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# Four Aspects of Dogwood Pollination: Insect Visitation, a Novel approach to Identify Pollen, Floral Volatile Emission, and Tracking Parentage

Paul Raymond Rhoades
*University of Tennessee - Knoxville*, prhoades@utk.edu

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To the Graduate Council:

I am submitting herewith a thesis written by Paul Raymond Rhoades entitled "Four Aspects of Dogwood Pollination: Insect Visitation, a Novel approach to Identify Pollen, Floral Volatile Emission, and Tracking Parentage." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

John A. Skinner, Major Professor

We have read this thesis and recommend its acceptance:

William E. Klingeman, Robert Trigiano

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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**A Thesis Presented for the**
**Master of Science**
**Degree**
**University of Tennessee, Knoxville**

**Paul Raymond Rhoades**
**August 2010**

# Dedication

This Thesis is dedicated to my parents.  Without their love and support this would not have been possible.

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I would like to thank the following people for their contributions to this project:

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# Abstract

Part 1: Visitation of potential pollinators to *Cornus florida* and *C. kousa* flowers was assessed in East Tennessee in 2008 and 2009. Data regarding insect visitation rates to multiple trees per location were gathered throughout the flowering period. Diurnal and seasonal variations in visitation were recorded. Pollen coverage was assessed on portions of captured insect exoskeletons that were most likely to contact the stigma. Eleven families in four insect orders were collected from *C. florida* and 26 families in five orders from *C. kousa*. The most important pollinators in eastern Tennessee were bees in the Andrenidae and Halictidae. The most common visitors to *C. kousa* flowers were scarab, cerambycid and cantharid beetles. Halictid bees were also frequent visitors to *C. kousa*.

Part 2: Dispersion of flowering dogwood pollen in an orchard was evaluated by performing parentage analysis on open pollinated seedlings collected from a single maternal tree. Pollen source for 45 seedling trees were established using three polymorphic simple sequence repeat (SSR) loci. The expected leptokurtic distribution was not seen. Although the majority of paternal trees were within the 12 m radius of the study area, the trees most likely to donate pollen were some of the furthest away. Unusual pollen movement may be a product of lack of synchronization of flowering times between the mother tree and potential pollen donors in the area. Additionally relative proportions of certain pollinating insects (andrenid and halictid bees) may have moved pollen further than expected.

Appendix 1: Pollen from 6 species in the genus *Cornus* was analyzed with Fourier Transform Infrared (FTIR) Spectroscopy to determine the utility of this tool to identify pollen

grains to species. Although there were differences visible in the spectra, principal components analysis coupled with cluster analysis could not consistently identify pollen grains.

Appendix 2: Floral volatile emissions from 6 species of *Cornus* were collected in a headspace chamber and analyzed with gas chromatography-mass spectrometry to determine if differences between emitted volatiles of *C. florida* and *C. kousa* explain differences in floral insect visitation. The relationship between floral volatile emission and the phylogeny of *Cornus* was also examined.

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# Introduction

Pollinators are vital for a variety of crops. The demand for pollinators is growing in the United States even as agricultural intensification and urbanization may be reducing the numbers of wild pollinators (Kremen et al., 2002; Torchio, 1990). Estimates of the value of insect pollination of agricultural crops in the United States range from $150 million paid for pollination services to $14.5-$18.9 billion in total crop value (Levin, 1983; Morse and Calderone, 2000; Rucker et al., 2005). Of this, native bees may contribute about $3 billion annually (Losey and Vaughn, 2006).

Beginning in October 2006, beekeepers began reporting unexplained colony death (Review Colony Collapse, 2007). Thirty to ninety percent losses in beekeeping operations across the United States have continued through the next three winters (CCD working group summary). This syndrome was termed colony collapse disorder (CCD) and characterized as a rapid loss of adult bees from a colony without dead bees found inside or nearby the colony (Oldroyd, 2007; Stokstad, 2007). Possible causes of this disorder include the following: novel complexes of mites or pathogens, pesticides, or stress on colonies produced by ‘migratory beekeeping’ (Review Colony Collapse, 2007).

The largest losses of resulting from CCD were in honey bee populations managed for the purposes of commercial pollination. This has fueled interest in utilizing of wild bees for pollination. Management and culture of formerly wild bees has had success. The alfalfa leaf cutter bee (*Megachile rotundata*) has been an effective pollinator of alfalfa (Peterson et al., 1992). Several mason bees (*Osmia lignaria*, *O. cornifrons*, *O. cornuta*) are valuable pollinators in orchards (Bosch and Kemp, 2002). However, little is known about the biology of native bees and information about their floral preference and seasonal pattern of activity may aid the eventual utilization of these bees for agricultural purposes. A survey of visitors to *Cornus florida* and *C. kousa*, flowering in April and May respectively, may reveal insects useful in pollinating important food crops flowering at a similar time, such as cherries, plums, pears and apples.

*Cornus florida* and *C. kousa* are members of the genus *Cornus*, and the Cornaceae. The trees, shrubs and few herb forms of *Cornus* have opposite or alternate, simple, entire, exstipulate leaves. Flowers are actinomorphic, 4 or 5 merous and may be unisexual or bisexual. Inflorescences are composed of cymes, panicles or heads and may have large bracts. Inflorescences of C. florida and C. kousa are composed of fifteen to thirty flowers sharing a capitate inflorescence subtended by four large bracts (Britton and Brown, 1970; Smith, 1977; Zomlefer, 1994).

*Cornus florida* produces fruit in the form of a drupe, which has high fat and calcium content and provides nourishment to many birds and mammals. Fruit is readily eaten by game birds, including wild turkeys, ruffled grouse and quail, as well as songbirds like cardinals, grosbeaks, robins, brown thrashers and cedar waxwings. Both the browse and the fruit are very important food items for white-tailed deer. Other mammals, including rabbits, foxes, black bears, chipmunks and squirrels often eat the fruit. (Eyde, 1988; Halls, 1977; Stiles, 1980).

Flowering dogwood is also considered a soil builder. The leaves have very high calcium content (2-3.5%) and decompose rapidly making these minerals quickly available (Hepting, 1971; Thomas, 1969). Positive impact on soil fertility is enhanced by its capacity to achieve maximum photosynthetic rate at low light levels found in the understory (Kramer and Decker, 1944).

In addition to its importance as a component in eastern forests, their interesting habit, attractive foliage and bracts has made *C. florida* a common part of the landscape in temperate regions of the country. The majority of dogwoods used in the landscape are produced in Tennessee with an estimated farm gate value of about 6.18 millions of dollars in 1998 (USDA-NASS, 1998). Unfortunately these receipts have been impacted negatively in recent years by the following two diseases caused by fungi: dogwood anthracnose and powdery mildew.

Since the late 1970s, dogwood anthracnose has affected *C. florida* (Daughtrey and Hibben, 1983). In 1991, *Discula destructiva* Redlin, was identified as the fungus responsible for the disease (Redlin, 1991). This fungus was suggested to be an introduced pathogen to the United States (Trigiano et al., 1995), but the origin of the pathogen has not been determined. Limb and trunk cankers, necrotic spots on leaves and bracts, and twig blights characterize the disease (Hibben and Daughtrey, 1988). Mortality in the wild can be as high as 97% (Mielke and Langdon, 1986).

Anthracnose, along with other causes like canopy closure and environmental stress, has caused significant recent decline in populations of native dogwoods in the northeastern US and along the Appalachian Mountains and associated highlands as far south as Alabama. An estimated 36% decline in total individuals in populations has occurred from 1990 – 2000 (McEwan et al., 2000).

Powdery mildew caused by *Erysiphe pulchra* (Cook & Peck) is another disease that has adversely affected dogwood production in Tennessee. Shriveled, discolored leaves, dead branches and stunted growth characterize this disease. Infection makes trees more susceptible to other stresses such as drought or insect problems.  Severe infections may kill the tree (Windham and Witte, 1998).

*Cornus kousa* Hance is another economically important member of this genus.  This small tree is native to China, Japan and Korea and grows up to 30 feet high and 40 feet wide, with many low horizontal branches.  *Cornus kousa* is considered to be the Asian counterpart to *C. florida*, and like flowering dogwood there are many ornamental cultivars available (Cappiello and Shadow, 2005).  Though *C. kousa* is not nearly as popular as *C. florida* in the landscape, it does display resistance to anthracnose and powdery mildew (Holmes and Hibben, 1989; Ranney et al., 1995).

Both *C. florida* and *C. kousa* have simple, open flowers that provide minimal nectar reward to arthropod pollinators.  Grant (1949) suggested that dogwoods could fall into a category of bee pollinated plants, bee and long tongue fly pollinated plants or promiscuously pollinated plants.  Lacking any floral impediment to nectar or pollen, a large diversity of insect visitors have been observed including, honey bees, native bees, syrphid flies, as well as other Dipterans, butterflies and beetles (Mayor et al., 1999; Barrett and Helenurum, 1987; Douglas, 1983; Lovell, 1898; Lovell, 1915; Gunatilleke & Gunatilleke, 1984).

The diversity of visitors engendered by the lack of floral complexity reduces the likelihood that a visit will result in fertilization (Schemske and Horvitz, 1984).  Though these flowers may attract a large variety of visitors, only a few of these visitors will forage in a way that pollinates the plant (Lindsey, 1984).  The relative importance of different species as pollinators is not clear.  However, Andrenid and Halictid bees appear to be the primary pollinators of *C. florida* and beetles and flies are of secondary importance (Eyde 1988).  Since *C.*

*kousa* is resistant to the major diseases of *C. florida*, an interspecific cross may be an effective way to produce a resistant cultivar. And so knowledge of insect visitors across a range of *Cornus* species may help produce this interspecific cross.

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# Part 1: Cataloging floral visitors to *Cornus florida* and *Cornus kousa*

# Abstract

Visitation of potential pollinators to *Cornus florida* and *Cornus kousa* flowers was assessed in East Tennessee in 2008 and 2009. Data regarding insect visitation to multiple trees per location were gathered throughout the flowering period and across a range of environmental conditions. Diurnal and seasonal variations in visitation were recorded. Pollen coverage was assessed on portions of captured insect exoskeletons that were most likely to contact the stigma.

In total, eleven families in four insect orders were collected from *C. florida* and 26 families in five orders from *C. kousa*. Although there was considerable variation in visitor assemblage for *C. florida* across sites and years, the most important pollinators in eastern Tennessee were bees in the Andrenidae and Halictidae. The most common visitors to *C. kousa* flowers were scarab, cerambycid and cantharid beetles. Halictid bees were also frequent visitors.

# Introduction

Flowering dogwood trees, *Cornus florida* L., are an important native plant component in the woodland ecology of eastern North America. Flowering dogwood fruit and foliage feed many birds and mammals (Eyde, 1988; Halls, 1977; Stiles, 1980). Leaves of flowering dogwood have very high calcium content (2-3.5%) and decompose rapidly, making these minerals quickly available to other plants (Hepting, 1971; Thomas, 1969). Soil fertility is further enhanced by the capacity of flowering dogwood trees to achieve maximum photosynthetic rate at low light levels found in the forest understory (Kramer and Decker, 1944).

In addition to the ecological importance of flowering dogwood to eastern forests, the interesting growth habit, attractive foliage and ornamental bracts have made it a common part of managed urban landscapes across temperate regions of the United States. Unfortunately, commercial production of flowering dogwood trees has been adversely affected in recent years by widespread outbreaks of the following two significant diseases caused by fungi: dogwood anthracnose and powdery mildew (Klingeman et al., 2001; Windham et al., 2005).

Chinese dogwood, *C. kousa* (Buerger ex Miq.) Hance, which is native to East Asia, is considered to be the Asian counterpart to *C. florida* (Cappiello and Shadow, 2005). Although both trees flower in spring, *C. florida* flowers in mid- to late- April and *C. kousa* flowers about a month later in Tennessee (Windham et al., 2005).

*Cornus kousa* is also an economically important member of this plant genus. Like flowering dogwood, many ornamental are cultivars commercially available (Cappiello and Shadow, 2005). However, *C. kousa* is not nearly as common as *C. florida* in managed urban landscapes, but the species does display resistance to dogwood anthracnose and powdery mildew (Holmes and Hibben, 1989; Ranney et al., 1995; Windham, et al., 2005).

Flowers without any impediments to floral nectar or pollen, like those of dogwoods, may be visited by a diverse array of insect species, including those that are ineffective as pollinators or that function as floral herbivores (Waser et al., 1996). In past survey efforts, many insects have been observed in association with *Cornus* species including honey bees, native bees, syrphid flies and other dipterans, butterflies and beetles (Barrett and Helenurum, 1987; Douglas, 1983; Gunatilleke and Gunatilleke, 1984; Lovell, 1915; Lovell, 1898; Mayor et al., 1999).

Diversity of visitors has been suggested to reduce likelihood that a floral visit results in fertilization (Schemske and Horvitz, 1984). For example, although flowers of *Thaspium* and *Zizia*, herbaceous perennial species in the Apiaceae, may attract a large variety of arthropod visitors, only a few visitors forage in a way that pollinates the plant (Lindsey, 1984). Indeed, pollination effectiveness is a complex function of floral visitor shape, relative pollinator abundances, and their interactive behaviors. Thus, relative effectiveness of each visitor should be assessed independently (Fishbein and Venable, 1996; Johnson and Steiner, 2000). By failing to do this, the most important pollinators risk being misclassified and a specialized pollination system may be interpreted as general (Fenster et al., 2004; Lindsay, 1984; Sugden, 1986).

A recent survey of insects interacting with *C. florida* (Mayor et al., 1999) reported large numbers of andrenid and halictid bees associated with trees, but did not differentiate between insects that were merely in close proximity to trees (e.g., to encounter a potential reproductive mate) from those that were true floral visitors. Furthermore, no data were gathered regarding diurnal or seasonal fluctuations in visitation of these potential pollinators (Mayor et al., 1999). Knowledge of floral visitors to *C. florida* is important both to better understand the ecology of flowering dogwood within its native range and to facilitate commercial and academic breeding of these popular urban landscape trees to increase disease resistance characteristics. Indeed, observing insect visitors across a range of economically important *Cornus* species may facilitate interspecific crosses that combine benefits of both of their positive attributes. To date, however, efforts to breed novel cultivars of *C. florida* resistant both to dogwood anthracnose and powdery mildew have been hindered, in part, by the intense labor requirements for producing a controlled cross. Effective hand pollination of flowering dogwood requires one to touch each stigma at least twice (Reed, 1999). Honey bees in screen cages have been used to cross pollinate dogwoods, although a solution containing sucrose and queen mandibular pheromone must be placed on the bracts to attract the honey bees to flowers, which produce very little nectar

(Craddock et al., 1997; Wadl et al., 2009). Natural pollinators may be more efficient because they seek pollen rather than nectar as a food, thus requiring no supplemental encouragement to forage. Therefore, the objective of this study was to determine which native pollinators are the most frequent visitors to flowering and Chinese dogwood trees in our region and which may be most efficient for purposes of controlled cross breeding.

