Theses and Dissertations   Graduate School

2013

REPRODUCTION AND FUNCTIONAL RESPONSE OF CORNUS FLORIDA ACROSS AN URBAN LANDSCAPE GRADIENT

Angela Redwine
Virginia Commonwealth University

REPRODUCTION AND FUNCTIOAL RESPONSE OF *CORNUS FLORIDA*
ACROSS AN URBAN LANDSCAPE GRADIENT

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

by

Angela H. Redwine

B.S., Virginia Commonwealth University, 2011

Major Adviser: Dr. Rodney J. Dyer
Associate Professor
Department of Biology

Virginia Commonwealth University
Richmond, VA
May 2013

Acknowledgements

I am grateful to my thesis adviser, Dr. Rodney Dyer, for his support and encouragement throughout this entire process. I would like to also thank my committee members Dr. Christopher Gough, Dr. Don Young, and Dr. Matthias Leu for all of their time and contributions. I thank the VCU Rice Center and Environmental Studies Department for financial support during my time in the program. I certainly could not have gotten very far without the valuable conversations and excellent examples set by my fellow graduate students and friends James Deemy and Dan Carr. I am so thankful for the love and support of my family. Thank you to my husband Joseph, my rock throughout my time in college. And most importantly, thank you to my father Curtis J. Hutto for instilling in me the deepest love and appreciation for the natural world. It is because of him that I am here at all, and I dedicate this research to the legacy of his life's work in natural resources protection.

Table of Contents

List of Tables

List of Figures

Abstract

REPRODUCTION AND FUNCTIONAL RESPONSE OF *CORNUS FLORIDA* ACROSS AN
URBAN LANDSCAPE GRADIENT

By Angela Hutto Redwine

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

Virginia Commonwealth University, 2013

Major Adviser: Dr. Rodney J. Dyer, Department of Biology

Urbanization greatly alters plant and pollinator communities and can affect pollinator movement
and subsequent gene flow. Plants persisting in urban areas must adjust to local environmental
conditions often different from those in which they naturally evolved, and cultivation techniques
for landscaping species have developed traits suitable for existence in urban habitats. Cultivated
varieties and native conspecifics often exist in geographic proximity, and if pollinator movement
is not blocked by urban structural components, functional differences may negatively impact
spatially proximate native populations. I used spatial analysis of successful pollination of *Cornus
florida* to estimate how pollinator movement is affected by urban features. My results suggest
that buildings and canopy are the most important components which influence reproductive
success in urban habitats. Additionally, I compared functional responses of both adult and
offspring *C. florida* cultivars and native plants to differential light environments in the urban and
natural understory habitats, and we found differences in physiology and morphology that could

lead to negative fitness consequences for native populations should gene escape from urban

cultivar to native populations occur via pollinator movement.

Introduction

Urbanization can alter pollinator and plant assemblages, as well as the ways in which these organisms interact. Alteration of host plant assemblages and habitat fragmentation are the key factors leading to reductions in pollinator populations in North America (Ahrnè *et al.* 2009). Functional differences caused by anthropogenic landscape manipulation, however, have been largely context specific to the kinds of modifications being made and the species examined. For example, in addition to reducing native pollinator presence, Dick *et al.* (2003) found that agricultural fragmentation resulted in a complete change in pollinators from native bees to invasive species for the tree *Dinizia excelsa* (Fabaceae). However, green spaces within the intensively urbanized areas of New York City maintained a widely diverse pollinator assemblage, with invasive or non-native species comprising less than 20% of the total species (Matteson *et al.* 2008). Plants that persist in urban habitats must adjust to their altered environmental conditions, and cultivation techniques artificially select for traits such as high light and drought tolerance, longer-lasting and higher floral output, and desirable plant size and shape. Cultivated species in the Southeastern U.S. consist of a wide variety of native taxa including flowering dogwood (*Cornus florida*), redbud (*Cercis canadensis*), cherry (*Prunus spp.*), elm (*Ulmus spp.*), and many species of oak and maple (*Quercus spp.* and *Acer spp.*; Flint 1997). Cultivated species in these urban altered habitats are often situated within a larger matrix of surrounding suburban, agricultural, and second-growth forest habitat where native conspecific populations exist. There may be functional differences in how cultivated plants respond to their urban environmental conditions when compared to their native conspecifics. The larger context within which how gene escape influences native populations has generally been applied to

1

agriculturally important species and their native counterparts, particularly as a result of genetic engineering (e.g., Mannion and Morse 2012, Uwimana *et al.* 2012). However, should functional differences exist and given the accelerating pace of urbanization, cultivated plants in urban areas may also prove problematic for native conspecific population persistence, particularly if they are connected through gene flow within and between urban and natural environments.

Natural and artificial landscape features within urban areas may affect gene flow by altering plant – pollinator, as well as pollinator - landscape, interactions. Physical structures within the urban environment can negatively impact gene flow by modifying spatial patterns of pollinator movement. Road corridors represent barriers to insects (Bhattacharya *et al.* 2003), and increased impervious surface coverage reduces habitat for several beetle species (Deichsel 2006). Overall, increases in infrastructure and vegetation loss associated with urbanization negatively affect bee and wasp populations in urban areas (Zanette *et al.* 2005). Where urban structural features block gene flow, cultivars in urban environments may be reproductively isolated. Hennig and Ghazoul (2011) found that plant patch size can influence pollinator visitation, with smaller patches receiving fewer visits. Across urban landscapes, this could lead to reduced genetic connectivity, not only within urban areas but between urban and spatially proximate native populations, as well as between native populations separated by urban landscapes. If features of the urban environment do minimize gene movement, this isolation can result in limited pollen movement. Broadly applied, restrictions in gene flow may result in inbreeding, isolation, and subsequent inbreeding depression, as pollinators sample from smaller pollen pools with fewer potential pollen donors (Becker *et al.* 2011). This is particularly problematic for species that are self-incompatible and highly reliant upon insect pollination (Aguilar *et al.* 2006), such as most urban cultivars.

Green spaces and urban parks play a potentially important role in pollinator movement and gene flow, providing desirable habitat islands for pollinators (Faeth and Kane 1976). The openness of landscape matrix surrounding parks can also be positively correlated with bumblebee abundance, suggesting that the structural composition of urban areas is important for pollinator movement (e.g., McFrederick and LeBuhn 2006). Green space corridors could facilitate urban gene escape by supporting pollinator movement from urban areas into the surrounding native populations. Gene flow could also move into cultivated populations, but seed recruitment is minimal in the often highly managed and manicured urban landscaped areas. Even urban parks are mowed regularly, limiting the potential of offspring recruitment. Given increasing urbanization and use of cultivars, the possibility for gene escape also increases, with potential negative effects on native plant population fitness should functional differences exist between the cultivar and native populations. The net effect of both plant - pollinator and pollinator - landscape interactions will determine the immediate genetic ramifications and long-term fitness consequences of urban expansion.

This project evaluated the impact of urban context on the physiology, reproductive success, and offspring morphology of a cultivated species, using a nearby native population as a baseline indicator of functional differences between natural and urban cultivar populations. The following two research questions were addressed: First, does the urban environment with its green spaces and man-made structural components inhibit pollinator movement and reproductively isolate spatially proximate native conspecifics, i.e. is gene flow limiting potential urban gene escape? To address this question, I performed spatial analysis on a sample census of potential reproductive output and realized reproductive success in urban and natural environments. Second, this research examined functional differences between urban cultivar and

native populations, with respect to physiological and reproductive variations created through selective breeding and cultivation. The offspring of these populations were collected to evaluate how these differences translate into early seedling fitness, which may lead to altered subsequent fitness for native populations.

Methods

Study Sites & Sampling

The study species is the flowering dogwood (*Cornus florida* L., Cornaceae). Its natural range covers much of the East Coast, from southern Ontario to Florida and west to Oklahoma and eastern Texas. The natural habitat is primarily the understory of eastern deciduous and coniferous forests, and it is listed in twenty-two of the ninety recognized forest cover types by the Society of American Foresters (Eyre 1980). Flowering dogwood blooms from March through June depending on location within its range, and the blooms can persist for up to two weeks. The inflorescences contain 28-32 perfect flowers, subtended by four large showy white (rarely pink) bracts. Fertilized ovules develop into bright red berries by October and provide a valuable food source for songbirds (Wennerberg 2004, McLemore 1990). Dogwoods in the Piedmont region of Virginia are pollinated by a variety of bees from the Halictidae and Andrenidae families, as well as several species of small beetles and flies (Mayor *et al.* 1999, Carr 2010). A common cultivar in the eastern U.S., several inbred varieties have been developed and are frequently used as an ornamental in both public and private settings. As such, flowering dogwoods represent a model study system for analysis of how pollination is influenced by urban context. Given that native conspecific populations exist in sympatry with planted cultivars, this species is also ideal for investigating the potential fitness consequences of urban gene escape.

