

Pollination Biology of *Ailanthus altissima* (Mill.) Swingle (Tree-of-Heaven) in the Mid-Atlantic United States

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ABSTRACT

To date little information has been collected on the pollination biology of *Ailanthus altissima* (Mill.) Swingle (tree-of-heaven), an invasive exotic in the U.S. This study was conducted to determine the insect pollinator fauna visiting *A. altissima* and to study general pollinator visitation patterns associated with the tree's nectar profile. A list of taxa visiting trees within each of three sites was developed from collected insects. Overall, visitor assemblage was dominated by the soldier beetle *Chauliognathus marginatus* with large numbers of ants in the genera *Formica*, *Prenolepis*, and *Camponotus*. No major diurnal pattern was found for visitation of insect pollinators using instantaneous counts. The nectar composition, concentration, and amount of total sugars in the flowers of *A. altissima* and how these are related to tree gender and time of day were determined. Nectar was found to be sucrose-dominant with lower, but nearly equal amounts of fructose and glucose. Total amounts of sugar in male and female blossoms were not statistically different, however higher concentrations of sugar were found in males (40.7%) than in females (35.3%). No difference was found over time. Nectar production and removal in trees was studied by comparing bagged flowers with flowers open to insect visitation. Bagged flowers were higher in overall sugar than open flowers, however, this was not constant across all times and gender.

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CHAPTER 1. Introduction.

With an increase in global trade and travel, the introduction of nonindigenous species has steadily risen over time. The cost of preventing and controlling these invasive species has likewise risen with estimated costs of \$137 billion per year (Pimentel et al. 2000). Knowledge of the basic biology and ecology of these organisms can lead to more efficient management and control. Often forgotten, mutualisms such as those between plants and pollinators provide numerous valuable services, including aiding in the pollination of about a third of the world's crop production. Research on these mutualisms is necessary for understanding and preventing the establishment and spread of invasive plant species.

Classification

Ailanthus altissima (Miller) Swingle is a member of the Simaroubaceae family, a fairly heterogeneous taxon and comprised of about 30 genera and 200 species (Brizicky 1962). The genus *Ailanthus* is primarily tropical with the range including southeastern Asia and the Pacific islands (Davies 1942, Brizicky 1962, Hu 1979). The name *Ailanthus* is derived from the native Moluccan name of *ailanto*, meaning “sky tree” or “tree of heaven”, referring to its height and rapid growth. *Ailanthus* has many common names, including tree-of-heaven, heaven-wood, false varnish tree, Chinese sumac, paradise tree, copel tree, and stink tree (Davies 1942).

History

Ailanthus altissima is native to China where it is known as chu'un shu. The Chinese name for *Ailanthus* is found as far back as the first encyclopedia on natural history and cultural objects in China (Hu 1979). It is deeply intertwined in the country's cultural history and is a sign of the coming spring. While commonly thought of as a pest in the United States today, in China *Ailanthus* is grown purposely. It is harvested for its wood, but also for its roots, leaves, and bark, which are used to make medicine for a variety of ailments. It has been used for centuries to treat dysentery and intestinal hemorrhage, and has recently been found to contain compounds effective against malaria (Peigler 1993).

The pollen of *Ailanthus* was shown as a possible allergenic source in one study (Ballero et al. 2003). *Ailanthus* is also used as a food source for the ailanthus silk moth or cynthia moth, *Samia cynthia* Drury (Lepidoptera: Saturniidae), for silk production.

The seeds were first carried from Peking to Paris in the 1740s by Pierre d'Incarville, a Jesuit priest in the China mission. d'Incarville was in search of economical plants to grow in the colonies in the Americas, which have a similar climate to China. The exportation of *A. altissima* was accidental, as d'Incarville mistook the tree from a distance for the Chinese varnish tree (*Rhus verniciflua*) of the Yangtze region (Hu 1979). This mistake would confuse the botanical literature in Europe for several decades. Herbarium specimens and seeds were sent to Bernard de Jussieu, the Superintendent of the Jardin Royal des Plantes, Paris in 1751 (Swingle 1916).

Once in Paris, some of the seeds of *A. altissima* were planted by Jussieu while the rest were sent on to be planted in England by Philip Miller, Superintendent of the Physic Garden at Chelsea, and Philip C. Webb in Busbridge in 1751. *Ailanthus* first came to the United States from England in 1784 when it was planted in the garden of William Hamilton in Philadelphia. Made popular by its luscious foliage, pollution tolerance, and rapid growth, *Ailanthus* was commonly planted in industrial centers such as New York City, Baltimore, and Boston. While it was at first only planted as an ornamental, it was later used in certain areas for afforestation. *Ailanthus* may also have been imported into California and other western states by way of Chinese immigrants during the building of the western rail systems (Feret 1985). The ailanthus silk moth (*Samia cynthia*) was first introduced to North America in Philadelphia in 1860 in an attempt to use it for silk production with its introduced host tree. This endeavor was unsuccessful, and the moth has declined significantly (Peigler 1993). This decline is possibly due to parasitoids, pollution, and the introduction of multiflora rose (*Rosa multiflora*), which provides habitat for avian predators of the moth (Frank 1986).