## Materials and Methods

Insect visitors to *C. florida* trees at three eastern Tennessee locations and visitors to *C. kousa* trees maintained at the University of Tennessee Forest Resources Research and Education Center Arboretum (UT Arboretum) in Oak Ridge, TN were captured during flowering periods in 2008 and 2009, identified, and assessed for pollen. Sampling of visitors commenced as soon as flowers began to open and continued until there were no unopened flowers on any of the trees included in each of the study sites.

### Study Sites

**Site 1.** The University of Tennessee Forest Resources Research and Education Center Arboretum (UT Arboretum) (Lat. 35° 59.8” N, Long. 84° 13” W) is a 250-acre facility that manages over 2,500 native and exotic woody plant specimens representing 800 plant species, varieties and cultivars. These ornamental plantings are surrounded by native temperate woodlands. There are extensive plots of species and named cultivars of both *C. florida* and *C. kousa* trees. Trees used for sampling insect visitors were between 10 and 20 years old and had been planted in an orchard configuration on five meter centers.

**Site 2.** The historic Island Home subdivision is located in south Knoxville, TN (Lat. 35° 57.5” N, Long. 83° 53” W). This suburban neighborhood contains many mature hardwoods and diverse ornamental plant species in mature landscapes. Insect visitors were sampled from mature 15- to- 35- year old flowering dogwood specimens located within a park-like median strip on a lightly traveled residential road.

**Site 3.** Ijams Nature Center (Lat, 35° 57.4” N, Long. 83° 52.2” W) is a woodland park located in southeastern Knox County on the perimeter of urban Knoxville. Trees sampled were approximately 20-30 years old and were adjacent to the wooded border of 0.25-0.5 acre mown grass fields.

### Monitoring Insect Visitors

In 2008, all arthropod visitors to flowers that could be captured were collected whereas in 2009, a subset of visitors were captured for identification and others were observed without capture. The technique was changed to accommodate much higher rates of floral visitation in 2009. Observation was chosen over capture to prevent distortion of diurnal and seasonal variation in visitation caused by sampling bias. As a consequence, the subset of visitors captured in 2009 was similar in number to the entire collection taken in 2008.

Whether engaged in collection or observation, a single data period averaged 45 minutes. To assess diurnal variation in activity by location, individual trees were sampled at all times of the day and in all weather conditions that permitted insect flight. Time and location of visitation was recorded and captured insects were stored individually in glass vials and placed on ice, to prevent pollen transfer between individuals.

Insects were identified using keys in ‘Bees of the Eastern United States’ (Mitchell, 1960), ‘Bees of the World’ (Michener, 2000), ‘Discover Life’ (http://www.discoverlife.org/mp/20q?search=Apoidea ) and with the assistance from Adriean Mayor (Taxonomic curator, Great Smoky Mountains National Park Collection).

### Analysis of pollen coverage on floral visitors

Due to the small size of individual dogwood flowers, small size of many insect visitors and brief time spent at each flower, foraging behavior could not be adequately characterized at the time of visitation. In both years, the wide variety of potential vectors and low rates of visitation made single visit pollination efficiency impractical. Pollination efficacy was therefore measured by evaluating coverage of pollen on insects captured on those body regions most likely to contact the stigma (Beatty, 1978). This evaluation tool is a good proxy for more direct measurements of pollination efficacy due to the open geometry of dogwood flowers and the fact that anthers and stigma are held at similar orientations and at a similar height above the inflorescence surface. If an insect visitor contacts an anther when foraging, it is likely that they will contact the stigma as well.

Insects were observed under a dissecting scope and pollen coverage was evaluated by estimating pollen grain densities on the head, lower thorax, leg and lower abdominal regions of each insect. Pollen coverage on each region was ranked as no pollen (0) or few scattered pollen grains (1) to high coverage (5) on each sampled insect body region (data not shown). Estimates were then pooled to create a per-species or per-family value designed to represent the pollination efficiency of that species or family **(Table 1.1)** (after Beattie, 1978).

# Results

## Insect visitation to *Cornus florida* flowers

Insect representatives of five orders and 15 families were collected from *C. florida* flowers with hymenopterans accounting for nearly 70% of floral visitors. Andrenid and halictid bees were the two most common families captured and visitation by members of these two families combined comprised more than 50% of total visitation at all sites. Flies and cerambycid beetles were also frequently encountered **(Table 1.1)**.

Andrenid bee visitors to *C. florida* included 18 species with 10 represented by a single specimen. The two most common andrenid visitors to *C. florida* were *Andrena fenningeri* Viereck and *A. miserabilis* Cresson. Although andrenid visitation was higher in 2009, species diversity was lower. Fifteen different species were gathered in 2008 whereas only nine species were collected in 2009 **(Table 1.2)**.

Sweat bee (Hymenoptera: Halictidae) species diversity was similar in both 2008 and 2009 **(Table 1.2)**. Nine species were captured. Eight individuals could only be categorized as belonging to the taxonomically contentious *Lasioglossum viridatum* group, in which *L. perspicuum* Knerer and Atwood, *L. admirandum* Sandhouse, *L. paradmirandum* Knerer and Atwood, and *L. viridatum* Lovell are generally considered to be indistinguishable. Regardless, the most common halictid bee visitor to *C. florida* was *L. imitatum* Smith in both years of the study **(Table 1.2)**.

**Table 1.1:** All visitors to flowers of *C. florida* and *C. kousa* at 3 sites in east Tennessee in 2008 and 2009.

| | | Insect visitation by site and year* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Family, genus | 2008 | | *C. florida* | | 2009 | | *C. kousa* | | Pollen |
| Order | or subfamily | ar | ij | ih | ar | ij | ih | 2008 | 2009 | Coverage† |
| Coleoptera | Cantharidae | - | - | - | - | - | - | 67 | 150 | 0.9 |
| | Cerambycidae | 1 | 5 | - | 1 | 8 | 2 | 17 | 231 | 1.4 |
| | Cleridae | - | - | - | - | - | - | - | 2 | 0 |
| | Coccinellidae | - | - | - | - | - | - | 2 | - | 0 |
| | Curculionidae | - | - | - | - | - | - | - | 5 | 0.5 |
| | Elateridae | - | - | - | - | - | - | - | 5 | 0 |
| | Trichiinae | - | - | - | - | - | - | - | 91 | 0 |
| | Mordellidae | - | - | - | - | - | - | - | 4 | 0 |
| | Scarabaeidae | - | - | - | - | - | - | 129 | 399 | 1.45 |
| | Unknown | 5 | 2 | 1 | - | - | - | - | 49 | - |
| Diptera | Anisopodidae | - | - | - | - | - | - | 3 | 1 | 0 |
| | Anthomyiidae | - | - | - | - | - | - | 1 | - | 0 |
| | Bombyliidae | 5 | - | - | 40 | 17 | 2 | - | - | 0.6 |
| | Calliphoridae | - | - | - | 1 | - | - | - | 7 | 0.3 |
| | Dolichopodidae | - | - | - | - | - | - | 2 | - | 0 |
| | Phoridae | - | - | - | - | - | - | - | 3 | 0 |
| | Sarcophagidae | 1 | 1 | - | - | - | - | 52 | 20 | 0.2 |
| | Scathophagidae | 3 | - | 3 | - | - | - | - | - | 0.73 |
| | Sciaridae | - | - | - | - | - | - | 3 | - | 0 |
| | Syrphidae | 7 | - | 1 | 41 | 10 | 2 | 20 | 1 | 0.62 |
| | Tachinidae | - | - | - | - | - | - | - | 1 | 0 |
| | Unidentified | 2 | - | - | 34 | 11 | 25 | - | 52 | - |
| Hemiptera | Miridae | - | - | - | - | 2 | 1 | - | 42 | 0.3 |
| | Reduviidae | - | - | - | - | - | - | 12 | - | 0.4 |
| | Unknown | - | - | 1 | - | 1 | - | - | - | - |
| Hymenoptera | Braconidae | - | - | - | - | - | - | - | 2 | 0 |
| | Chalcididae | - | - | - | - | - | - | - | 1 | 0 |
| | Crabronidae | - | - | - | 2 | 11 | - | - | 4 | 0.8 |
| | Halictidae | 25 | 2 | 3 | 431 | 53 | 25 | 29 | 163 | 1.1 |
| | Megachilidae | 5 | - | - | 20 | 1 | - | - | - | 3.1 |
| | Andrenidae | 14 | 6 | 10 | 168 | 99 | 36 | 1 | 6 | 1.5 |
| | *Apis* | 3 | - | - | 3 | 26 | - | 1 | - | 1.5 |
| | *Bombus* | - | - | - | 11 | 4 | 1 | 1 | - | 1.7 |
| | Colletidae | 1 | - | - | - | - | - | 2 | 2 | 1 |
| | Xylocopidae | 2 | 1 | - | - | 5 | - | - | - | 0.8 |
| | Unknown | 4 | - | - | - | - | - | - | - | - |
| Lepidoptera | Hesperiidae | 1 | - | - | - | - | - | 7 | 4 | 0 |
| | Papilionidae | - | - | - | - | - | - | - | 2 | 0 |
| | | | | | | | | | | |
| Total insects observed | | 77 | 17 | 19 | 752 | 248 | 94 | 348 | 1247 | |
| Total Orders observed | | 4 | 3 | 4 | 4 | 5 | 5 | 5 | 5 | |
| Total Families observed | | 11 | 5 | 5 | 11 | 13 | 8 | 16 | 22 | |
| Total observation hours | | 18 | 4.6 | 5.4 | 17 | 7.2 | 2.5 | 18.5 | 25.5 | |
| Average visitation per hour | | 4.4 | 3.1 | 3.7 | 44 | 35 | 38 | 19 | 48.9 | |

*For this table data on collection and observation are combined. Each specimen collected or observed visiting is considered one visit.

† Pollen coverage was assessed on the head, legs, lower portions of the thorax and abdomen on a scale of 1-5 with 1 = few scattered grains; 2 = less than 20% coverage of area; 3 = between 20% and 70% coverage 4 = greater than 70% coverage and 5 = total coverage with pollen many layers deep. Scores were averaged for portions of the body and for all visitors.

**Table 1.2:** Hymenopteran visitors to flowers of *Cornus florida* and *C. kousa* at three sites in east Tennessee in 2008 and 2009.

| | | | **Number collected** | | **Number collected** | |
|---|---|---|---|---|---|---|
| | | | C. florida | | C. kousa | |
| Family | Species | | 2008 | 2009 | 2008 | 2009 |
| Andrenidae | *Andrena* | *barbara* | 1 | 0 | 0 | 0 |
| | *Andrena* | *confederata* | 1 | 0 | 0 | 0 |
| | *Andrena* | *crateagi* | 1 | 1 | 0 | 0 |
| | *Andrena* | *cressonii* | 1 | 3 | 0 | 0 |
| | *Andrena* | *erythroni* | 0 | 1 | 0 | 1 |
| | *Andrena* | *fenningeri* | 5 | 7 | 0 | 0 |
| | *Andrena* | *fragilis* | 1 | 0 | 0 | 0 |
| | *Andrena* | *hilaris* | 1 | 0 | 0 | 0 |
| | *Andrena* | *hirticincta* | 1 | 0 | 0 | 0 |
| | *Andrena* | *ilicis* | 1 | 0 | 0 | 0 |
| | *Andrena* | *illini* | 0 | 2 | 0 | 0 |
| | *Andrena* | *imatatrix* | 1 | 0 | 1 | 0 |
| | *Andrena* | *miserabilis* | 5 | 4 | 0 | 0 |
| | *Andrena* | *nasoni* | 1 | 0 | 0 | 0 |
| | *Andrena* | *nivalis* | 1 | 1 | 0 | 0 |
| | *Andrena* | *personata* | 1 | 0 | 0 | 0 |
| | *Andrena* | *sayi* | 1 | 1 | 0 | 0 |
| | *Andrena* | *vicina* | 0 | 2 | 0 | 0 |
| Apidae | *Apis* | *mellifera* | 3 | 2 | 1 | 0 |
| | *Bombus* | *griseocollis* | 0 | 0 | 1 | 0 |
| | *Ceratina* | *calcarata* | 3 | 4 | 0 | 0 |
| Colletidae | *Hylaeus* | *mesillae* | 1 | 0 | 0 | 0 |
| Halictidae | *Augochlora* | *pura* | 0 | 2 | 0 | 1 |
| | *Augochlorella* | *persimilis* | 0 | 0 | 1 | 2 |
| | *Augochlorella* | *striata* | 2 | 1 | 1 | 0 |
| | *Augochloropsis* | *metallica* | 0 | 2 | 0 | 0 |
| | *Halictus* | *confusus* | 1 | 0 | 0 | 2 |
| | *Halictus* | *rubicundus* | 1 | 1 | 0 | 0 |
| | *Lasioglossum* | *fuscipenne* | 2 | 1 | 0 | 0 |
| | *Lasioglossum* | *imitatum* | 18 | 29 | 14 | 34 |
| | *Lasioglossum* | *versatum* | 2 | 0 | 6 | 0 |
| | *Lasioglossum* | *viridatum** | 1 | 7 | 7 | 3 |
| Megachilidae | *Osmia* | *georgica* | 3 | 0 | 0 | 0 |
| | *Osmia* | *lignaria* | 2 | 0 | 0 | 0 |
| | *Osmia* | *opumila* | 0 | 1 | 0 | 0 |

* *L. perspicuum*, *L. admirandum*, and *L. paradmirandum* are considered indistinguishable, thus are referred to collectively as *L. viridatum* group

Overall visitation was much higher in 2009 than in 2008. Insect visitation to *C. florida* flowers was highest around mid-day between 11:30 and 14:00 hours, due in large part to an increase in activity by bee species **(Figs. 1.1, 1.2)**. The peaks of activity of *Bombus* spp. visitation are staggered against the higher activity periods of andrenid and halictid bees **(Fig. 1.2)**. Beetles, flies, and butterflies were most active on flowers later in the day, between 14:00 and 15:00 hours. By contrast insects in the Syrphidae, Cerambycidae, Apidae, Scathophagidae, and Andrenidae did not share an early afternoon visitation peak, but were more evenly distributed throughout the day (data not shown). Although diurnal visitation to *C. florida* in 2009 did have greatest intensity between 11:00 and 13:00 hours, there was greater visitation throughout the afternoon finally drooping off at about 17:00 hours. Seasonal variation in visitation to *C. florida* closely followed temperature, with dips in temperature clearly reflected in lower visitor abundance **(Figs. 1.3, 1.4)**. This is especially true for the smaller halictid bees that made up a large part of the assemblage of visitors.