Research was conducted at three locations along a human-altered landscape gradient in Richmond, Virginia, from a native understory habitat to a highly impacted urban industrial area. Research in the minimally disturbed native habitat took place at the Inger and Walter Rice

5

Center for Environmental Life Sciences in Charles City County, Virginia (37° 19' N; 77°12' W).

This 200 ha research facility for Virginia Commonwealth University is located along the James

River, approximately 50 kilometers southeast of the urban study location, and consists of tidal

wetlands, riverine habitat, mixed upland deciduous forest, and pine plantation. *Quercus* spp.,

Acer rubrum, and *Pinus taeda* comprise the primary canopy, while the subcanopy is made up of

Cornus florida, *Ilex opaca*, and *Foresteria acuminata* (Carr 2010).

Two levels of human land-use were represented by the urban study areas (Figure 1). The

intermediately disturbed urban area is represented by a historical residential area known as the

Fan, consisting of closely-spaced homes and apartments with mature hardwood trees lining the

streets. There are many urban parks within this area, as well as numerous landscaped private

spaces. The most highly disturbed and human-altered study area, classified as *Industrial*,

contains warehouses, train yards, and manufacturing facilities. There is a high percentage of

impervious surface coverage and few landscaped gardens or urban parks. Trees located in the

urban areas, both *Industrial* and *Fan*, were assumed to be of cultivar origin specifically bred for

certain desirable physiological and morphological traits, though no genetic assessment as to their

provenance has been made at this time. These trees are referred to as "*cultivars*." The study trees

located at the Rice Center were assumed to be wild-type specimens naturally regenerated and are

hereby referred to as "*native*."

These two urban sampling locations were chosen because they represent significantly

different habitat through which potential pollinators must cross while traversing the urban

landscape. Preliminary sampling of flowering dogwood trees has shown that Fan trees are 2.25

times closer to other urban canopies (ANOVA; df=1,45; F=8.713, P=0.005) and 1.78 times

closer to physical structures (ANOVA; df=1,45; F=10.11, P=0.003) than Industrial study trees,

though there is no covariance between these variables (Pearson correlation r=0.190, df=45, t=1.301, P=0.2).

The number of successfully pollinated flowers per inflorescence was counted on at least ten inflorescences per sampled tree between 6-10 June 2011. Observations were made such that flowers were past pollen receptivity and had initiated ovule expansion. The census was then repeated in autumn, between 26-28 September 2011 to observe total seed set and calculate seed abortion rates for each maternal individual.

Drupes were collected in early October from the forty-seven *cultivar* and ten *native* trees (mean of 40.1 drupes per tree, Range=2-163). The germination protocol as described by Carr (2010) was followed. Seeds were cold stratified for 90 days. After stratification, seeds were depulped and rinsed in a 10% bleach solution to remove contaminants, then rinsed three times in deionized water to remove bleach residue. Growth medium was mixed by hand with 2/3 fine milled sphagnum moss and 1/3 medium grained perlite. The growth medium was sterilized with 10% bleach solution and rinsed three times in deionized water. Seeds were placed horizontally relative to their major axis 1cm deep in ~10cc of growth medium and heat sealed in individual chambers of five-chamber gas permeable bags (Garner Enterprises, Willis, TX). They were then stored upright in a ventilated refrigerator at 5.5^{o} C for secondary stratification. After 60 days, bags were monitored daily for signs of germination. Once epicotyl extension was noted, the individual seed was removed from the gas-permeable bag and planted ~10cm deep in 15cm x 10cm plastic azalea garden pots containing Sta-Green Potting Soil Mix with Fertilizer. Seedlings were watered immediately after planting and placed in the VCU greenhouse under full sun.

Adult Physiology

To measure potential differences in physiological functioning, leaf-level gas exchange, water-use efficiency, and ambient light conditions were assessed for 19 study trees in the urban (*Fan*, N=6; *Industrial*, N=5) and natural understory (N=8) habitats in mid-May 2011 using a Li-Cor 6400 Portable Photosynthesis System (Li-Cor Inc., Lincoln, NE, USA). Incident photosynthetically active radiation (PAR) and leaf-level maximum photosynthesis (A_{max}), stomatal conductance, and transpiration were recorded on fully developed, mid-crown leaves at a common light-saturating actinic light intensity of 1200 µmol m^{-2} s^{-1}. A minimum of 12 and a maximum of 25 subsamples for each parameter were recorded for each tree between 9:00 AM and 3:00 PM. Instantaneous leaf-level water-use efficiency (WUE) was calculated by dividing maximum photosynthesis by transpiration. WUE and A_{max} of *cultivar* and *native* leaves were evaluated using difference of means Student's t-test.

Reproductive Output: Cultivar v. Native

Relative reproductive output was quantified by measuring pollination success, seed set, fruit abortion, and germination rates for both cultivar and native trees. Differences were examined at three levels: differences due to provenance (*cultivar* vs. *native*), differences due to features immediately surrounding the focal tree (at-site landscape features), and differences due to the makeup of features between focal trees (between-site features). Relative reproductive output between *cultivar* and *native* trees were assessed by quantifying differences in both the mean response as well as the variance (heteroscedasticity) of reproductive output with ANOVA and

TukeyHSD (R, ver. 2.15, R Development Team). Residuals were examined and transformations were executed when necessary.

Reproductive Output: At-Site Landscape Influence

The extent to which site-specific features influence reproductive success was explored using spatially explicit analyses. Geographic location for each study tree was recorded using a Garmin GPS 60 handheld GPS unit. Using DNR Garmin (Minnesota Department of Natural Resources), GPS points were uploaded and transformed from decimal degrees to meters and reprojected into State Systems Virginia Lambert Conformal Conic in ArcCatalog (version 10.0, ESRI Redlands, CA) using the Project tool. A bounding polygon containing the location of all study trees was generated using the Minimum Bounding Geometry tool with Rectangle by Area option in ArcToolbox (ArcMap version 10.0, ESRI Redlands, CA). The resultant polygon was buffered by 50m. Additional spatial data for Richmond, Virginia was obtained from two sources, the publicly available Richmond City Government FTP GIS site, consisting of raster layers representing building structure, forest canopy, transportation surface, and city boundaries, and public forestry data supplied by the City Forester of Richmond with data covering the location and species of all recorded public trees within City limits (N=17,827 total trees of which N=96 were dogwood). All shapefiles were reprojected to Virginia Lambert Conformal Conic and clipped to the size of the buffered minimum bounding polygon to reduce processing area. Shapefiles for the *native* trees sampled at the Rice Center were provided by W. Shuart (Center for Environmental Studies, VCU). From these data sources, the Euclidean distance from each focal tree to surrounding at-site landscape features was estimated using the Generate Near Table function in ArcMap.

9

Euclidean distance was measured from each sampled tree to the closest building structure, road edge, proximate flowering dogwoods not in the study, and canopy, separated into groups of species pollinated primarily by insects and those species pollinated via wind movement. The relationship between these at-site features with measures of both mean and variance in reproductive output (as above) was ascertained using a correlation test (Pearson product moment) in R.

Reproductive Output: Intervening Landscape Influences

Habitat between focal trees may also influence reproductive success if features either promote or prevent pollinator movement. To identify potential influences of intervening habitat, a cost-surface resistance approach was adopted. Shapefiles were converted to raster images with 3m x 3m grid resolution using ArcMap. Grid cells were then assigned resistance values, representing a cost associated with traversing this cell for potential pollinators. Distances between focal trees, measured using the cumulative costs on cost surfaces, was estimated using CircuitScape 3.5 (Shah and McRae 2008). Since the cost allocation to each cell is a parameter of interest rather than something known *a priori*, a range of cost values was utilized for each landscape feature. For physical structures (e.g., buildings), costs were calculated as an increasing series of relative traversal weights, 1:5, 1:10, 1:20, 1:50, 1:100, and 1:500, representing relative costs of 5X, 10X, etc. to move through a building as opposed to around a building. The remaining landscape features were assigned cost values of 20:1, 10:1, 5:1, 2:1, 1:2, 1:5, 1:10, and 1:50 allowing both preferences to either move within or to avoid the feature. Correlations between pairwise circuit-

distance across these cost surfaces and reproductive output was determined using a Mantel matrix correlation test (Mantel 1967) in R using the *ecodist* package (Goslee and Urban 2007).