First reports of *Ailanthus* becoming invasive in Virginia are found in 1888, and it had become a pest in North Carolina by 1955 where it was concentrated in the piedmont,

mountains, and northern coastal plain (Curtiss 1888, Patterson 1976). Currently, *Ailanthus* is found in the U.S. in 42 states and is naturalized in much of the temperate and meridional zones of the world (Kowarik 1995, Brizicky 1962, Hu 1979). American *Ailanthus* seedlings have been shown to be genetically different from Chinese *Ailanthus* seedlings and are as genetically variable. Genetically different subpopulations can be distinguished in American *Ailanthus* (Feret et al. 1974). Inbreeding depression has not been found to occur in American *Ailanthus* seedlings (Feret and Bryant 1974).

Morphological Characteristics

Ailanthus is a deciduous tree that can reach heights of 70 or more feet when mature and is characterized by smooth, pale grey bark and heavy foliage. It has large pinnately compound leaves of 1-4 feet in length with 11-35 alternate, ovate-lanceolate, smooth-edged leaflets. *Ailanthus* can grow as a single-stemmed tree with a broad crown and sometimes slightly drooping branches. It can also occur as groups of small stems from sprouting, often growing over 6 feet in a season. Some dieback of sprouts may occur in the winter but resprouting occurs (Adamik & Brauns 1957, Feret 1985). Repeated freezing damage can lead to a reduction in seedling survival, however (Miller 1990). Appearance is similar to the staghorn sumac (*Rhus typhina*). Easy identification can be made from the large, specialized glands found at the basal margins of each leaflet. Smaller simple hair-like glands arising from outgrowths of epidermal cells are also scattered over the surface of young leaflets, particularly along the veins (Davies 1943-1944a). The root system of *Ailanthus* is shallow and spreading, and roots near the stem thicken into a storage structure. No true taproot occurs as most roots grow only in the upper 46 cm (18.1 inches) of the soil (Miller 1990). This characteristic allows for high drought resistance, but causes difficulty surviving in areas of poor drainage, as is evidenced by its lack of occurrence in swamps and wetlands (Davies 1943-1944b).

Ailanthus is most commonly found on soils in the orders Ultisols, Inceptisols, and Entisols (Miller 1990). *Ailanthus* is usually described as shade intolerant and has been shown to respond poorly to decreasing light intensity (Grime 1965, Bourdeau & Laverick 1958). However, it has been reported in an old-growth forest in New York as a gap-

obligate species (able to reach canopy height through a single period of release) (Knapp and Canham 2000). It is extremely pollution tolerant and is an aggressive colonizer of disturbed habitats, suppressing the growth of other species and often forming nearly pure stands (Feret 1985, Mergen 1959). It is common in urban settings and can grow up through cracks in sidewalks and on roadsides. In a survey in Baltimore, Maryland, *Ailanthus* was found to have significantly higher annual mortality rates in a transportation land use category than in high density, low to medium density, or residential land use types (Nowak et al. 2004).

Reproductive Structures

Ailanthus is chiefly dioecious though sometimes monoecious. Flowers are mostly imperfect, but perfect flowers do occur (Feret 1973). Both pistillate and staminate flowers are small, yellowish-green and arranged in large panicles at the ends of new shoots (Hu 1979). The male plant commonly produces three to four times as many flowers as the female plant, and is more conspicuous, particularly due to the odor staminate flowers emit as an attractant to insects. The flowers are usually regular, formed by a very small copular and lobed calyx, an annular and lobed gland, and five petals in the corolla that are pubescent on the inner surface (Davies 1943, Hu 1979). The ten stamens on the male flower are spreading with a globular anther and a glandular green disc. The female flower can be distinguished by the ten (or occasionally five) sterile stamens with heart shaped anthers as well as the five carpel pistil and star-shaped stigma (Hu 1979). Flowers do not open all at once. In a study by Davies (1943) the staminate flowers were found to bloom for an average of 27 days and the pistillate flowers were found to bloom for an average of 12 days. The first staminate flowers opened three days before the first pistillate flowers, and continued to open 12 days after the last pistillate flower had opened. Individual staminate flowers were only retained on the panicle for an average of 2.9 days.

