2010

Canopy Disturbance and Reproduction in Cornus florida L.

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CANOPY DISTURBANCE AND REPRODUCTION IN *CORNUS FLORIDA* L.

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

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August 2010

Acknowledgements

I thank my thesis director, Dr. Rodney J. Dyer, for his expertise, patience and support through this learning process. I thank my committee members, Dr. James R. Vonesh, Dr. D'arcy P. Mays, Dr. Mary Harris, and Dr. Leonard Smock for their time, patience and expertise. I thank Dr. Dyer, the VCU Rice Center, the College of Humanities and Sciences and the Department of Graduate Studies of Virginia Commonwealth University for their gracious financial support during my time in this program. I thank Dr. Elwin Orton of the department of Plant Pathology at Rutgers University for his invaluable assistance with my germination protocol. I am deeply indebted to the members of my laboratory and my fellow graduate students for their patience, good humor and confidence. Special thanks to Vicki Gardiakos for her willingness to share her laboratory expertise, to Candace Dillon and Crystal Meadows for their support and assistance in the classroom and the field, and to Dr. Ryan Garrick, Steven A. Baker and Morgen Gostel for all sorts of things. I extend my sincere thanks to my mother, Mary Lou Carr, for her support and encouragement, and to my father, Dr. Francis F. Carr, Jr., for his fine example and sound advice.

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Abstract

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Virginia Commonwealth University, 2010

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This study examined aspects of local reproductive variation in the flowering dogwood (*Cornus florida* L.) coincident with recent differences in primary canopy architecture. The dogwood trees in this study were impacted by a hurricane that created numerous treefall gaps, which created fine scale heterogeneity in the primary canopy. Fine scale disturbances in a forest can result in changes for multiple members of the forest community, including the reproductive responses of the trees and interspecific pollination mutualisms. Previously determined differences in offspring genetic structure suggested that pollen movement among genetically unstructured maternal individuals was significantly impacted between open, or disturbed, canopy and closed, or undisturbed, types. To further understand mechanisms by which this nonrandom mating occurred, this study examined both intrinsic and extrinsic reproductive factors for *C. florida*.

The first chapter examines several parameters of the reproductive phenology of sample groups of dogwood between the canopy types as intrinsic factors. The parameters observed included initiation, course and termination of flowering, seed set and seed germination ratios. The results showed some significant differences between the sampling areas in flowering intensity, although the majority of the variation was between maternal individuals throughout the entire sample. The second chapter examines insect community composition across areas of differential canopy disturbance. Community analysis showed that the open canopy contained a larger and more diverse assemblage of insects than the closed canopy or the field, which represented an area of complete canopy removal. This shift in insect community composition may have created functional differences in the local pattern of pollen flow by altering the functional composition of local potential pollinator assemblages.

The major finding of this study was that the impact of intermediate natural disturbance on the mating systems of understory plant populations may be more indirect than direct. In conclusion, the observed differences in insect community structure within these habitat types in this study were consistent with predetermined patterns of pollen flow; this structure can explain some of the previously observed genetic structure within locally proximate understory tree populations.

Keywords: reproductive phenology, disturbance, *Cornus florida* L., community analysis, pollen movement

CHAPTER 1

FLORAL SYNCHRONY AND MATERNAL REPRODUCTIVE OUTPUT IN *CORNUS FLORIDA* L.

Introduction

Plant reproductive output can be partitioned into a series of successive steps. Reproduction begins with the formation of flower buds and proceeds through the maturation of floral structures, pollination, fertilization, dispersal of seeds, and finally germination of a seed. Resources must be allocated by the parental plant to accomplish sexual reproduction; buds develop into flowers in which gametes and numerous accessory compounds such as nectar and oils are produced. Next, the pollen must be transferred from an anther, usually on another individual, to a receptive stigma. Pollen competition and interactions with the maternal plant facilitate the fertilization of an ovary, which matures to become the seed. Finally, seeds are either passively or actively dispersed away from the maternal tree and depending upon the condition in which they land, may germinate into a seedling. Each of these steps in the sequence present many opportunities for either stochastic events, individual variation, or selection to operate resulting in differential maternal reproductive output (e.g., Schemske 1977, Gomez 1993, Goubitz *et al.* 2002, Davis 2004, Devoto *et al.* 2005, Galloway 2005, Hegland and Totland 2007).

Plants that rely on insect pollinators for reproduction must produce flowers that attract animals that can effectively accomplish pollen transfer resulting in seed set. There are many

mechanisms that plants have evolved to attract pollinators. Visual cues such as the size, shape or color of the floral display are the most obvious mechanisms. However, in addition to visual cues, plants have also evolved chemical attractants such as nectar rewards high in sugars and amino acids (Baker and Baker 1973), volatile organic compounds (Brodmann *et al*. 2009), insect mating hormones (Handel and Peakall 1993), and even mimics of rotting flesh (Kite and Hetterschieid 1997). Once the pollen grain has reached a receptive stigma, the successful extension of the pollen tube can be affected by such factors as the intensity of pollen grains per stigma or competition between pollen from different paternal individuals (Davis 2004, Hegland and Totland 2007). Under resource limitation, maternal individuals may selectively abort seeds based upon the level of inbreeding (Karkkainen *et al.* 1999). Abortion of fertilized ovules of early spring flowering species in the understory may also increase as the primary canopy develops and carbon fixation through photosynthesis is reduced (Schemske 1977). Finally, as embryos develop into mature seeds, they are subject to seed predation and abortion by the maternal plant.

Seed dispersal is typically facilitated by either passive dispersal with gravity, wind, or water, or as an active dispersal mechanism often with the aid of an animal. The success of a dispersed seed depends upon the local characteristics of the site into which it was dispersed. The Janzen-Connell Hypothesis (Janzen 1970, Schupp 1992), states that seeds dispersed too close to the maternal individual may be subject to both density dependent seed predation and competition for light from the maternal individual resulting in a decreased probability of germination and subsequent establishment. Conversely, seeds dispersed too far from the maternal individual may reach a habitat that is poorly suited to the particular set of species requirements for

establishment. As a result, it is hypothesized that there is an optimal dispersal distance, with respect to establishment probability, which is dependent upon environmental heterogeneity. Once dispersed, germination of a seed depends on a correct combination of light, water, temperature and oxygen to end the period of dormancy and begin the development of the embryonic plant. To reach the correct combination of factors for proper development to adulthood, seeds must be dispersed to areas of suitable environmental conditions.

Pollination, fertilization, dispersal, and establishment do not occur in isolation, rather each can be influenced by site-specific environmental factors. For example, temperature has been determined to be a major factor in the onset of flowering for many temperate woody species, including members of the Cornaceae (Smithberg and Weiser 1968, Collins *et al.* 1985). While temperature is broadly viewed as a function of the overall seasonal changes in an ecosystem, some fluctuations occur due to variation in the local environment (Schemske *et al.* 1978). For example, heterogeneity in the topography of the landscape can have significant influences on local temperatures (Ashcroft 2006). Water and light levels are also subject to these local fluctuations, and plant phenology can be affected accordingly (Andrew and Ustin 2009). Canopy cover and cardinal aspect both influence temperature and light availability.

For any particular plant, flowering intensity may be influenced by the available amount of light (Schemske *et al.* 1978). For example, it has been shown in dogwood that plants at the edge of a clearing will flower more often than will plants within the canopy (Sork *et al.* 2005). For individual flowers, pollinator attraction may be enhanced by direct sunlight or elevated temperatures on the flowers on a south facing slope relative to the shading and cooler temperatures on a north facing slope (Kilkenny and Galloway 2008). These different levels of

foraging frequency could result in different patterns of pollen movement and therefore impact the parental composition of the seedling cohort on a local scale. Changes in the phenology, or timing, of flowering between populations can lead to selection by reproductive incompatibility due to varying degrees of temporal isolation between individuals (Hall and Willis 2006, Levin 2009). Changes in humidity or amount of available water in the soil or humus layer may also contribute to variation in reproductive phenology and plant-pollinator interactions across multiple spatial scales (Devoto *et al.* 2005).

The research organism for this study was *Cornus florida* L., the flowering dogwood. This is a small, deciduous understory tree native to the eastern United States that can be found ubiquitously distributed between northern Mexico and New England, and as far west as eastern Texas. It is common in the understory of hardwood forests and is a typical component of the secondary growth after natural and anthropogenic disturbance. These trees are monecious with compound inflorescences subtended by four large showy white bracts. Each inflorescence is composed of approximately 20-30 perfect, protandrous flowers that open sequentially over three to four weeks in the early spring. This species has a single seasonal flowering period, in the early spring, so all gamete transfer is effectively concentrated into a short time period. This makes quantification of the flowering effort more straightforward than if it flowered over the summer or the entire year. It flowers before most of the understory has begun to leaf out, and the inflorescences are visible at a distance because the large white bracts are fully present for a week or more before anthesis. These characteristics allow this small understory tree to be easily identified and located reliably at a distance, especially towards the end of the flowering season when visibility is reduced by many heterospecifics producing leaves. Since this species typically

is very common in the understory, many trees can be sampled. Finally, this species is relatively short, generally less than 3 meters in height, which allows the flowers to be easily accessible from the ground (McLemore 1990, Mayor *et al.* 1999).

Previous mating system analysis (Gardiakos 2009) showed differences in the genetic structure of offspring that were correlated with the degree of primary canopy openness above the maternal individual. Seedlings in open canopy areas were three times more likely to be the result of biparental inbreeding (mating among relatives) than seedlings from the closed canopy area. Moreover, the number of effective fathers per maternal individual was higher in the closed canopy than in the open canopy, suggesting that trees in the open areas were mating with fewer individuals than trees in the undisturbed canopy even though the floral output was greater in the open canopy. Overall, that work suggested that pollen dispersal in the open canopy is more restricted than in the closed canopy. One potential mechanism for these results could be asynchrony in phenology with open canopy sites flowering earlier than the rest of the forest, resulting in temporal isolation.

In this study, individual *C. florida* trees were surveyed to quantify the extent to which site-specific factors influenced flowering phenology and relative female reproductive output. Flowering phenology was characterized as the overlap in available flowers across trees sampled in a 2x2 factorial design to determine the importance and interaction of topography and canopy openness. Female reproductive output was characterized by seed set (potential reproductive output) and germination percentage (realized reproductive output). A germination protocol was compiled to measure the actualized reproductive output by collecting and germinating all seeds produced from the observed flowers.

Methods

Sampling Location

Research was conducted at the Virginia Commonwealth University Inger and Walter Rice Center for the Environmental Life Sciences in Charles City County, VA, USA (37° 19' N; 77°12' W). This 343 acre facility is located on the north bank of the James River, approximately 50 miles upriver from the Chesapeake Bay. The majority of the Rice Center is representative of the Southern Mixed Hardwood forest type common in the eastern Mid-Atlantic region (Braun 1950, Ware 1970, Plunkett and Hall 1995). Sampling areas in this study were characterized by a primary canopy of *Quercus* spp, *Carya* spp., *Acer rubrum* and *Pinus taeda*, with a secondary canopy of *Ilex opaca*, *Cornus florida*, *Prunus* spp. and *Forestiera acuminata*.

The Rice Center, along with much of the James River corridor, was among the areas affected by Hurricane Isabel in September of 2003. Much of the eastern Mid-Atlantic coast of the United States was subjected to severe storm conditions for several days. Isabel came ashore in North Carolina as a Category 2 hurricane, losing strength as it proceeded north-northeast through western Pennsylvania to dissolve in Canada about 48 hours later. It made landfall with wind speeds around 100 mph, and reached central Virginia several hours later as a tropical storm with wind speeds in excess of 70 mph. Rainfall in central Virginia for the duration of the storm was 4-7 inches. This hurricane was the most significant storm event in the region since Hurricane Hazel in 1954 (Beven and Cobb 2003). The existing canopy community of the study area was altered as multiple small groups of primary canopy trees were felled during the storm (Figure 2.1). Due to the severity of the storm, these gaps represent an intermediate level of primary

canopy heterogeneity, between periodic senescence of canopy members and the complete removal of vegetative growth (Pickett and White 1985).

Sampling Design and Data Collection

Twenty-four trees were selected in a haphazard fashion within four habitat types in approximately three hectares of riparian forest. These habitat types were subjectively classified as Upper Forest, Lakeside, Bottom Clearing and North End. Flowering phenology was measured for each maternal *C. florida* individual through one flowering season. The unit of observation within each habitat type was the individual tree with several inflorescences identified as replicates samples. Several branches were selected per tree to approximately represent the entire tree, rather than a single branch that would represent just one side. On each inflorescence, the number of reproductively active pollen producing flowers was recorded at 3-4 day intervals. This interval was calculated to minimize the chance of counting the same flower twice. Reproductive activity was defined by the presence of pollen on at least one anther per flower. Time of flowering commencement, average number of receptive flowers per inflorescence, the end of flowering and the duration of the total flowering period were recorded for each tree from early April until late April 2007. Maternal trees were resampled in August to quantify both seed set and seed abortion for each individual.

Potential maternal reproductive output was determined by measuring seed set, or potential reproductive output, and germination percentages, or actualized reproductive output. Seeds were classified into two categories: seeds that were fully mature were categorized as "full" seeds; those seeds that were obviously initiated (e.g., ovaries that were obviously fertilized) but

had development halted for some reason were counted as "aborted" seeds. Seeds were surveyed at one month and four months following the end of the flowering season.

Actualized reproductive output was measured by percent germination of the collected seeds. A germination protocol was compiled based upon personal conversations with Dr. Elwin Orton of the Department of Plant Biology and Pathology at Rutgers University. Inflorescences were bagged with fine mesh on the trees after the end of the flowering period to prevent predation or dispersal of the developing seeds. The seeds were collected upon maturation in the fall and were placed in a ventilated refrigerator at 5.5 C° until preparation for germination. As *C. florida* seeds require a 90-120 day period of cold-stratification for germination, the seeds were stored in a refrigerator for two months to maintain the natural period of dormancy prior to planting. Growing media was prepared by combining 2/3 fine-milled sphagnum moss and 1/3 medium grained perlite, rinsed with water and sterilized with a 10% bleach solution. The media was rinsed and drained three times with deionized water after sterilization to remove all bleach. Each seed was depulped, cleaned in 10% bleach solution and rinsed three times with deionized water. Seeds were each placed laterally 1 cm below the surface of ~ 10 cc moist growing media and heat sealed in individual gas permeable bags (Garner U.S. Enterprises, Willis, TX). Bags were labeled by tree and placed upright in ventilated flats to allow for adequate gas exchange around each bag. Bags were stored at 5.5 C° in the refrigerator and observed weekly for results. Seedlings were collected for subsequent genetic analysis upon the extension of the epicotyl.

Data Analysis

Flowering synchrony was calculated following Augspurger (1983) and Gomez (1993) for each

individual and for the sample population as a whole. The individual synchrony was the number of days that each individual flowered that overlapped with the rest of the population, and the population synchrony was the number of days that the whole population flowered. The following equations were used to estimate the synchrony of each individual with every other individual:

$$X_i = \frac{1}{n-1} \frac{1}{f_i} \sum_{j \neq 1}^{n} e_{j \neq i}$$

and of the sample population:

$$Z = \frac{1}{n} \sum_{i=1}^{n} X_i$$

For these equations, N is the number of individual trees in the population, f_i is the number of sample days that the i^{th} individual is in flower and e_j is the number of sample days that individuals j and i are flowering synchronously. The flowering synchrony statistic (Z) was further calculated between individuals in subsets of the trees by sampling area. These values were used to qualitatively assign flowering synchrony differences between open and closed canopy areas and upper and lower elevation.