Relative Offspring Fitness

Performance related differences in seedling fitness components between *cultivar* and *native* types were assessed by comparing survival and growth characteristics in a controlled greenhouse study. Following development of cotyledons, the seedlings were assigned to one of two light treatments. A shade treatment was created using 80% shade cloth over constructed PVC hoop structure to approximate a natural understory light environment, and the urban light environment was represented by full sun exposure. Family groups were evenly distributed between treatments, with seedlings from the same family group randomly placed throughout the treatment area to reduce site-specific effects. Seedlings were monitored continuously throughout the growing season and watered as needed.

Differential seed survival was examined at three temporal scales: 1) the fraction of seeds that germinated following cold stratification, 2) the fraction that survived to the first census date of June 30, and 3) seedlings surviving the entire greenhouse experiment of June 30 to September 10. Student *t*-tests were used to assess differences between *cultivar* and *native* seedlings in both percent germination and survival. A two-way ANOVA was used to estimate the interaction of shade treatment and seed origin on germination and survival percentages. The number of days elapsed between the germination protocol for each seed and visible extension of the epicotyl was counted using Julian calendar days, and ANOVA was used to evaluate differences due to origin (*native*, *Fan*, and *Industrial*) of study trees.

For the duration of the experiment, physical characteristics of all seedlings were measured every two weeks – a total of six times – for plant height from ground level to the apical meristem, length and width of the largest leaf, number of leaves, and number of secondary branches, and plants were given a grade based on overall appearance (Figure 2). Leaf width and length measurements were used to estimate elliptical leaf area. Shade treatment seedlings of *cultivar* and *native* origin were compared using ANOVA for all measured growth parameters: elliptical leaf area, plant height, number of branches, number of leaves, and grade. Again, two-way ANOVA was utilized to test interaction effects of treatment and seed origin. To account for differences in age due to potting date (e.g., elongated time to germination), germination period was regressed out of the model for the first measurement date. For all subsequent ANOVA tests, change in growth parameter beyond the previous measurement was used to eliminate age as a factor.

At the end of the growing season, seedlings were harvested to quantify plant biomass. Leaves, including petioles, were removed from each seedling, stems were cut to soil level, and roots were separated from soil using a #10 sieve (2mm opening) and deionized water with gentle massage. Total leaf area for each seedling was measured within one week of harvest using a LI-COR LI-3100 Area Meter (LI-COR, Lincoln Nebraska). Separated tissues were dried in standard paper lunch bags for 24 hours at 62 C^o in a Fisher Scientific Isotemp Standard Lab Oven (Thermo Fisher Scientific) and then weighed. Specific leaf area (SLA) was calculated by dividing total leaf area by total leaf dry mass. Root to shoot ratios were determined using below-ground dry mass divided by above-ground dry mass (sum of leaf and stem dry mass). Differences were assessed using difference of means Student's *t*-test on SLA and root to shoot ratio, and interactions of treatment and origin were assessed with two-way ANOVA.

Leaf chemistry analysis was performed using dry leaf material of twenty shade-treated seedlings, one from each of fifteen *cultivar* and five *native* randomly selected family groups. Individuals with high dry leaf mass were chosen to assure adequate leaf material for analysis. Approximately 70mg of dried leaf material from each seedling was pulverized in 2mL PCR vials using a Qiagen Tissue Lyser with titanium pellets for sixty seconds at a frequency of 30 vibrations/s. Samples were processed at the University of Michigan Biological Station for bulk and stable isotope concentrations of carbon and nitrogen. Specifically, 13C isotope measures are used as a temporally integrated proxy for leaf-level WUE. Nitrogen leaf area was found by dividing percent nitrogen by total leaf area of the plant. Carbon to nitrogen ratios were also calculated for each leaf sample. Differences in mean nitrogen leaf area, nitrogen to carbon ratios, 13C isotopes, and total nitrogen and carbon between *cultivar* and *native* seedlings were tested using difference of means Student's *t*-tests.

Results

Study Sites and Sampling

Within the urban study areas, forty-seven flowering dogwoods (*Fan*: N=35, *Industrial*: N=12) were selected haphazardly. Preference was given first to trees in publically accessible locations and then to trees on private spaces whose branches overhung sidewalks for ease of access and observation. Study trees from the Rice Center consisted of 13 *native* adult individuals, which were randomly selected from the western portion of the Rice Center property.

Adult Physiology

Light conditions and leaf-level physiology differed significantly between *cultivar* and *native* dogwood. Mid-day light intensity in the urban study areas (186 μmol m^{-2} s^{-1}) averaged > 7 times greater than that in the natural understory habitat of flowering dogwood (26 μmol m^{-2} s^{-1}). When urban *Industrial* and urban *Fan* study areas were considered separately, *Industrial* trees were exposed to higher light intensities than *Fan* trees (325 μmol m^{-2} s^{-1} vs. 132 μmol m^{-2} s^{-1}), however the *Fan* trees experienced a broader range of PAR than the *Industrial* area trees.

Maximum photosynthetic rates at a common saturating light level were also significantly different between *cultivar* and *native* leaves (t=2.05, df=15.01, P=0.05). The instantaneous WUE of urban *cultivar* leaves was also significantly higher than those of *native* leaves (t=3.06, df=15.09, P=0.008). Stomatal conductance was not different between *cultivars* and *natives*.

Successful pollination of the flowering dogwoods in the urban areas of Richmond was significantly higher than in the natural habitat of the Rice Center (ANOVA, df=1,151, F=33.868, P = 7.06e-13; TukeyHSD mean differences μ_{urban} - μ_{rural}=1.412 seeds/inflorescence, P<1e-15, $\mu_{industrial}$ - μ_{rural} = 0.826, P=0.017. When pollination censuses were compared for abortion rates in the *Fan* and *Industrial* study areas, there were no significant differences between spring pollination and fall seed set (*Fan*: t= -0.669, df=55.338, P=0.507; *Industrial*: t=0.004, df=7.94, P=0.997), suggesting that pollinators may be selecting from a broad genetic pool of potential pollen donors rather than a limited set of related *cultivar* pollen parents.

At-site landscape features for each study tree were estimated as Euclidean distances to the nearest building structure, road edge, inset-and wind-pollinated canopy, and total canopy. Mean spring pollination rates were negatively correlated with proximity to buildings (Pearson correlation r= -0.42, df=36, t= -2.749, P=0.009). Successful pollination was related to proximity to insect-pollinated canopy (Pearson correlation r= -0.58, df=36 , t= -4.34, P=0.0001) and wind-pollinated canopy (Pearson correlation r= -0.51, df=36, t= -3.53, P=0.001), where cultivar trees nearer to these canopies had higher rates of spring pollination and fall seed set (Table 1).

The best-fit cost surface resistance model was determined by the magnitude of the Mantel correlation. The model explaining the highest amount of variance in reproductive output contained distance to canopy (weight 1:10, partial ρ = 0.13) and avoidance of buildings (weight 1:100, partial ρ = 0.10) (Figure 3). These resistance values suggest that in the urban environment, both tree canopy and physical structures influence pollinator movement.

Approximately 2,005 seeds were collected from forty *cultivar* and ten *native* trees. A mean of

83% ± 3% germination occurred in seeds from *cultivar* mothers, while seeds from *native*

mothers had a mean germination of 65% ± 10%. While percent germination was not significantly

different between origins (*cultivar* vs. *native*: t=1.62, df=7.23, P=0.148), *cultivar* offspring seeds

germinated sooner than *native* offspring by almost six days (F=29.94, P<0.0001; Figure 4).

Additionally, significant differences in germination timing existed between mothers of the same

origin (P<F<0.001).

Further comparison of *cultivar* seedlings by *Fan* or *Industrial* location origin indicated no

significant difference in germination time between urban land uses. *Fan* seeds germinated after a

mean of 90 ± 0.75 days of secondary cold stratification, *Industrial* seeds germinated after 95 ±

1.79 days, and *native* seeds were the latest to germinate after 96 ± 1.44 days of cold

stratification. Hartigans' Clip Test for Unimodality (Hartigan and Hartigan 1985) was used to

determine if the number of days to germination showed bimodal distribution for any seedling

origin. The test showed that all three study populations had a significant bimodal distribution of

days to germination: *Fan* seeds (P<0.002), *Industrial* seeds (P<0.003) *native* (P<0.001; Figure

4).