A basal gland in the staminate flowers may be responsible for the characteristic odor of the tree in flower. Davies (1943) found that the odor produced by the pistillate flowers was as great or greater than that of the staminate flowers, but this is in disagreement with

other reports (Hu 1979, Peigler 1993, Dalby 2000). The discrepancy may be due to Davies using individual flowers while others generally are referring to the flowers while on the tree. As there are significantly more staminate flowers than pistillate, it would be logical to assume that if the odor was equal, the staminate panicles would be more noticeable. *Ailanthus* is also able to reproduce asexually through root sprouts.

The carpel of the pistillate flowers develops into five or less samaras when mature which may be green or reddish in color. The fruits are usually retained on the trees through the winter but can be dispersed starting as early as October. *Ailanthus* produces the most seeds in trees aged 12 to 20 years (Miller 1990). Germination is epigeal in nature (Miller 1990). *Ailanthus* seeds may be stored in soil seed banks for some period of time as they have been found with no parent trees present in the overstory (Zasada & Little 2002).

Pollination

Little information on the pollinators of *Ailanthus* is available. Brizicky (1962) noted that small flies and beetles have been recorded as pollinators, but did not state by whom. Honeybees have also been recorded as pollinators, particularly as the honey produced from *Ailanthus* nectar is fairly unique. It is reportedly foul in flavor at first, but improves after several months (Melville 1944, Dalby 2000).

Allelopathy

Another important factor in the success of *Ailanthus* is found in the release of compounds that negatively affect surrounding vegetation. These possible allelopathic effects have been the subject of the vast majority of experimental studies on *Ailanthus*. The first report of potentially allelopathic effects found that an extract made from the leaves of *Ailanthus* produced an adverse response in 35 species of gymnosperms and 11 species of angiosperms (Mergen 1959). A considerable amount of work on toxicity in *Ailanthus* has been done by R.M. Heisey using bioassays with garden cress (*Lepidium sativum* L.) seedlings. His experiments demonstrated that the phytotoxin in *Ailanthus* is relatively

polar, as a methanol extraction reduces herbicidal activity while a dichloromethane extraction does not reduce activity appreciably. A number of secondary metabolites have been identified in *Ailanthus* including β -carbolines, canthin-6-one alkaloids, coumarins,

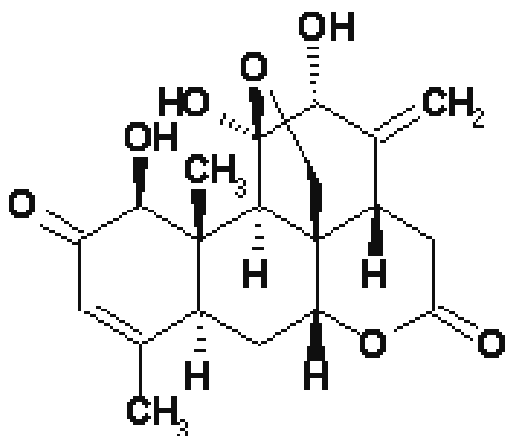


Fig. 1.1. Chemical structure of ailanthone, the most toxic compound produced by *A. altissima*.

and quassinoids. The most phytotoxic of these is ailanthone, a water soluble quassinoid (Fig. 1.1) (Heisey 1996). The highest levels of toxicity in *Ailanthus* occur in the bark of the roots. A medium level of toxicity is found in the leaflets, rachises, shoots of young seedlings. Low levels of toxicity are found in wood, outer bark of the trunk, and young inflorescences. The roots of young seedlings and the stemflow of rainwater off *Ailanthus* foliage, branches, and trunk

both have a stimulatory effect (Heisey 1990a, 1990b). Seasonal changes are also observed with leaflets showing decreasing toxicity with age, corresponding with a decrease in nutrient content (Heisey 1990a, Lawrence et al. 1991). This finding provides evidence that the phytotoxic compounds may act as a feeding deterrent.

Root bark has been shown to reduce both emergence of seedlings and vigor of emerged seedlings in multiple species (Heisey 1990b, 1997, Lawrence et al. 1991). Bioassays using soil and eluants of soil collected near *Ailanthus* roots in the field have also resulted in a reduction of radicle growth of seedlings. The phytotoxins in *Ailanthus* roots and leaflets do not appear to persist for a long period of time when in warm soil, possibly due to soil microbial degradation. Toxicity is reduced with high rainfall and moderate temperatures and is increased with low rainfall and elevated temperatures (Heisey 1997, Lawrence et al. 1991). In bioassays, the loss of toxicity over time is followed by a stimulatory effect on radical growth. This phenomenon seems to be due to the inhibitory effect at higher concentrations and a stimulatory effect at low concentrations (Heisey

1990b, Tsao et al. 2002). Ailanthone may have antifungal effects as well as herbicidal effects (Heisey 1997).