Data analysis to detect differences in potential reproductive output was conducted employing a series of linear mixed effects models using the `lmer` function in R (R Development Core Team 2009). Maternal individuals were treated as a random effect (e.g., only a haphazard sample of potential maternal individuals was sampled). Other factors such as habitat (canopy openness and location), and the maternal specific covariates such as maternal dbh and canopy size were treated as fixed effects. Response variables for potential reproductive output were the total number of flowers per inflorescence per maternal tree, the number of flowers showing

reproductive activity, the number of full seeds produced per tree, and the number of aborted seeds per tree. Since these variables are counting variables, the error term was assumed to be Poisson distributed. A stepwise approach was used to determine the best subset of factors and covariates in describing each aspect of reproductive output. The best fit linear model was determined as the model with the lowest estimated AIC (Akaike's Information Criterion). Models whose estimated AIC was within 2 units (e.g., $\delta AIC \equiv |AIC_{model\ 1} - AIC_{model\ 2}| < 2$) were considered as putatively acceptable models.

The response variable for actual reproductive output was the germination percentage of seeds per tree, which was appropriately transformed prior to analysis. Germination ratios for each tree were compared with a Kruskal-Wallis non-parametric rank sum test, to account for different sample sizes across the four sampling areas. This procedure tested the experimental hypothesis that the mean germination percent per maternal individual was different between at least two of the sampling areas.

Results

Flowering Phenology

Flowering phenology measurements were recorded on six days between April 4 and April 25, 2007. Flowering was recorded for all six days for twenty-two of the twenty-four trees. Two trees, 647 and 519, were removed because of incomplete sampling data. The twenty-two trees measured contained 5086 total flowers in 302 inflorescences. The Lakeside group contained eight trees with 2888 flowers in 182 inflorescences, the Bottom Clearing contained three trees with 344 flowers in 17 inflorescences, the Upper Forest contained eight trees with 1416 flowers in 75 inflorescences, and the North End contained three trees with 438 flowers in 28 inflorescences. All trees exhibited a high level of flowering synchrony, with the values for X_i, essentially average degree of overlap, for all individuals being between 0.893 and 0.952 (Table 1.1). The flowering synchrony was not different between the trees in the four sampling areas, nor was the flowering synchrony for all individuals ($Z_{all} = 0.911$) different from the flowering synchrony between sample areas: $Z_{lakeside} = 0.914$, $Z_{bottom} = 0.905$, $Z_{upper} = 0.911$, $Z_{north} = 0.913$ (Table 1.2). The flowering synchrony between these sampling areas was not significantly different (Table 1.1). Missing data for one sampling date for three trees in the Bottom Clearing were extrapolated for the synchrony measurement because of the confounding presence of nesting *Haliaetus leucocephalus* (Bald Eagle). The trees in question were subjectively recorded to be flowering on the non-sampled date because they were flowering on the dates directly before and after the non-sampled date, and other trees in the immediate vicinity were observed to be flowering concurrently.

Potential Reproductive Output

All models fit to reproductive output parameters were fit with the maternal individual as a random effect and all models retained the maternal effect with high significance ($P < 2e^{-16}$, Table 1.3). As a result, in the discussion of potential reproductive output, the identity of the fixed effects is of primary concern. The best fit model describing the number of flowers per inflorescence across maternal trees contained the habitat type term as a significant fixed effect (Table 1.3). The effect sizes for alternate habitats were all similar. There were two models whose δAIC = 2.0, containing either dbh or canopy diameter along with habitat. Both of these models had identical AIC but the effect size for the non-habitat term was very small, particularly in relation to the coefficients for the habitat levels. Overall, these additional models were assumed to be not as informative as the model containing only habitat. Consequently, mothers appear to be producing different numbers of flowers and the mothers in the north and upper habitat locations appear to have the largest difference in floral production (Tables 1.3 – 1.5).

The number of aborted seeds per mother included terms for both the number of flowers and a term for habitats type (Table 1.4), with habitat being an order of magnitude more important in terms of effect size. Across habitats, more seeds were aborted in the Bottom Clearing habitat type than in the other (Figure 1.1). Two additional models, adding either dbh or canopy size, had δAIC less than 2.0 although the effect sizes of the additional terms were rather small in comparison to those for habitat. As a result, it appears that the number of seeds aborted is a function of at least habitat type, with maternal trees in the Bottom Clearing aborting the most seeds.

Total seed set of the twenty-two trees in the four sampling habitats was analyzed (two of the original twenty four trees, 645 and 702, were removed due to sampling error). Again, for all the candidate models, the maternal individual was fit as a random effect and was highly significant (Table 1.4). For both seed set and number of aborted seeds, the number of flowers per mother was used as an additional fixed effect covariate in the model selection procedure. There were three models whose δAIC was less than 2.0, each of which contained the flower term either alone or in combination with canopy size or with canopy size and dbh (Table 1.5). For the putatively best of these models, containing flowers, dbh, and maternal canopy diameter, the relative effect of the number of flowers was an order of magnitude greater than the effect of canopy diameter. Moreover, the effects of the diameter of the maternal individuals trunk was in the opposite direction as that for both number of flowers and canopy diameter. This model suggests that smaller maternal individuals that set more flowers and have larger canopies will set more seeds than larger individuals with smaller canopies and less dense floral displays.

Actual Reproductive Output

A total of 698 seeds were collected from 19 individuals across all four environmental areas. Of these offspring, 446 seeds germinated with an average germination rate for all trees of 64%. The germination ratios for the four sampling areas ranged from 50% in the Bottom Clearing to 80% in the North End. The analysis of actualized reproductive output showed that mean germination ratios among sampling areas were not significantly different (df = 16, $\chi^2 = 13.00$, $P = 0.673$; Figure 1.5).

Discussion

By far the most surprising finding in this study was that canopy opening and topographic location influenced both flower number and seed abortion in *C. florida*, though not seed set or phenology. Unfortunately, for both flowering and aborted seeds, the observed differences due to habitat were not consistent. For floral output, the North End (closed canopy) and Upper (disturbed site with open canopy) were the most important factors, whereas the consequences of habitat for aborted seeds was partitioned between the Bottom Clearing (open canopy) and the remaining sites (both open and close canopy). Despite habitat location being important for those two components of maternal reproductive output, the most important factor, seed set, was independent of habitat type and tied specifically to characteristics of the maternal tree.

The high degree of flowering synchrony ($Z_{all} = 0.911$) observed among all trees suggests that any reproductive differences were not due to temporal isolation (Table 1.2). Canopy opening caused by periodic storm events did not appear to affect the initiation, course, peak or termination of flowering in these *C. florida*. This lack of a shift in the initiation of the overall course of flowering may have been due to the size of the changes in light and temperature levels in the openings. Temperature has been shown to affect some aspects of flowering phenology, including initiation and intensity, among recently disturbed forest gaps (Smithberg and Weiser 1968, Collins *et al.* 1985). Elevation in local temperature in a canopy gap due to increased light may have allowed plants to reach the minimum temperature needed to initiate flowering sooner than in an area without canopy opening (Schemske *et al.* 1978). This temperature difference may not have been present before the majority of primary canopy species put out leaves because

the gap did not represent significant canopy heterogeneity, or the gap did not functionally exist without the canopy. To test this, a Kruskal-Wallis rank sum test was used to determine if the date of initiation and/or termination of flowering were significantly different among sampling areas. The results did not suggest that the mean dates of initiation (X^2 = 3.952, df = 3, *P*-value = 0.267) or termination (X^2 = 1.406, df = 3, *P*-value = 0.704) for each tree were likely to be significantly different among sampling areas. The lack of flowering variation suggests that there was not ecologically significant temperature variation between the undisturbed canopy and the openings in this study. The lack of flowering asynchrony is most likely due to the relatively homogenous elevation profiles across the study site.

The regression analysis showed that the number of flowers produced per inflorescence differed by maternal individual and habitat, though independent of dbh or canopy size (Figure 1.2). In general, trees in the other open canopy location (Bottom Clearing) were uniformly smaller in stature and while located within a windfall gap, the previous canopy tree density was less than that observed in the Upper sampling location. As a result, floral output may have been a function of a much larger increase in light levels and/or reduced competition in the Upper Forest site as compared to the Bottom Clearing. It is also possible that factors other than just light influenced floral output (Andrew and Ustin 2009). For example, Kudo *et al.* (2007) showed that the flowering initiation and output of some understory plants may be highly influenced by snowmelt. This overall canopy heterogeneity may have allowed for sufficient elevation of light levels to positively influence the number of flowers that certain trees produced, without raising the temperature enough to affect the overall initiation of reproductive activity in any of the sampling areas.

The abortion of seeds was primarily driven by increased abortion in the Bottom Clearing. This site was of relatively low density of dogwoods, with individuals primarily of smaller stature than at the remaining sites. In other species maternal individuals selectively abort offspring based upon inbreeding. Given the positive spatial autocorrelation among adults throughout the study areas, mating among individuals in low density stands may result in an increase in consanguineous mating thus explaining the increase in aborted seeds. The low germination results for the Bottom Clearing area may complement the higher seed abortion rate in this area. The Bottom Clearing appeared to have less competition for light and water than any other of the sampling areas as the canopy opening was most pronounced there, and it was the closest area to the open water (pers. obs.). The high seed abortion rate and somewhat lower seed germination in this area may reflect higher rates of self-fertilization; the pollinators may have spent more time foraging among flowers on single individuals versus multiple individuals in this area. The resulting increase in the levels of consanguineous mating per individual seed crop would be reflected in the levels of spontaneous seedling abortion (Reed 2004) or lowered germination rates. This is in agreement with Gardiakos (2009), though future research should test these ideas. More robust sampling ($n > 3$) of the habitats might have yielded a more precise comparison of these areas. Also, while the reproductive processes examined here were the result of putative mating events, environmental conditions surrounding the maternal individual can influence the postzygotic development of the seeds (Galloway 2005).

Seed set, the most salient parameter measured here in quantifying maternal reproductive habitat, was independent of habitat type though it did respond to maternal specific factors. These results suggest that perhaps differential allocation of resources is most important to reproductive

success. Trees that set more flowers and have larger canopies attract more pollinators resulting in higher levels of pollination success. More flowers per inflorescence across an individual tree could represent an area of relatively concentrated resource, which could lead to pollinators spending more time foraging among the more dense floral display relative to less dense floral display (Spaethe *et al* 2001). Herrera (1987) showed that foraging intensity of some generalist pollinators can be highly influenced by the density of floral display in their immediate vicinity, within a meter, rather than at any larger scales.

The maternal individuals in this study varied widely in their reproductive output for each measured parameter. Despite the differences in flower output and abortion rates, the lack of clear differentiation among habitat for seed set suggests that the variation seen here is not consistent with what was seen in the mating system analyses. It is unlikely that characteristics of maternal reproductive output, as measured here, differentially influenced mating in open canopy versus closed canopy locations. One possible explanation for the observed differences is that it wasn't characteristics of the trees that changed as a function of canopy openness but rather it was a change in the community of insects that provide pollination services to those maternal individuals. If characteristics of canopy openness change either the behavior or the composition of the pollinator community and those changes influence the distances and manner in which pollen is transferred from one plant to the next, then this could explain the genetic differences observed by Gardiakos (2009).

Works Cited

Andrew, M. E. and S. L. Ustin. 2009. Effects of microtopography and hydrology on phenology of an invasive herb. *Ecography*. 32: 860-870.

Ashcroft, M. B. 2006. A method for improving landscape scale temperature predictions and the implications for vegetation modelling. *Ecological Modelling* 197:(3) 394-404.

Augspurger, C. K. 1983. Phenology, flowering synchrony and fruit set of six neotropical shrubs. *Biotropica*. 15:(4) 257-267.

Baker, H. G. and I. Baker. 1973. Amino acids in nectar and their evolutionary significance. *Nature* 241: 543-545

Beven, J. and H. Cobb. "Tropical Cyclone Report: Hurricane Isabel". 19 December 2003. National Hurricane Center, National Weather Service. June 17, 2009. http://www.nhc.noaa.gov/2003isabel.shtml.

Braun, E. L. 1950. *Deciduous Forests of Eastern North America.* The Blakiston Company, Philadelphia.

Brodmann, J., R. Twele, W. Francke, L. Yi-bo, S. Xi-qiang, and M. Ayasse. 2009. Orchid mimics honey beee alarm pheromone in order to attract hornets for pollination. *Current Biology* 19:(16) 1368-1372.

Collins, B. S., K. P. Dunne and S. T. A. Pickett. 1985. Responses of forest herbs to canopy gaps. in *The ecology of natural disturbance and patch dynamics* (S. T. A. Pickett and P. S. White, eds.) pp. 17-33. Academic Press, Orlando.

Davis, S. L. 2004. Natural levels of pollination intensity and effects of pollen loads on offspring

quality in females of Thalictrum pubescens (Ranunculaceae). *Plant Systematics and Evolution*. 244:(1) 45-54.

Devoto, M., Medan, D. and Montaldo N. H. 2005. Patterns of interaction between plants and pollinators along an environmental gradient. *Oikos*. 109: 461-472.

Galloway, L. 2005. Maternal affects provide phenotypic adaptation to local environmental conditions. *New Phytologist* 166:(1) 93-99.

Gardiakos, V. A. 2009. *Pollen-mediated gene movement in flowering dogwood,* Cornus florida *L.* Master's Thesis. Virginia Commonwealth University, Richmond, VA.

Gomez, J. M. 1993. Phenotyic selection on flowering synchrony in a high mountain plant, *Hormathophylla spinosa* (Cruciferae). *Journal of Ecology* 81: 605-613.

Goubitz, S., M. J. A. Werger, A. Schmida and G. Ne'eman. 2002. Cone abortion in *Pinus halepensis*: the role of pollen quantity, tree size and cone location. *Oikos* 97: 125-133.

Hall, M. C. and J. H. Willis. 2006. Divergent selection on flowering time contributes to local adaptation in *Mimulus guttatus* populations. *Evolution* 60(12): 2466-2477.

Handel, S. N. and R. Peakall. 1993. Thynnine wasps discriminate among heights when seeking mates: tests with a sexually deceptive orchid. *Oecologia* 95: 241-245.

Hegland, S. J. and O. Totland. 2007. Pollen limitation affects progeny vigour and subsequent recruitment in the insect pollinated herb *Ranunculus acris*. *Oikos* 116: 1204-1210.

Herrera, C. M. 1987. Components of pollinator "quality": comparative analysis of a diverse insect assemblage. *Oikos* 50: 79-90.

Janzen, D.H. 1970. Herbivores and the Number of Tree Species in Tropical Forests. *The American Naturalist*. 104(940): 501-528.

Karkkainen, K., O Savolainen and V. Koski. 1999. Why do plants abort so many developing seeds: bad offspring or bad maternal genotype? *Evolutionary Ecology* 13:(3) 305-317.

Kilkenny, F. F. and Galloway, L. F. 2008. Reproductive success in varying light environments: direct and indirect effects of light on plants and pollinators. *Oecologia* 155: 247-255.

Kite, G. C. and W. L. A. Hetterschieid. 1997. Inflorescence odours of *Amorphophallus* and *Pseudodracontium* (Araceae). *Phytochemistry* 46:(1) 71-75.

Kudo, G., T. Y. Ida and T. Tani 2007. Linkages between phenology, pollination, photosynthesis and reproduction in deciduous forest understory plants. *Ecology* 89(2): 321-331.

