time*Site	8	2.129	1.85	0.070	1.470	2.24	0.027	0.524	0.99	0.444

Table 7 – Pollinators trapped while visiting flowers and pollinators caught in pan traps.

	Flowers	Pan Traps
<i>Augochlorella striata</i> Prov.	1	1
<i>Augochlorella gratiosa</i> (Smith)	2	3
<i>Lasioglossum</i> spp.	12	8

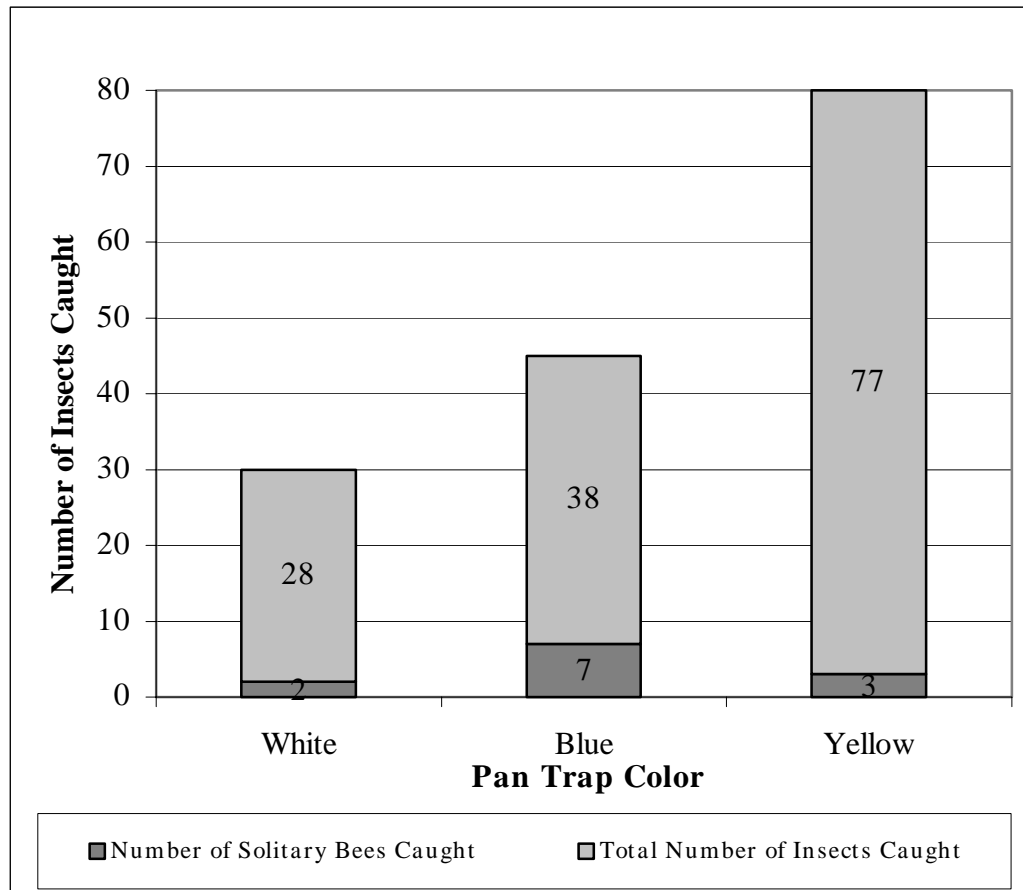


Figure 2.5. Number of solitary bees in pan traps compared with total insects caught in traps of each color.

Table 8 - Percent of flowering stems naturally producing one or more capsules at each study site.

	2000	2001
C	0.11	0.06
H - Group A	0.00	0.00
H - Group B	0.00	0.00
H - Group C	0.72	0.08
H - Group D	0.44	0.00
M	0.03	0.08

Table 9. Results of two-way ANOVA performed on percent pollen stainability.

Source	df	Type I SS	F	Pr>F
Site	2	7109.59	40.05	<.0001
Year	1	92.20	1.04	0.3092
Site*Year	2	44.44	0.25	0.7788

Table 10. Mean percent pollen stainability, standard deviations, and Tukey groupings for 2000 (preburn) and 2001 (postburn) averaged across both years.

Site	Tukey Grouping	Mean Across Years	2000			2001		
			n	Mean	Std. Dev.	n	Mean	Std. Dev.
HR	B	83.286	26	81.81	13.29	44	84.16	10.67
C	C	74.623	13	74.85	8.28	56	74.57	7.77
M	A	87.946	33	87.00	11.77	61	88.46	6.15

Table 11. Results of three-way ANOVA performed on square-root transformed seed and fruit production data.