The strongly herbicidal effect exhibited by *Ailanthus* on a variety of species has led to investigations into its potential commercial use. Current problems to be overcome before this can be achieved include the short-lived toxicity of the extract, which results in the need for frequent applications. In addition, ailanthone is considered a broad-spectrum herbicide, and is as injurious to many crop species as it is to weed species. Bioassays to assess insecticidal activity have shown that *Ailanthus* extracts are not particularly toxic to yellow fever mosquito larvae (*Aedes aegypti*), taking levels of 778 ppm and 195 ppm of a methanol extract to cause mortalities of 100% and 50%, respectively, after 24 hours of exposure (Tsao et al. 2002).

Control Methods

Current control options for *Ailanthus* employ both physical and chemical methods. Young seedlings of *Ailanthus* can be hand-pulled or dug before a full root system develops or seeds are produced (Hoshovsky 2005). All roots and stems must be removed in order to prevent root sprouting. Girdling the cambial tissue, including heat girdling, kills the tree stem but results in significant sprouting. Burning and grazing have been used to kill *Ailanthus* stems, but control is short term due to root sprouting. Sterrett et al. (1971) found that applying 5000 mg/L of 2-chloroethylphosphonic acid (68-240) in early July defoliated *Ailanthus* by 80-100%. A 2003 study compared the effectiveness of manual cutting to several mixtures of low volume basal applications of herbicide at controlling *Ailanthus* (Burch and Zedaker 2003). Manual cutting alone was found to stimulate root sprouting and increase the overall stand density. While all treatments reduced stem count after 2 years compared to cutting alone, a combination of 5% Tordon K (picloram @ 240 gae/L) with 20% v/v Garlon 4 (triclopyr @ 480 gae/L) was the most effective at controlling *Ailanthus*.

Biological control methods for *Ailanthus* are not currently available as very few diseases, parasites, and insect pests are known to affect the tree. There are reports in Japanese

literature of indigenous insects living on *Ailanthus*, and in India, *Atteva fabricella* is known as an *Ailanthus* defoliator (Hoshovsky 2005). Insect damage is minimal in North America and is primarily limited to three main insects (Feret 1985). The larvae of the *Ailanthus* webworm (*Atteva punctella*) feed on foliage as do the larvae of the *Ailanthus* silk moth, which reportedly can defoliate the tree in a few days (Pirone et al. 1988). The Asiatic garden beetle (*Maladera castanea*) also includes *Ailanthus* in its wide host selection. Occasional attacks by the fall webworm (*Hyphantria cunea*), whitemarked tussock moth (*Hemerocampa leucostigma*), oystershell scale (*Lepidosaphes ulmi*), and citrus whitefly (*Dialeurodes citri*) occur (Pirone et al. 1988). Deer also have been reported as occasional browsers, and a number of birds in Texas feed on the seeds of *Ailanthus* (Miller 1990). A number of fungi including *Cercospora glandulosa*, *Phyllosticta ailanthi*, and *Gloeosporium ailanthi* have been found to attack *Ailanthus*'s stems, foliage, vascular system, and decaying wood, but most of these cause little major impact (Pirone et al. 1988). In Italy, *Ailanthus* seedlings damaged by cold were weakly parasitized by *Placosphaeria* spp. (Hoshovsky 2005). Verticillium wilt (*Verticillium albo-atrum*) may potentially become a major fungal disease and was responsible for the death of many trees in Philadelphia in 1936. Shoestring root rot (*Armillaria mellea*) infections have also been reported in the northeastern United States (Pirone et al. 1988, Hepting 1971). Another vascular wilting disease caused by *Fusarium* spp. is currently being investigated as a possible biocontrol treatment for *Ailanthus* (Stipes 1995). Other potential biocontrol agents currently under investigation include two weevils from the native range of *Ailanthus*, *Eucryptorrhynchus brandti* and *E. chinensis* (Ding et al. 2006).

Objectives

Research on invasive species has in the past focused primarily on their effect on competitive interactions for nutrients, space, water, and light (Grace and Wetzel 1981, Hacker 1984, Mandujano et al. 1998). However, in recent years more attention has been placed on the competition for pollinators. Invasive plants that rely on out-crossing are