Levin, D. A. 2009. Flowering-time plasticity facilitates niche shifts in adjacent populations. *New Phytologist*. 183: 661-666.

Mayor, A. J., J. F. Grant, M. T. Windham and R. N. Trigiano. 1999. Insect visitors to flowers of flowering dogwood, *Cornus florida* L., in eastern Tennessee: Potential pollinators. *Southern Nurseries Association Research Conference* 44: 192-196.

McLemore, B. F. 1990. *Cornus florida* L. flowering dogwood. *in* R. M. Burns and B. H. Honkala [eds.], *Silvics of North America*, 278–283. Agriculture Handbook 654. US Department of Agriculture Forest Service, Washington D.C., USA.

Pickett, S. T. A. and P. S. White. 1985. in *The ecology of natural disturbance and patch dynamics* (S. T. A. Pickett and P. S. White, eds.) pp. 17-33. Academic Press, Orlando.

Plunkett, G. M., and G.W. Hall. 1995. Vascular flora and vegetation of western Isle of Wight

County, Virginia. *Castanea* 60: 30-59.

R Development Core Team. 2009. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna Austria. http://R-project.org

Reed, S. M. 2004. Self-incompatibility in *Cornus florida. HortScience* 39:(2) 335-338.

Schemske D.W. 1977. Flowering phenology and seed set in *Claytonia virginica* (Portulacaceae). *Bulletin of the Torrey Botanical Society* 104(3): 254-263.

Schemske, D. W., M. F. Willson, M. N. Melampy, L. J. Miller, L. Verner., K. M. Schemske and L. B. Best. 1978. Flowering ecology of some woodland herbs. *Ecology* 59(2): 351-366.

Schupp, E.W. 1992. The Janzen-Connell Model for Tropical Tree Diversity: Population Implications and the Importance of Spatial Scale. *The American Naturalist* 140(3): 526-530.

Sork, V. L., P. E. Smouse, V. Apsit, R. J. Dyer, and R. D. Westfall. 2005. A Two-Generation analysis of pollen pool genetic structure in flowering dogwood (*Cornus florida*, Cornaceae) in the Missouri Ozarks. *American Journal of Botany*. 92(2): 262-271.

Spaethe, J., Tautz J. and L. Chittka. 2001. Visual constraints in foraging bumblebees: Flower size and color affect search time and flight behavior. *Proceedings of the National Academy of Sciences* 98: 3898-3903.

Smithberg, M.H. and C. T. Weiser. 1968. Patterns of variation among climatic races of Red-Osier Dogwood. *Ecology* 49:(3) pp. 495-505.

Ware, S. A. 1970. Southern mixed hardwood forest in the Virginia coastal plain. *Ecology* 51(5): 921-924.

Tables

Table 1.1: Phenological overlap (X_i) of each tree partitioned by sampling habitat.

Sampling Area	Tree	*X*
lakeside	711	0.924
lakeside	646	0.893
lakeside	645	0.905
lakeside	643	0.924
lakeside	712	0.905
lakeside	648/9	0.905
lakeside	717	0.952
lakeside	719	0.905
bottom	600	0.905
bottom	599	0.905
bottom	598	0.905
upper	478	0.905
upper	498	0.905
upper	513	0.905
upper	705	0.929
upper	706	0.924
upper	320	0.905
upper	323	0.905
north	701	0.917
north	702	0.905
north	558	0.924
north	559	0.905

Table 1.2: Phelological overlap (*Z*) among all groups of maternal individuals across habitat types.

Sampling Area	***Z***
Bottom Clearing	0.905
Lakeside	0.91
North End	0.913
Upper Forest	0.911
All	0.911

Table 1.3: Mixed effects models used to test the significance of environmental variables as predictors for the number of flowers per inflorescence. The maternal individual was the random effect, and the environmental and reproductive parameters were included as fixed effects: dbh = diameter at breast height (cm), canopy = largest canopy diameter measurement (cm), habitat = sample area. In all cases, the significance of the maternal individual (a random effect) was highly significant ($P < 2e^{-16}$, not included in the Table but a component of every model). The best model was assumed to be the one with the lowest AIC (denoted with *) although other plausible models were those whose difference in AIC was less than 2 (e.g., $\delta AIC < 2$) for the putatively identified best model (denoted with †).

Fixed Effects	**Fixed Effect Coefficients**	**AIC**	**Deviance**
dbh	$\beta_{dbh}=0.35$	288.2	11.19
canopy	$\beta_{canopy}=0.01$	265.4	11.14
habitat	$\beta_{bottom}= 3.05$, $\beta_{lakeside}=3.04$, $\beta_{north}=3.01$, $\beta_{upper}=3.08$	165.7*	0.97
dbh + canopy	$\beta_{dbh}=-0.08$, $\beta_{canopy}=0.01$	266.2	11.13
dbh + habitat	$\beta_{dbh}=-0.001$, $\beta_{bottom}=3.06$, $\beta_{lakeside}=3.05$, $\beta_{north}=3.02$, $\beta_{upper}=3.09$	167.7†	1.08
habitat + canopy	$\beta_{canopy}=0.001$, $\beta_{bottom}=3.05$, $\beta_{lakeside}=3.04$, $\beta_{north}=3.01$, $\beta_{upper}=3.08$	167.7†	0.97
habitat + canopy + dbh	$\beta_{dbh}=-0.0002$, $\beta_{canopy}=0.00$, $\beta_{bottom}=3.04$, $\beta_{lakeside}=3.04$, $\beta_{north}=3.01$, $\beta_{upper}=3.07$	169.6	0.73

Table 1.4: Mixed effects models used to test the significance of environmental variables as predictors for the number of aborted ovules per inflorescence. The maternal individual was the random effect, and the environmental and reproductive parameters were included as fixed effects: flowers = number of flowers per inflorescence, dbh = diameter at breast height (cm), canopy = largest canopy diameter measurement (cm), habitat = sample area. In all cases, the significance of the maternal individual (a random effect) was highly significant ($P < 2e^{-16}$, not included in the table but a component of every model). The best model was assumed to be the one with the lowest AIC (denoted with *) although other plausible models were those whose difference in AIC was less than 2 (e.g., $\delta AIC < 2$) for the putatively identified best model (denoted with †).

Fixed Effects	**Fixed Effect Coefficients**	**AIC**	**Dev**
flowers	$\beta_{flowers}=0.03$	497.9	3.19
flowers + dbh	$\beta_{flowers}=0.04$, $\beta_{dbh}=-0.07$	496.9	2.39
flowers + canopy	$\beta_{flowers}=0.04$, $\beta_{canopy}=-0.001$	498.2	2.42
flowers + habitat	$\beta_{flowers}=0.05$, $\beta_{bottom}=0.68$, $\beta_{lakeside}=-0.81$, $\beta_{north}=-0.76$, $\beta_{upper}=-0.68$	494.0*	1.25
flowers + dbh + canopy	$\beta_{flowers}=0.04$, $\beta_{dbh}=-0.10$, $\beta_{canopy}=0.001$	498.7	2.64
flowers + dbh + habitat	$\beta_{flowers}=0.05$, $\beta_{dbh}=0.02$, $\beta_{bottom}=0.61$, $\beta_{lakeside}=-0.95$, $\beta_{north}=-0.91$, $\beta_{upper}=-0.85$	495.8†	1.13
flowers + habitat + canopy	$\beta_{flowers}=0.05$, $\beta_{bottom}=0.15$, $\beta_{lakeside}=-1.38$, $\beta_{north}=-1.28$, $\beta_{upper}=-1.43$, $\beta_{canopy}=0.002$	494.7†	1.06
flowers + dbh + habitat + canopy	$\beta_{flowers}=0.05$, $\beta_{dbh}=-0.04$, $\beta_{bottom}=0.02$, $\beta_{lakeside}=-1.42$, $\beta_{north}=-1.29$, $\beta_{upper}=-1.5$, $\beta_{canopy}=0.003$	496.3	1.21

Table 1.5: Mixed effects models used to test the significance of environmental variables as predictors for the the number of seeds set per inflorescence.The maternal individual was the random effect, and the environmental and reproductive parameters were included as fixed effects: flowers = number of flowers per inflorescence, dbh = diameter at breast height (cm), canopy = largest canopy diameter measurement (cm), habitat = sample area. In all cases, the significance of the maternal individual (a random effect) was highly significant ($P < 2e^{-16}$, not included in the table but a component of every model). The best model was assumed to be the one with the lowest AIC (denoted with *) although other plausible models were those whose difference in AIC was less than 2 (e.g., $\delta AIC < 2$) for the putatively identified best model (denoted with †).

Fixed Effects	**Fixed Effect Coefficients**	**AIC**	**Dev**
flowers	$\beta_{flowers}= 0.04$	$157.9^{\dagger}$	0.56
flowers + dbh	$\beta_{flowers}=0.04$, $\beta_{dbh}=0.01$	159.5	0.54
flowers + canopy	$\beta_{flowers}=0.03$, $\beta_{canopy}=0.001$	$156.9^{\dagger}$	2.89
flowers + habitat	$\beta_{flowers}=0.02$, $\beta_{bottom}=0.94$, $\beta_{lakeside}=0.35$, $\beta_{north} = 0.44$, $\beta_{upper} = 0.58$	159.1	0.94
flowers + dbh + canopy	$\beta_{flowers}=0.03$, $\beta_{dbh}= -0.07$, $\beta_{canopy}=0.003$	156.5*	3.67
flowers + dbh + habitat	$\beta_{flowers}=0.02$, $\beta_{dbh}=-0.002$, $\beta_{bottom}=0.94$, $\beta_{lakeside}=0.37$, $\beta_{north}=0.47$, $\beta_{upper}=0.60$	161.0	0.96
flowers + habitat + canopy	$\beta_{flowers}=0.02$, $\beta_{bottom}=0.76$, $\beta_{lakeside}=0.15$, $\beta_{north} = 0.26$, $\beta_{upper}=0.31$, $\beta_{canopy}=0.000$	160.5	1.08
flowers + dbh + habitat + canopy	$\beta_{flowers}=0.02$, $\beta_{dbh}=-0.04$, $\beta_{bottom}=0.63$, $\beta_{lakeside}=0.12$, $\beta_{north}=0.26$, $\beta_{upper}=0.25$, $\beta_{canopy}=0.002$	161.6	1.64

Figures

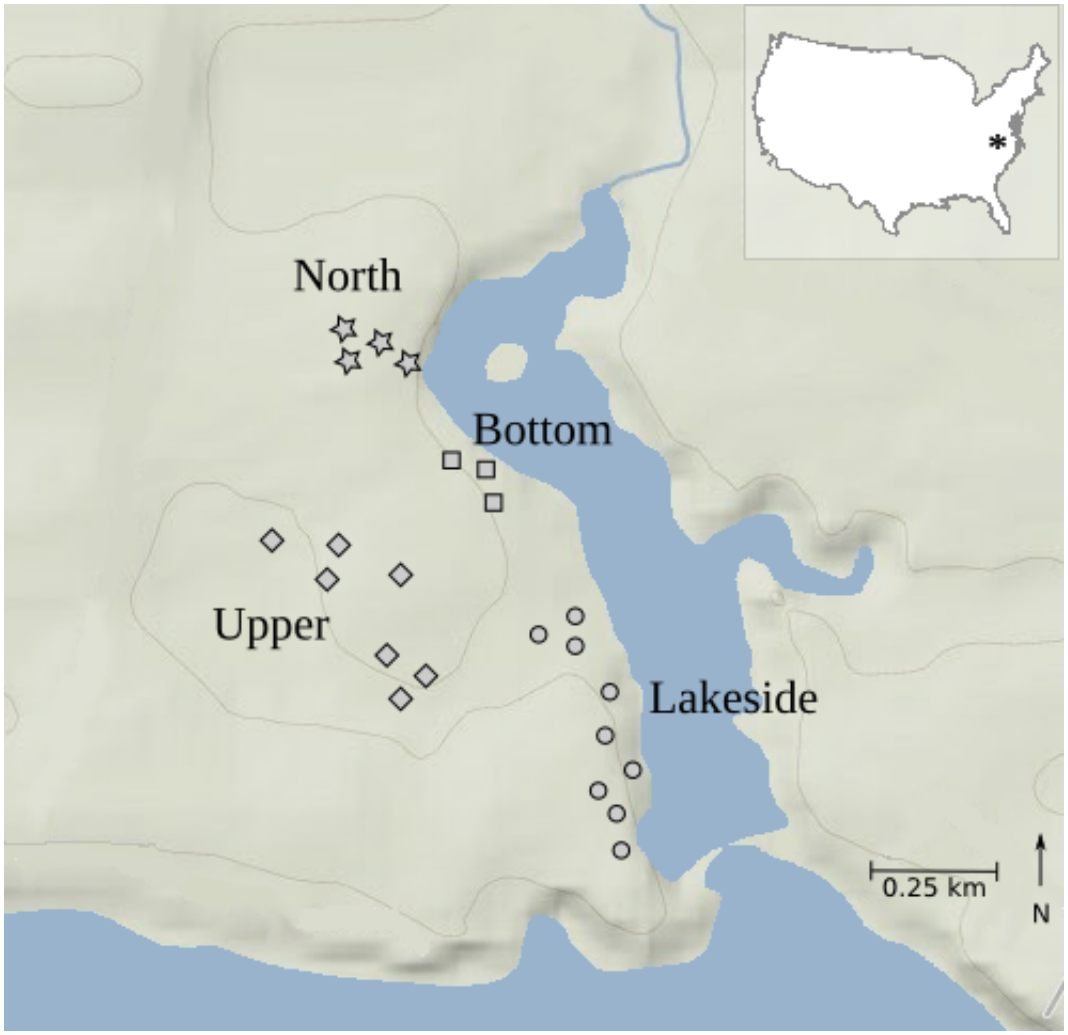

Figure 1.1: Map of the study site, the Inger and Walter Rice Center for Environmental Education. The inset shows the relative location of this site in the eastern United States. The tree locations are denoted by stars (North End), squares (Bottom), diamonds (Upper Forest) and circles (Lakeside).

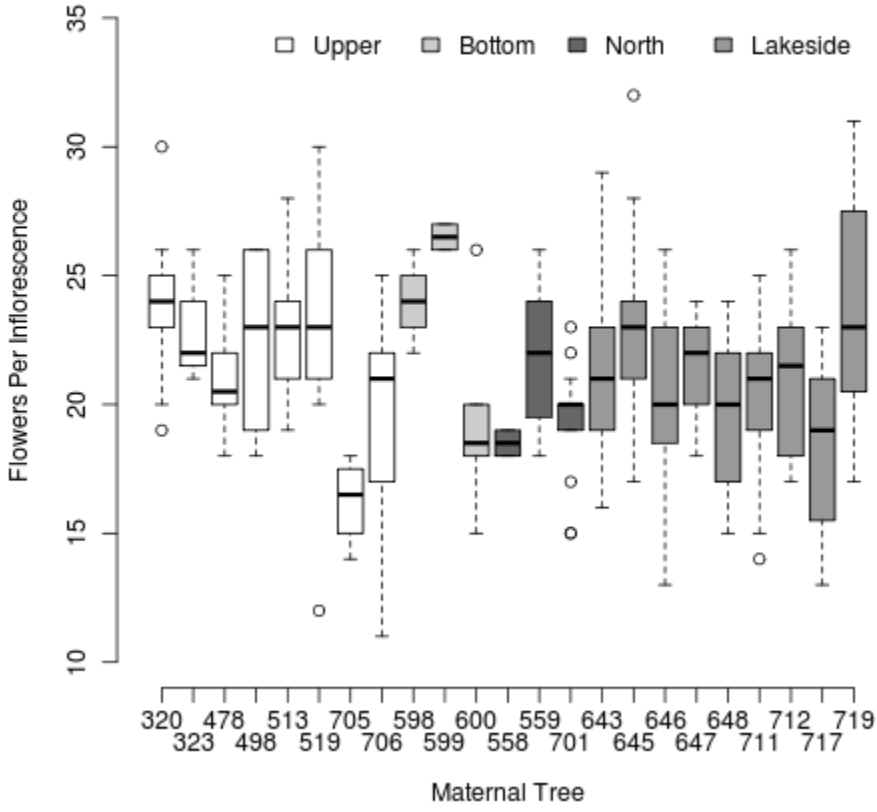

Figure 1.2: Flowers per inflorescence for the four sampling areas.