Andrenid visitors to *C. florida* were not affected as much by variations in temperature **(Fig. 1.3)**. Though visitation by syrphid flies was low, these insects did not seem affected by changes in temperature. With variations in temperature taken into consideration, visitation was relatively constant throughout the flowering period on *C. florida.*

### Insect visitation to *Cornus kousa* flowers

Insect visitors to the flowers of *C. kousa* comprised five orders and 30 families; a more diverse assemblage of insect visitors than to *C. florida* **(Table 1.1)**. A single species of scarab beetle, *Macrodactylus subspinosus* F. (Coleoptera: Scarabaeidae), and soldier beetle,

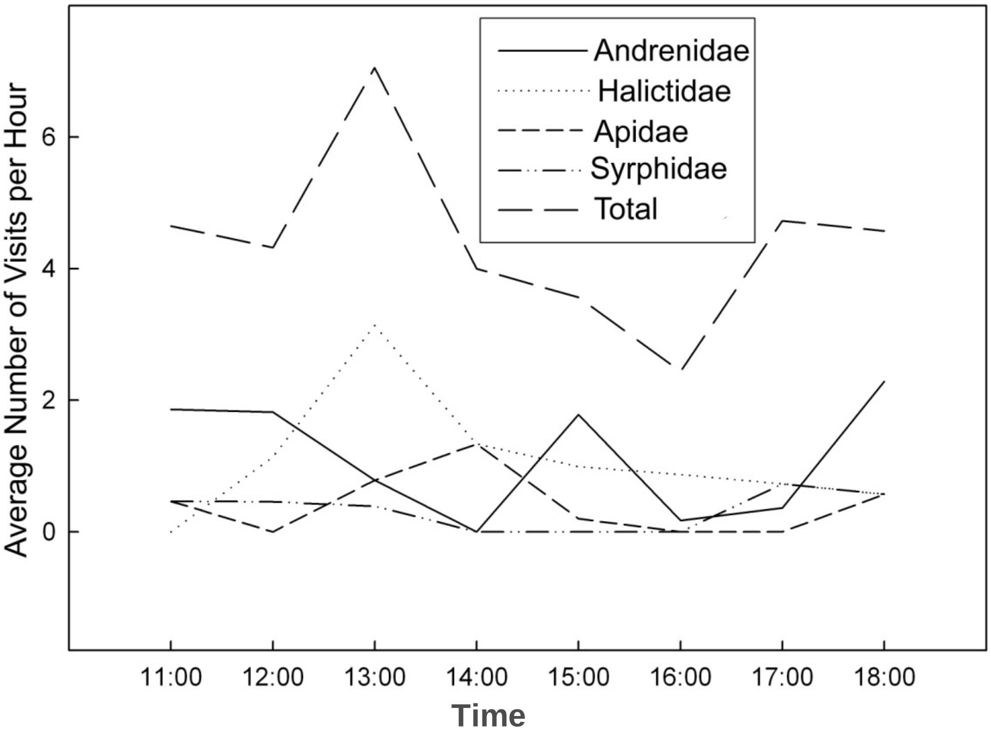

**Figure1.1:** Diurnal variations in visitors to *Cornus florida* in east Tennessee in 2008

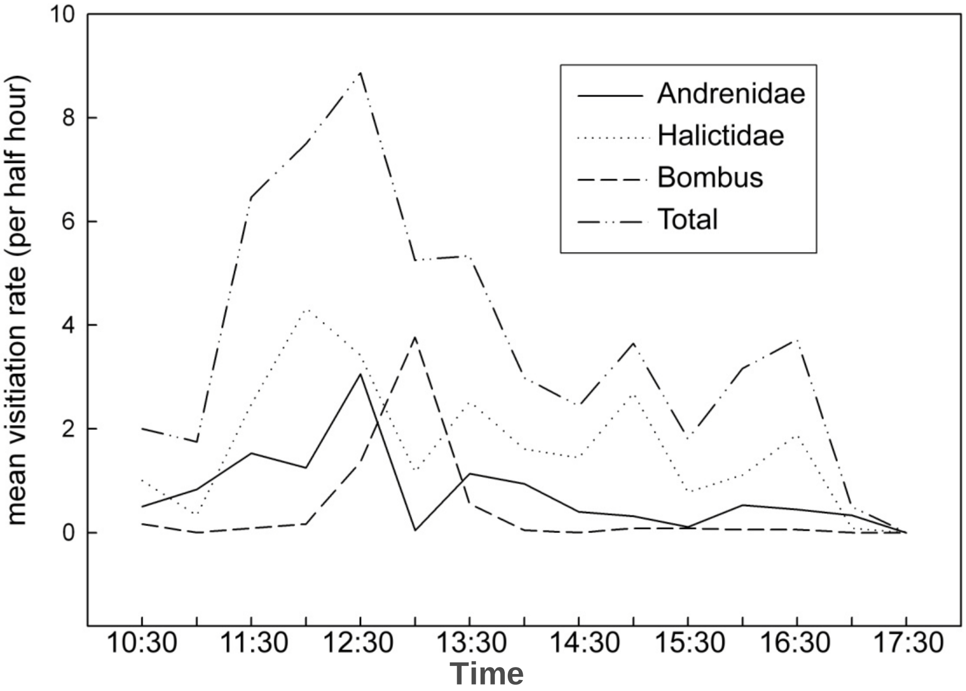

**Figure 1.2:** Diurnal variation of visitors to *Cornus florida* in east Tennessee in 2009

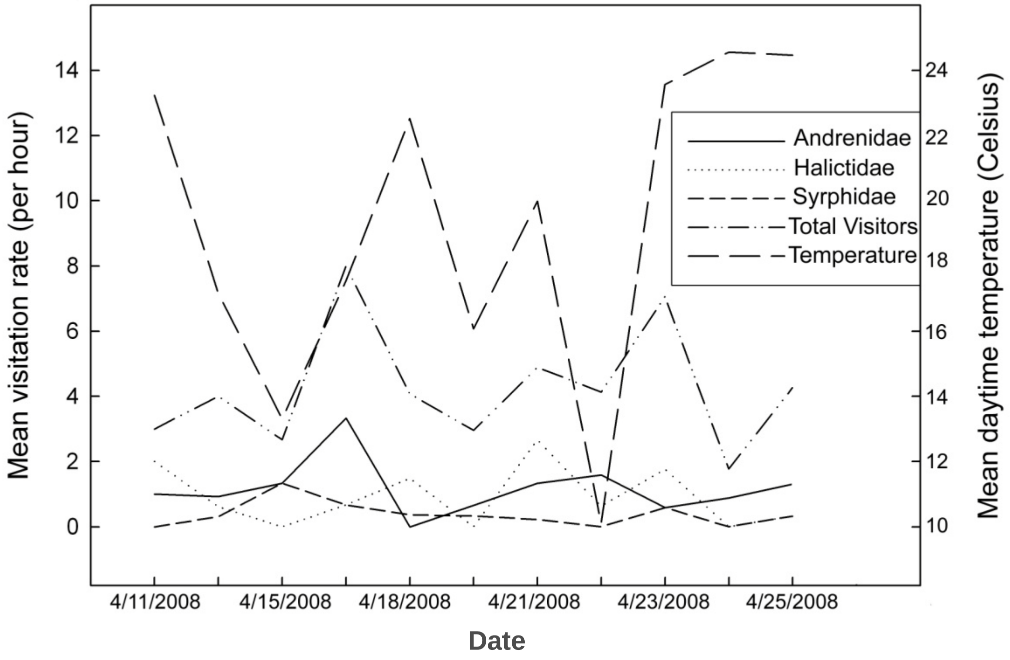

**Figure 1.3:** Variation in visitation of potential pollinators to *Cornus florida* during seasonal flowering period at the UT Arboretum in east Tennessee in 2008; by family with temperature.

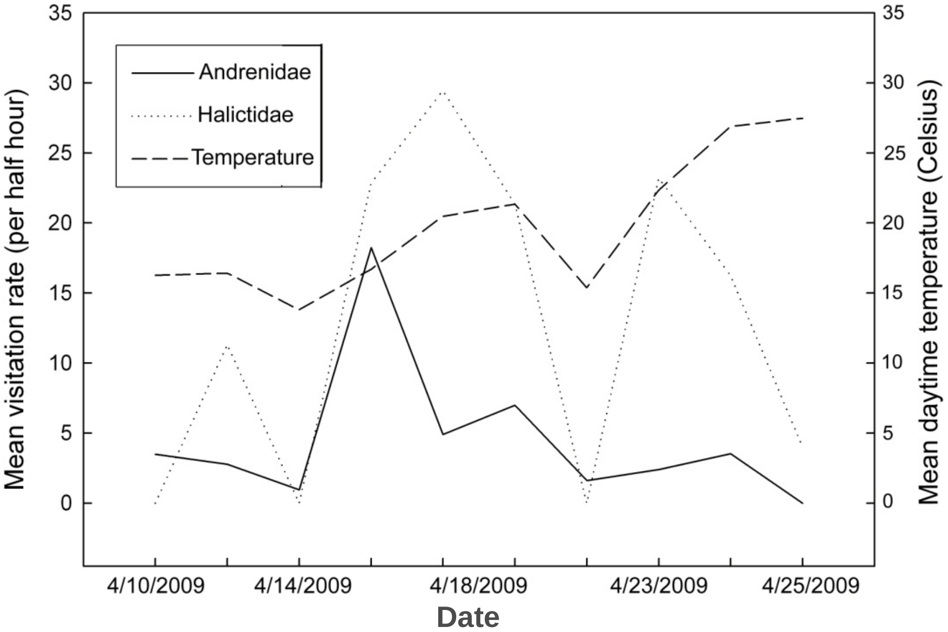

**Figure 1.4:** Variation in visitation of potential pollinators to *Cornus florida* during its seasonalflowering period for three sites in east Tennessee in 2009; by family with temperature.

*Chauliognathus marginatus* F. (Coleoptera: Cantharidae) were extremely abundant on Chinese dogwood flowers. Insects in the Halictidae, Sarcophagidae, Syrphidae, and Cerambycidae were also frequent visitors **(Table 1.1)**. From the *C. kousa* trees, 29 halictid specimens were taken representing at least seven species. As in *C. florida*, the most common halictid bee was *L. imitatum*. Representatives of the *L. viridatum* group were also well represented, which are most likely to be *L. admirandum* and *L. viridatum* bee species.

Overall diurnal variation in visitation to *C. kousa* followed a similar pattern to visitation on *C. florida*. Activity peaked at roughly 13:00, despite the warmer temperatures. Flesh flies and soldier beetles, which, while common on *C. kousa*, were absent from *C. florida*, had peaks of activity between 14:00 and 16:00 hours with both families were active until 16:00 hours. The most common visitor to *C. kousa* flowers, *Macrodactylus subspinosus* (Scarabaeidae), was most active just after noon, with another apparent period of activity after 16:00 hours **(Fig. 1.5)**. As in *C. florida*, visitation in 2009 continued at greater intensity throughout the afternoon with several later peaks **(Fig. 1.6)**.

Seasonal variations in visitation to *C. kousa* are also, to some extent, affected by temperature **(Fig. 1.7)**. Visitation by most families is lower after a temperature decrease. The period of highest visitation by the rose chafer is in the middle of the flowering period for *C. kousa* and did not seem to depend closely on temperature. Many of the other groups (Sarcophagidae, Scarabaeidae, other Diptera) had higher rates of visitation during the latter half of the flowering period.