met with a distinct problem in their new habitat. These plants must either have brought their pollinators with them from their native region, wait for the appropriate pollinating mutualists to be independently introduced, or compete for resident pollinating species (Parker and Haubensak 2002). When the other two options are unavailable, establishing mutualistic associations with native pollinators may greatly enhance an invading species' ability to establish (Simberloff and Von Holle 1999, Jesse et al. 2006). Pollination and the competition for pollinators has been studied for a number of invasive plants (Grabas and Laverty 1999, Barthell et al. 2001, Chittka and Schürkens 2001, Brown et al. 2002, Larson et al. 2002, Parker and Haubensak 2002, Jesse et al. 2006, Morales and Aizen 2006). Invasive plants that have showier flowers, higher quality nectar, or more nectar may cause problems for native species in two ways. First, this competition can cause a decrease in the quantity of pollinator visits or in the amount of pollen received by the stigma (Waser 1978, 1983). Second, there can be a decrease in the quality of pollination due to interspecific pollen transfer (Waser 1983, Brown et al. 2002). This decrease occurs when deposited pollen loads are from mixed plant species and from pollinators wasting and losing pollen (Waser 1983, Campbell and Motten 1985). More florally competitive invasive plants may harm reproduction of native species as surely as any other form of competition.

Ailanthus altissima is considered to be a weed tree to many in the United States and an effective method of control for it has yet to be found. The aggressive reproductive nature of *Ailanthus* is one important factor in its success in colonizing and dominating habitats. Eradication of the tree by cutting it down is difficult due to the asexual root sprouts that develop. Herbicides are relatively effective against the above ground portions of the tree, but care must be taken to destroy the root system. To date little information has been collected on the pollination ecology of *Ailanthus* in the U.S. A detailed knowledge of the sexual reproductive biology of *Ailanthus* may provide information that will help control this method of propagation.

Knowledge of a tree's normal reproductive efforts provides a baseline by which changes can be measured. The continued investigation of new control methods, including the

possibility of a native fungus being used as a biocontrol agent, is important in order to have these baseline data. These data can then be used in comparison to determine the tree's reproductive response to a decline in health so as to prepare for an increase or decrease in fecundity. When under stress, many plants may increase or decrease reproductive effort. A decrease is explained by the plant focusing its remaining resources on survival. An increase is often explained as a "last ditch" effort to pass on the plant's genes. Either situation has a ramification towards the control of *A. altissima*.

The overall goal for this research was to describe the pollination biology of *A. altissima* for the mid-Atlantic region of the U.S. Survey work and laboratory analysis were used to determine what was pollinating *Ailanthus*, when those pollinators were visiting, and why or for what rewards.

The goal of the first set of experiments was to determine the pollinators of *Ailanthus* and their visitation patterns. A complete list of the insect pollinators for *Ailanthus* has yet to be assembled for any region in the United States. A survey of insect floral visitors was performed to make this information available. Visitation rates are a useful measure of the relative abundance of pollinators as well as an aid for predictions of ovule fertilization and seed set (Kearns and Inouye 1993). In addition, they often are correlated with the availability of floral rewards (Fell 1986). A count survey was conducted to determine the visitation patterns of insect pollinators on *Ailanthus*.

Pollinator behavior such as visitation time is commonly associated with both environmental factors and the rewards offered by the plant. Rewards can be available at different times of the day, often peaking at specific times. Plants and their pollinators have coevolved towards a mutually beneficial arrangement involving a number of different factors for both parties involved. These include the types of rewards offered, their availability for the pollinators, and getting the most effective pollen transfer for the least amount of energy input for the plants. The goal for the second set of experiments was to determine the nectar profile of *A. altissima*, which will aid in predicting the timing of pollinator visitation. Several studies were conducted to describe the nectar rewards

offered by *A. altissima* to pollinators. These were to 1) determine the nectar composition and amount of total sugars in *A. altissima* and how these are related to tree gender and time of day, and 2) determine nectar production and removal throughout the day.

CHAPTER 2. Floral visitors and visitation patterns of *Ailanthus altissima*.

Introduction

Numerous factors affect a species' ability to colonize a new habitat. Although it has received little attention until recently, an invading plant that requires outcrossing has three main options for success. The plant can either have been introduced to the new habitat along with its associated pollinators from its native region, it can wait for those previously associated pollinators to be independently introduced, or it can attempt to compete for resident pollinating species (Parker and Haubensak 2002). The establishment and competition for mutualistic associations with native pollinators has been studied for a number of invasive plants (Grabas and Laverty 1999, Barthell et al. 2001, Chittka and Schürkens 2001, Brown et al. 2002, Larson et al. 2002, Parker and Haubensak 2002, Jesse et al. 2006, Morales and Aizen 2006). With costs of preventing and controlling invasive species estimated at \$137 billion per year, it is increasingly important to understand the reproductive biology of these pest species (Pimentel et al. 2000).