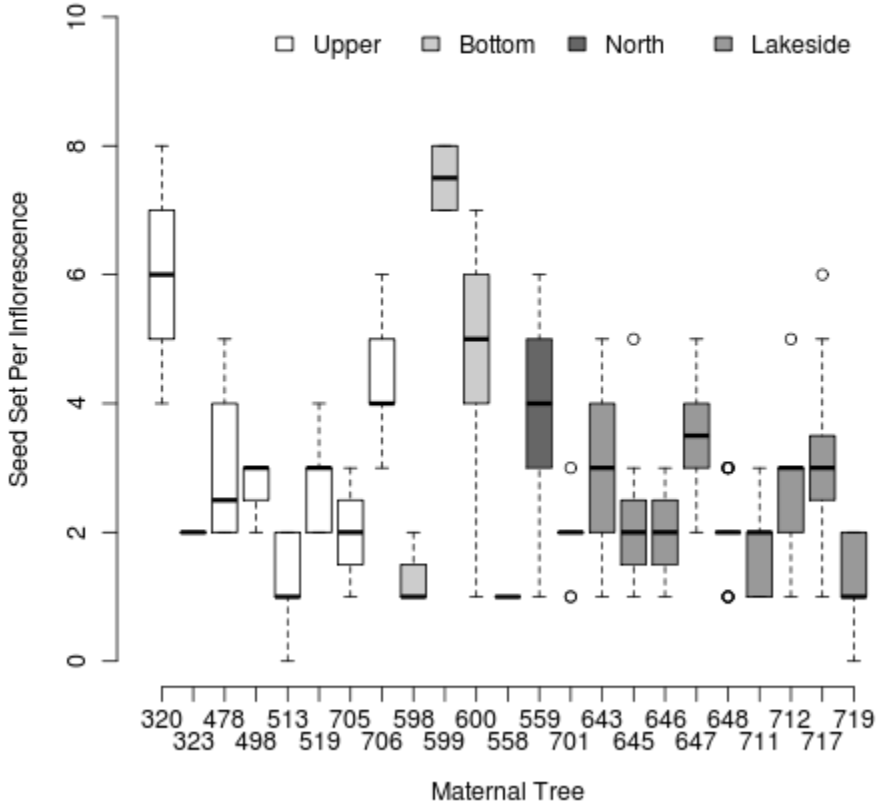

Figure 1.3: Seeds set per inflorescence for the four sampling habitats.

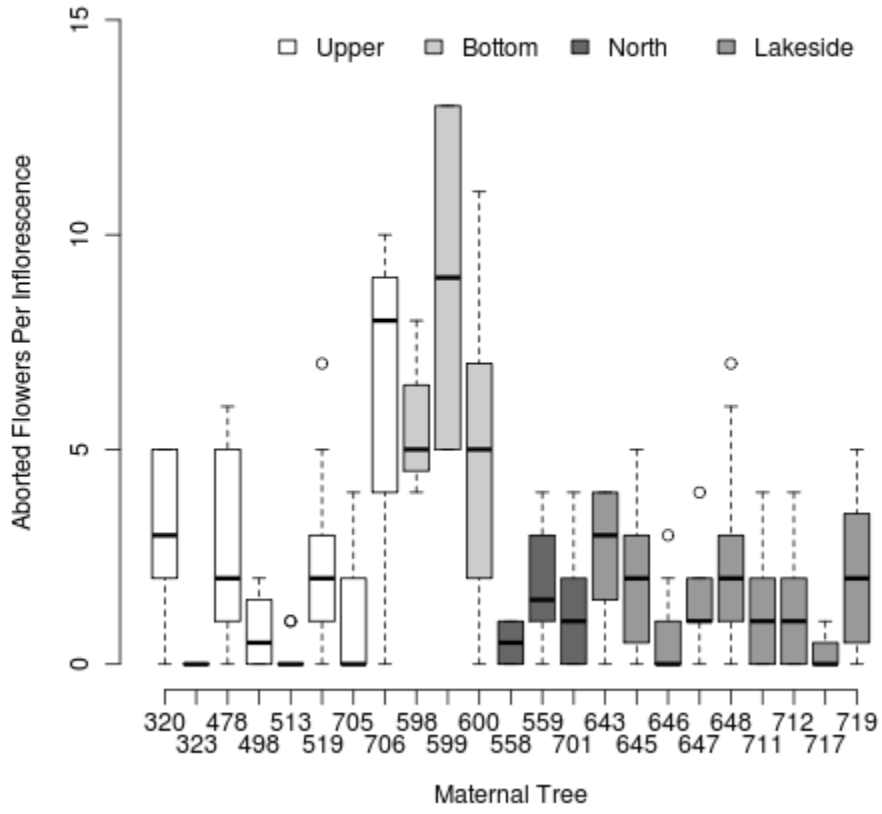

Figure 1.4: Aborted seeds per inflorescence for each of the four sample areas.

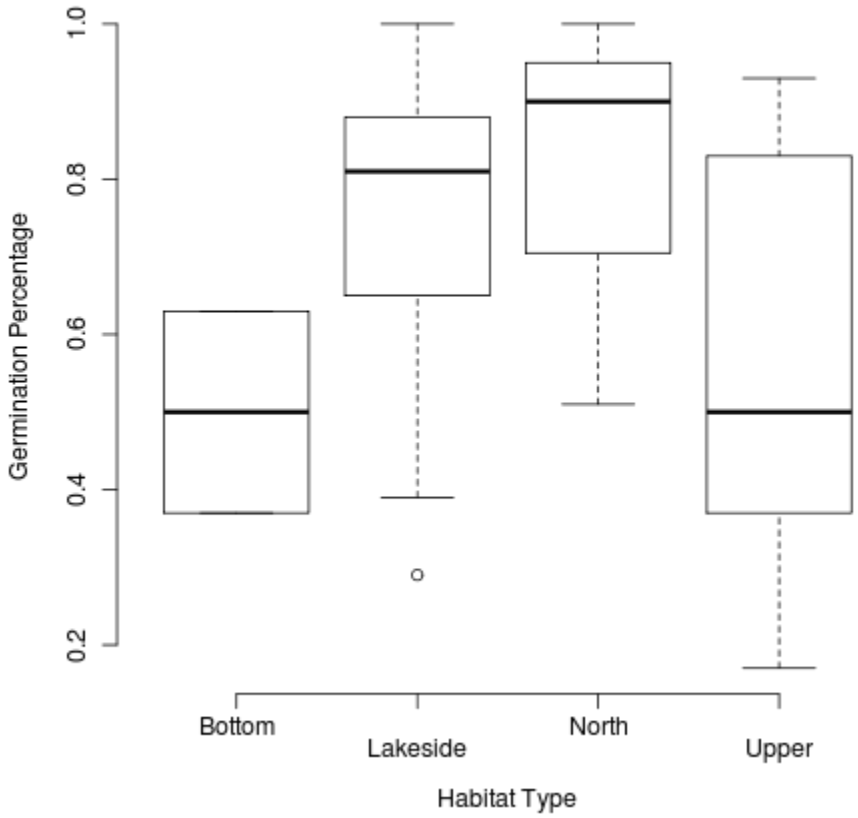

Figure 1.5: Differences in percent germination per maternal individual for the four sampling habitats (X^2 = 13.003, df = 16, P = 0.672).

CHAPTER II

INSECT COMMUNITY COMPOSITION RESPONSE TO FINE-SCALE DISTURBANCE

Introduction

Changes in composition of the local insect communities can impact reproductive parameters of plant populations by changing the manner in which pollen is dispersed between reproductive individuals, either directly by influencing pollinator species identities or indirectly by changing community dynamics (e.g., Dick 2001). Such changes can occur as insect communities respond to such natural fluctuations in habitat quality as seasonal changes in climate, periodic disturbance, and successional processes. While broad macroenvironmental factors outline the overall composition of a system, the microenvironmental factors in the immediate vicinity of the plants themselves dictate the individual response of an organism to its environment (Barbour *et al.* 1998). Changes in these microenvironmental factors can influence the reproduction of insect-pollinated plants by affecting the composition of the local pollinator community as well as the species with which they interact. Recent studies have outlined how generalist pollinators may be important to ecosystem health by offsetting the impact of disruption on more specialized pollination mutualisms (e.g., Memmott *et al.* 2004, Fortuna and Bascompte 2006). This study examined the insect communities in three habitat types defined by disturbance to determine if differences in insect community composition existed, and if so, whether they were consistent with previously observed patterns of genetic structure within the offspring of an understory tree population in these areas.

Natural disturbances in a forest can affect microenvironmental conditions by producing fine scale heterogeneity in the primary canopy architecture (Runkle 1985). The removal of

canopy trees by periodic storms or senescence contributes in varying degrees to this localized heterogeneity, with wind as a primary factor in gap formation (Hart and Grissino-Mayer 2009). Such localized alterations in habitat have been linked to changes in relative arthropod abundance and diversity (Schowalter 1985, Cartar 2005, Hatfield and LeBuhn 2007; Hirao *et al* 2008), which can change local plant-pollinator interactions. Gorham *et al.* (2002) found both higher abundances and morphotaxonomic richness of insects in primary canopy gaps than in interior or undisturbed forest areas. Although periodic flooding also affected these data, the canopy gaps were found to provide more and different arthropod habitat due to increased subcanopy and herbaceous biomass. Significant consequences of gap formation events on the mating patterns of insect pollinated species in such landscapes are density-dependent pollen pool composition (Murawski and Hamrick 1991), pollen flow limitation (Nason and Hamrick 1997), and differential reproductive phenology between conspecifics in fragmented areas (Fuchs *et al.* 2003).

Reproductive output for plants may be indirectly modified by heterogeneity in local canopy structure through changes in the composition of the potential pollinator communities (Aizen and Feinsinger 1994, Roubik 2002; Dick *et al.* 2003; Hatfield and Lebuhn 2007). The Intermediate Disturbance Hypothesis (IDH) suggests that a community experiencing intermediate disturbance regime will contain the most diverse assemblage of members. These differences are putatively ascribed to a wider variety of available niches provided by intermediate levels of disturbance (Sousa 1979). Alterations in the potential pollinator community can result in functional changes in gene flow between otherwise reproductively capable individuals (Didham *et al.* 1996). Both Schemske and Horvitz (1984) and Herrera

(1987) used fruit set data to show that different insect taxa function at different levels of pollination efficiency independent of floral visitation frequency. However, canopy alteration and subsequent switching of pollinators may not always result in reduced reproductive output. Dick et al. (2003) found that the formation of pastures surrounding *Dinezia excelsa* individuals resulted in pollinator switching from native honey bees to introduced, Africanized honey bees, and the reproductive output was actually increased. Because the impact of natural disturbance on local insect communities has been correlated with fluctuations in plant reproductive success through altered gamete transfer and seed dispersal (Ghazoul and McLeish 2001; Potts *et al.* 2003), and the local abundance of generalist pollinators varies according to the level of disturbance in a forest (Cartar 2005), this study sampled the available insect community as representative of the potential pollinator community.

Cornus florida is pollinated by a wide variety of insects due in part to its early flowering time when it is one of the few sources of pollen available for early emerging insects. Current work in the Dyer laboratory at Virginia Commonwealth University has identified several quantitative changes in reproductive characteristics of this species that are coincident with differences in local canopy structure. First, maternal individuals located within windthrow gaps left by Hurricane Isabel in 2003 (Beven and Cobb 2003) show more consanguineous mating (e.g., mating among relatives; $s_{b;\ open\ canopy} = 0.16$; MLTR Ritland 2000) when compared with adjacent individuals under closed canopies ($s_{b;\ closed\ canopy} = 0.11$). Randomly selected pairs of offspring from mothers in openings are more than three times as likely to be full siblings ($r_{p;\ open\ canopy} = 0.21$; MLTR Ritland 2002) than those in closed canopies ($r_{p;\ closed\ canopy} = 0.06$). Second, when examining the set of fathers contributing to the next generation, maternal individuals are

sampling from a more differentiated set of pollen donors (AMOVA; Φ_{PT} = 0.22 for fathers pollinating mothers in open canopies versus Φ_{PT} = 0.17 for mothers in closed canopies; Gardiakos 2009). These observed differences in the genetic structure of the offspring exist even though adults are not structured across the landscape (e.g., these results are not due to existing spatial genetic structure), which suggests that the mating process is being influenced by the recent primary canopy opening.

In the previous chapter, differences in tree-specific factors such as timing of reproduction, and relative maternal reproductive output were examined. It was hypothesized that canopy openings caused by Hurricane Isabel caused either temporal partitioning of the mating population (e.g., trees in open areas flowered earlier) or that competitive release of light and moisture increased reproductive output. While maternal trees showed wide variance in flowering time, number of flowers produced per inflorescence, rate of seed set, number of aborted seeds and germination rate, these factors were not coincident with canopy opening. Consequently, those data suggest that it is unlikely that tree-specific factors drove the differences observed in the genetics of the offspring. As a result, this chapter focuses on potential differences that exist in insect communities across fine scale forest canopy structure which may contribute to observed reproductive differences in *C. florida* (Gardiakos 2009). Differences in the morphotaxonomic composition of insect communities, and therefore potential pollinator communities, were sampled across alternate canopy architectures; closed canopy, open canopy due to disturbance, and no canopy.

Methods

Sampling

This work was conducted in the same location as the study in the previous chapter, the Virginia Commonwealth University's Inger and Walter Rice Center for the Environmental Life Sciences in Charles City County, VA, USA. This 343 acre facility is located on the north bank of the James River, approximately 50 miles upriver from the Chesapeake Bay (Figure 2.1; see also Chapter 1 for description of vegetation).

Insect communities were sampled in three different habitats representative of a decreasing gradient of primary canopy across the study area. The no disturbance sites consisted of closed canopy mixed hardwood stands. Fallen trees in this habitat type consisted of mostly single individuals due to normal mortality rather than groups of trees caused by windthrow (Carr pers. obs.). Openings in the primary canopy were relatively large and not coincident with high stem biomass on the ground; the primary canopy in this area was nearly complete. The disturbed habitat was characterized by groups of fallen trees on the ground coinciding with large openings in the primary canopy above. These fallen trees were positioned approximately parallel, with the crown and some roots still attached, indicative of windthrow damage (Runkle 1985). The third area was a periodically maintained old field, representing a heavily and repetitively disturbed habitat. This field was livestock pasture until 2000, after which time it has been mown annually and burned once in 2005 (Smock, L.A., pers. conv.). These habitat types are hereafter referred to respectively as the canopy, the blowdown, and the field.