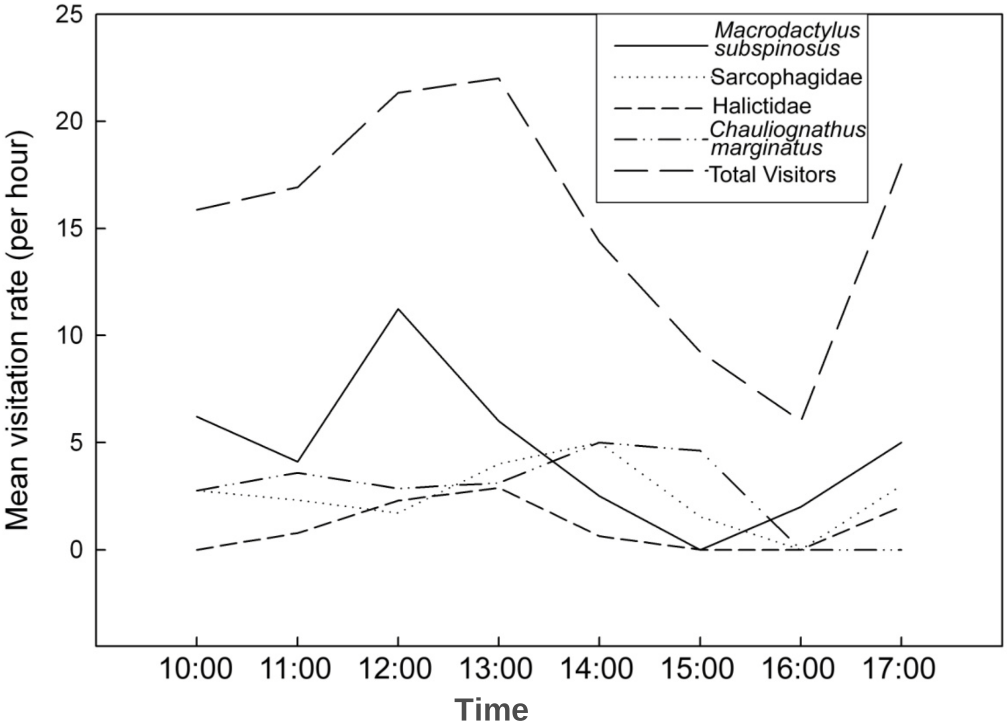

**Figure 1.5:** Diurnal variation of visitors to *Cornus kousa* in east Tennessee in 2008

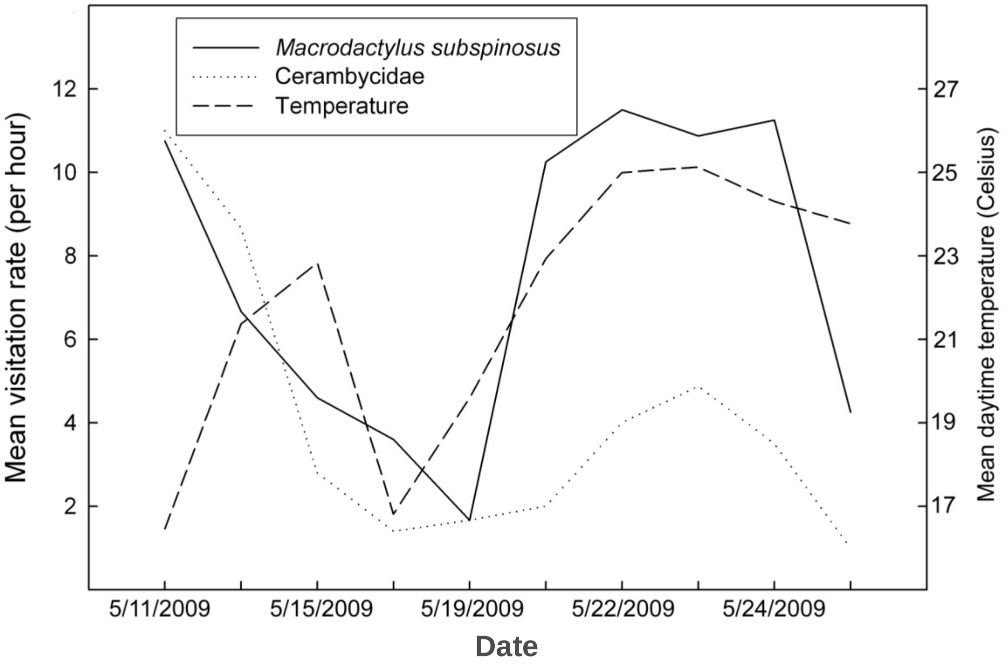

**Figure 1.6:** Variation in visitation of potential pollinators to *Cornus kousa* during its seasonal flowering period at one location in east Tennessee in 2009 by families with temperature.

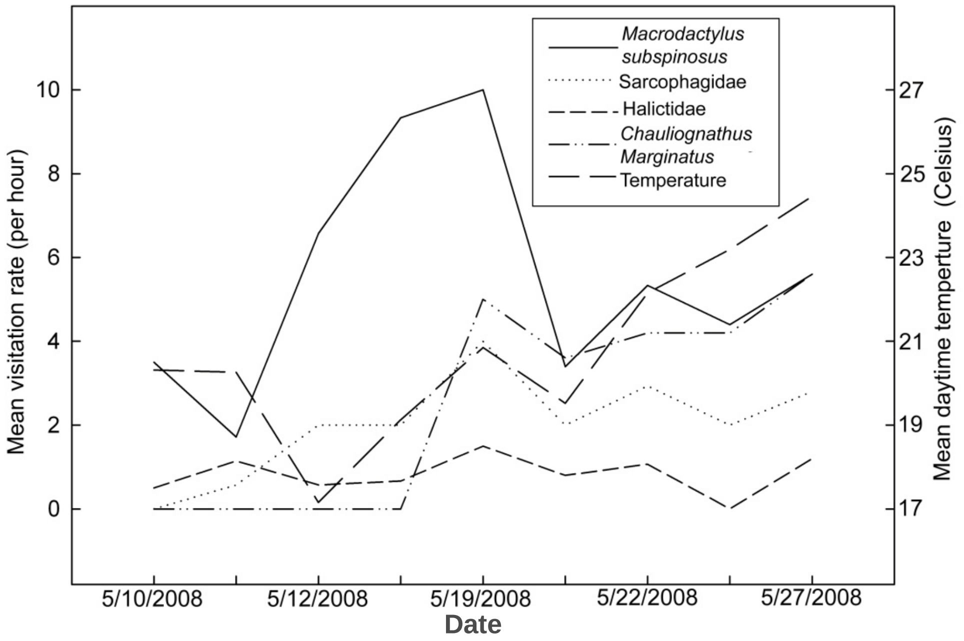

**Figure 1.7:** Variation in visitation of potential pollinators to *Cornus kousa* during seasonal flowering period at one site in east Tennessee in 2008; by family with temperature

Visitation by cerambycid beetles and *Macrodactylus subspinosus* to *C. kousa* did not follow increased temperature during the first half of the flowering period in 2009 **(Fig 1.6)**. Visitation by halictid bees this year seems divergent from temperature. As noted before, fly visitation has little relationship with temperature.

### Analysis of pollen coverage on floral visitors

Generally, bees carried more pollen than all other insect visitors and hymenopteran visitors were more likely to carry more pollen than dipteran visitors **(Table 1.1)**. Among visitors to *C. florida,* megachilid bees carried much more pollen than any other family of visitors. In general, visitors to *C. kousa* flowers tended to carry less pollen than visitors to *C. florida*, yet, coleopteran visitors to *C. kousa* tended to carry nearly as much pollen as bees (**Table 1.1**).

## Discussion

### Variations in visitation

Composition of floral visitors can change dramatically with differences in both time and space (Herrera, 1988), so differences between different years and sites observed in visitor assemblage and rates of visitation were expected. A decline in visitation and reductions in diversity are sometimes attributed to low temperatures or to drops in temperature just before or during the time of flowering (Boyle and Philogene, 1983; Buide, 2006; Navarro, 2000). However, low temperatures recorded at the UT Arboretum do not explain lower visitation that was observed in 2008. In 2008, the mean temperature for the 17 days preceding flowering was 13.8°C with no significant drops in the 2 weeks before flowering. In 2009, temperatures 17 days pre-flowering averaged 11.4°C with a freeze just before flowers opened, the opposite of what would be suggested by visitor abundance. Similarly, average daytime temperature during flowering was 18.8°C in 2008 and 19.3°C in 2009, a seemingly negligible difference. Instead, we hypothesize that visitor abundance was lowered in 2008 due to three days of hard freezing weather following several weeks of warm temperatures. Indeed, freezing weather following warm weather has been cited as a cause of local annual extinction of pollinating insects (Navarro, 2000). These local extinctions or reductions in population size may be related to availability of floral resources (Bowers, 1985). The freeze of 2008 destroyed the flowers of

*Acer* species, present at all sites. Both andrenid and halictid bees are known to forage on *Acer* species and this plant may be a very important early season resource (Krombien et al., 1979).

There were also large differences in visitor composition between sites, which have often been reported (Dorr, 1981; Kearns and Inouye, 1994; Lindsay, 1984; Miller, 1978; Spears, 1987; Willson et al., 1979). In 2008, diversity of visitors to flowers at site 1 was greater than at sites 2 or 3. In 2009, Sites 1 and 3 had high levels of diversity whereas diversity at site 2 was still quite low. Such differences may result either from increased habitat degradation or fragmentation at site 2, which lowers habitat diversity (Kremen et al., 2002; Tomimatsu and Ohara, 2003).

Additionally, many visitors to *C. florida* and *C. kousa* may be generalists foraging on convenient plant resources. Community-wide surveys of seasonal variation in bee abundance revealed that spring populations of pollinators in California and Ithaca, New York tend to be composed mostly of *Andrenid*, *Dialictus*, *Osmia*, and *Calcarata* bee species (Ginsberg, 1983; Wojcik et al., 2008). The varying assemblage of floral visitors at different sites may also be a product of differing habitat preference (Tomimatsu and Ohara, 2003).

### Pollen coverage

Pollen coverage scores combined with visitation rate indicated that andrenid and halictid bees are the most important pollinators of *C. florida* in east Tennessee. As previous work has suggested, bees tend to carry the most pollen among floral visitors (Galloni et al., 2008; Talavera et al., 2001), a trend also observed on *C. florida*.

While megachilid bees were most likely to carry copious amounts of pollen, low floral visitation rates by this family made them mostly irrelevant as pollinators of *C. florida* at sites where sampling took place. Similarly, while visitation by syrphid, scathophagid and bombyliid flies was moderately high, low amounts of pollen carried by these visitors suggest they are inefficient pollinators. Andrenid and halictid bees are both numerous enough and carry enough pollen to be considered important pollinators of *C. florida* in east Tennessee.

### Diurnal visitation

Foraging by insects on *Cornus* species was influenced by time of the day. The activity of the halictid bee, *L. imitatum*, has been observed to decrease in the afternoon (Michener and Wille, 1966). Moreover, these bees were less likely to collect pollen in the afternoon. Because *C. florida* flowers produced little nectar, species of Halictidae may have moved to another plant by early afternoon (Batra, 1966; Michener and Wille, 1966). Peaks of visitation occurring at mid-day have been noted before for trees and shrubs (Döll et al., 2007; Voigt et al., 2004). In contrast, an afternoon resurgence of floral visitors has also been reported (Brown and McNeil, 2009; Tangmitcharoen et al., 2006).

### Seasonal visitation

Most visiting families experienced drops in visitation corresponding to drops in temperature. The relationship between temperature and overall visitor abundance is well established (Figueroa-Castro and Cano-Santana, 2004; McCall and Primack, 1992). Flies, beetles and andrenid bees seem to be less influenced by temperature. Flies forage in cooler weather (Ngamo, 2005). Given the early spring flight season of Andrenid bees, this lack of influence of temperature on visitation rate should have been expected.

## Conclusions

Andrenid and halictid bees are the most consistent and important pollinators of *Cornus florida* in east Tennessee. They were frequent visitors both years at every site and carried pollen on parts of their bodies likely to contact the stigma and deposit pollen. In some, though not all years, cerambycid beetles may have played an important role in pollination of this tree. Flies visited frequently in one year, although the amount of pollen carried by them tended to below compare to the bees. The assemblage of visitors varied substantially by site for *C. florida* though the presence of andrenid and halictid bees was consistent across all sites.

Halictid bees are also effective pollinators of *C. kousa*, but their importance is eclipsed by greater visitation by beetles. Scarab, soldier, and in one year, cerambycid beetles, functioned as substantial pollinators. However, we caution about making inferences regarding the relative importance of the beetle species as pollinators we observed on *C. kousa* trees. Additional research will be needed to quantify the presence of this beetle at multiple sites external to the UT Arboretum and the efficacy by which they transfer pollen to floral structures. Flies were also common visitors one year, but the lack of pollen found on their bodies implied that they probably function poorly as pollinators.

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# Part 2: Using Simple Sequence Repeats (SSRs) to Measure Pollen Flow in an Orchard of Flowering Dogwood

# Abstract

Dispersion of flowering dogwood pollen in an orchard was evaluated by performing parentage analysis on open pollinated seedlings collected from a single maternal tree. Pollen source for 45 seedling trees were established using three polymorphic simple sequence repeat (SSRs) loci. The expected leptokurtic distribution, where the likelihood pollen transfer will occur between trees decreases quickly with distance, was not seen. Although the majority of paternal trees were within the 12 m radius of the study area, the trees most likely to donate pollen were some of the furthest away. Unusual pollen movement may be a product of lack of synchronization of flowering times between the mother tree and potential pollen donors in the area. Additionally relative proportions of certain pollinating insects (andrenid and halictid bees) may have moved pollen further than expected.

# Introduction

Dogwoods (*Cornus* species) have interesting growth habits, attractive foliage and bracts making them popular landscape trees in the United States, Europe and Japan. Although several dogwood species are important to the nursery industry (*C. elliptica, C. kousa*, *C. mas*, *C. nuttallii*, *C. sericea* and (formerly *C. angustata*), flowering dogwood (*C. florida*) is by far the most popular. In 1998, total U.S. sales of flowering dogwood exceeded 26 million dollars with Tennessee producing more trees than any other state (USDA-NASS, 1998).

Flowering dogwood is a medium- sized tree growing 8 to 12 m tall. Leaves are acuminate, cuneate or rounded near the base, may be 7.5 to 15 cm long and offer deep red to purple fall coloring. *Cornus florida* is self-incompatible (Reed, 2004; Wadl et al., 2009).

Insects, not wind, are primary movers of pollen (Personal observation). In a previous experiment various *C. florida* cultivars sequestered together in screen insect exclusion cages failed to set fruit. Cages containing an insect vector (honey bees) had high rates of fruit set, suggesting wind is an ineffective vector for pollen transfer (Trigaino and Wadl pers. com.).

Inflorescences of flowering dogwood are composed of 15 to 30 flowers contained within an umbel 2 cm across that is typically subtended by four large bracts. True flowers are 6-9 mm in length, may be yellow or green are rarely fragrant to humans (Cappiello and Shadow, 2005), but emit distinctive floral volatiles that may attract insect pollinators (Zhuang et al., 2008). Regarding floral development and pollen maturation, an individual flower within a *C. florida* inflorescence has a bloom sequence that may be divided into five stages. At stage one, flowers are green and unopened. At stage two, anthers have dehisced under unopened yellow–green petals. Stage three begins as the flowers open; anthers are erect and covered in pollen. At stage four, the anthers are empty and the petals have recurved. By stage five, the petals and anthers have abscised. Stigmas are receptive to pollination at any stage, but fertilization and fruit set is slightly more likely if pollination occurs in stages three and four. Time required for an individual flower to pass from stage three to stage five is temperature dependent. In 1996, when ambient air temperatures averaged 16.9 C, about 1.8 days were needed for flowers to progress from stage 3 to stage 5. In 1997, when ambient air temperature means were 11.9 C, the progression from stage 3 to stage 5 took about 3.7 days (Malueg, 1998).

Costs associated with nursery production of dogwoods have increased in recent years because of the following two diseases caused by fungi: dogwood anthracnose and powdery mildew (Klingeman et al., 2001; Windham et al., 2005). Cultivars have been introduced that are resistant to either anthracnose or powdery mildew (Windham et al., 2003; 1998; Windham, 1996). However, efforts to breed *C. florida* cultivars resistant to both these diseases are hindered in part by the labor intensive nature of performing controlled crosses. Traditional plant breeding involves sequestering trees from unwanted sources of pollen by bagging flowers, placing entire trees in screened cages and, if necessary, emasculating flowers, to prevent self-pollination by removing anthers prior to anthesis. Pollen is then collected from the anthers of one tree and applied to the stigma of another tree typically using a paint brush or q-tip, although an eyedropper, straw, syringe, or other tool may be used on some plants. Effective pollination of flowering dogwood by hand requires the breeder to touch each receptive stigma at least twice with viable pollen (Reed, 1999).