Source	DF	Seeds			Fruits		
		SS	F	PR>F	SS	F	PR>F
Site	2	1.7711	10.4	<0.001	0.524	9.88	<0.001
Treatment	4	0.8981	2.63	0.040	0.45	4.24	0.0037
Pollen Donor	21	5.3021	2.96	<0.001	2.128	3.82	<0.001

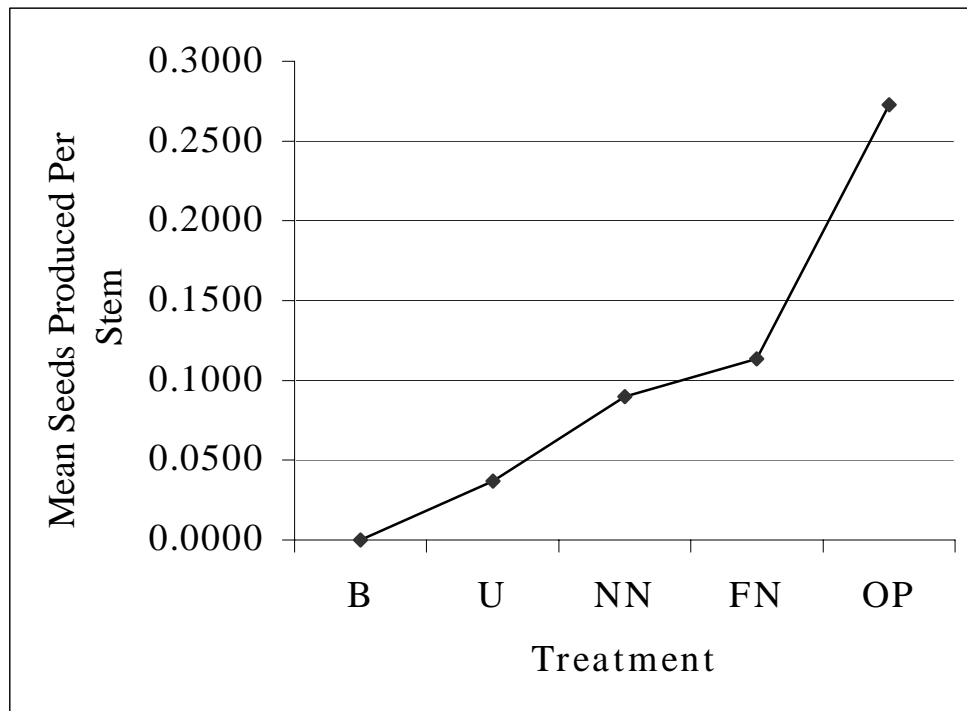


Figure 2.6. Mean number of seeds produced per stem in each treatment category.

Table 12. Production and germination trends for seeds produced by genetic compatibility treatments in 2000 and 2001.

Treatment	2000			2001		
	Total Seeds Produced	% of Total Seeds That Germinated	% Containing Endosperm That Germinated or Stained Viable	Total Seeds Produced	% of Total Seeds That Germinated	% Containing Endosperm That Germinated or Stained Viable
NN	12	0	58	0	na	na
FN	13	0	54	0	na	na
OP	38	18	47	10	30	30
U	7	0	14	0	na	na

Table 13. Production and germination trends for seeds produced by pollinator limitation treatments in 2001.

Treatment	Total Seeds Produced	% of Total Seeds That Germinated	% of Total Seeds That Germinated or Stained Viable
Unbagged Unmanipulated	24	21	46
Unbagged Artificially Pollinated	6	17	50

## APPENDIX A – Co-flowering species

*Aletris farinosa*  
*Calopogon pallida*  
*Calopogon pulchellus*  
*Cleistes divaricata*  
*Coreopsis falcata*  
*Coreopsis gladiata*  
*Dionaea muscipula*  
*Drosera capillaris*  
*Eriocaulon septangulare*  
*Hypericum reductum*  
*Hypericum galioides*  
*Lachnanthes caroliniana*  
*Lobelia nutallii*  
*Magnolia virginiana*  
*Pogonia ophioglossoides*  
*Polygala lutea*  
*Pyxidantha barbulata*  
*Rhexia alifanus*  
*Rhexia lutea*  
*Rhynchospora colorata*  
*Rhynchospora pallida*  
*Sarracenia flava*  
*Sarracenia purpurea*  
*Sarracenia rubra*  
*Sisyrinchium mucronatum* var. *atlanticum*  
*Tofieldia racemosa*  
*Zigadenus densus*

## APPENDIX B – T-Square Methods

In each study population, we selected random points along a transect. At each random point, we measured the following distances: 1) the point to the nearest stem and 2) that stem to its nearest neighbor outside of the half-plane falling between the first stem and the transect.

We derived an index of spatial pattern from the T-square sampling distances, as a ratio of squared point-to-stem distances,  $x_i$ , and squared stem-to-nearest-neighbor distances,  $y_i$ , as

$$C = \frac{\sum_{i=1}^N \left[ x_i^2 / \left( x_i^2 + \frac{1}{2} y_i^2 \right) \right]}{N}$$

where  $N$  is the total number of sample points. According to this method, the value of  $C$  is approximately one-half for random patterns, less than one-half for uniform patterns and greater than one-half for clumped patterns. To test the significance of any departure of  $C$  from one-half, a value,  $z$ , is computed as

$$z = \frac{C - 0.5}{\sqrt{\frac{1}{12N}}}$$