The locations of the insect collection transects were selected haphazardly in areas subjectively identified as representative of the three different habitat types. Sampling transects

consisted of two different passive collection methods: pan traps and hanging Malaise traps (Dafni *et al*, 2005, Stephen and Rao 2005, Campbell and Hanula 2007). The pan trap collection method included plastic bowls (Solo Cup Company, Highland Park, Illinois, USA) deployed in single transects of 12 blue and 12 yellow in each habitat (following Droege 2009). The collection bowls were approximately 5 cm deep and 20 cm in diameter. Each bowl was placed on wire stands raised 0.25 m above the ground to preferentially collect low-flying rather than crawling insects. Each bowl was half-filled with mildly soapy water to reduce the surface tension of the water such that small insects would sink upon contact and be unlikely to escape. The water was changed during each observation period.

The aerial trapping employed two hanging Malaise traps (Sante Traps, Lexington, Kentucky, USA), one each in the undisturbed canopy and the blowdown (Campbell and Hanula, 2007). These traps were suspended 2m above the ground and were positioned at least four meters from the nearest tree. The traps collected samples from all four sides, in 1L jars at the top and bottom of each trap. Insects collected from pan traps and canopy traps were treated as random samples of each local habitat type. These sampling methods were used in the closed canopy and blowdown, and only the pan traps were deployed in the old field.

All insects collected were stored in plastic shell vials or 2.0 mL PCR tubes in the field. Each insect was rinsed with 30% ethanol to remove any debris and soapy water remaining and to separate smaller taxa from larger taxa. Insects were sorted into morphotaxonomic groups using the following method. Specimens were identified to order using Marshall (2006), and then further subdivided into groups consisting of taxa that could not be further subdivided by difference in appearance or morphology (Oliver and Beattie 1996, Nansen *et al*, 2004).

Classification was done with an Olympus SZ40 dissection microscope (Olympus America Inc., Center Valley, PA, USA). Each insect was then placed individually into a 2.0 mL PCR tube with 70% ethanol, labeled by trap, location and date, and stored at -20° C. Voucher specimens of all morphotaxa and the identification key were prepared and stored along with all specimens collected at Virginia Commonwealth University, Richmond, Virginia.

Statistical Analysis

Preliminary analysis was conducted to characterize sampling effort and to determine if observed collection differences between habitats were a function of relative abundance. The following analyses were conducted on the pan trap samples; the data from the Malaise trap collections are presented in summary as they were deployed without replication across habitats. The mean values for abundance, richness, Pielous's evenness and Shannon's diversity were calculated per pan for each habitat without subdivision through time. A rarefied sample accumulation curve was plotted for collections by habitat type to control for differences in sample size (Gotelli and Colwell 2001). This curve was plotted to examine the change in rarefied morpho-taxonomic richness as a function of individual accumulation due to differences in habitat heterogeneity (Gotelli and Colwell 2001). The `specpool` function in R (R Development Core Team 2009) using the BiodiversityR package (Kindt and Coe 2005) was used to provide a jackknife estimate of the standard error associated with species richness (Heltshe and Forrester 1983).

Samples were analyzed for differences in community structure at three different spatio-temporal scales. At the largest scale, samples were pooled by habitat to test for differences in community structure as a function of overall habitat differences. Next, the samples in each

habitat were further subdivided by pan trap color to test for differences attributed to pan color. Finally, temporal differences within each habitat were examined by comparing collections across sampling intervals.

Using these three levels, community structure was first characterized using the following summary statistics: abundance, richness, Pielou's evenness (*J*) and Shannon diversity (*H*). Abundance was quantified as a count of how many insects of each morphotaxa were collected in each sampling unit. Richness was recorded as the number of distinct morphotaxonomic groups in each sampling unit (Oliver and Beattie 1996). Shannon's diversity index provides values between 1.5 (low diversity) and 3.5 (high diversity) (Magurran 1988). Pielou's evenness is a ratio of observed diversity and maximum possible diversity. Each of these parameters was estimated using the BiodiversityR package (Kindt and Coe 2005) for R 2.8.1 (R Development Core Team 2009). Quantitative differences in these parameters were determined using a two-way Analysis of Variance (ANOVA) approach following Gorham *et al.* (2002). Sample allocation for differences attributable to habitat was tested as a two-way ANOVA for the pan trap data. Temporal variation was analyzed separately for each habitat with sampling date as the primary treatment. Tukeys post-hoc tests were used to determine individual treatment differences as a function of both sampling time and pan color. Interaction between sample time and pan color was tested as well as any interaction between habitat and pan color.

Morisita's index of similarity was calculated for the species/area matrices for each habitat. This index uses relative values of abundance and richness of individual count data to generate a similarity index value between zero and approximately one, with higher values indicating more similarity (Krebs 1989). Linear discriminant analysis (LDA) was conducted to determine the

extent to which samples could be successfully partitioned by habitat type using abundance, richness and evenness as factors (after McCune and Grace, 2002). Order-level classification was used to perform a Chi-square test between the proportions of Coleoptera, Diptera, Hymenoptera, Araneae and other insects in each habitat. All calculations were performed using the BiodiversityR (Kindt and Coe 2005) package for R 2.8.1 (R Development Core Team 2009).

Malaise collections were described by reporting the abundance, richness, Pielou's evenness (*J*) and Shannon diversity (*H*) for each collection and overall in each habitat. (Table 2.2).

Results

Sampling was carried out on ten days between April 16 and April 28, 2008. The blue pan traps were deployed on April 15, 2008, and the yellow pan traps were deployed on April 16, 2008. Two of the thirteen days were not sampled, and three of the remaining ten days yielded no results. The three days with zero results, 4/17, 4/18, and 4/22, were during or immediately after large rain events. A total of 1134 individuals representing 234 morphotaxa were collected comprising ten orders in Class Insecta as well as multiple Collembola. The collembolids were included in the Other Insects category. The mean values of abundance, morphotaxonomic richness, Pielou's evenness and Shannon diversity per sample were all highest in the blowdown and lowest in the canopy, with the field collection intermediate (Table 2.2). The blowdown (39.6 +/- 18.4) and the field (24.2 +/- 11.4) were not statistically different in abundance. This rank ordering of the raw data (Figures 2.2-2.5) indicated that some structuring was present within these collections.

Habitat Differences

Total arthropod abundance consisted of 1134 individual specimens separated into 234 morphotaxa. Individuals collected in pan traps (747 specimens in 158 morphotaxa) contributed to approximately two thirds of the total number of specimens and morphotaxonomic groups (65% and 67% respectively). By habitat, 386 specimens in 100 morphotaxa were collected from the undisturbed canopy, 498 specimens in 143 morphotaxa from the blow down, and 250 specimens in 60 morphotaxa from the field. The pan trap collections represent the majority of the collected samples by individual abundance, 66%, and by richness, 68%. Of the pan trap samples,

119 specimens in 66 morphotaxa were collected in the undisturbed canopy, 378 specimens in 96 morphotaxa were collected in the blow down, and 250 specimens in 60 morphotaxa were collected in the field. The remaining 387 specimens were collected over the four days in the Malaise traps, with 267 specimens in 44 morphotaxa in the canopy and 120 specimens in 59 morphotaxa in the blowdown. Mean arthropod abundance per sample (pan) was highest in the blowdown (40 +/- 18), followed by the field (24 +/- 11), and was lowest in the canopy (15 +/- 9) (Figure 2.2). The raw abundance was significantly different between habitat types (two-way ANOVA, $F_{2,66} = 24.8$, $P < 0.0001$), as were the raw values for morphotaxonomic richness between habitat types (two-way ANOVA, $F_{2,66} = 20.9$, $P < 0.0001$). Mean morpho-taxonomic richness in the pan collections was highest in the blowdown (12.5 +/- 3.9), lower in the field collections (8.5 +/- 3.0) and lowest in the canopy (7.2 +/- 2.8; Figure 2.3). The rarefied morphotaxonomic richness supports this, showing that the blowdown sample yields more types of morphotaxa than the other two habitats over 1000 resampling iterations (Figure 2.6). The jackknife estimate of taxonomic richness in the pan samples yielded an estimate of 230 +/- 10 taxa, compared to 158 collected taxa.

The mean values for morphotaxonomic evenness per pan sample (Figure 2.4) were similar between habitat types, rather than following the rank order displayed in the results for abundance and richness. The mean number of insects per type of morphotaxa present per pan sample was somewhat more evenly distributed in the canopy (1.99 +/- 0.53) and blowdown (2.08 +/- 0.25) than in the field (1.80 +/- 0.46). Analysis of Variance showed that these results were marginally significant (two-way ANOVA, $F_{2,66} = 3.16$, $P = 0.049$). The mean values for the Shannon diversity index (Figure 2.5) per pan sample for the canopy (1.10 +/- 0.7) and field (1.27

+/- 0.62) collections were similar, while the blowdown (1.94 +/- 0.42) yielded higher values for both, and Analysis of Variance showed that there were significant differences in diversity values between habitat types (F = 14.38, P = 6.56 e^{-06}).

Trap color

Analysis of Variance showed significant differences per pan between the two trap colors, blue and yellow, in richness (F = 12.29, P = < 0.001) and diversity (F = 6.51, P = 0.013), and a marginally significant difference in abundance (F = 3.63**,** P = 0.061). Values referred to as marginally significant indicate that these results may be open to interpretation. Tukey's HSD post hoc testing revealed that the differences in each of the three community characteristics followed similar patterns. The yellow pan traps in the blowdown contained more samples with higher diversity than all other combinations of trap and habitat.

Habitat and trap type interactions

Two-way Analysis of Variance was also used to test for significant interactions in sampling response summary statistics between each habitat and pan trap type. The interaction between habitat type and trap color per pan was significant for abundance ($F_{1,2}$ = 6.52, P = 0.0026), richness ($F_{1,2}$ = 5.87**,** P = 0.0045) and evenness ($F_{1,2}$ = 4.43, P = 0.0156). The differences in abundance indicate that the number of insects per pan sample in different habitats depended on the trap type. Tukey's post hoc testing showed that this was due to the higher numbers of insects caught in the yellow traps in the canopy. The significant interaction in richness suggests that the

number of types of insects per pan sample in different habitats depended on the trap type.

Morisita's index of similarity estimated between habitats yielded values of 0.65 between the blowdown and canopy, 0.53 between blowdown and field and 0.13 between the canopy and field (Table 2.2). These values suggest that the composition of the sampled habitats was different and contributed to differences in community composition. The proportions of Diptera and Hymenoptera in the blowdown and Coleoptera in the field were the likely drivers of this result.

The linear discriminant analysis did not result in meaningful support for the specified groups based upon habitat (Figure 2.11). Despite explaining 99% of the observed variation in the first two axes (80% for the first axis and 19% for the second), clustering based on habitat types was not apparent.

Temporal variation

The most pervasive pattern through time was due to evening thunder storms on 4/16, 4/17 and 4/21. The three days following the storms yielded no insects or debris of any type in any pan. A few traps on each of these days were observed to have been overturned, presumably due to heavy rainfall or runoff from nearby plants because no traps were found out of place at any other time than the day after these storms (Figures 2.7-2.10). Traps were not checked on two days, April 20 and April 26, due to sampling error. This resulted in compounded sampling yield with the previous day. The blowdown appeared to show the highest values of abundance, richness and diversity. The abundances in the samples appeared to peak twice, about two days after each

storm event. The blowdown yielded the most individuals collected in the first peak, from 4/16 until 4/23, and the field yielded the most in the second peak, from 4/23 to 4/27. The highest abundance recorded was on 4/19 in the blowdown, and was concurrent with a similar increase in richness. Richness and diversity were generally highest for the blowdown collections for each nonzero collection day until 4/27. The field collections were higher for richness, evenness and diversity on 4/27, the last day of sample collection, corresponding with the cessation of flowering in *C. florida*.

The ten days of sampling not directly affected by the rain were analyzed by two-way ANOVA to determine if there were significant differences in abundance, richness, evenness and diversity among sample composition between days. The results indicate that there was significant variation among sampling dates and habitat types for all summary statistics (abundance: $F_{2,9} = 8.32$, $P < 0.0001$, richness: $F_{2,9} = 8.12$, $P < 0.0001$, evenness: $F_{2,9} = 5.88$, $P < 0.0001$, diversity: $F_{2,9} = 7.18$, $P < 0.0001$). However, the short duration of the sampling period limits the potential significance of the analysis of temporal variation, primarily because it did not include the entire course of flowering.

Summary of Malaise Traps

A total of 387 individuals in 87 morphotaxonomic groups were collected from the Malaise traps over four sampling days. The trap in the blowdown yielded 120 individuals in 59 morphotaxa, and the trap in the canopy yielded 267 individuals in 44 morphotaxa. The higher abundance in the canopy was largely due to a hatching event which resulted in 135 early instar larvae in the trap collector on 4/25. As these larva were completely non motile, they were removed as

unrepresentative of flying mid-canopy insects. The adjusted canopy trap number was 132 individuals in 43 morphotaxa (Table 2.2).

Discussion

The most important result in this study was that significant differences in morphotaxonomic abundance and richness existed between the insect pan sample collections of the three habitat types. The abundance and richness values were rank ordered, from highest to lowest, as the blowdown, the field, and the undisturbed canopy. The insect community pan sample from the undisturbed canopy had the most diverse and most evenly distributed taxa, followed by the blowdown. The collection of more individuals of more types of insects in the area of intermediate disturbance, or blowdown, demonstrates that the insect community in this area is distinct relative to the other two. Further, the rarefied morphotaxonomic richness (Figure 2.6) of all pan sample collections by habitat type suggests that these results were not due to sampling effort. The hanging traps collected more types of insects in the blowdown, but more overall numbers of individuals in the closed canopy. These results may reflect functional dissimilarities in pollen resource availability or usage between the habitat types for potential pollinators. Such heterogeneity in these insect communities is likely to result in differences in the pollen movement between the insect pollinated trees within these habitats. The consequences of such changes in pollen movement can be used to explain the previously observed structure within the offspring of *C. florida* L. between habitat types (Gardiakos 2009).

The different summary statistical values for the insect community pan samples from the blowdown suggest a different type of community structure in this habitat type. Morisita's index of similarity showed that the blowdown was also likely to contain more of the overall types of insects sampled than the other two habitats. These summary statistics indicated that a larger community was present in the blowdown than in the canopy or the field, and that this community

was more representative of the available morphotaxa than the others. However, the significantly different values for evenness and Shannon's diversity between the field and the canopy, and the blowdown and the canopy, demonstrated that the sampled insects were more equitably distributed among the taxa present in the canopy than in either of the other two habitats. Because rarefaction of the pan sample collections did not appear to reach an asymptote, the sampling effort may have been too low rather than too high. This may have resulted in under-representation of the available morphotaxonomic richness in all areas. Such under-representation may have led to the canopy having the highest values of morphotaxonomic diversity because Shannon's diversity incorporates the evenness of the representative species into the value. The collections from the malaise traps appear to support this.

The composition of the insect communities in these habitats can be modified by the resulting changes in available resources after periodic disturbance (Shelley 1988, Didham *et al.* 1996, Cartar 2005). The degree to which these gaps affect arthropod habitat can depend on the size or severity of the disturbed area (Shure and Phillips 1990). Because the level of natural disturbance in this study was relatively small (Runkle 1985), the concurrent changes in the composition of the insect communities were expected to be less than extreme. The analysis of similarity (Table 2.2) showed that the most morphotaxonomically rich habitat type, the blowdown, was composed of members that represented at least half the groups found in the other habitat types rather than groups found nowhere else. The percentage of the total morphotaxa sampled found in the blowdown, 61%, was higher than those found in the canopy, 42% or in the field, 38%. These values suggested that the composition of the insect community in the blowdown represented a concentration of the available morphotaxa, rather than an entirely

separate suite of insects. These data also support the idea that the larger size of the insect community in the blowdown may have been indicative of relatively high quality arthropod habitat in comparison to the other sampled habitat types, consistent with the Intermediate Disturbance Hypothesis (Canham and Marks 1985). A larger and more varied insect community in an area of recent intermediate disturbance could include more potential pollinators than a smaller and less differentiated community, due to more available opportunities for forage and habitat. Also, a higher degree of interaction may be expected between the insects in the blowdown community due to the greater density of insects per pan sample and the higher number and type of morphotaxa present relative to the other habitats.