Insect-mediated pollination offers an alternative to hand-pollination. Honey bees in screened cages are commonly used to cross trees for breeding purposes and have been successful in controlled crosses involving flowering and kousa dogwoods (Wadl et al., 2009; Wang et al., 2009). Naturally foraging honey bees seldom visit inflorescences of *C. florida* because of the miniscule nectar reward offered by the flower. To overcome this limitation a solution containing sucrose and honey bee queen mandibular pheromone can applied to the base of bracts to entice honey bees to visit the flowers (Craddock et al., 1997). Native bees may be more efficient for pollinating flowers of dogwood because they seek pollen rather than nectar as food and therefore do not need encouragement in the form of pheromones and sugars to forage. Regardless, techniques have not been developed for culture and utilization of andrenid and halictid bees, which are the most likely flowering dogwood pollinators (Mayor et al., 1999; See Part 1).

Prior data suggest pollen movement in a natural setting is typically limited to very short distances. In a previous study on *C. florida*, DNA amplification fingerprinting [DAF (Caetano-Anollés et al., 1991)], was used to determine the parentage of seedlings taken from a single maternal tree. Eighty-seven percent of the seedlings were the result of pollen originating from the nearest tree, located less than 3 m from the recipient plant (Ament et al., 2000).

Natural pollen transfer is widely considered to occur over short distances. Distance of pollen movement in plants has been estimated by evaluating the distance pollination vectors travel between flowers and by more direct genetic analysis of seedlings to determine parentage. Insects are more likely to move between flowers that are close together (Beattie, 1976; Heinrich, 1979; Levin and Kerster, 1969ab; Olesen and Warncke, 1989; Pyke, 1978; Richards, 1997; Schaal, 1980; Schmitt, 1980; Waddington, 1981; Wilson, 1983). Genetic analysis of actual gene movement within populations has indicated a similar pattern in which the likelihood of pollen transfer decreases quickly as dispersal distance increases. Long distance dispersal events are more rare. Isozyme, allozyme and SSR markers have been common ways to assess gene movement by determining parentage (Fenster, 1991; Jackson and Clarke, 1991; Karron et al., 1995; Krauss, 1994; Rust, 1980; Schaal, 1980; Smyth and Hamrick, 1987).

SSR markers have been developed for a variety of fruit and ornamental plants such as apple (Liebhard et al., 2002), rose (Esselink et al., 2003), almond (Mnejja et al., 2005; Shiran et al., 2007), peach (Chen and Huang, 2009; Dirlewanger et al., 2002; Sosinski et al., 2000), grape (Gaspero, 2000; Thomas and Scott, 1993), and kiwi (Zhen et al., 2004). SSRs markers are useful in determining parentage for a number of reasons. Most importantly SSR markers are co-dominant, meaning at a certain locus one allele is derived from each parent. This makes parental determination much easier. Additionally, SSRs often display high polymorphism between individuals. Although SSRs libraries are difficult to make initially, once developed SSRs are relatively cheap and easy to use, requiring only small amounts of moderate quality DNA.

The goals of this study were to identify the pollen sources for seedlings obtained from a single tree and determine whether native pollinators in an open orchard setting carry pollen only short distances. This information will validate the utility of using open pollination to perform controlled crosses in flowering dogwood.

## Materials and Methods

Parentage analysis of flowering dogwood seedlings was conducted on seeds harvested from a single tree growing in the Dogwood Clonal Orchard at the University of Tennessee Forest Resources Research and Education Center's Arboretum in Oak Ridge, Tennessee (N 36.01526, W 84.18993). The orchard contains many cultivars of several *Cornus* species that range in age from five to 20+ years **(Fig. 2.1)**. Most flowering dogwood trees ranged from 4-5 m tall and 3-4 m wide, except 'Double White', which was about 2 m tall and 1.5 m wide, and 'Pygmy' which was about 1.5 m tall and 1 m wide. The study site was limited to an area encompassing a 15 m radius from the maternal tree, 'Sweetwater Red'. The numbers of inflorescences on trees surrounding 'Sweetwater Red' were similar except for 'Pygmy' and 'Double White', which had fewer inflorescences. Though essentially overlapping, the flowering period was not identical for each tree in the study area.

To find unique alleles for the putative parents, unopened flower buds were collected from *C. florida* cultivars **(Fig. 2.1)** during the fall of 2008. Flower buds were crushed with tweezers, placed into 1.5 ml microcentrifuge tube containing 2.3 mm silica beads (Biospec Inc., Bartlesville, OK, USA), frozen in liquid nitrogen and homogenized using a fastprep FP 120 at speed four for 20s (ThermoSavant, Holbrook, NY, USA). Genomic DNA was isolated using a DNeasy extraction kit (Qiagen,Valencia, CA, USA) following manufacturer's instructions. Three SSR primer pairs [CF 213, CF 585 and CF 581 (Wang et al. 2008; Wadl et al. 2008)] were used in this study **(Table 2.1)**. The final 10 μl polymerase chain reaction (PCR) contained 1μl 25mM $MgCl_2$ (Applied Biosystems, Foster City, CA, USA), 1μl 10× buffer, 1μl 2.5mM dNTPs 1μl 2.5 mM of each primer, 1μl DNA (4 ng), 0.06μl (0.3 units) Taq DNA polymerase (Applied Biosystems), and 4.94μl sterile water. The PCR conditions consisted of an initial denaturation at 94 °C for 10 min, followed by 35 cycles of 94 ºC for 40 sec, annealing at 56 ºC for 40 sec, 72 ºC for 30 sec and finally 72 ºC for 4 min. The amplified PCR products were separated and visualized on the QIAxcel Capillary Electrophoresis System (Qiagen). SSR regions amplified with PCR primers were screened for polymorphisms between the cultivars included in **Fig. 2.1**. A 25 bp size marker was used to determine the size of the amplicons.

For parentage analysis, 85 single-seeded fruits were collected in fall 2008 from 'Sweetwater Red' and the fleshy exocarp removed manually. Cleaned seeds were placed into re-sealable zipper storage bags containing a moist peat moss and sand mixture in a ratio of 1:1. These seeds were stratified at 4°C until germination, which occurred after four to seven months. Germinated seeds were planted into 7.5 $cm^2$ pots containing peat moss (Sun Grow Horticulture, Bellevue, WA, USA) between March and May 2009. Seedlings were kept in a greenhouse with ambient photoperiod and fertilized weekly with 150 ppm nitrogen using a liquid 20N-8.7P-16.6K fertilizer solution (J.R. Peters Inc. Allentown, PA, USA). The third pair of true leaves was

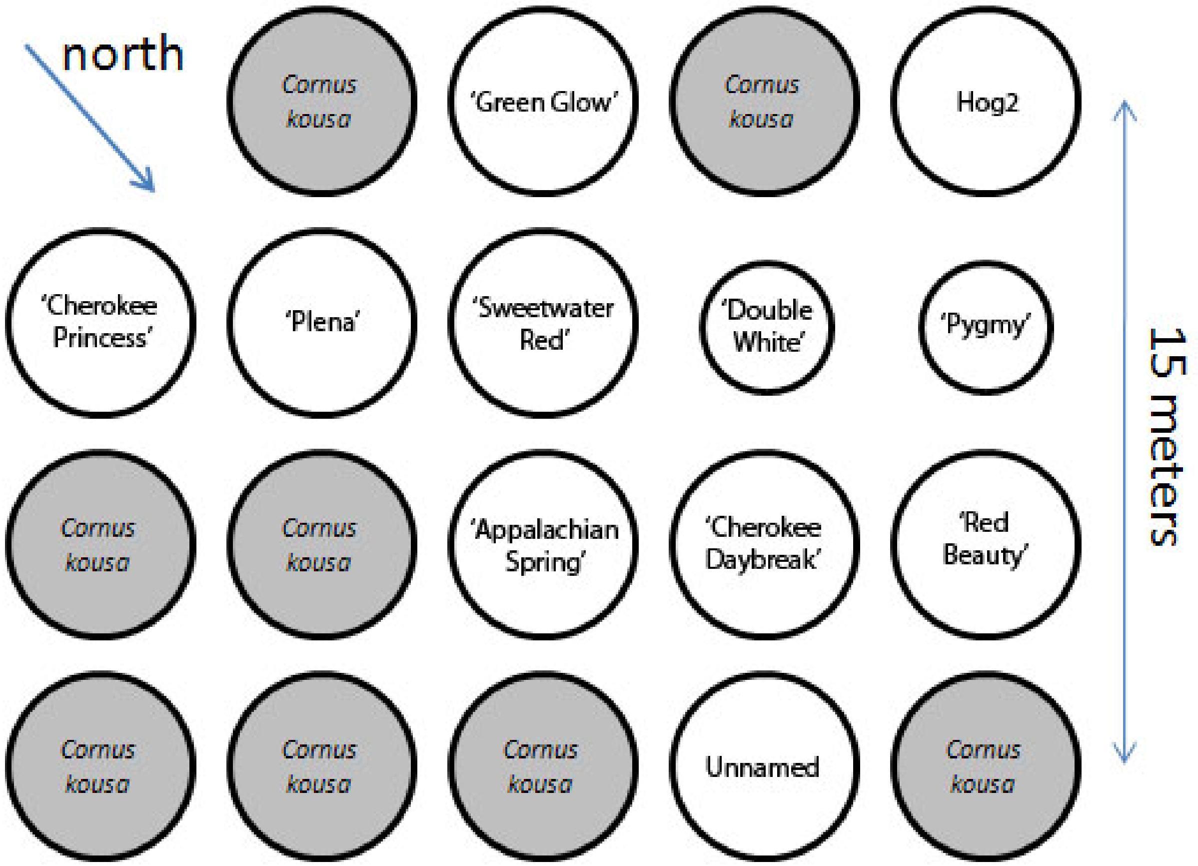

**Figure 2.1**. Schematic of the study area in the Dogwood Clonal Orchard at the University of Tennessee Forest Resources Research and Education Center's Arboretum in Oak Ridge, TN. Trees are planted on 5-m centers. Other than *C. kousa*, nearby species of *Cornus* include: *C. amomum*, *C. drummondii*, *C. mas*, *C. racemosa*, or *C. kousa* × *florida*.

**Table 2.1.** Genbank accession numbers and sequences for the primers used to amplify Simple Sequence Repeat regions and determine parentage of 45 *Cornus florida* seedlings.

| Primer | Genbank accession number | Primer sequence forward | Primer sequence reverse |
|---|---|---|---|
| CF 213 | ED651874 | 5′TTC TTG CAA ATG GTT ATT GAT TG 3′ | 5′ATA GGC GTC CAT TTG TTT CC 3′ |
| CF 581 | ER870603 | 5′GGG GCA GTA AGA AAA CAC ATT C 3′ | 5′TGT AAC CTG CAC ATA GAC AGC A 3′ |
| CF 585 | ER870607 | 5′AAC GAA GCA AGC AAA ACA ATC 3′ | 5′ACC CCA CCA CTT CAT CTC TCT 3′ |

harvested for DNA extraction. Leaves were placed into 1.5 ml centrifuge tube containing 70% ethanol for at least 24 h to remove chlorophyll prior to DNA extraction. Leaves were then removed from the ethanol and dried completely before tissue homogenization, as previously described. DNA was then extracted using the DNeasy extraction kit following the manufacturer's instructions except that 1.5% (w/v) polyvinylpyrrolidone (PVP) (Acros Organics, Geel, Belgium) was added to the AP1 buffer.

Seven seedlings were completely consumed by rodents and could not be analyzed. Although 16 other seedlings were damaged, DNA was recovered from the hypocotyls and remnants of the cotyledons. Following elution, 7.5µl of 5M NaCl was added for every 100µl of elute to limit co-precipitation of polysaccharides. DNA was than precipitated using 100% ethanol, the pellet was washed using 80% ethanol and then re-suspended in 0.1 TE (Tris-EDTA) buffer. All SSR loci were amplified using PCR with the primers listed in **Table 2.1**. PCR was performed in a 10µl reaction as listed above except primer concentration was increased from 2.5 µM to 5 µM and DNA concentration was decreased from 4 ng/µl to 2 ng/µl. The cycling conditions were identical to those listed above. PCR products were separated and visualized using a QIAxcel Capillary Electrophoresis System (Qiagen). SSR amplicon sizes in the seedlings were compared to those of the putative parents **(Table 2.2)**. Parental exclusion was used to determine parentage or conclude that the pollen came from a foreign source.

## Results

Sixty-seven (79%) of the 85 seeds harvested from ‘Sweetwater Red’ in fall 2008 germinated by June 2009. Post germination mortality attributed to rodent injury, thrips, and damping off disease left 45 seedlings from which DNA was extracted. Genotyping confirmed that 29 seedlings (65%) shared alleles with nine pollen donors that were located within 12 m of ‘Sweetwater Red’. Although the parentage of nine seedlings (20%) could not be conclusively determined with the SSR primers used, the parentage of these nine seedlings could be assigned to two or three other possible pollen donors located within 12 meters. Thus, in all cases it is likely pollen was donated from trees located nearby. Genotyping of the remaining seven (15%) seedlings demonstrated that pollen was carried in from dogwood trees located beyond the perimeter of the study area. Some trees were more likely than others to be pollen donors **(Table 2.3)**. More than half of the seedlings tested were attributed to two trees. ‘Pygmy’ sired no seedlings. Between one and three seedlings were associated with each of the remaining trees at the study site.