This study focused on the non-indigenous invasive *Ailanthus altissima* (Miller) Swingle (Simaroubaceae) in the mid-Atlantic region of the United States. *Ailanthus* is native to China and was first introduced to the United States in 1784 when it was planted in the garden of William Hamilton in Philadelphia, Pennsylvania. *Ailanthus* was first reported as invasive in Virginia in 1888 and currently is found in the U.S. in 42 states (Curtiss 1888). It is now naturalized in much of the temperate and meridional zones of the world (Kowarik 1995, Brizicky 1962, Hu 1979). *Ailanthus* is a deciduous tree that can grow to heights of 70 or more feet at maturity. While usually described as shade intolerant, *Ailanthus* is well adapted as a colonizer of disturbed habitats. Its aggressive reproductive nature, its rapid vegetative growth (often over 6 feet in a season), and its ability to release potentially allelopathic chemicals into the soil allows it to commonly form nearly pure stands (Feret 1985, Mergen 1959). Its shallow and spreading root system allows for high drought resistance, but low tolerance to flooding (Davies 1943/1944b). Though perhaps better known for its ability to reproduce asexually and tenaciously through stump and

root sprouts, *Ailanthus* is a primarily dioecious tree and reproduces sexually through small, yellowish-green flowers arranged in large panicles at the ends of new shoots (Hu 1979). Pistillate flowers produce up to five green or reddish samaras that can be stored in soil seed banks for some time (Zasada & Little 2002).

Currently there is very little information available on the pollination or pollinators of *Ailanthus*. Anecdotal reports of small flies and beetles as pollinators exist, as well as reports of honeybees in response to the supposedly strange flavor of *Ailanthus* honey (Melville 1944, Brizicky 1962, Dalby 2000).

Though physical and chemical methods are currently used to control *Ailanthus*, an economically and ecologically efficient control has yet to be found. An understanding of the tree's normal reproductive efforts provides a baseline by which changes can be compared. When stressed, many plants will respond with an increase or decrease in reproductive effort. This has been explained by either a last chance attempt to pass on genes, or as a focusing of energy on survival. These changes may become evident with continued research on new control methods, particularly with potential biological control agents, and will likely have an impact on management.

The main goal of this study was to describe the pollination biology of *A. altissima* in the mid-Atlantic U.S. As part of this goal I identified the insects pollinating *Ailanthus*, when those pollinators were visiting, and why or for what rewards. These questions were addressed by designing a study to determine the pollinators of *Ailanthus* and their visitation patterns. As a complete list of the insect pollinators for *Ailanthus* has yet to be assembled for any region in the United States, a survey of insect floral visitors was performed. The visitation patterns of the pollinators of *Ailanthus* were determined using an instantaneous count survey. Count surveys of pollinators are often correlated with the availability of floral rewards and can be good measures of relative abundance, predicted ovule fertilization, and seed set (Fell 1986, Kearns and Inouye 1993). We examined these patterns across time of day, tree gender, and location.

Materials and Methods

Studies were conducted at three sites along highway right-of-ways. These were located in Ruckersville, VA on Rt. 29 (38.22N -78.39W), in Harpers Ferry, WV on Rt. 340 (39.32N -77.76W), and in Newport, VA on Rt. 460 (37.29N -80.48W). At the Ruckersville site, sampling was conducted from June 11-13 in 2005 and from June 1-6 in 2006 with 18 trees both years. The Harpers Ferry site was sampled from June 16-18 in 2005 with 18 trees and from June 8-10 in 2006 with 8 trees. Many of the original trees from 2005 at this site were removed by 2006 for highway maintenance. At the Newport site sampling was conducted from June 22-24 in 2005 with 11 trees and from June 15-17 in 2006 with 13 trees. Trees were chosen randomly within the parameters of at least 150 meters apart and with flowering branches within safe reach of the researcher. The geographic coordinates of trees selected for sampling were recorded with GPS and the trees were tagged.

Floral Visitor Collection

A sweep net was used to collect insects found on flowers over two 30 sec. intervals. All insects captured were collected, including any incidental pollinators. This sampling was performed before any other activities in order to avoid disturbing the insects on the blooms. Each tree was sampled at 3 different times of day: morning (8-10 a.m.), midday (11-1 p.m.), and afternoon (3-5 p.m., Eastern Daylight Savings Time). Insects were identified and a list was developed of taxa visiting trees within each site and year.

Visitation Patterns

Insect visitation patterns were studied at the Newport site only in 2005 from June 28-30 on 2 trees due to time constraints. In 2006, the study was conducted in Ruckersville from June 4-5 on 4 trees, in Harpers Ferry from June 10-11 on 2 trees, and in Newport from June 18-23 on 4 trees. Instantaneous counts (counts made only on insects actually foraging at the time of observation) were taken on 10 randomly selected panicles on each tree (Fell 1986, El Shafie et al. 2002, Goulson & Derwent 2004). Trees were sampled

every 2 hours of daylight (6 times of day) from 8:00a.m. to 6:00p.m., EDST within peak bloom. In 2006, the numbers of the soldier beetle *Chauliognathus marginatus* observed on each panicle were also recorded. After the first year of the study, we noticed a large number of these beetles, which are known to be pollen feeders, visiting *Ailanthus* flowers. Our overall research objectives were primarily concerned with nectar rewards, so in the second year of the study the beetles were excluded for part of the analysis.