Such fine-scale increases in habitat quality could also affect the pattern of pollen dispersal by functional differences in the available forage resource between habitat types. The floral composition within a gap initially increases in diversity resulting in an increased availability of different types of forage resource for pollinators (Runkle 1985, Cartar 2005). Optimal foraging theory suggests that this broadening of the available forage resource will result in a decrease in breadth of foraging by pollinators (MacArthur and Pianka, 1966). This theory suggests that there will be a shift from more opportunistic foraging patterns in the less resource rich habitats to a less opportunistic foraging pattern in the more resource rich habitat as foragers maximize use of fewer, high yield sources. Pollen foraging insects in the habitat types that supported lower levels of insect abundance and richness may have been expected to forage among all available resources, while those pollen foragers in the habitat types that support higher levels of insect abundance and richness may have been expected to forage only among the most readily available resource. In this study, the primary pollen resource in each area was *C. florida*,

likely due to the early flowering habit of the dogwood. Canopy gaps did appear to contain more types of shrub and ephemeral vegetation than the closed canopy (pers. obs.).

Optimal foraging theory may explain some aspects of the previously observed structure of *C. florida* offspring between the maternal individuals in these habitat types (Gardiakos 2009). The higher levels of full-siblings on maternal trees in the disturbed, or open canopy, areas suggest that pollinators spend more time moving back and forth between neighboring trees. *Cornus florida* is a predominately outcrossing species (Sork *et al.* 2005, Gardiakos 2009) and is therefore incapable of producing selfed offspring. Adult trees exhibit positive spatial autocorrelation throughout the study area (Dyer, unpub.) indicating that these close neighbors are most likely genetic relatives. Increased pollen movement between related trees would also result in higher levels of full siblings and can increase the granularity of differentiation among sampled pollen pools.

Fine-scale structure between local understory conspecifics can have implications for the larger population. Small, localized natural disturbances such as those in this study represent areas of increased heterogeneity in the composition of a forest plant community, as opposed to large disturbances that can divide or restructure plant communities by complete removal of vegetation or major disruption of the existing species composition. For populations of plants within continuous forests, areas of low to intermediate disturbance are important to the overall genetic structure because they can provide opportunities for increased localized genetic diversity. While the heterogeneity resulting from individual disturbance events is temporary, the effects of multiple fine-scale disturbance events through time can contribute to the overall structure of a plant community. For example, trees on a high spot in the forest will likely be blown down more

often than trees in a low spot, resulting in an area of frequent disturbance. The resulting structure may yield areas of genetic heterogeneity that may contribute to greater overall genetic variance, a vital component for adaptation and persistence.

Works Cited

Aizen, M. A. and P. Feinsinger. 1994. Forest fragmentation, pollination and plant reproduction in a chaco dry forest, Argentina. *Ecology*. 75: 2, 330-351

Barbour, M. G., J. H. Burk, W. D. Pitts, F. S. Gilliam and M. W. Schwartz. 1998. Terrestrial Plant Ecology. 3rd ed., 1998. Addison Wesley Longman, Menlo Park.

Beven, J. and H. Cobb. "Tropical Cyclone Report: Hurricane Isabel". 19 December 2003. National Hurricane Center, National Weather Service. June 17, 2009. < http://www.nhc.noaa.gov/2003isabel.shtml >.

Canham, C. D. and P. L. Marks. 1985. The response of woody plants to disturbance: patterns of establishment and growth. in “The ecology of natural disturbance and patch dynamics” (S. T. A. Pickett and P. S. White, eds.) pp. 197-216. Academic Press, Orlando.

Campbell, J. W. and J. L. Hanula. 2007. Efficiency of Malaise traps and colored pan traps for collecting flower visiting insects from three forested ecosystems. *Journal of Insect Conservation*. 11: 399-408.

Cartar, R.V. 2005. Short-term effects of experimental boreal forest logging disturbance on bumble-bees, bumble-bee pollinated flowers and the bee-flower match. *Biodiversity and Conservation*. 14: 1895-1907.

Dafni, A., P. G. Kevan and B. C. Husband, eds. 2005. Practical Pollination Biology. Enviroquest, Cambridge.

Dick, C. 2001. Genetic rescue of remnant tropical trees by an alien pollinator. *Proceedings of the Royal Society of London B.* 268: 2391-2397.

Dick, C. W., G. Etchelecu and F. Austerlitz. 2003. Pollen dispersal of tropical trees (Dinizia excelsa: Fabaceae) by native insects and african honeybees in pristine and fragmented Amazonian rainforests. *Molecular Ecology* 12: 753-764

Didham, R. K., J. Ghazoul, N. E. Stork, and A. J. Davis. 1996. Insects in fragmented forests: a functional approach. *Trends in Ecology and Evolution* 11:6, 255-260.

Droege, S. 2009. "A Very Handy Bee Manual." June 2009. National Biological Information Infrastructure. June 17, 2009. http://www.nbii.gov/portal/community/Communities/Ecological_Topics/Pollinators/.

Fuchs, E. J., J. A. Lobo, and M. Quesada. 2003. Effects of forest fragmentation and flowering phenology on the reproductive success and mating patterns of the tropical dry forest tree *Pachira quinata. Conservation Biology* 17(1): 149-157.

Fortuna, M. A. and J. Bascompte. 2006. Habitat loss and the structure of plant-animal mutualistic networks. *Ecology Letters* 9: 278-283.

Gardiakos, V. A. 2009. Pollen-mediated gene movement in flowering dogwood, *Cornus florida L.* Master's Thesis. Virginia Commonwealth University, Richmond, VA.

Ghazoul, J. and M. McLeish. 2001. Reproductive ecology of tropical forest trees in logged and fragmented habitats in Thailand and Costa Rica. *Plant Ecology* 153: 335-345.

Gorham, L. E., S. L. King, B. D. Keeland and S. Mopper. 2002. Effects of canopy gaps and flooding on Homopterans in a bottomland hardwood forest. *WETLANDS* 22(3): 541-549.

Gotelli, N. J. and R. K. Colwell. 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters* 4: 379-391.

Hart, J. L. and H. D. Grissino-Mayer. 2009. Gap-scale disturbance processes in secondary hardwood stands on the Cumberland Plateau, Tennessee, USA. *Plant Ecology*. 201: 131-146.

Hatfield, R. G. and G. LeBuhn. 2007. Patch and landscape factors shape community assemblage of bumble bees, Bombus spp. (Hymenoptera: Apidae), in montane meadows. *Biological Conservation* 139: 150-158.

Herrera, C. M. 1987. Components of pollinator “quality”: comparative analysis of a diverse insect assemblage. *Oikos* 50: 70-90.

Heltshe, J.F., and N. E. Forrester. 1983. Estimating species richness using the jackknife procedure. *Biometrics* 39: 1-11.

Hirao, T., M. Murakami, and H. Oguma. 2008. Functional spatial scale of community composition change in response to windthrow disturbance in a deciduous temperate forest. *Ecological Research* 23: 249-258.

Kindt, R. and Coe, R. 2005. Tree diversity analysis: A manual and software for common statistical methods for ecological and biodiversity studies. World Agroforestry Center, Nairobi, Kenya.

Krebs, C. J. 1989. Ecological Methodology. Harper Collins, New York, 654 p.

MacArthur, R. H. and E. R. Pianka. 1966. On optimal use of patchy environment. *The American Naturalist.* 100. 603-609.

Marshall, S. A. 2006. Insects: Their Natural History and Diversity with a Photographic Guide to Insects of Eastern North America. Firefly Books, Ontario.

Magurran, A. E. 1988. Ecological diversity and it's measurement. Princeton University Press, Princeton, New Jersey.

McCune, B. and J. B. Grace. 2002. Analysis of Ecological Communities. MjM Software Design, Gleneden Beach, Oregon.

Memmott J., N. M. Waser and M. V. Price. 2004. Tolerance of pollination networks to species extinctions. *Proceedings of the Royal Society B-Biological Sciences* 271: 2605-2611.

Murawski, D. A. and J. L. Hamrick. 1991. The effect of the density of flowering individuals on the mating systems of nine tropical tree species. *Heredity* 67: 167-174.

Nansen, C., T. W. Phillips and M. W. Palmer. 2004. Analysis of the insect community in a stored maize facility. *Ecological Research* 19: 197-207.

Nason, J. D. and J. L. Hamrick. 1997. Reproductive and genetic consequenses of forest fragmentation: two case studies of neotropical canopy trees. *Journal of Heredity* 88: 264-276.

Oliver, I. and A. J. Beattie. 1996. Invertebrate morphospecies as surrogates for species: a case study. *Conservation Biology* 10(1): 99-109.

Potts, S., B. Vulliamy, P. Willmer, A. Dafni and G. Ne'eman. 2003. Linking bees and flowers: how do floral communities structure pollinator communities? *Ecology* 84(10): 2628-2642.

R Development Core Team. 2009. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna Austria. http://R-project.org

Ritland, K. 2000. Marker-inferred relatedness as a tool for detecting heritability in nature.

Molecular Ecology 9: 1195-1204.

Ritland, K. 2002. Estimation of gene frequency and heterozygosity from pooled samples. *Molecular Ecology Notes* 2:3 370-372.

Roubik, D. W. 2002. The value of bees to the coffee harvest. *Nature* 417: 708.

Runkle, J. R. 1985. Disturbance regimes in temperate forests. in "The ecology of natural disturbance and patch dynamics" (S. T. A. Pickett and P. S. White, eds.) pp. 17-33. Academic Press, Orlando.

Schemske, D. and C. Horvitz. 1984. Variation among floral visitors in pollination ability: a precondition for mutualism specialization. *Science* 225: 519-521.

Schowalter, T. D. 1985. Adaptations of Insects to Disturbance. in "The ecology of natural disturbance and patch dynamics" (S. T. A. Pickett and P. S. White, eds.) pp. 235-252. Academic Press, Orlando.

Shelley, T. E. 1988. Relative abundance of day-flying insects in treefall gaps va shaded understory in a neotropical forest. *Biotropica* 20:2 114-119.

Shure, D.J. and D.L. Phillips.1990. Patch size of forest openings and arthropod populations. *Oecologia* 86: 325-334.

Sousa, W. P. 1979. Disturbance in marine intertidal boulder fields: The nonequilibrium maintenance of species diversity. *Ecology.* 60: 6, 1225-1239.

Sork, V. L., P. E. Smouse, V. Apsit, R. J. Dyer, and R. D. Westfall. 2005. A Two-Generation analysis of pollen pool genetic structure in flowering dogwood (*Cornus florida*, Cornaceae) in the Missouri Ozarks. *American Journal of Botany*. 92(2): 262-271.

Stephen, W. P. and S. Rao. 2005. Unscented color traps for non-Apis bees (Hymenoptera: Apiformes). *Journal of the Kansas Entomological Society* 78(4): 373-380.

Tables

Table 2.1: Summary of results for community composition metrics by sample collection and habitat for the 13 day sampling period.

	Sampling Date									
Canopy	4/16	4/17	4/18	4/19	4/21	4/22	4/23	4/24	4/25	4/27
Abundance	19	0	0	24	8	0	16	11	26	15
Richness	9	0	0	21	7	0	13	9	20	15
Evenness	0.37	0	0	0.26	0.14	0	0.2	0.17	0.27	0.19
Shannon	1.59	0	0	2.93	1.73	0	2.27	1.89	2.81	2.62
Blowdown										
Abundance	29	0	0	177	17	0	39	47	54	15
Richness	8	0	0	54	14	0	29	25	31	11
Evenness	0.47	0	0	0.61	0.21	0	0.34	0.35	0.4	0.2
Shannon	1.42	0	0	3.28	2.43	0	3.22	2.58	3.30	2.21
Field										
Abundance	0	0	0	55	9	0	19	67	58	42
Richness	-	0	0	18	6	0	16	21	15	28
Evenness	0	0	0	0.59	0.35	0	0.98	0.23	0.36	0.35
Shannon	0	0	0	1.68	1.3	0	2.65	1.94	1.44	2.99
Total										
Abundance	48	0	0	256	34	0	74	125	138	72
Richness	11	0	0	73	23	0	49	44	57	45
Evenness	0.30	0	0	0.43	0.14	0	0.23	0.28	0.31	0.23
Shannon	0.73	0	0	1.84	0.44	0	0.90	1.08	1.26	0.86

Table 2.2: Summary statistics for abundance, density, richness, evenness, and diversity by habitat and trap type. Morisita's Index of Similarity between habitats is indicated below. Cells with '-' indicate parameters that could not be calculated for the particular combination of parameter and sampling location.

	Canopy				**Blowdown**				**Field**				**Total**		
	Blue	Yellow	Malaise	Total	Blue	Yellow	Malaise	Total	Blue	Yellow	Malaise	Total	Pan	Malaise	Total
Abundance	63	56	267	386	176	202	120	498	173	77	-	250	747	387	1134
Density	0.53	0.52	-	-	1.47	1.87	-	-	1.44	0.71	-	-	1.09	-	-
Richness	40	34	44	100	47	76	59	153	38	42	-	60	158	87	234
Evenness	0.90	0.95	-	-	0.73	0.86	-	-	0.49	0.94	-	-	0.77	-	-
Diversity	3.32	3.35	-	-	2.79	3.71	-	-	1.81	2.51	-	-	3.91	-	-
Similarity Index					0.654				0.532						
									0.127						

Figures

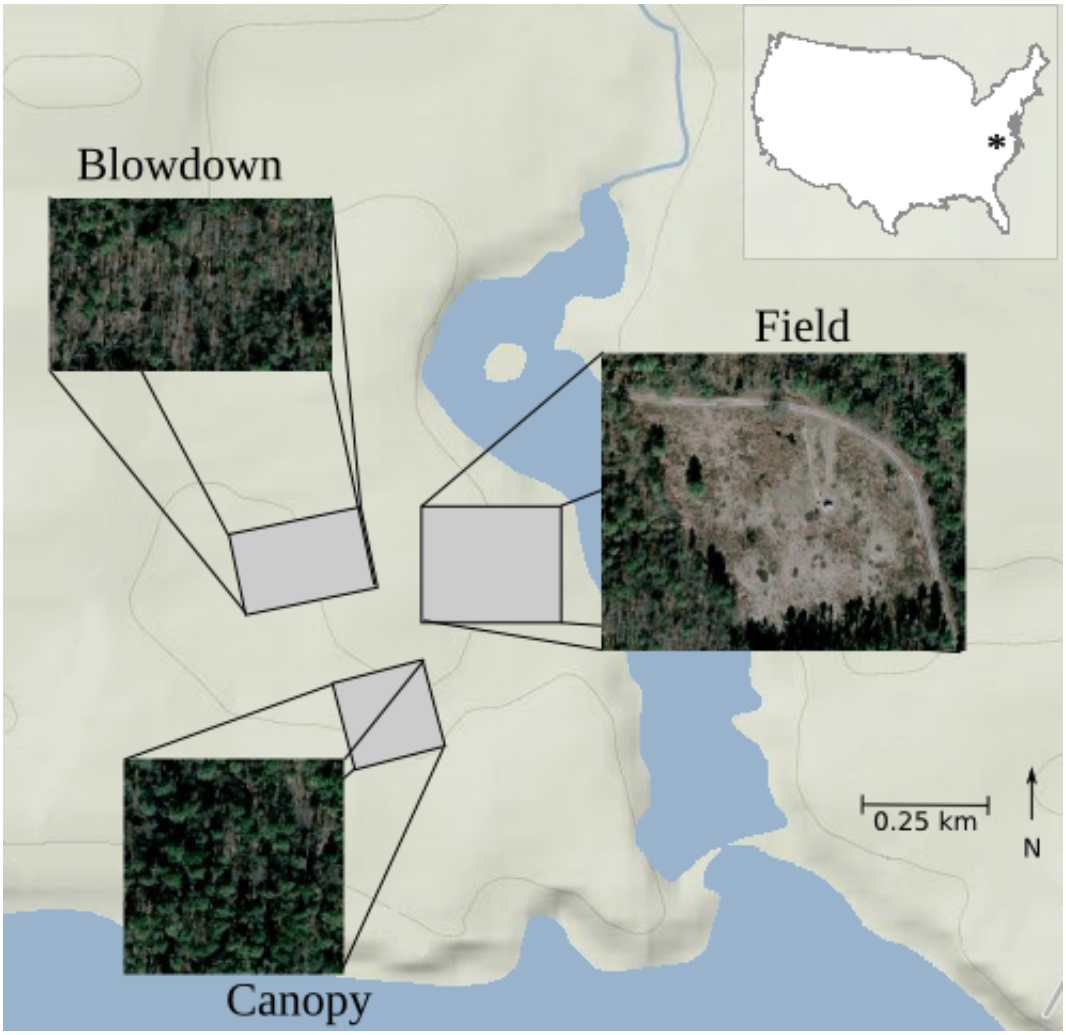

Figure 2.1: VCU Rice Center, with areas indicated for alternate habitats. The inset shows the relative location of this site in the eastern United States.