## Discussion

The majority of pollen was donated from trees located within the delineated study site, supporting prior evidence natural movement of *C. florida* pollen occurs over distances of 3 m or less (Ament et al., 2000). However, the most common pollen donors were over 11 m away. Two possible contributing factors may explain this result. The first possibility is that the stage of flowering of the pollen donor was more synchronized with stigma receptivity of ‘Sweetwater Red’ than the other potential pollen donors. Flowering times for dogwood trees used in this study were not identical. Asynchronous flowering between self-incompatible pear cultivars may reduce seed set (Sharifani and Jackson, 2001). Additionally, differences in flowering phenology can increase distances pollen is moved (Kitamoto et al., 2006). The trees most likely to donate pollen were those whose flowering period lagged slightly behind that of the maternal tree. In our study, difference in flowering period may be a contributing factor to successful pollen donation due to the protandrous nature of *C. florida* flowers. At the time petals open, anthers have already dehisced and are thick with pollen. Within a few hours pollen will be removed from the anthers by foraging insects and weather, though the stigma may remain receptive for several days (Malueg, 1998). So the period each tree is receptive to fertilization lasts longer than the period during which it has copious amounts of pollen to donate. Therefore, trees most likely to donate pollen to a breeding tree target are those that flower shortly after the maternal tree. The second possible source of discrepancy between observed pollen movement in this study as opposed to the previous study may be due to behavior and biology of potential pollinators of the tree at the study site. The most important pollinators of *C. florida* are probably andrenid and halictid bees (Mayor et al., 1999; See Part 1). Although these two families of bees are common visitors to flowering dogwood in east Tennessee, the proportions of these two families vary across locations (See Part 1). Halictid bees are more likely to display social behavior, which may lead to transfer of pollen between foraging individuals. For example, *Lasioglossum imitatum,* a very common visitor to the trees at the study site, is known to have multiple foraging females sharing the same nest (Michener and Wille, 1961). By contrast, Andrenid bees are very rarely social. A single solitary female typically excavates and provisions her own nest, rarely coming into contact with other conspecifics (Osgood, 1989). Bees sharing a communal nest may pass

**Table 2.2.** Alleles used in primary identification and expected size of amplicon produced by three simple sequence repeat loci in ten individuals of *Cornus florida*.

| Cultivar | Locus | | |
|---|---|---|---|
| | CF 213 | CF 581 | CF 585 |
| ‘Sweetwater Red’ | 168 | 159 | 177:191 |
| Unnamed | 157:175 | 159 | 178:194 |
| ‘Red Beauty’ | 160:167 | 159 | 184 |
| ‘Hog 2’ | 152:152 | 159 | 180:193 |
| ‘Cherokee Daybreak’ | 169:177 | 150:158 | 171 |
| ‘Cherokee Princess’ | 156:156 | 159 | 171 |
| ‘Double White’ | 168:168 | 152:159 | 178:194 |
| ‘Appalachian Spring’ | 165:188 | 150:163 | 177:191 |
| ‘Green Glow’ | 168 | 159 | 171 |
| ‘Pygmy’ | 168 | 159 | 182:194 |

**Table 2.3.** Numbers of *Cornus florida* progeny attributed to each possible father, their relative flowering state and the distance from the mother plant.

| Tree | Number of attributed progeny | Flowering state | Distance (m) from ‘Sweetwater Red’ |
|---|---|---|---|
| ‘Sweetwater Red’ | Mother tree | Just past peak | 0 |
| Unnamed | 10 | Peak | 11.2 |
| ‘Red Beauty’ | 6 | Peak | 11.2 |
| ‘Hog 2’ | 3 | Early peak | 11.2 |
| ‘Cherokee Daybreak’ | 3 | Past peak | 7.1 |
| ‘Cherokee Princess’ | 3 | Beginning flowering | 10 |
| ‘Double White’ | 2 | Early peak | 5 |
| ‘Appalachian Spring’ | 1 | Beginning flowering | 5 |
| ‘Green Glow’ | 1 | Well past peak | 5 |
| ‘Pygmy’ | 0 | Well past peak | 10 |

pollen between individuals through direct and indirect contact (Dag et al., 2001; Degrandi-Hoffman et al., 1992). Halictid bees were more common at the UT Arboretum than any other site surveyed (See Part 1). Thus, pollen collected from the paternal tree may be transported back to the communal nest by one bee and then moved to the maternal tree by another bee, having picked it up from her nest mate. The relative lack of highly social halictid bees at other sites may limit distance of pollen flow.

Although less numerous than andrenid and halictid bees, cerambycid beetles also visited the flowers of *C. florida* at this site, (See Part 1). Pollen carried by bumble bees (*Bombus* spp.) has been shown to have lower genetic diversity than pollen carried by flower beetles (Mordellidae), possibly because of the grooming action of bees (Matsuki et al., 2008). Bumble bees often groom their bodies during foraging, packing pollen into transport structures on their hind legs and making it unavailable for pollination (Thorp 2000). The relatively higher populations of cerambycid beetles may contribute to greater distances of pollen movement observed in this study.

Thirty-five percent of pollen donations were from two paternal trees reflects the short duration in which ‘Sweetwater Red’ was receptive to fertilization due to high temperatures (averaging 16.6 ºC) that occurred during flowering. In a previous study involving flowering dogwood, the flowering period was shortened from 23 to 10 days when temperatures were increased from 11.9 ºC to 16.9 ºC The length of time individual flowers remained receptive also decreased (Malueg, 1998). As a consequence of higher temperatures, trees that provided newly opened flowers at a time just after the peak flowering time for the maternal tree are the most physiologically probable pollen donors.

The distance pollen moved was greater in this study than recorded in previous work (Ament et al., 2000) and can be attributed to differences in flowering period between trees at the site and differences in pollinator assemblage. Despite the complications suggested by this report, using ‘open pollinated controlled crosses’ to breed flowering dogwood may be preferable to current alternatives. This method could be used by selecting trees for a controlled cross and sequestering them away from other sources of dogwood pollen. Most, if not all of the trees produced by this union will be the desired cross. The self-incompatiblity of *C. florida* makes this practice feasible because far fewer seedlings will need to be discarded, even without emasculating flowers. Successful utilization of this method will require the breeder to account for the relative flowering period between the trees to be crossed. Flowering time should be as close to overlapping as possible and seedlings should be taken from the first tree to flower. If possible, pollinators should be surveyed and a site chosen with a low abundance of halictid bees.

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# Conclusion

Andrenid and halictid bees are the most consistent and important pollinators of *Cornus florida* in east Tennessee. They were frequent visitors both years at every site and carried pollen on parts of their bodies likely to contact the stigma and deposit pollen. In some, though not all years, cerambycid beetles may have played an important role in pollination of this tree. Flies visited frequently in one year, although the amount of pollen carried by them tended to below compare to the bees. The assemblage of visitors varied substantially by site for *C. florida* though the presence of andrenid and halictid bees was consistent across all sites.

Halictid bees are also effective pollinators of *C. kousa,* but their importance is eclipsed by greater visitation by beetles. Scarab, soldier, and in one year, cerambycid beetles, functioned as substantial pollinators. However, we caution about making inferences regarding the relative importance of the beetle species as pollinators we observed on *C. kousa* trees. Additional research will be needed to quantify the presence of this beetle at multiple sites external to the UT Arboretum and the efficacy by which they transfer pollen to floral structures. Flies were also common visitors one year, but the lack of pollen found on their bodies implied that they probably function poorly as pollinators.

The distance pollen moved was greater in this study than recorded in previous work (Ament et al., 2000) and can be attributed to differences in flowering period between trees at the site and differences in pollinator assemblage. Despite the complications suggested by this report, using ‘open pollinated controlled crosses’ to breed flowering dogwood may be preferable to current alternatives. This method could be used in this way: trees could be selected for a controlled cross and sequestered away from other sources of dogwood pollen. Most, if not all of the trees produced by this union will be the desired cross. The self-incompatiblity of *C. florida* makes this practice feasible because far fewer seedlings will need to be discarded, even without emasculating flowers. Successful utilization of this method will require the breeder to account for the relative flowering period between the trees to be crossed. Flowering time should be as close to overlapping as possible and seedlings should be taken from the first tree to flower. If possible, pollinators should be surveyed and a site chosen with a low abundance of halictid bees Utilizing native bees for an open pollinated controlled cross of dogwoods may be feasible.

# Appendices

# Appendix A: Using Fourier Transform Infrared (FT-IR) Spectroscopy to Identify and Characterize Pollen from Six species of *Cornus*

## Introduction

Identification of pollen may be useful for a variety of reasons including forensics, (Horrocks et al., 1999) plant taxonomy, (Erdtman, 1952), allergy studies, paleontology and paleoclimatology (Jacobs, 1985), melissopalynology (Louveaux et al., 1978) as well as pollination biology (O'Rourke and Buchmann, 1991).

The pollen exine, or outer shell, has a wide range of shapes, sizes and sculpturing. Examination of morphology by light microscopy, sometimes supplemented with SEM, is the traditional method of pollen identification. Features of the exine that may be used to identify pollen include, apertures, or pores in the wall, sculptural elements or texture of the surface of the pollen, overall shape, size and presence and arrangement of furrows. A dichotomous key is used for tentative identification followed by comparison with a known sample for positive identification. The strong points of visual identification are the low cost of implementation, and general efficacy. However, it requires a great deal of skill and experience to use such identification keys properly and even when employed by a skilled operator, unknown pollen is often only resolvable to family or genus. Environmental conditions can make pollen grains shrink or swell, obscuring features used for identification. Even with the addition of expensive and time consuming high-resolution SEM images, many species may not be differentiated using visual methods.

There are three concentrically arranged layers to the angiosperm pollen grain. The central or deepest part is the protected living cell that germinates on the stigma. The middle part is known as the intine. It envelops the entire central cell in a sheath of consistent thickness. There is contention as to the composition and structure of the intine, though it is generally thought to consist of cellulose, callose, pectin, other pollysacharides, protein and small amounts of lipids and enzymes. (Fang et al., 2008; Castells et al., 2003) The protective, outer layer is known as the exine. The exine is exceptionally resistant to degradation and will frequently outlast the intine and cellular cargo within. Different species of pollen have varying, but extraordinary resistance to heat as well as concentrated acids and bases.

The substance responsible for this resistance of the exine is known as sporopollenin. Sporopollenin is an oxygenated hydrocarbon with a chemical composition between $C_{90}H_{134}O_{20}$ and $C_{90}H_{150}O_{33}$ (Zetzsche et al., 1937). Though the incredible resistance of this substance has aroused curiosity for quite some time, the actual structure and formation of sporopollenin remains a mystery. There is evidence that sporopollenin could be formed by oxidative polymerization of carotenes and carotene esters (Brooks and Shaw, 1968). However, inhibiting carotinoid biosynthesis in *Curcubita pepo* had little effect on the sporopollenin coat (Prahl et al., 1985). Regardless of the structure and formation of sporopollenin, it has been demonstrated through $^{13}C$ NMR that sporopollenin is a class of substances and not a single unique molecule (Guilford et al., 1988). Differing levels of resistance to corrosion may be a product of differing qualities of sporopollenin like differing proportions of different monomers or different degrees of polymerization. It is the structure and composition of the exine that is represented in FTIR spectra. Unique spectra suggest unique structures and compositions.

Laucks et.al. (1999) made the first attempts to chemically characterize pollen grains using Raman spectroscopy. Pollen electrodynamically trapped from the atmosphere may be differentiated using a 785nm laser to reveal differences in the 400-1600 $cm^{-1}$ range. Pappas et al. (2003) were the first to use Fourier Transform Infrared Spectroscopy (FT-IR) to identify unknown samples based on library spectra collected from known pollen. When compared to traditional morphological identification techniques, spectroscopy offers the benefits of easier, cheaper sample preparation and higher throughput sample analysis with similar accuracy.

The first attempt to identify pollen using Raman spectroscopy identified broad, obvious differences in the spectra, easily visible to the eye. As differentiation was attempted between larger sets of more similar spectra, two broad approaches have been used to elucidate and characterize this data. One method is to use a computer program to give a direct estimation of which spectra is the closest match to the unknown (Gottardini, 2007; Pappas, 2003) and the second approach is the use of principal components analysis (PCA) in conjunction with hierarchical cluster analysis (HCA) (Dell'anna, 2009; Ivleva, 2005; Schulte, 2008).

It is unknown if different species within the genus *Cornus* may be differentiated using FT-IR spectroscopy in conjunction with PCA and cluster analysis. Furthermore it's possible the chemical construction and composition of the pollen exine will reflect the phylogeny of the genus and may be more similar in closely related species.

## Materials and Methods

In the spring of 2009, flowers were gathered as blooming occurred from 6 species of dogwoods and 19 individuals from the following three locations:

The UT arboretum is a 250-acre facility that has over 2,500 native and exotic woody plant specimens representing 800 species, varieties, and cultivars. This facility is on the outskirts of Oak Ridge, TN. Woodlands with mixed hardwoods surround the ornamental plantings. There are extensive plots of *C. florida, C. kousa* and *C. mas*. The trees used for sampling were arranged in an orchard configuration on five meter centers.

The UT Gardens are a collection of a wide variety of ornamental plants spread out over 7 acres on the Agriculture Campus of the University of Tennessee in Knoxville, TN. Semi-urban areas as well as woodlands surround the gardens.

The Urban Landscape and Nursery Research (ULNR) facility, also on the Agriculture campus of the University of Tennessee in Knoxville, houses container grown dogwood specimens. The ULNR compound is a one-half acre production facility comprised of bow houses, enclosed greenhouses and open ground. In addition to dogwoods used in breeding efforts, a variety of woody ornamental and perennial plant species are grown here. Dogwood trees from which inflorescences were collected were being grown in a 100 percent pine bark soilless substrate in three gallon (11.4L) nursery containers under 70 percent shadecloth and irrigated as needed by overhead irrigation.

After sampling, flowers were placed in a desiccator and left there for 5 days until dry. Flowers were then sealed and stored at -20°C until spectra were gathered. Samples were frozen to inhibit fungal growth that can occur, even at low temperatures. (Baxter and Illston, 1980)

### Microscopic Analysis

Samples of pollen were viewed on a light microscope to compare their shape, size and other morphological features such as pore placement and exine sculpturing. Pollen was taken from unopened but nearly mature flowers. Flowers were opened with forceps and the pollen was allowed to fall onto a clean glass microscope slide. A drop of silicon oil was used as a matrix for embedding. Pollen was photographed at 400x.

### FT-IR sampling

FT-IR spectra were gathered on a Spectra One (Perkin Elmer; Waltham, MA) equipped with the 'golden gate' sampling accessory. Stored flowers were allowed to come to room temperature before opening. Pollen was taken from unopened flowers exhibiting a range of developmental states, though flowers nearest opening produced much more pollen. To sample pollen, flowers were removed from inflorescences. Petals were then peeled back with forceps and the pollen was tapped into the sampling chamber. A 10x hand lens and a single hair brush were used to remove any debris from the pollen such as shards of petals and anthers. Absorbance in the 4000-650cm$^{-1}$ range was sampled. Eight scans were averaged top produce each spectra. A minimum of five spectra were gathered for each individual.