Seedling survival was similar between *cultivar* and *native* plants. Of 2,005 seeds

collected from all populations, 70% germinated after cold stratification. Of those that

germinated, 993 were potted due to resource and space constraints, of which 738 were *cultivar*

origin and 254 were *native* origin. Seedling survival declined before the first morphology

measurement date of June 30, at which time 276 *cultivar* seedlings (38%) and 51 *native*

seedlings survived (20%). However, of those that survived to the first measurement date, 211 *cultivar* seedlings (76%) and 41 *native* seedlings (80%) survived through the duration of the greenhouse experiment. Considering the shade treatment group, *cultivar* seedlings had a 67% survival rate during the time from the first measurement date to harvest, while *native* seedlings had a 70% survival rate (Figure 5). Survival over the course of the entire study did not differ by seed origin (t= -0.78, df=10.03, P=0.45), with *cultivar* seedlings experiencing a mean overall survival rate of 27% survival and *native* seedlings a 34% mean survival rate.

Seedling growth was assessed using several different indicators: number of leaves on the seedling, plant height, length and width of largest leaf, and overall appearance. Within the shade treatment group, none of the growth indicators were significantly different between cultivar and native origin. There were no differences between *Fan*, *Industrial*, and *native* seedlings when plant age was regressed out using a linear model and residuals (Table 2).

The shading treatment showed significant differences in all biomass measures. Sun treatment seedlings had a mean total biomass more than five times higher than the mean total biomass of shade treatment seedlings (F=112.09, P<0.0001; Table 3). In the shade, seedlings of *cultivar* and *native* origins had similar leaf, stem, and root mass. SLA and root to shoot ratio were also not significantly different (Table 4). However, the leaf morphology of *cultivar* seedlings adjusted minimally in response to light conditions, exhibiting similar SLA in both sun and shade treatments (t=0.66, df=73.98, P=0.51). When only family group was considered regardless of treatment, *cultivar* offspring dedicated fewer resources to leaves and more to stem growth than *native* offspring (F=1.64, P=0.021).

Mean leaf δ13C concentrations, an integrated proxy of leaf-level WUE, were marginally higher in *cultivar* than in *native* seedlings (P=0.12; Table 5), a finding that is consistent with gas

exchange measurements demonstrating significantly higher water use efficiency in *cultivar* leaves.

Finally, if the differences observed between *cultivar* and *native* seedlings have the potential to result in relative seedling fitness differences, these changes must be heritable. Broad sense heritability (H^2) of morphological traits was evaluated using a random-effects ANOVA with maternal individual as the treatment, appropriate for analysis of half sibling data. The period of cold stratification exhibited the highest potential heritability (Table 4; H^2=0.6186), with growth traits such as branching, plant height, and number of leaves showing slightly less. Leaf and root biomass measures had the lowest potential heritability (H^2=0.278 and H^2=0.256, respectively).

Discussion

My study examined how an urban environment can impact reproduction and functional response of a common cultivar of *C. florida*. Urban structural features significantly affected pollinator movement and the subsequent reproductive success of plants within urban habitats, and while pollinators may meet greater resistance from urban landscapes than natural ones, gene flow is substantial within urban populations of the flowering dogwood. This suggests that urban gene escape into natural ecosystems may be possible, and even probable. Furthermore, selective breeding for traits suitable for persistence in urban environments could lead to functional differences between urban and native varieties, suggesting a potentially negative impact on the long-term fitness of native plant populations if urban gene escape is occurring.

Pollinator movement in the urban environment

I found that urban *cultivars* had higher successful pollination than *native* trees in the natural forest understory habitat, most likely due to higher light exposure. Niesenbaum (1993) observed higher fruit set when an understory shrub was grown in full sunlight, and insects are more active on sunlit flowers (Kilkenny and Galloway 2008). Additionally, *cultivars* may more strongly attract pollinators as a result of selection for increased floral output (Witte *et al.* 2009). Dogwoods have the ability to abort seeds successfully pollinated by highly related pollen donors (Karkainnen *et al.* 1999), and given this inherent inbreeding avoidance and the genetic relatedness among cultivar individuals (Dyer *et al.* 2012), it was surprising that there was no indication of differential abortion in sampled cultivated study trees. Either the selective breeding

process has broken the incompatibility system or pollinators are sampling from a much larger population of non-cultivar genotypes, suggesting that urban habitats are not blocking pollinator movement and gene flow within cultivated populations.

Pollinators forage and move within their habitat based upon the structure and composition of their environment, whether natural or urban. Structural variability within natural habitat affects pollinator flight paths and ultimately genetic covariance of a flowering dogwood population. Pollinators follow open edges and paths with other *C. florida* while avoiding canopy gaps such as roads (Dyer *et al.* 2012). Population genetic structure is often defined by natural barriers to pollinator movement and dispersal (Leidner and Haddad 2007), but Cranmer *et al.* (2011) also demonstrated that plant reproductive success was affected by connectedness via linear landscape features of man-made hedgerows. Similarly, I found that close proximity to features such as tree canopies and buildings increased pollination success of the cultivated flowering dogwood. Optimal foraging theory states that pollinators will preferentially visit closer pollen sources over more distant sources (Pyke 1978), and this could result in higher visitation for nearby flowering dogwoods. While little research has investigated how physical urban "hardscape" structures alter pollinator flight paths, our findings suggest that buildings in the urban environment represent significant impediments to pollinator movement, the spatial arrangement of which may funnel and alter pollination trajectories at the landscape level. Flowers adjacent to these physical structures provide a resting place and food source, and subsequently see higher pollination rates and seed set than those dogwoods located further from buildings.

Surface resistance modeling is valuable in estimating gene flow through an environment, and my spatial analysis of reproductive success provides insight into how pollinators move throughout urban areas, a fundamental process that has yet to be examined at any depth. The

results presented herein suggest that urban structural components do not block gene flow within *cultivar* populations but allow transmission of genes broadly throughout the urban landscape. Therefore, it is highly probable that urban gene escape is occurring, with pollinators moving between urban *cultivar* and *native* conspecific populations with varying degrees of success. Looking forward, the development of multivariate resistance surfaces using a combination of structural and natural features in urban areas will prove helpful in understanding how pollinators respond to multiple variables along their flight paths.

Cultivar seedling survival and growth

In the greenhouse experiment, *cultivar* and *native* seeds germinated at similar overall rates, but the number of days needed for cold stratification was significantly lower in *cultivar* seeds. *Industrial* seeds germinated before the *Fan* and *native* seeds. Many individual factors of the mother tree can influence germination timing: growth patterns, available resources during flowering and fruit development (Hernandez-Verdugo *et al.* 2010), and specific location and climate in which the mother exists (Munir *et al.* 2001, Meyer *et al.* 1995). Light conditions of the mother tree also play a role in seed traits, and Galloway (2005) saw that germination rates and timing were directly affected by a mother's light environment in the herb *Campanula americana*. Furthermore, seeds from plants adapted to cold, harsh winters require longer stratification before germinating, while those from plants originating in warmer areas require a shorter stratification period (Meyer *et al.* 1995). However, *cultivars* are propagated by commercial growers in many different geographical locations and climates, and without knowledge of the source of our

21

cultivar study trees, it is difficult to predict how climatic adaptation in previous generations may have influenced germination timing of our study seeds.

Although percent germination was similar, survival beyond germination differed between *cultivar* and *native* seedlings. Early survival was higher in *cultivars*, but once established, *native* seedlings had a greater tendency to survive throughout the entire greenhouse experiment. This may be a tradeoff in allocation, where *cultivars* focus greater resources on early germination of a larger number of seeds and fewer resources dedicated to long-term seedling survival and growth.

Growth in the sun and shade treatments was different, with sun treatment seedlings responding positively in leaf size, height, and branching to increased light exposure. Although growth responses are highly species specific, many shade tolerant species have increased total biomass, total leaf area, and photosynthetic levels when grown in high light environments (e.g., Rebbeck *et al.* 2011; Huante and Rincón 1998). In the shade treated seedlings, growth parameters and biomass were similar in the *cultivars* and *natives*. Environmental conditions in the early stages of seed germination and growth can have significant long-term effects on fitness measures (Verdúe and Traveset 2005), and the slight differences we found between seedlings of the two origins could broaden as seedlings developed and matured.