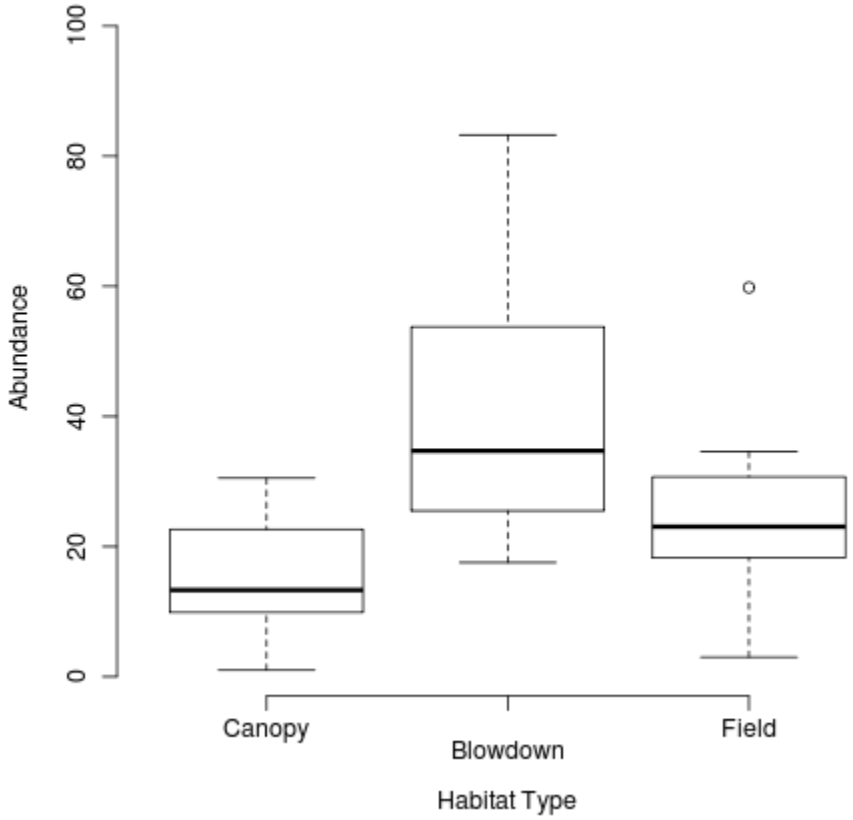

Figure 2.2: Mean number of individuals per sample unit (pan) in each habitat type.

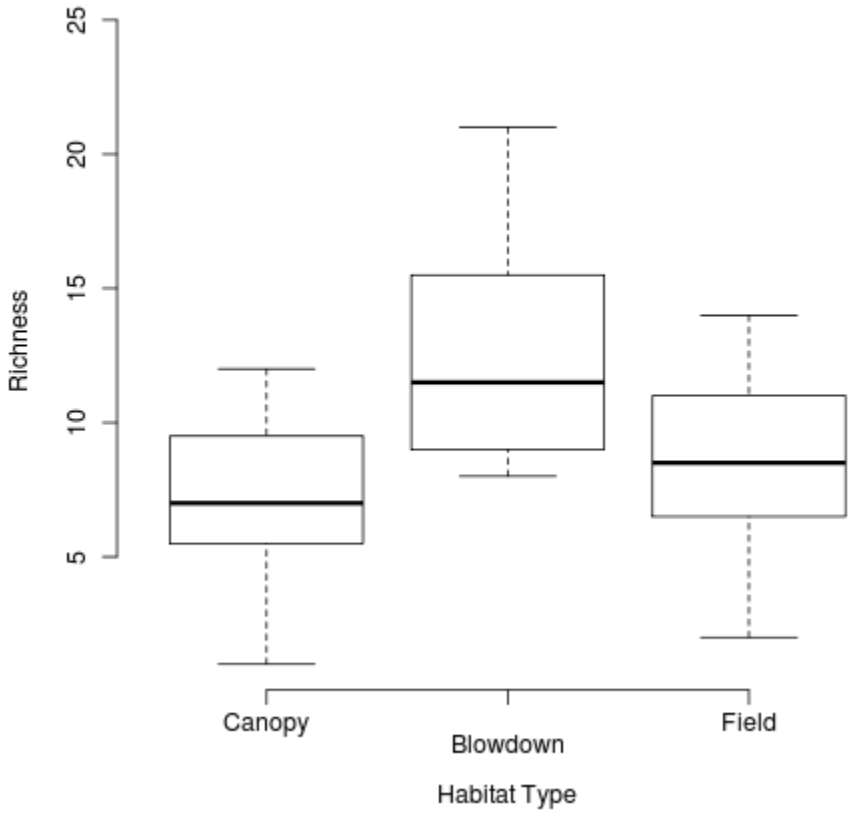

Figure 2.3: Mean number of morphotaxa (richness) per sample unit (pan) across three sampling habitats.

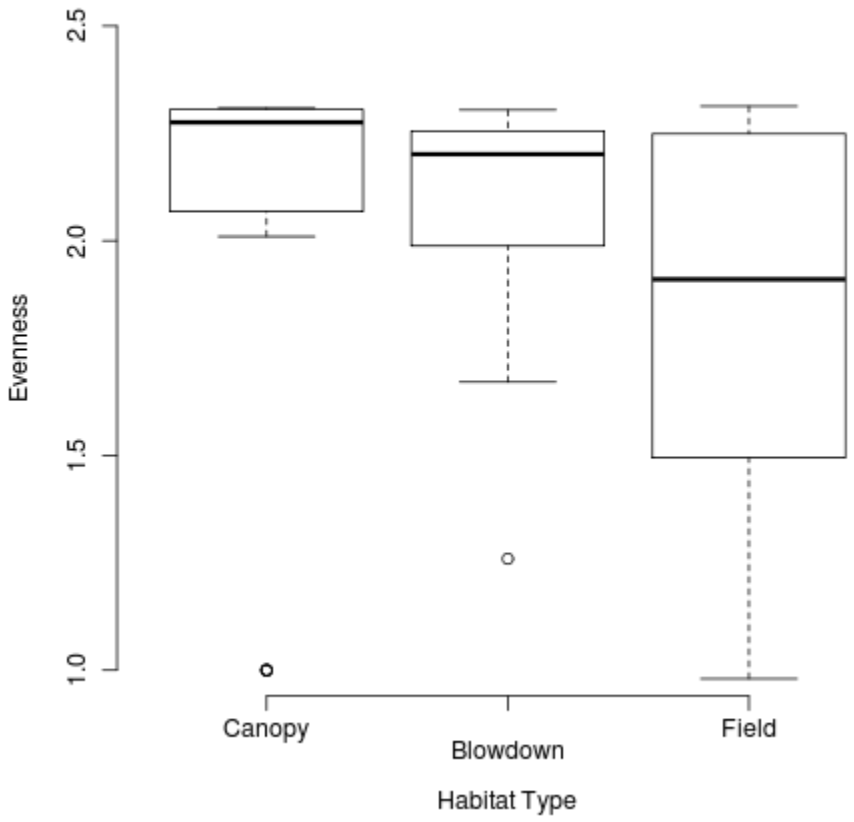

Figure 2.4: Mean evenness (J) of taxa per sample unit (pan) across habitat types.

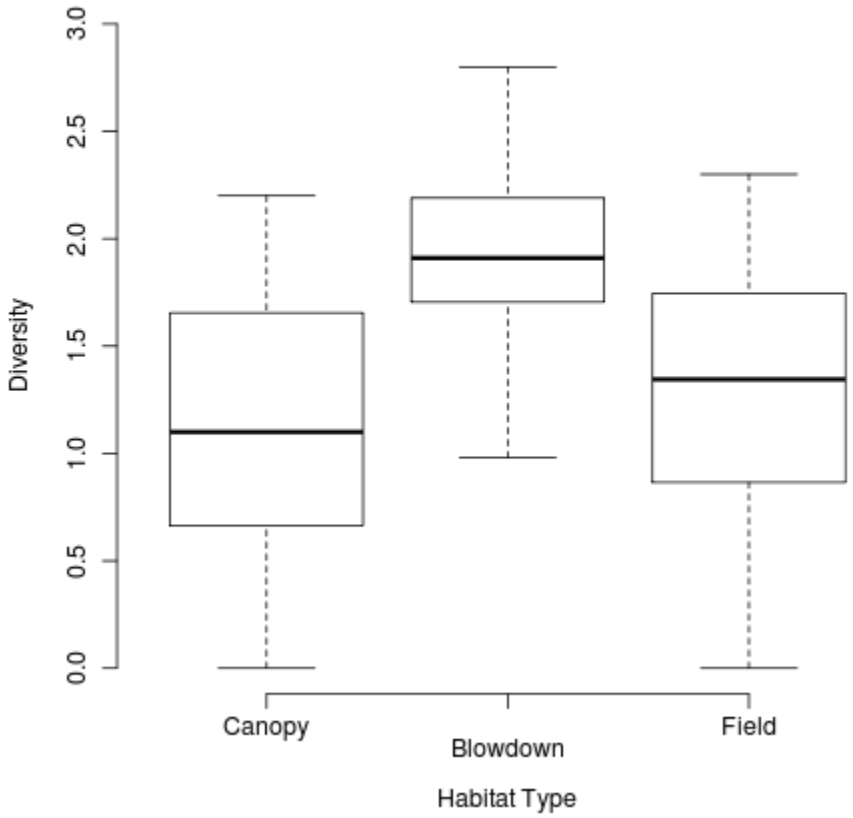

Figure 2.5: Shannon diversity per sample unit (pan) in each habitat type.

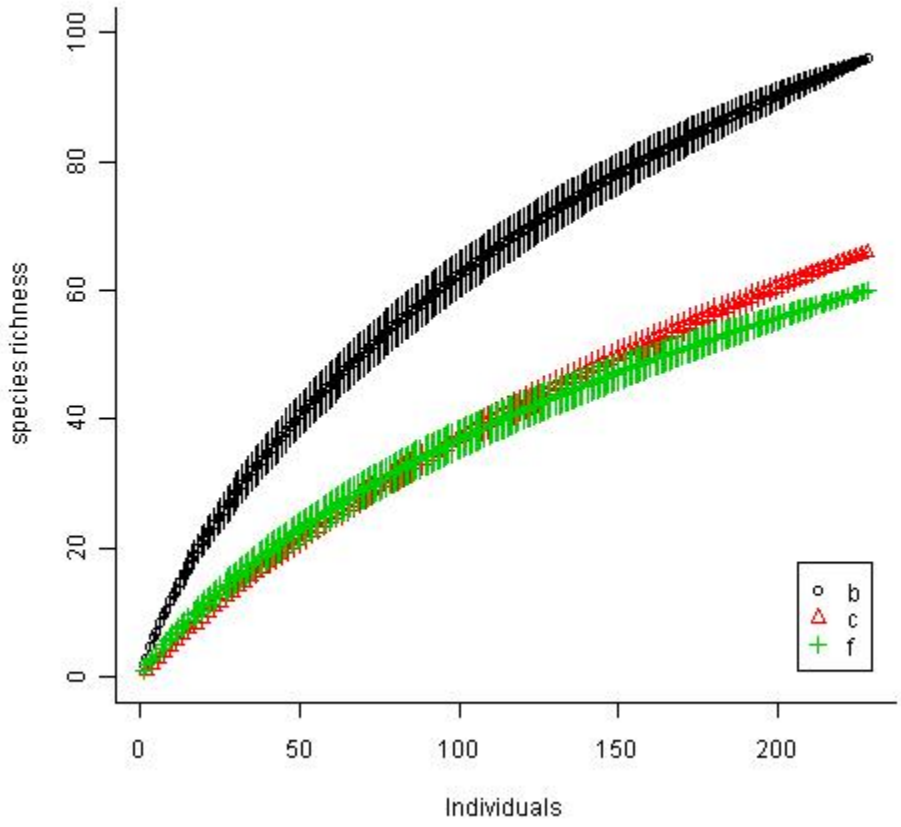

Figure 2.6: Rarefied taxonomic richness (morphotaxa) as a function of the number of individuals collected in each habitat type. Rarefaction was determined by 1000 replicate re-sampling runs. Areas around each series represent 95% confidence intervals.

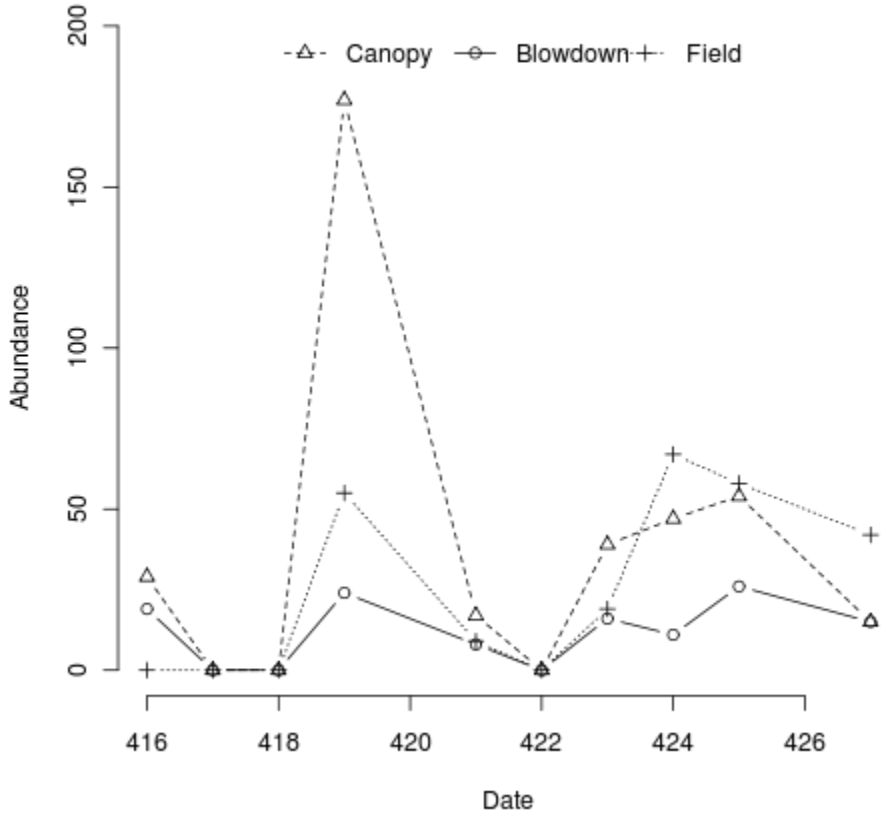

Figure 2.7: Abundance of insects collected in the pan traps in each sampling area on each sampling date.