Data transformation and analysis was done using Unscrambler computing software (Camo, Princeton, NJ). Data were normalized using mean normalization. Scatter effects were then removed using multiplicative scatter correction. Individual spectra were than observed visually and anomalous spectra were removed from further analysis. PCA was then performed on the remaining spectra.

# Results

### Microsopic observation

**Figure A.1** displays photographs of dry pollen from six *Cornus* species averaging about 40x30 microns. All species are tricolporpate (three elongated-round apertures situated equatorially) and subtringular in polar view, oval compressed (prolate) in equatorial view. Sculpturing was not visible at this magnification. There is a clear similarity between pollen grains of morphologically similar dogwood species.

### FT-IR Spectroscopic study

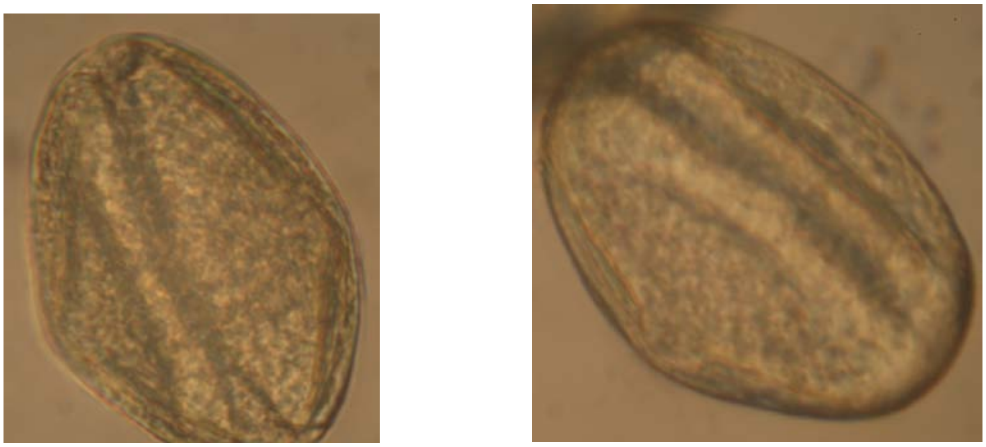
FT-IR spectra produced by averaging all samples for a given species are displayed in **figure A.2.** Though they are similar, differences are evident in several peaks (**Table A.1**). Differences between species may be a product of differences in relative abundance or structure of various precursors to sporopollenin or differing degrees of polymerization.

Though differences in average spectra are evident between species of *Cornus*, individual sample variation was quite high. Therefore differences in peak intensity, visible in the graph above, were not useful in differentiating the spectra of one species from another. Furthermore the visual aspects of average spectra are not more similar in closely related species.

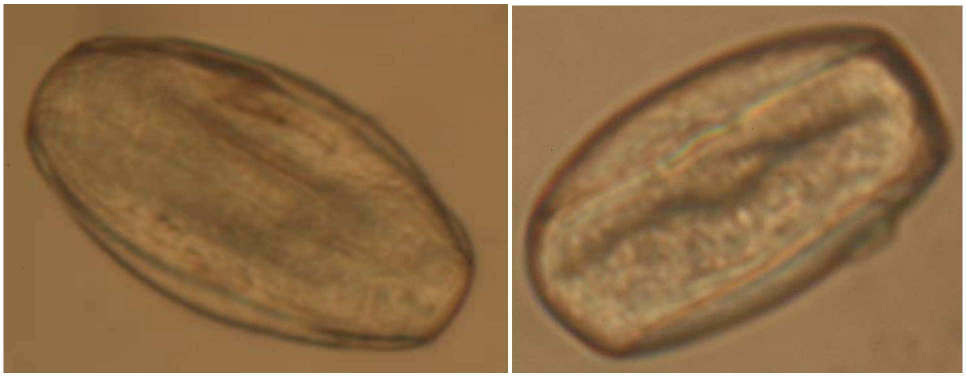
### PCA and Cluster Analysis

Principal components analysis was performed on spectral data to determine if any subtle, consistent differences in spectra could be elucidated. Sample separation was greatest on PCs 1, 2 and 5 (**Figure A.3**). There was some clustering by both species and morphologic group. However, hierarchical cluster analysis did not present any real meaningful groupings. (data not displayed) This may suggest the chemical composition and construction of the exine is broadly similar across the genus *Cornus*. It is also possible individual sample variation was too high for cluster analysis to work; possibly because samples were taken from trees growing differently in different places. Disease pressure, nutritional discrepancies, and environmental differences could have all affected the pollen in ways that could have changed the spectra.

# Figures and Tables



*C. amomum* *C. asperfolia* var. *drumondii*



*C. florida* *C. kousa*

*C. mas* *C. officianalis*

**Figure A.1.** Microscopic photographs of pollen from the six *Cornus* species (400x).

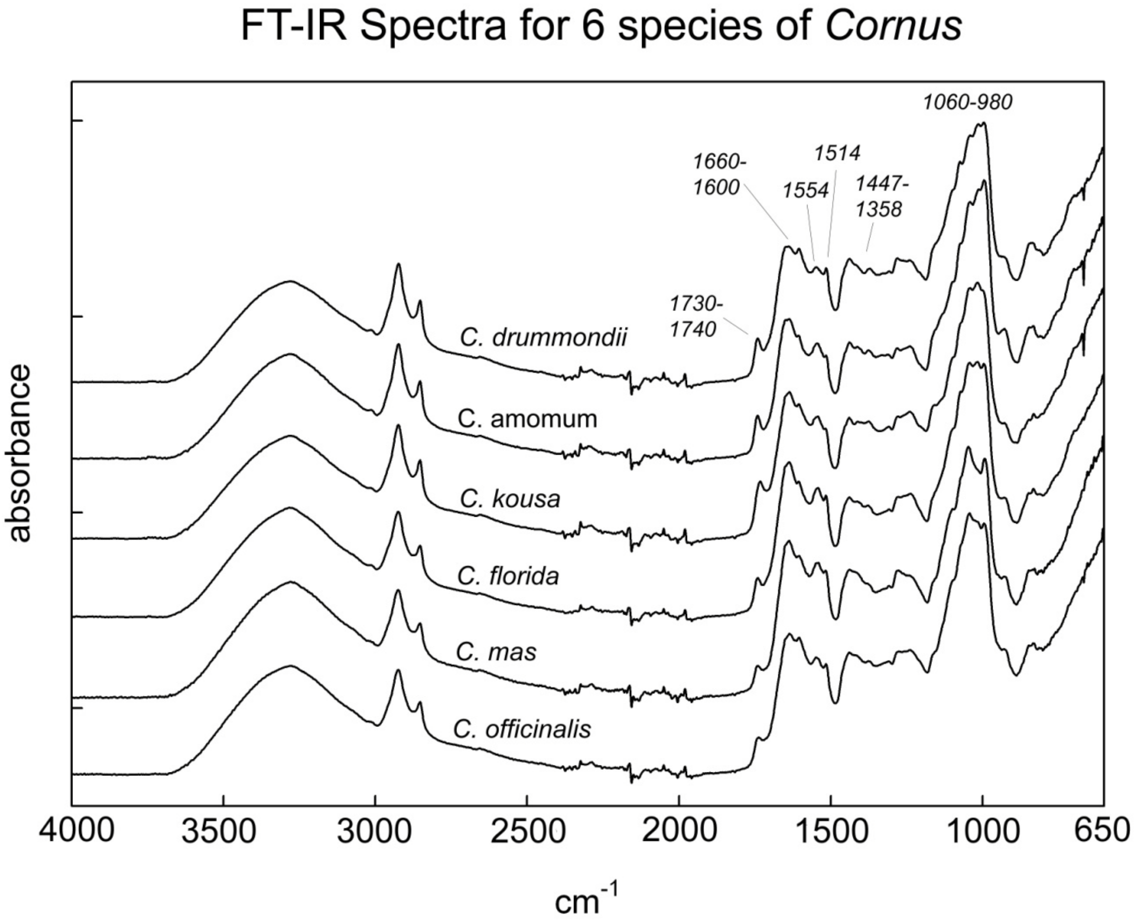


**Figure A.2.** Spectra representing an average of all samples for a given species of *Cornus*

**Table A.1.** Regions where differences were detected in spectra of dogwood pollen and the chemical bonds attributed to differences in spectra.

| Regions of interest | Chemical bonds possibly responsible |
|---|---|
| 1740-1730 | C=O |
| 1660-1600 | C=C |
| 1554 | Aromatic rings or conjugated bonds |
| 1514 | C-C |
| 1447-1358 | C-H; methyl or methylene |
| 1060-980 | C-O |

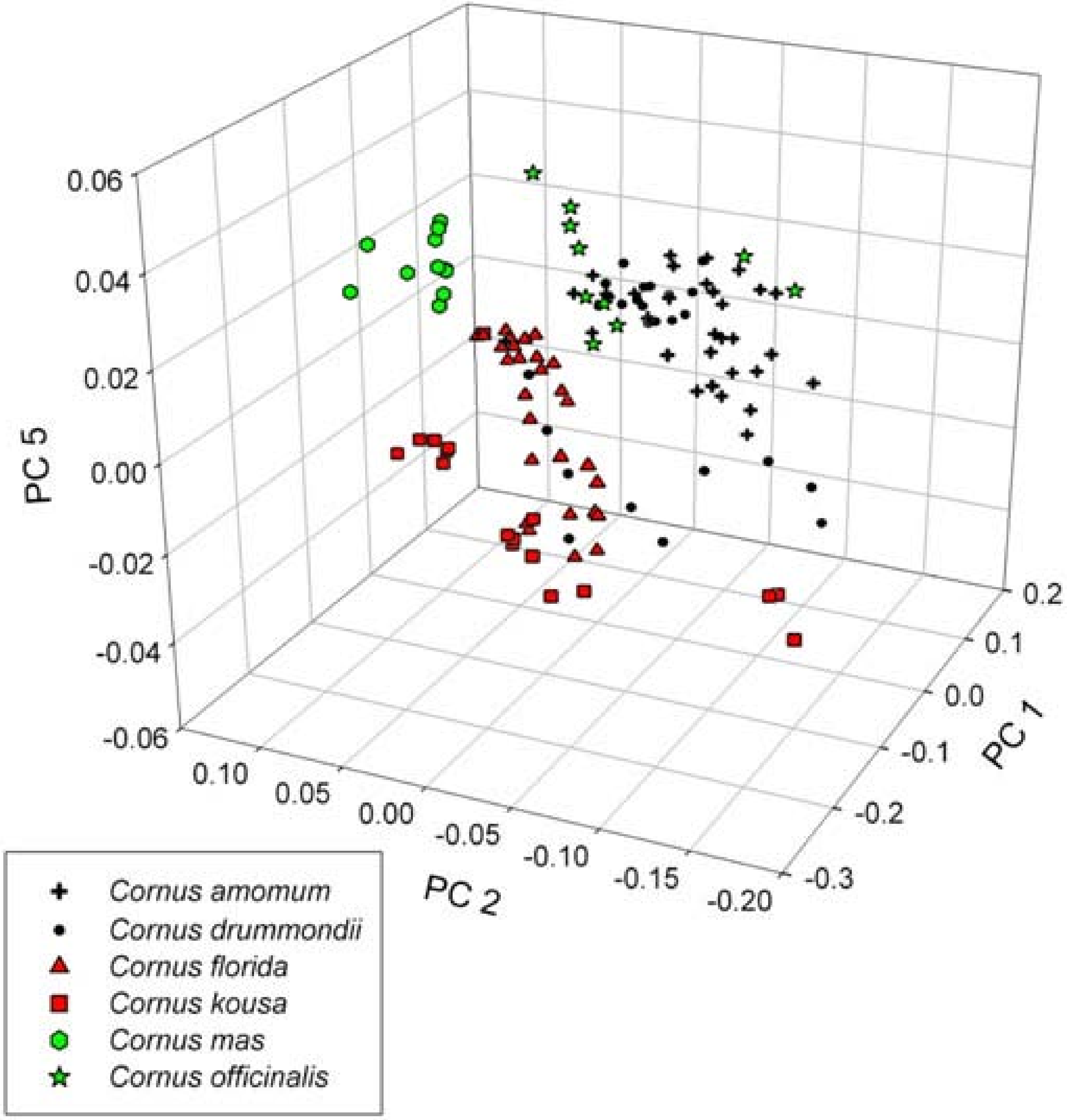


**Figure A.3.** Scores for principal components 1, 2, and 5 by species of *Cornus*.

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# Appendix B: A Survey of Floral Volatiles Emitted From Six Species of *Cornus*

## Introduction

Floral scent, which is expressed in the form of volatile chemicals, is known to affect insect visitation to flowers (Faegri and van der Pijl, 1979; Pellmyr, 1986). Even plants with no discernible scent to human olfaction may emit volatiles in large enough quantities to be detected by a potential pollinator (Chen et al., 2003; Zhuang et al., 2008).

Floral scent is an extremely variable aspect of the plant phenotype with flowers emitting from a very few to over one hundred volatile compounds. Despite this chemical diversity, emitted compounds all presumably share the same function of attracting pollinators (Knudsen and Gershenzon, 2006).

Volatile chemicals originate from epidermal cells of various floral parts, especially petals. In the case of snapdragon (*Antirrhinum)* there is evidence that concentrations of volatiles increase along the path that bees follow to reach a nectar source, suggesting one possible role scent plays in guiding pollinators (Pichefsky and Gershenzon, 2002).

When different plant species exploit the same group of pollinators, convergent evolution is thought to produce similar floral features. Floral scent is seen as a component, along with floral color and shape, of the pollination syndrome that is characteristic of a particular pollinator group. A pollination syndrome is the unique combination of floral features attractive to a certain group of pollinators (Faegri and van der Pijl, 1979). Differences in volatiles emitted may help explain the taxonomic differences in floral visitors to *C. kousa* and *C. florida.*

Within the genus *Cornus*, floral volatiles have only been sampled from *C. florida*. Nothing is known about volatiles from *C. kousa* or other *Cornus* species. Six major compounds (E-beta-ocimene, linalool, decanal and nonanal, ketoisophrone, and 3-formyl-pyridine) were found emanating from floral units of *C. florida*. Floral units include flowers, bracts, pedicles, peduncle and a portion of the stem (Zhuang et al., 2008).