Plant functional characteristics of cultivar and native flowering dogwood

Urban *cultivars* possessed the physiological and morphological features of sun-adapted plants, suggesting functional consequences for shade-adapted native flowering dogwood populations if gene escape is occurring. Light intensity in the urban areas was much greater than that in the understory of second-growth forests. Light-saturated photosynthesis of *cultivar* leaves was

greater than that of *native* leaves in full sunlight, indicating that *cultivars* are bred to perform optimally in high light environments, which is in distinct contrast to the high shade tolerance of natural populations.

Cultivar leaf morphology retained features of sun-acclimated leaves with low SLA when grown in low light, suggesting that *cultivars* will produce "sun leaves" regardless of light conditions. Previous research found that sun-adapted plants grown in shaded environments show plasticity in newly developing leaf morphological response to light by increasing SLA (e.g., Avramov *et al.* 2007; Awada *et al.* 2002; Pires *et al.* 2011), but this response is dependent upon individual species characteristics and genetic diversity. *Native* leaf morphology exhibited sensitivity to changes in light conditions, and we would expect to see some plasticity in *cultivar* response if they were well-adapted to shade environments like their *native* conspecifics. However, *cultivars* and *natives* appear to be functionally different in this regard, and our results point to *cultivars'* limited genetic, physiological, and morphological variation and an inability to adjust to changing environmental conditions.

Our gas exchange and leaf chemistry measures of WUE suggest differences in how efficiently *cultivars* and *natives* utilize available water. High light increases water loss from transpiration and, along with reduced water access in generally poor quality and shallow soil, causes water stress in urban *cultivars,* likely prompted breeders to select for high WUE. Sun-acclimated leaves have higher WUE measured by gas exchange than do shade leaves, even when located on the same tree at varying canopy levels (Stokes *et al.* 2010). The offspring WUE, examined through bulk $\delta^{13}C$ stable isotope, also revealed marginally significant differences in *cultivar* and *native* seedlings grown in the shade treatment and provides further evidence that *cultivars* are ultimately sun-adapted plants. Taken together, leaf physiological, morphological,

23

and chemistry results suggest that *Cultivars* are diverging quantitatively from *native* conspecific populations as a result of breeding selection for desirable physical and functional traits that allow *cultivars* to thrive in urban habitats, including high light tolerance.

I found that there are several heritable traits of the flowering dogwood upon which adaptation can potentially work, with germination timing showing the highest potential heritability (Table 4). This supports our finding of significant differences in germination timing between *cultivar* and *native* seeds. Growth patterns were also highly heritable, while biomass measures showed the lowest potential heritability. Adaptation to new environments can work upon these heritable traits, and our results suggest that germination and growth patterns may be important subjects of future research investigating *cultivar* and *native* tree response to urban and natural habitats. Building upon the heritable traits we studied, a genetic parentage analysis could identify pollen donors for all offspring, as well as pure *cultivars* and *cultivar-native* hybrids.

Conclusions

Overall, my results suggest that existence in urban environments can greatly affect reproductive success and functional response of *cultivars*, which could have a negative impact on *native* plant populations if urban gene escape is occurring. Pollinators appear to be highly mobile in their urban environment, and green spaces could promote urban gene escape into *native* populations when those urban areas are situated within a surrounding natural landscape. Although seedling establishment and growth rates were similar in *cultivars* and *natives*, physiology and other morphology measures were significantly different, indicating that the sun-adapted cultivated varieties could fill ecological niches, such as sunny gaps in the subcanopy, which native plants

typically not currently occupy. With recent climate models predicting warmer temperatures and drier conditions during the growing season in the mid-Atlantic (IPCC 2007), and conversion of second-growth forest to urban land uses, *cultivars* may continue to flourish and dilute the gene pool of *native* populations, with as yet unknown consequences.

Literature Cited:

Aguilar R, Ashworth L, Galetto L, Aizen MA (2006) Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecology Letters* 9: 968-980. doi: 10.1111/j.1461-0248.2006.00927.x

Ahrnè K, Bengtsson J, Elmqvist T (2009) Bumble bees (*Bombus* spp) along a gradient of increasing urbanization. *PLoS One* 4(5): e5574. doi:10.1371/journal.pone.0005574

Avramov S, Pemac D, Tucic B (2007) Phenotypic plasticity in response to an irradiance gradient in *Iris pumila*: adaptive value and evolutionary constraints. *Plant Ecology* 190: 275-290. doi: 10.1007/s11258-006-9207-3

Awada T, Radoglou K, Fotelli MN, Constantinidou HIA (2002) Ecophysiology of seedlings of three Mediterranean pine species in contrasting light regimes. *Tree Physiology* 23: 33-41.

Becker T, Boss N, Durka W (2011) Pollen limitation and inbreeding depression in an 'old rare' bumblebee-pollinated grassland herb. *Plant Biology* 13: 857-864. doi:10.1111/j.1438-8677.2011.00452.x

Bhattacharya M, Primack RB, Gerwein J (2003) Are roads and railroads barriers to bumblebee movement in a temperate suburban conservation area? *Biological Conservation* 109: 37-45.

Carr D (2010) Canopy disturbance and reproduction in *Cornus florida* L. Masters Thesis, Virginia Commonwealth University. http://hdl.handle.net/10156/3078

Cranmer L, McCollin D, Ollerton J (2011) Landscape structure influences pollinator movements and directly affects plant reproductive success. *Oikos* 121: 562-568. doi: 10.1111/j.1600-0706.2011.19704.x

Deichsel R (2006) Species change in an urban setting – ground and rove beetles (Coleoptera: Carabidae and Staphylinidae) in Berlin. *Urban Ecosystems* 91: 161-178. doi: 10.1007/s11252-006-8588-3

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

Dyer RJ, Chan DM, Gardiakos VA, Meadows CA (2012) Pollination Graphs: Quantifying pollen pool covariance networks and the influence of intervening landscape on genetic connectivity in the North American understory tree, *Cornus florida* L. *Landscape Ecology* 27: 239-251. doi: 10.1007/s10980-011-9696-x

Eyre FH (1980) Forest Cover Types of the United States and Canada. *Society of American Foresters*.

Faeth SH, Kane TC (1978) Urban biogeography: City parks as islands for Diptera and Coleoptera. *Oecologia* 32: 127-133.

Flint HL (1997) *Landscape plants for Eastern North America* Second Edition. John Wiley and Sons, New York, New York.

Galloway LF (2005) Maternal effects provide phenotypic adaptation to local environmental conditions. *New Phytologist* 166: 93-100. doi: 10.1111/j.1469-8137.2004.01314.x

Goslee SC and Urban DL (2007) The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software* 22: 1-19.

Hartigan JA and Hartigan PM (1985) The dip test of unimodality. *The Annals of Statistics* 13(1): 70-84.

Hennig EI and Ghazoul J (2011) Plant-pollinator interactions within the urban environment. *Perspectives in Plant Ecology, Evolution, and Systematics* 13: 137-150. doi:10.1016/j.ppees.2011.03.003

Hernández-Verdugo S, López-Espana RG, Porras F, Para-Terraza S, Villareal-Romera M, Osuna-Enciso T (2010) Variation in germination amond populations and plants of wild chili pepper. *Agrociencia* 44: 667-677.

Huante P and Rincón E (1998) Response to light changes in tropical deciduous woody seedlings with contrasting growth rates. *Oecologia* 113:53-66.

Intergovernmental Panel on Climate Change. IPCC Fourth Assessment Report: Climate Change 2007. Cambridge University Press: Cambridge, United Kingdom and New York, NY.

Kilkenny FF and Galloway LF (2008) Reproductive success in varying light environments: direct and indirect effects of light on plants and pollinators. *Oecologia* 155: 247-255. doi: 10.1007/s00442-007-0903-z

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

Leidner AK and Haddad NM (2010) Natural, not urban, barriers define population genetic structure for a coastal endemic butterfly. *Conservation Genetics* 11: 2311-2320. doi: 10.1007/s10592-010-0117-5

Mannion AM and Morse S (2012) Biotechnology in agriculture: Agronomic and environmental considerations and reflections based upon 14 years of GM crops. *Progress in Physical Geography* 36: 747-763. doi: 10.1177/0309133312457109

Mantel NA (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research* 27: 209-220.