Data Analysis

Data of visitation rates were square root transformed and analyzed using a univariate analysis of variance to test for significance of time of day and tree gender. Counts were compared within locations and years. Separate ANOVAs were performed with counts of *C. marginatus* removed. Means were separated with Tukey's HSD to show differences between treatments. Treatment differences were considered significant when $P \leq 0.05$.

Results

Floral Visitors

Results for the floral visitor collection for year and location are shown in Table 1. The most common visitors for all sites included a variety of ant species, and with the exception of Newport in 2006, all sites at both years were dominated by the soldier beetle *C. marginatus* (Cantharidae). At Newport in 2006 ants in the genus *Formica* (Formicidae) were the most common visitors (15.25% of the total number of visitors), with *C. marginatus* following (6.78%). Other prevalent ant genera were *Prenolepis* and *Camponotus*. *Apis mellifera* and *Bombus* sp. numbers were generally low (with the exception of Newport in 2005 with *Bombus* sp. at 13.64%).

Visitation Patterns

Visitation patterns were described by mean number of insects observed per tree. Data from each location showed no apparent diurnal pattern in visitation rates. Only male trees were sampled at the Harpers Ferry site, so no comparison was made between tree sexes for that location. No significant difference was found in counts between sampling

periods for Ruckersville (Fig. 2.1) or Harpers Ferry (Fig. 2.2) in 2006 ($F = 0.575$, d.f. = 5, 36, $P = 0.719$; $F = 2.41$, d.f. = 5, 18, $P = 0.077$). A significant difference was found between gender at the Ruckersville location ($F = 10.094$, d.f. = 1, 36, $P = 0.003$). At Newport in 2005 (Fig. 2.3), only male trees were sampled, and no significant differences were found between sampling times ($F = 0.405$, d.f. = 5, 18, $P = 0.839$). At the Newport site in 2006 (Fig. 2.4), a significant difference was found for both gender ($F = 833.30$, d.f. = 1, 102, $P \ll 0.05$) and sampling period ($F = 3.72$, d.f. = 5, 102, $P = 0.004$). Female trees were found to have higher average number of visitors in Newport while male trees were found to have a higher average number of visitors in Ruckersville.

When the counts for the soldier beetle *C. marginatus* were removed from the visitation analysis for 2006, results from the ANOVA for Harpers Ferry ($F = 0.531$, d.f. = 5, 18, $P > 0.05$) (Fig. 2.5) and Newport (sampling period: $F = 4.53$, d.f. = 5, 102, $P = 0.001$; gender: $F = 633.66$, d.f. = 1, 502, $P \ll 0.05$) (Fig. 2.6) were similar to those with all insects included. However, for the Ruckersville site, removal of soldier beetles resulted in a loss of significance between tree gender compared with the ANOVA with all counts included ($F = 1.490$, d.f. = 1, 36, $P > 0.05$) (Fig. 2.7). As in the analysis for all insect counts, sampling periods for Ruckersville were not found to be significantly different ($F = 0.295$, d.f. = 5, 36, $P > 0.05$).

Discussion

The presence or absence of various insect visitor species can be explained by their known ecological roles. *Ailanthus* blossoms are small, whitish-green, fairly primitive flowers with nectar and pollen both easily accessible to a variety of visitors. It does not require any specialization in the pollinator to obtain rewards, and this was demonstrated in the range of species collected. In general, the type of flower produced by *Ailanthus* is expected to be particularly attractive to beetle and fly pollinators. The syndrome of cantharophily is characterized by flowers with dull colors that are frequently greenish or off-white. Most beetles are relatively primitive insects with chewing mouthparts and can only handle the simplest of blossoms, such as the dish and bowl type, with pollen and

nectar openly accessible (Faegri & van der Pijl 1971). The most numerous species collected overall, *C. marginatus* is a member of the Cantharidae family, which has predacious larvae, and adults usually found on flowers (Triplehorn & Johnson 2005). The genus *Chauliognathus* contains species that feed on pollen and nectar of many flowers, and *C. marginatus* Fabricius is known to be abundant on flowers of linden, wild hydrangea, Jersey tea, and other plants (Dillon & Dillon 1972).