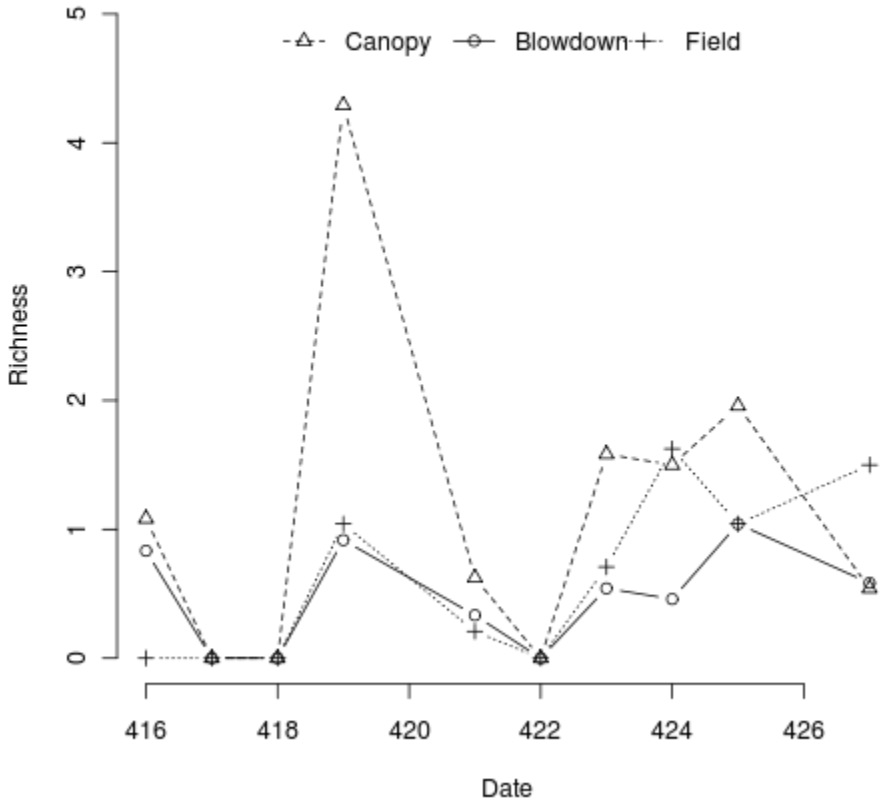

Figure 2.8: Relative morphotaxonomic richness of insects collected in the pan traps in each sampling area on each sampling date.

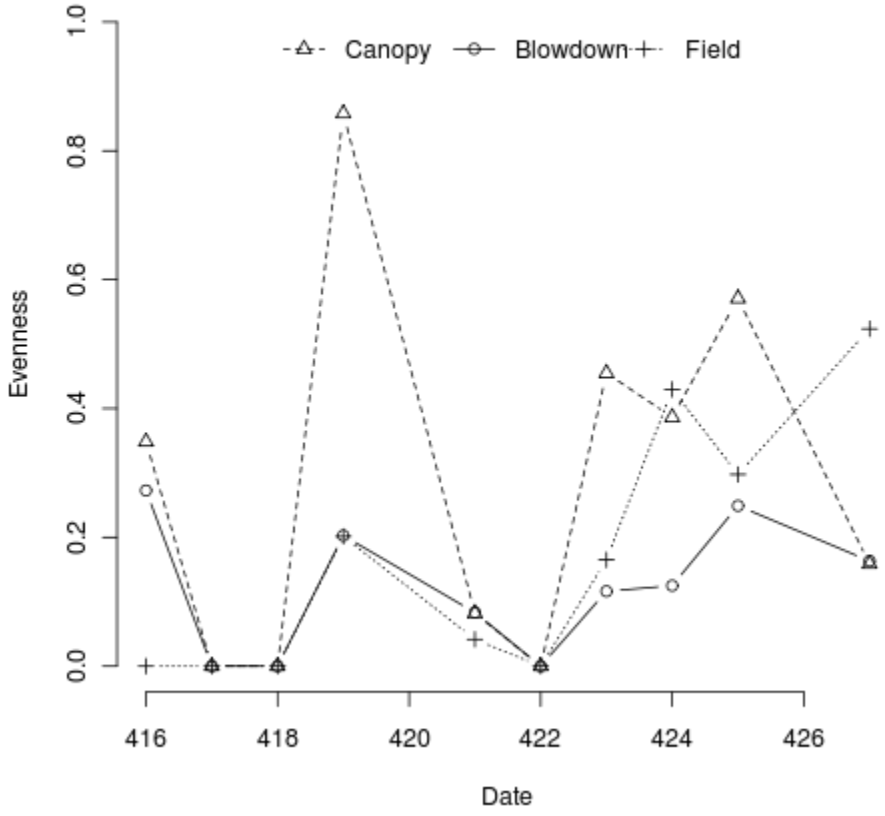

Figure 2.9: Pielou's evenness of insects collected in the pan traps in each sampling area on each sampling date.

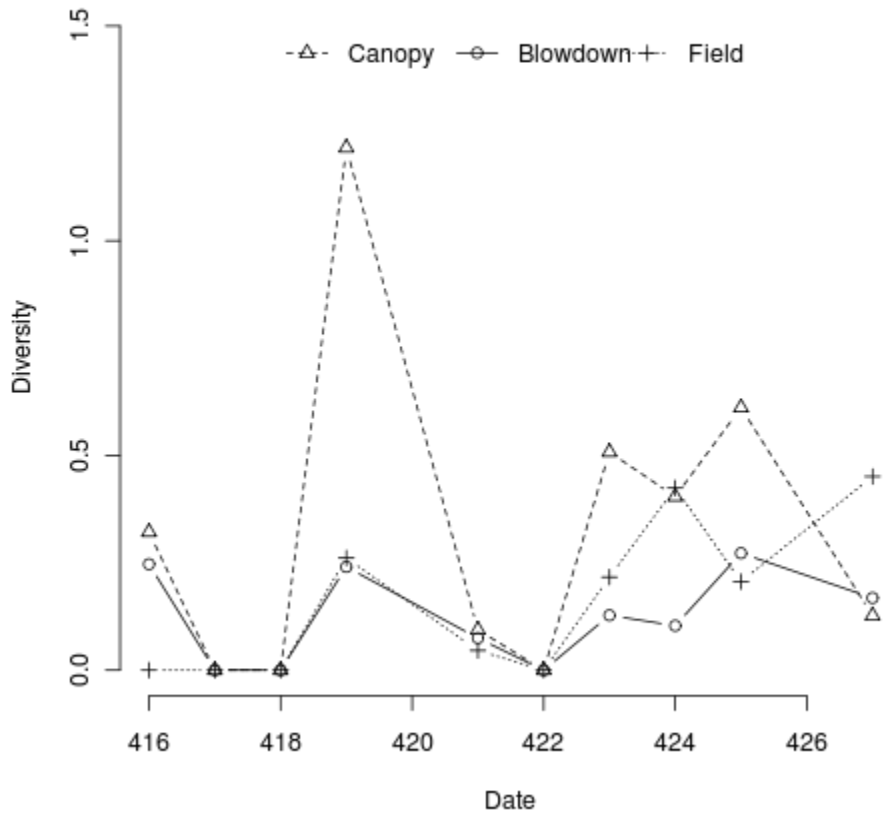

Figure 2.10: Shannon's diversity of insects collected in the pan traps in each sampling area on each sampling date.

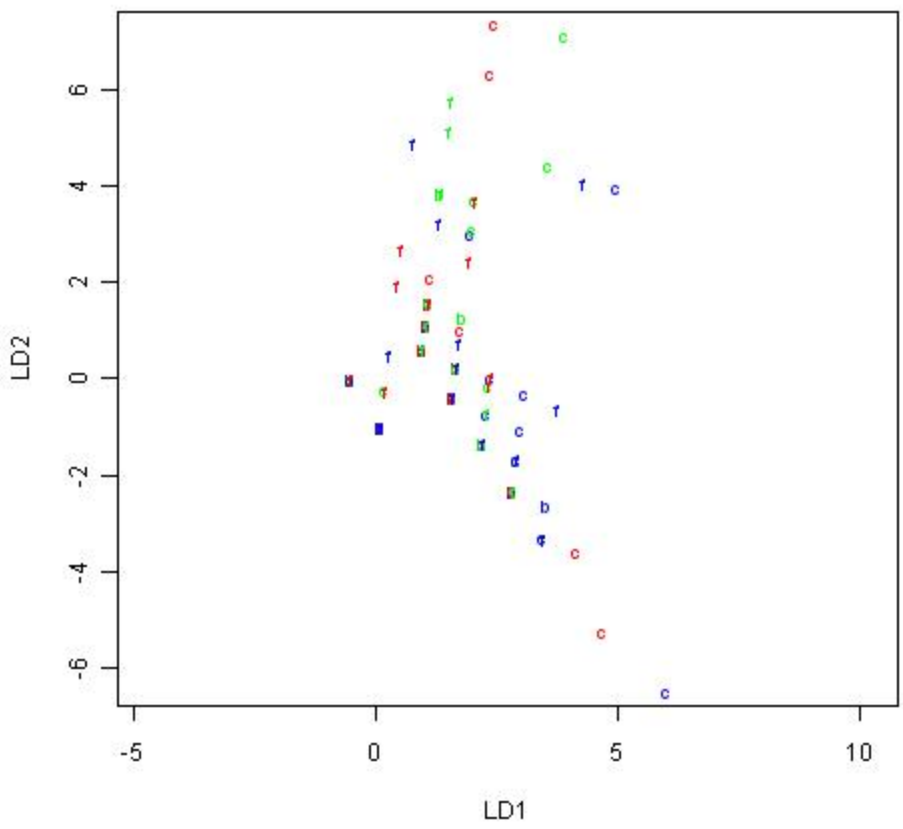

Figure 2.11: Linear discriminant analysis of the three community composition parameters, abundance, richness and evenness, by habitat type. The first axis explains 80% and the second axis 19%.

Conclusion

The major finding of this research was that the impact of intermediate natural disturbance on the mating systems of understory plant populations may be more indirect than direct. The disturbance event here appeared to impact the potential pollen dispersers more than the trees. The lack of flowering asynchrony between sampling sites suggests that the environmental parameters in each were not sufficiently different to cause any temporal reproductive isolation between habitats. Further, the lack of any clear correlation between the sampling groups and the measured reproductive parameters suggests that reproduction efforts of *C. florida* may be affected by environmental factors at a different scale than was measured here. The indirect effects on the dogwood mating system may have been more substantial. The insect community composition was quite different between areas of differential canopy opening. These results suggest that multiple assemblages of potential pollinators coexist in the forest coincident with the variation in fine scale canopy architecture. It is possible that these different insect assemblages move pollen in different ways across the landscape as the members of each respond to different localized environmental factors.

The lack of measured variation in flowering parameters observed here likely reflects the functional similarity of the local environments around these *C. florida* individuals. Although the trees in the open canopy areas were qualitatively judged to have been flowering more intensely per individual during sampling, the analyses did not support this observation. The overall reproductive phenology of these plants does vary over wider geographic and elevation scales (pers. obs.). For example, dogwoods in the Blue Ridge Mountains on the border of Virginia and

West Virginia begin to flower approximately two to three weeks after those in the coastal plain, two hundred miles east and two thousand feet lower. These changes are likely due to differences in temperature on a scale beyond that of the local variation observed at the VCU Rice Center. This large scale temperature dependence is beneficial to plants by timing the release of gametes with the emergence of pollinators across multiple environmental types. The variation in floral density per tree in sample groups at this scale may have been due to the health of the trees, or some other cryptic factor such as nutrient limitation or interspecific competition.

The insect communities studied here provide several important observations about pollination and disturbance and how they relate to forest health. First, the insect communities observed here were composed of varying combinations of insects found in all areas and insects found only in each specific area. This describes a dynamic balance of habitat-generalist and habitat-specific insects which changes through space and time as the forest is affected by disturbance events. Generalist insects are ubiquitous in and around the forest, and they only change in frequency and abundance as the landscape as a whole is modified. Specialists are found only within certain types of environmental conditions, for example, short-lived opportunistic species that take advantage of optimal conditions for foraging and mating presented by the resource supplementation of canopy gaps. These species likely migrate from among new disturbances as the forest changes with succession. A broadening of the insect community composition is beneficial to the insects themselves as they have more opportunities to compete and mate. Such interactions at the community level describe a trophic web of greater complexity as more predators, parasites and competitors interact with the existing pollen foragers. The maintenance of successful generalized pollination mutualisms is dependent on

healthy insect populations, and natural disturbance may improve the overall health of local insect populations by resource supplementation.

In conclusion, the observed differences in insect community structure within these habitat types in this study were consistent with predetermined patterns of pollen flow. This insect community structure could possibly explain some aspects of the previously observed genetic differentiation within spatially proximate tree populations. The more robust insect community assemblage in the open canopy area was consistent with observations about the higher quality of insect habitat in this area, as predicted by the Intermediate Disturbance Hypothesis. Qualitative estimation of differential floral display within these areas supported the conclusion provided by Optimal Foraging Theory that pollen foragers would not travel as far or forage as widely in areas of elevated resource. With these conclusions, the significant differences in insect community structure may be used to explain differential reproductive parameters observed in *C. florida* offspring samples by habitat type. The more abundant insect community in the area of intermediate disturbance was consistent with the previously observed genetic structure characterized by locally limited pollen movement. The higher levels of relatedness between offspring in the open canopy indicated that the paternal pollen pool was contributed to by more individuals within 15 meters, which is consistent with the limited pollen foraging behavior explained by foraging theory. These reproductive implications of natural disturbance in the understory flora provide insight into the mechanisms by which fine scale genetic diversity is maintained in natural populations.

Vita

Daniel Foster Carr was born in Richmond, Virginia. He graduated from Benedictine High School in 1993, and received a B.A. in History from Virginia Commonwealth University in 1998. After several years as a landscaper and stonemason, he returned to VCU and received a B.S. in Biology in the spring of 2007. He entered the Master of Science program in the fall of that year, and received a Research Assistantship for the 2008-9 academic year. During graduate school Daniel taught introductory biology laboratory classes for Biology majors and non-majors, as well as the practical component of a collaborative learning program between the Department of Biology and the School of the Arts. Daniel served as the president of the VCU Department of Biology's graduate student organization for 2008-9, organized a celebration of Charles Darwin's 200th birthday, and graduated in the summer of 2010.