Matteson KC, Ascher JS, Langellotto GA (2008) Bee richness and abundance in New York City

urban gardens. *Annals of the Entomological Society of America* 101(1): 140-150. doi:

0013-8746/08/0140D0150$04.00

Mayor AJ, Grant JF, Windham MT, Trigiano RN (1999) Insect visitors to flowers of flowering

dogwood, *Cornus florida* L., in eastern Tennessee: Potential pollinators. *Southern*

Nurseries Association Research Conference 44: 192-196.

McFrederick QS and LeBuhn G (2006) Are urban parks refuges for bumble bees *Bombus* spp.

(Hymenoptera: Apidae)? *Biological Conservation* 129: 372-382.

doi:10.1016/j.biocon.2005.11.004

McLemore BF (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.

Meyer SE, Kitchen SG, Carlson SL (1995) Seed germination timing patterns in intermountain

Penstemon (Scrophulariaceae) *American Journal of Botany* 82(3): 377-389.

Munir J, Dorn LA, Donohue K, Schmitt J (2001) The effect of maternal photoperiod on seasonal

dormancy in *Arabidopsis thaliana* (Brassicaceae). *American Journal of Botany* 88(7):

1240-1249.

Niesenbaum R (1999) Light or pollen – seasonal limitations on female reproductive success in

the understory shrub *Lindera benzoin*. *Journal of Ecology* 81: 315-323.

Pires MV, Almeida AF, Figueiredo AL, Gomes FP, Souza MM (2011) Photosynthetic

characteristics of ornamental passion flowers grown under different light intensities.

Photosynthetica 49(4): 593-602. doi: 10.1007/s11099-011-0075-2

Pyke GH (1978) Optimal foraging: Movement patterns of bumble bees between inflorescences. *Quarterly Review of Biology* 52: 137-154.

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

Rebbeck J, Gottschalk K, Scherzer A (2011) Do chestnut, northern red oak, and white oak germinant seedlings respond similarly to light treatments? Growth and biomass. *Canadian Journal for Forest Research* 41: 2219-2230. doi:10.1139/X11-124

Shah V and McRae B (2008) Circuitscape: A tool for landscape ecology. *Proceedings of the 7th Python in Science conference (SciPy 2008)*, G Varoquaux, T Vaught, J Millman (Eds.), pp. 62-65.

Stokes VJ, Morecroft MD, Morison JIL (2010) Comparison of leaf water use efficiency of oak and sycamore in the canopy over two growing seasons. *Trees* 24: 297-306. doi: 10.1007/s00468-009-0399-8

Uwimana B, D'Andrea L, Felber F, Hooftman DAP, den Nijs HCM, Smulders MJM, Visser RGG, van de Wiel CCM (2012) A Bayesian analysis of gene flow from crops to their wild relatives: cultivated (*Lactuca sativa* L.) and prickly lettuce (*L. serriola* L.) and recent expansion of *L. serriola* in Europe. *Molecular Ecology* 21:2640-2654. doi: 10.1111/j.1365-294X.2012.05489.x

Verdúe M and Traveset A (2005) Early emergence enhances plant fitness: a phylogenetically controlled meta-analysis. *Ecology* 86(6): 1385-1394.

Wennerberg, S (2004) Plant Guide: Flowering Dogwood *Cornus florida* L. USDA NRCS National Plant Data Center, Baton Rouge, LA.

Witte WT, Windham MT, Windham AS, Hale FA, Fare DC, Clatterbuck WK (2009) Dogwoods

for American gardens. Agricultural Extension Service, University of Tennessee.

Zanette LRS, Martins RP, Ribeiro SP (2005) Effects of urbanization on Neotropical wasp and

bee assemblages in a Brazilian metropolis. *Landscape and Urban Planning* 71: 105-121.

doi:10.1016/j.landurbplan.2004.02.003

Table 1: P-values for correlation between Euclidean distance to nearest urban feature and pollination census mean and variance. Census means for spring and fall represent the mean number of successfully pollinated flowers per inflorescence on each study tree and mean number of ripened drupes per inflorescence, respectively.

Census	Sample Measure	Canopy	Buildings	Road Edge	Insect Pollinated Canopy	Wind Pollinated Canopy
Spring	Mean	0.5684	0.0092*	0.7420	0.0001*	0.0012*
	Variance	0.9982	0.2168	0.7614	0.0063*	0.0071*
Fall	Mean	0.1899	0.3233	0.1563	0.02989*	0.0062*
	Variance	0.7359	0.09447	0.2999	0.3992	0.2154

Table 2: ANOVA results (F and p) of selected growth parameters comparing Rice Center, Fan, and Industrial origins for shade treatment seedlings. Age was regressed out of the first census date ANOVA test. For the remainder of the census dates, Δmeasurement between dates was calculated and tested.

Measured Traits	Census Period					
	June 30	July 16	July 28	August 10	August 27	September 10
Height (cm)	F=1.111, p=0.333	0.73, 0.485	0.589, 0.557	2.176, 0.121	1.264, 0.289	0.147, 0.863
Leaf Area (cm^2)	1.922, 0.152	0.271, 0.763	0.559, 0.574	0.108, 0.898	2.32, 0.106	0.474, 0.625
Number of Leaves	0.6446, 0.527	1.035, 0.360	3.009, 0.0551	2.223, 0.115	1.05, 0.356	0.173, 0.842

Table 3: Mean biomass (mg) of leaf, stem, and root material of all cultivar and native offspring in both the sun and shade treatment groups.

Origin	Treatment	
	Sun	Shade
Cultivar		
Leaf	1.32	0.17
Stem	0.71	0.20
Root	1.00	0.24
Native		
Leaf	1.53	0.13
Stem	0.82	0.20
Root	1.17	0.27

Table 4: ANOVA results for cultivar and native offspring dry mass in the shade treatment. SLA is calculated with total leaf area divided by total leaf dry mass. Root: shoot is calculated with root dry mass divided by the sum of stem and leaf dry mass.

Dry mass	F-statistic	P-value
Leaf	0.763	0.385
Stem	0.021	0.884
Root	1.267	0.264
SLA	0.64	0.462
Root: shoot	1.051	0.3088

Table 5: Mean amount of δ13C for cultivar and native seedlings in the shade treatment.

Origin	δ13C %
Cultivar	40.40
Native	39.41

Table 6: Heritability of measured traits due to maternal stratification for all family groups.

Trait	H^2
Cold Stratification	0.6186
Branching	0.3721
Height	0.3417
Largest Leaf	0.3101
Number of Leaves	0.3026
Stem Biomass	0.2965
Total Leaf Area	0.2804
Leaf Biomass	0.2787
Root Biomass	0.2568

Appendix B - Figures

Figure 1: Location map of study trees in the urban area of Richmond, Virginia.

Figure 2: Seedling morphology measurements recorded every two weeks during the period between June 30 and September 16. Measurements were recorded a total of six times.

Figure 3: Mantel r values of varying landscape resistances. The best-fit models were chosen for the highest absolute r value, 100 and 10 for buildings and canopy, respectively.

Figure 4: Number of days to germination for Rice Center, Fan, and Industrial study trees. Unimodality tests showed that all populations have bimodal distributions.