Decanal and Nonanal, compounds most likely produced by stem cells, have been found in a narrow variety of plants (Knudsen et al., 1993). E-beta-ocimene and linalool are very common constituents of floral volatiles, found in many bee, beetle and butterfly pollinated plants (Raguso and Pichersky, 1999). Ketoisophrone, and 3-formyl-pyridine have not been previously recorded as flower volatiles (Knudsen et al., 1993).

The genus *Cornus* includes about 45 species of trees, shrubs and herbaceous plants. This genus, commonly referred to as dogwoods, is distributed primarily throughout eastern Asia, North America and Central America. Two species are native to the Andean region of South America and one species may be found in the mountains of East Africa (Dale and Greenway, 1961; Macbride, 1929). The species of this genus contrast remarkably similar floral and foliar morphology with widely varied growth habits and inflorescence structure (Murrell, 1993).

Both molecular and morphological evidence support the monophyly of the genus but the relationships of subgroups within the genus has been the subject of some contention (Eyde, 1988; Murell, 1993; Xiang, 2001; 1998; 1993). Most have divided dogwoods into a number of subgenera, but some have split *Cornus* into several distinct genera (Eyde, 1987). The four main morphological divisions in the genus are Blue- or White-Fruited Dogwoods, Cornelian Cherries, Big-Bracted Dogwoods and Dwarf Dogwoods.

To date, nothing is known about volatile compounds produced by *C. kousa* flowers or flowers of other ecologically important *Cornus* species. In order to address this knowledge gap, and to obtain a better understanding of how these chemical volatiles might interact with key arthropods that affect pollination and fruit set in dogwoods, we selected species representative of the Blue or White fruited Dogwoods (*C. asperifolia* Michx. var. *drummondii* C.A. Mey, *C. amomum* Mill. and *C. sericea* L.), Big Bracted dogwoods (*C. kousa* (Buerger ex Miq) Hance) and Cornelian Cherries (*C. officinalis* L**.** and *C. mas* L.). Flowers of these key dogwood species were sampled to determine how phylogenetic distance based on genetic and morphological evidence are related to emitted volatiles

Floral volatiles were sampled from 6, previously unsampled, species of *Cornus* in order to better understand the function scent has in shaping the pollinator assemblage of *Cornus florida* and *C. kousa* and to examine the role phylogenic similarity has on the volatile profile.

## Materials and Methods

Floral and stem volatiles were collected in 2009, from inflorescences of 6 species using 24 individual plants at 3 locations in eastern Tennessee.

Flowers of *C. mas C. amomum, C. asperfolia* var. *drummondii* and *C. kousa* were collected from at the UT Arboretum in Oak Ridge, TN (lat 35.9975 lon -84.2197). The UT Arboretum is a 250-acre facility housing more than 2,500 native and exotic woody plant specimens, representing 800 species, varieties, and cultivars as well as native woodlands comprised of mixed hardwood trees surrounding the ornamental plantings. There are extensive plots of C. *florida* and *C. kousa* and *C. mas*. Trees used for sampling were arranged in an orchard configuration with five-meter centers.

*Cornus officianalis* and *C. mas* and *C. asperfolia* var. *drummondii* flowers were collected from the UT Gardens, on the Agriculture Campus of the University of Tennessee in Knoxville, Tennessee. (lat 35.9446; lon -83.9383) These gardens house a wide variety of ornamental plants spread out over 7 acres. Semi-urban areas and woodlands surround the gardens.

*Cornus amomum*, *C. asperifolia* var. *drummondi*, and *C. sericea* flowers were collected from container grown specimens, maintained in the Urban Landscape and Nursery Research (ULNR) facility also on the Agriculture campus of the University of Tennessee in Knoxville. The ULNR compound is a one-half acre production facility comprised of bow houses, enclosed greenhouses and open ground. In addition to dogwoods used in breeding efforts, a variety of woody ornamental and perennial plant species are grown here. Dogwood trees from which inflorescences were collected were being grown in a 100 percent pine bark soilless substrate in three gallon (11.4L) nursery containers under 70 percent shadecloth and irrigated as needed by overhead irrigation.

Inflorescences, along with associated stems, were cut between 8:00a.m. and 9:00a.m., placed in flasks containing 250ml of distilled water and transported to the laboratory for volatile analysis. Each inflorescence contained 15-30 flowers, depending on species. At the time of collection about half of the flowers in each inflorescence were open. Approximately 8cm of stem tissue was included in every replicate. Samples of *Cornus amomum*, *C. drumondii*, and *C. sericea* included leaves in addition to the stem and floral units; *C. kousa* included bracts and leaves.

Emitted volatile compounds were collected in an open headspace sampling system (Analytical Research Systems, Gainesville, FL). The headspace collection system consisted of a 30cm tall by 10cm wide glass cylinder with a sealed removable lid. Charcoal purified air entered the chamber at 0.8L/min from the top through a Teflon hose. Volatiles were collected in four-hour periods by a SuperQ volatile collection trap (Analytical Research Systems, Gainesville, FL) that was attached to the outlet from the headspace collection chamber. At the conclusion of the collection period, the volatile collection trap was eluted into a vial with 100µl Methylene Chloride with 1-Octanol added as an internal standard.

GCMS analysis was done with a Shimadzu (Kyoto, Japan) 17A gas chromatograph coupled to a Shimadzu QP5050A quadrupole mass selective detector. The associated software tentatively identified any compounds detected. (GCMS operational SPECS)

## Results and Discussion

Although there was considerable variation in the assemblage of chemical volatile compounds detected among individuals of the same species, only compounds detected in all or nearly all of the individuals sampled are listed **(Table A.1)**.

Floral volatiles may be divided into seven broad categories based on their supposed biosynthetic origin. Five of the seven chemical classes were represented in the dogwood species sampled. Of these five, benzenoids, terpenoids and aliphatics were the best represented and presented the greatest diversity of compounds. Dominance of these three classes is typical as the vast majority of floral volatiles isolated in all plants fall into one of these three classes (Knudsen and Gershenzon, 2006).

Regardless, one chemical class will often compose the majority of floral volatile emission. This dominance of a certain chemical class is often different, not only between species, in a genus but often among sub-species and varieties (Dobson, 2006).

Terpenoids and Aliphatics were the only chemical classes to be found in every species sampled. Dominance of terpenoids in floral scent is common among bee pollinated plants as well as among flowers of plants preferred by some fly species (Dobson, 2006; Knudsen et al., 2001).

Dogwoods are considered to have an essentially generalist pollination strategy, flowers are readily available to any visitor and do not require any special behavioral adaptations or physical manipulations that influence some plant/pollinator strategies. However, bees seem more important as visitors to dogwood and may drive selection for scent chemistry more strongly. (See Part 1) Among bee pollinated plants, terpenoids and occasionally benzenoids tend to dominate floral scent (Dobson, 2006). In this respect the blue- and white-fruited and big-bracted dogwoods are unusual. In these dogwoods, bees appear to contribute significantly to pollination, yet only *C. kousa* emits a terpinoid/benzinoid mixture typical of bee pollinated plants.

Of all flowers from the species sampled, *Cornus mas* and *C. officianalis* flowers shared the most emitted compounds. All eight of the primary volatiles emitted by *C. mas* are shared by *C. officianlis* and 66% of the compounds found emanating from *C. officianalis* are shared by *C. mas*, more compounds than any other species. These two species were very similar in the assemblage of volatiles emitted as well as the compounds dominating floral scent. The largest component of floral volatiles emitted from both *C. officianalis* and *C. mas* is the terpenoid farnesene. Farnesene is a very common component of volatile emissions, often found in generalist pollination systems (Dobson, 2006). The aliphatic compound, pentatonic acid was also consistently present is significant amounts.

The similarity of the emitted floral volatiles between *Cornus officinalis* and *C. mas* may reflect their phylogenetic and geographic proximity. Fossil and molecular data analyses suggest the split between *C. mas* and *C. officinalis* was relatively recent (Xiang et al., 2006). Additional similarities in morphology, genetic sequences of 26s rDNA, *rbcL* and *matK*, suggests these two species are closely related; more so than any other pair of species examined (Xiang et al., 2006; Xiang et al., 1998; Fan and Xiang, 2001).

In contrast, differences in the assemblage of emitted volatiles do not reflect phylogeographic and phylogenetic similarity as clearly for the other five species sampled. This may be because none of these species are as closely related as *C. mas* and *C. officianalis*. Both ITS and *matK* sequence data place all the remaining species rather far apart with regard to phylogeny (Xiang et al., 2006). Although *C. asperfolia, C. serecea* and *C. amomum* are all North American species, they belong to distinctly different clades within the larger subgenus *Kraniopsis*. Similarly, *C. florida* and *C. kousa* are considered counterpart species and both fall into the 'Big-Bracted' division of *Cornus,* they also belong to separate subgenera and ITS and *matK* sequences are not overwhelmingly similar (Capiello and Shadow 2005, Xiang et al., 2006).

The collection of individual compounds do not serve to separate the blue-fruited and white-fruited dogwoods, however, there are consistent trends demonstrated within the classes of compounds emitted at the highest concentrations. All three of the white-fruited species emitted high volumes of an aliphatic compound. In *C. sericea* and *C. amomum,* 2-heptanol was the largest constituent of floral volatile emission. In most individuals of *C. asperifolia* var. *drummondii*, the closely related compound nonanol was emitted at very high concentrations, while in other individuals 3-Formyl-Pyridine was emitted at higher rates.

The phylogenic distance between *C. florida* and *C. kousa* is greater than any other grouping in this study (Xiang et al., 2006). This is clearly expressed in the volatile profiles of the two species for which there is little overlap of individual emitted compounds. Even when looking at compounds with high rates of emission, the only commonality is found in the terpenoids. Frequently, the most important emitted compounds in a species are entirely absent from its counterpart.

In conclusion, floral volatiles may change more quickly than other morphological features of the plant. Species very close together phylogenetically may share many volatiles but the volatile profile can quickly diverge, even in species where other aspects of the phenotype remain strikingly similar.

It's unknown what role volatile emission plays in the different pollinator assemblages found visiting *C. florida* and *C. kousa*. However, given the markedly different volatiles emitted, it's possible this does serve to encourage or discourage certain visitors.

**Table B.1.** A list of emitted compounds found in seven species of dogwoods growing in eastern Tennessee. Grey shading indicates compounds emitted in significantly higher amounts.

| | *C. officinalis* | *C. mas* | *C. sericea* | *C. amomum* | *C. drummondii* | *C. kousa* | *C. florida* |
|---|---|---|---|---|---|---|---|
| Aliphatics | **Y** | **Y** | **___Y___** | **___Y___** | **___Y___** | **Y** | **Y** |
| Pentanoic acid | Y | Y | N | N | N | N | N |
| 2-Pentadecyn-1-ol (CAS) | Y | N | N | N | N | N | N |
| 7-Tetradecenal, (Z)- | Y | N | N | N | N | N | N |
| Pentadecane | Y | Y | N | N | N | N | N |
| Pentanoic acid | Y | Y | N | N | N | N | N |
| Hexadecane | Y | Y | N | N | N | N | N |
| Eicosane | Y | Y | N | N | N | N | N |
| 2-Heptanol | N | N | ___Y___ | ___Y___ | Y | N | N |
| Nonanol | N | N | Y | Y | ___Y___ | Y | Y |
| Decanal (CAS) | N | N | N | Y | Y | Y | Y |
| (E)-4,8-Dimethyl-1,3,7-nonatriene | N | N | N | N | N | Y | N |

| Benzenoids | **Y** | **Y** | **Y** | **Y** | **Y** | **___Y___** | **N** |
|---|---|---|---|---|---|---|---|
| Benzoic acid | Y | Y | N | N | N | N | N |
| Benzaldehyde | N | N | Y | Y | N | N | N |
| Homomenthyl salicylate | N | N | Y | N | N | ___Y___ | N |
| 1,2-Benzenedicarboxylic acid | N | N | Y | Y | Y | N | N |
| Benzeneethanol | N | N | N | Y | N | N | N |
| 2-Ethylhexyl salicylate | N | N | N | N | N | ___Y___ | N |
| Benzene, 1,3,5-trimethoxy- | N | N | N | N | Y | N | N |

| Nitrogen containing compounds | **Y** | **Y** | **N** | **N** | **___Y___** | **N** | **___Y___** |
|---|---|---|---|---|---|---|---|
| L-Isoleucine, methyl ester | Y | Y | N | N | N | N | N |
| 3-Formyl-Pyridine | N | N | N | N | ___Y___ | N | ___Y___ |
| 2-Propenamide | Y | Y | N | N | N | N | N |

| **Terpeniods** | **___Y___** | **___Y__** | **Y** | **Y** | **Y** | **___Y___** | **___Y___** |
|---|---|---|---|---|---|---|---|
| Sabinene | Y | N | N | N | N | N | N |
| Farnesene | ___Y___ | ___Y__ | N | Y | N | ___Y___ | N |
| d-Nerolidol | Y | N | N | N | N | N | N |
| 2,6,10,14,18,22-Tetracosahexaene | N | N | Y | N | Y | N | N |
| 1,3,6-Octatriene, (Trans-Ocimene) | N | N | N | Y | N | ___Y___ | Y |
| Linalool | N | N | N | N | N | Y | ___Y___ |

|  | *C. officinalis* | *C. mas* | *C. sericea* | *C. amomum* | *C. drummondii* | *C. kousa* | *C. florida* |
|---|---|---|---|---|---|---|---|
| **Miscelanous cyclical compounds** | **N** | **N** | **N** | **Y** | **Y** | **N** | **___Y___** |
| alpha.-Cubebene | N | N | N | Y | N | N | N |
| Trans(.beta.)-Caryophyllene | N | N | N | N | Y | N | N |
| alpha.-Humulene | N | N | N | N | Y | N | N |
| Ketoisophorone | N | N | N | N | N | N | ___Y___ |

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# Vita

Paul Raymond Rhoades was born in Table Grove, Illinois and grew up in Kennesaw, Georgia, graduating from Harrison High School in 1998. He received a B.S. in Geology from The University of Georgia in 2003. He proceeded to hold a series of jobs including work on an organic farm, as a landscaper and as a whitewater river guide.

In 2007 he came to Knoxville Tennessee and worked as a Greenhouse manager for the department of Entomology and Plant Pathology, beginning master work shortly thereafter.