Figure 5: Percent survival of cultivar and native offspring at three time intervals 1) to germination, 2) germination to date of first morphology measurement, and 3) to harvest.
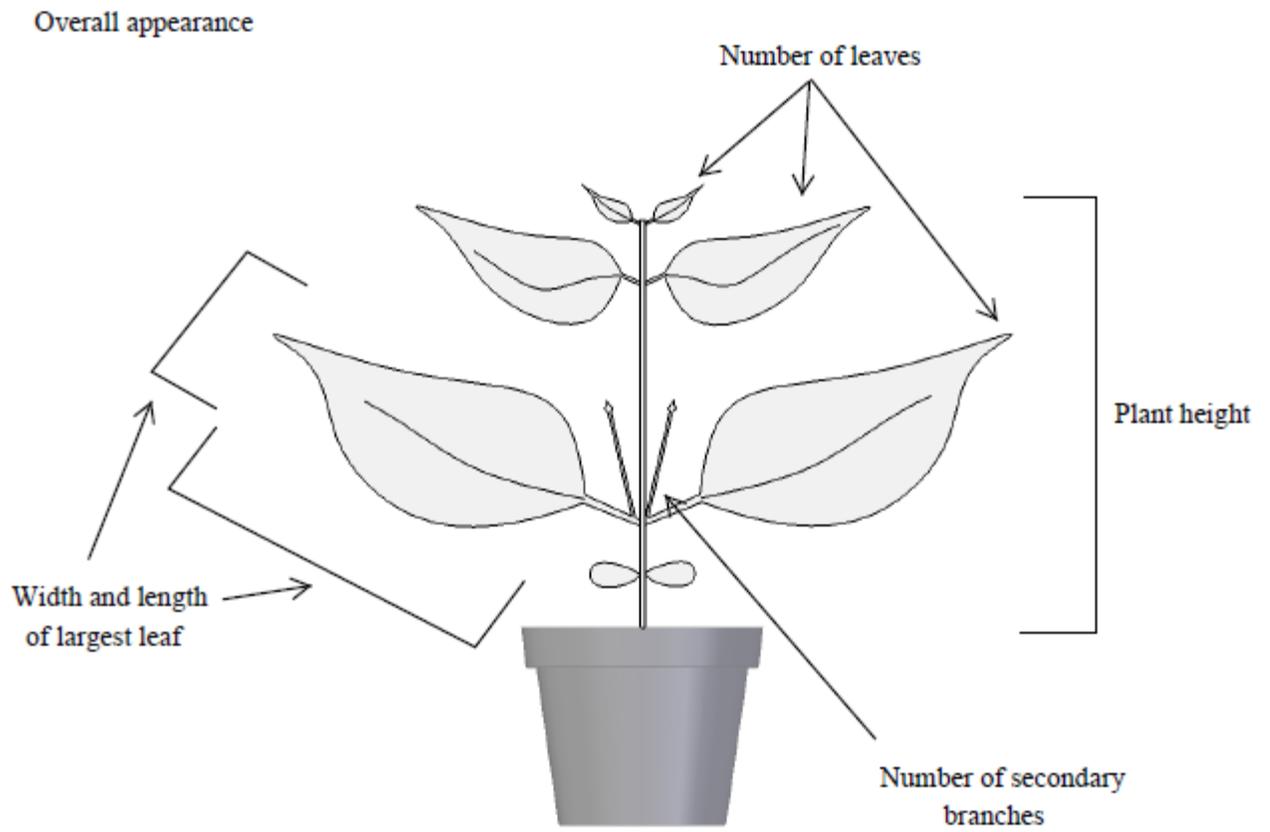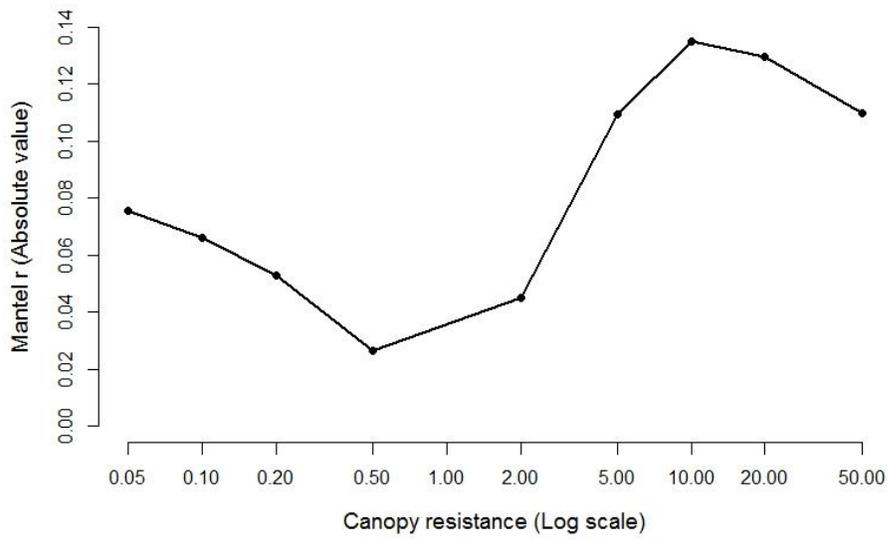
Figure 1.

Figure 2.

Overall appearance

Number of leaves

Plant height

Width and length
of largest leaf

Number of secondary
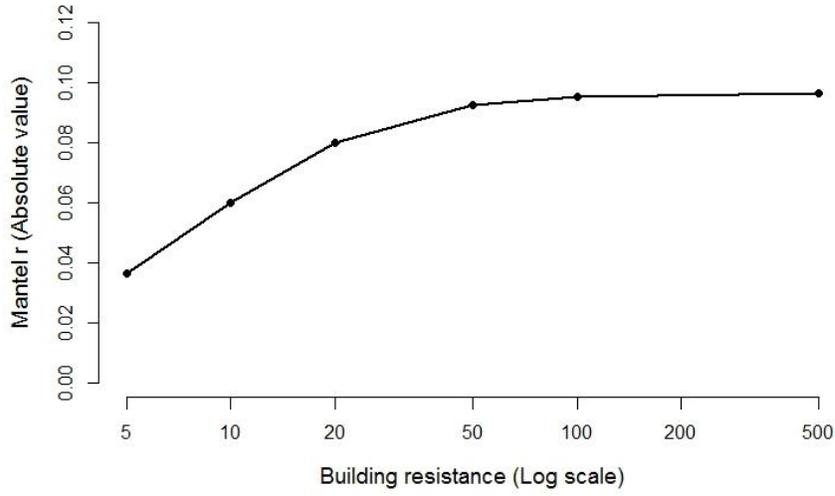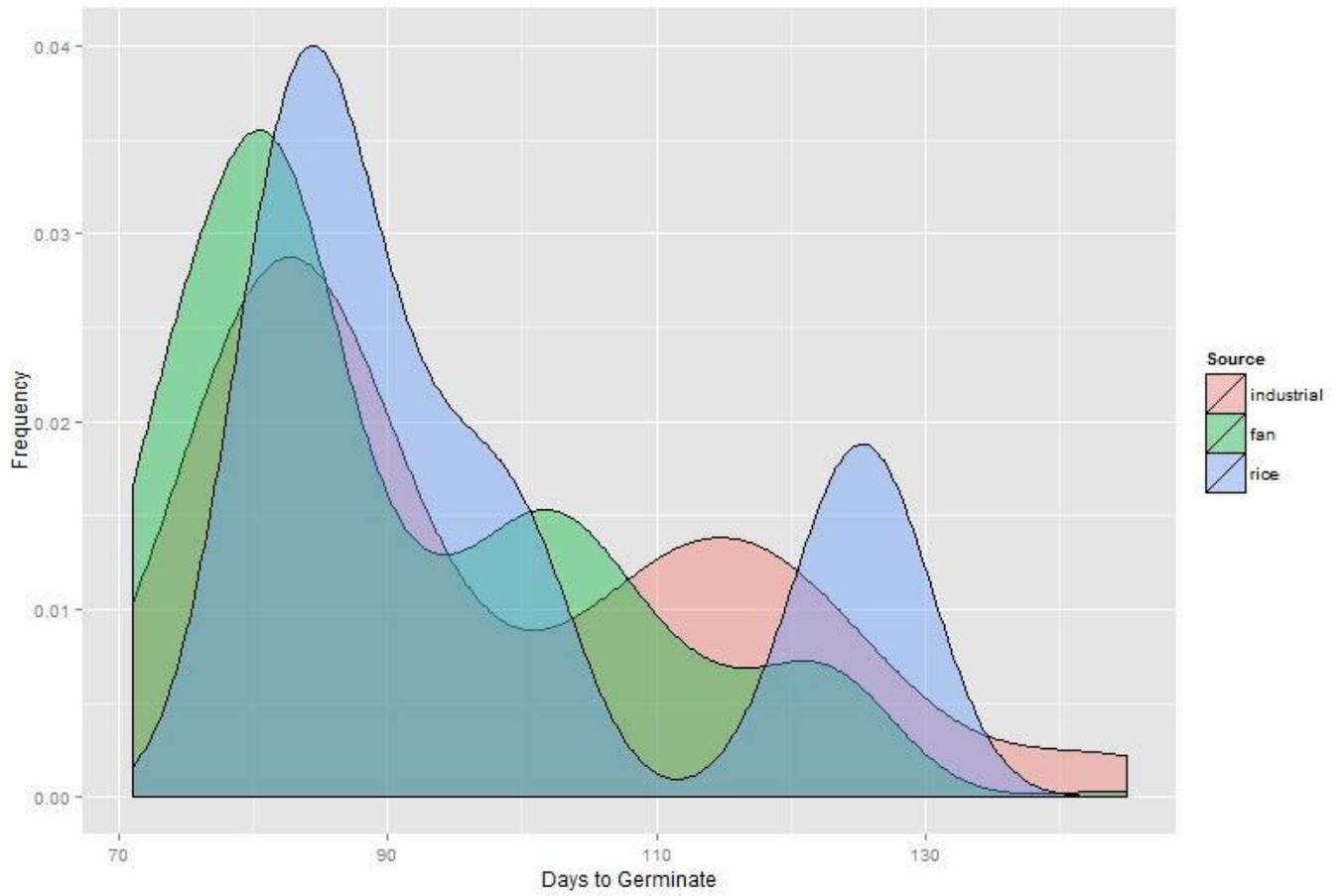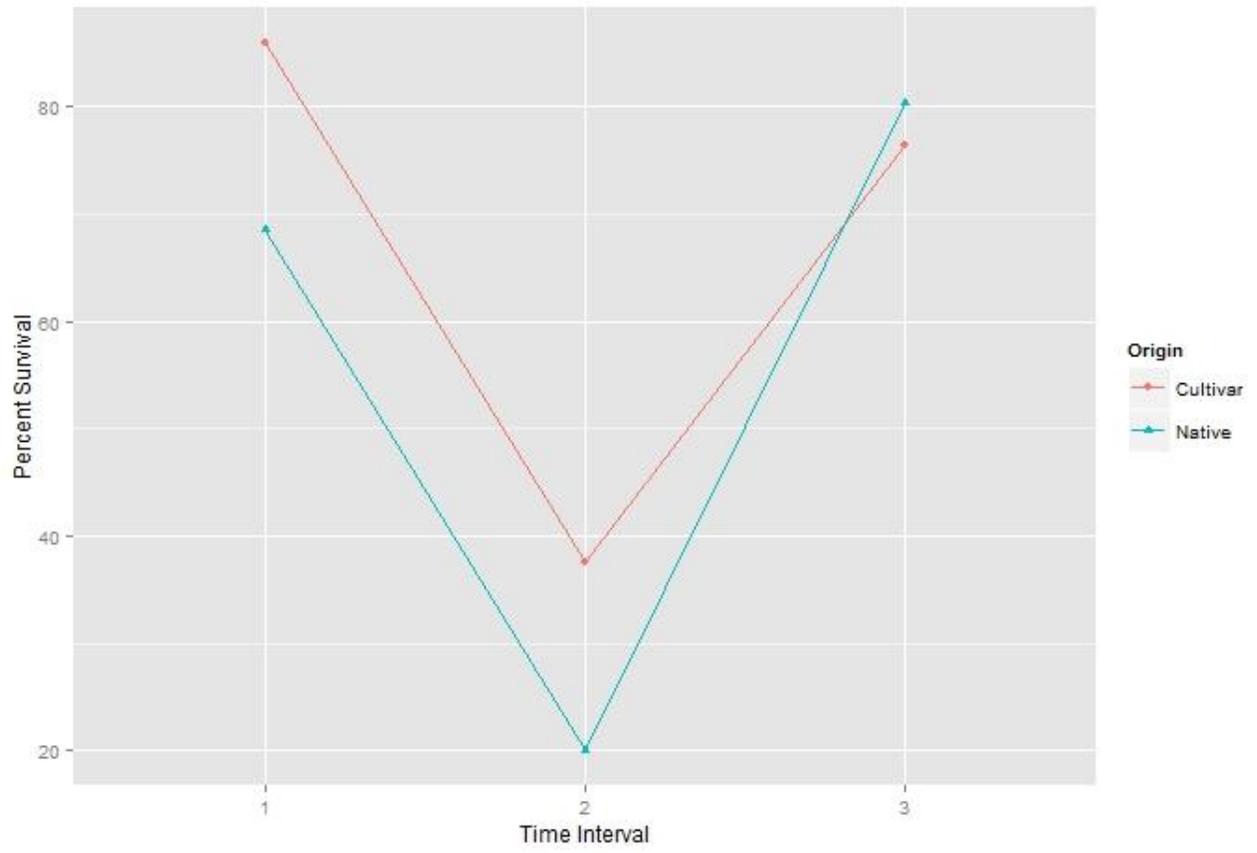branches
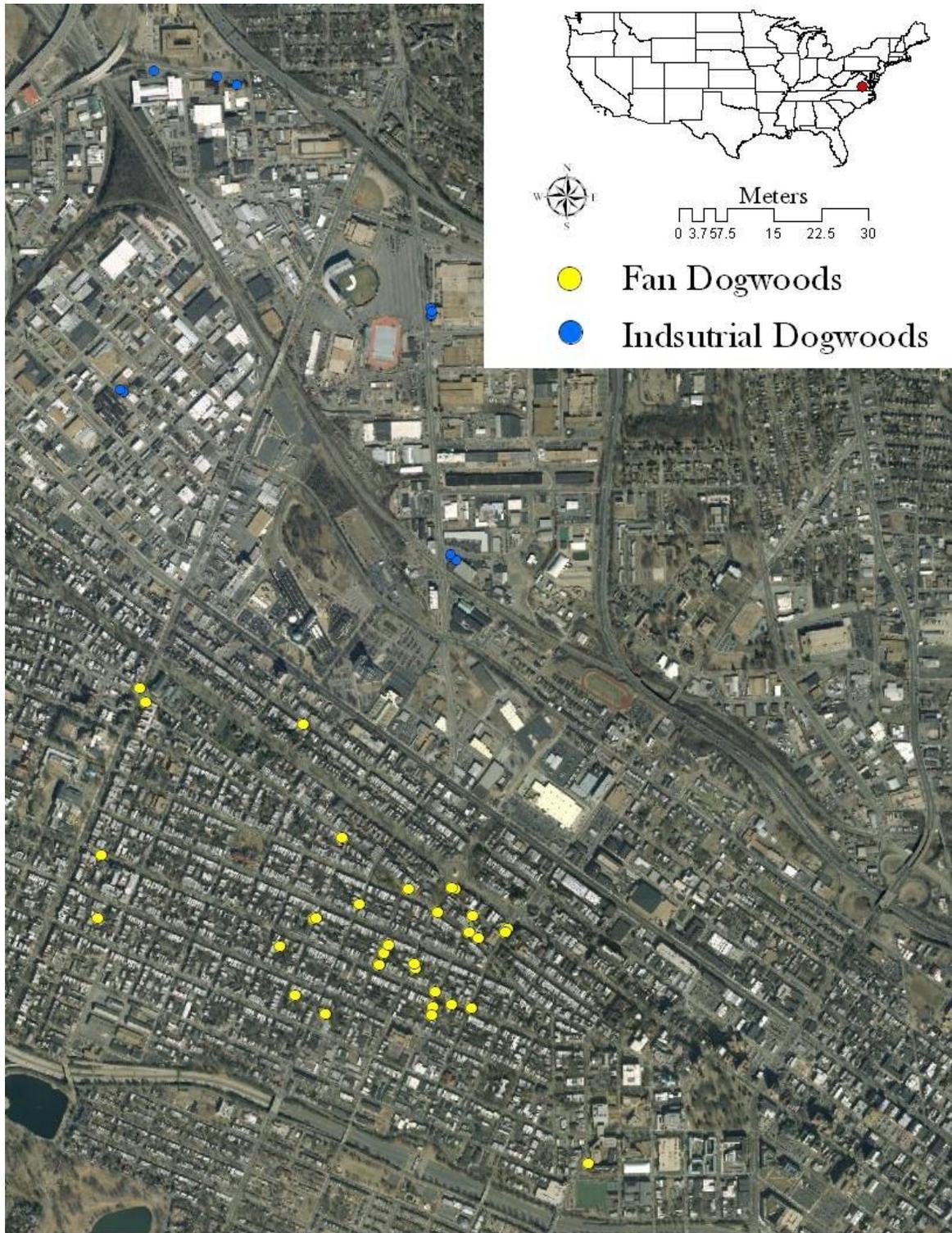
Figure 3.

Figure 4.

Figure 5.

Vita

Angela Hutto Redwine graduated with a B.S. in Environmental Studies from Virginia Commonwealth University in 2011, at which time she was also enrolled in the Accelerated Master of Environmental Studies at VCU. During her graduate studies, Angela taught the computer lab section of Earth Systems Science and maintained a position with the Department of Conservation and Recreation, Division of Natural Heritage as an environmental review assistant. She also worked to complete the Graduate Certificate in Geographic Information Systems through the L. Douglas Wilder School of Government and Public Affairs at VCU.