The reason for the presence of the other collected beetles is less clear. Species of *Melanotus* are important crop pests as larvae and are the most common of the Elateridae. The adults are phytophagous and live on flowers and vegetation, and are frequently found under bark (Dillon & Dillon 1972, Triplehorn & Johnson 2005). *Melanotus* is not known to feed on *Ailanthus*, but may possibly feed on floral tissues. The coccinellid species collected are common and predacious and were therefore likely present to feed on other insect visitors of *Ailanthus*. The buprestid *Agilus* is another visitor whose purpose is unclear. Adults are common on a variety of trees, especially those that are dead or dying (Triplehorn & Johnson 2005). Their presence at the Harpers Ferry site only may be related to the greater number of trees that appeared unhealthy, displaying chlorosis, flagging, and defoliation along the highway there relative to the other sites. While some incidental pollination may in fact occur with these beetle species, it is unlikely that they are important pollinators of *Ailanthus*.

There is a great deal of variation in the Diptera as pollinators. The syndrome of myophily is characterized similarly to the beetle blossoms in that flowers tend to be regular, simple, light and dull in color, and have nectar and pollen easily obtainable (Faegri & van der Pijl 1971). Odor of fly blossoms tends to be imperceptible, which is in contrast to *Ailanthus*'s strong scent. This syndrome does not include the sapromyophilous dipters which include the carrion and dung flies and are presumably not of importance to *Ailanthus*. Specialized flies provide some exception to the general fly blossom syndrome as well, ranging in proboscis length from highly evolved South African tabanids and nemestrinids, to the bombyliids and syrphids. Syrphid flies were collected in low numbers from *Ailanthus* (2.3% at Ruckersville in 2006, and 1.3% at

Harpers Ferry in 2005). These flies are common near flowers in a variety of habitats, have a short proboscis, and collect and chew pollen (Faegri & van der Pijl 1971, Triplehorn & Johnson 2005). The unspecialized flies are mostly omnivorous, but generally visit flowers primarily for nectar alone. As they do not nurse a brood, they take food for themselves only and require carbohydrates as their chief source of energy. However, many of the unspecialized flies use a great number of food sources and pollination tends to be irregular and unreliable (Faegri & van der Pijl 1971). Apart from the syrphids, sarcophagid flies were collected on *Ailanthus* in Ruckersville in 2006. These flesh flies are common insects that feed on a variety of sugary substances as adults including nectar, sap, fruit juices, and honeydew (Triplehorn & Johnson 2005). Flies from the family Muscidae were collected in the same amount as the sarcophagids from the same site and year. The Muscidae is a large and common group with many important pests. Adults feed on a variety of materials and may be predacious, saprophagous or pollenophagous depending on the species (Huckett & Vockeroth 1987). Dolichopodids were collected in Ruckersville in 2005, accounting for 3.7% of insect visitors. The adults of these flies are predacious on smaller insects and were likely on *Ailanthus* flowers for that purpose, similar to the coccinellid beetles collected.

The Hymenoptera contains many well-known and important pollinating species, and several were collected from *Ailanthus* blossoms. The apids *Apis mellifera* and *Bombus* species were collected in small numbers (at most, 13.6%) at Newport and Harpers Ferry. Flowers specialized for honey bee and bumblebee nectar collection are zygomorphic with great depth effect, generally yellow or blue in color, and often intricate with nectar hidden. However, while bees frequently collect pollen from the same blossom as they collect nectar, they also will visit more primitive blossoms for pollen collecting specifically. In addition, social bees will visit any type of flower that contains sufficient nectar and are restricted only from those flowers that are too deep for their mouthparts to reach rewards (Faegri & van der Pijl 1971). Bees from the Halictidae were collected in 2006 from the Newport site. These bees range in sociality from solitary, to nesting alone, to congregations, to primitive eusociality (Triplehorn & Johnson 2005). Small solitary bees, such as those in the Halictidae, tend to be oligolectic, restricting their occurrence,

but perhaps increasing chances of successful outcrossing for the plant of choice (Faegri & van der Pijl 1971).

A large portion of the collected insect species was made up of formicids. Ants are strongly attracted to whatever sugar source they can obtain, but also require protein for brood rearing. These needs make flowers such as those produced by *Ailanthus* an obvious potential target as they contain both a nectar source for carbohydrates and a pollen source for protein (Faegri & van der Pijl 1971). Their feeding habits are varied, but many are known to feed on sap, nectar, honeydew, or similar substances (Triplehorn & Johnson 2005). Ants have a bad reputation in the pollination world as the prototype for nectar thieves. Ants are often able to sneak in and out of flowers without touching the anthers or stigma due to their small size, and the fact that worker ants are flightless may limit their ability to facilitate cross pollination (Faegri & van der Pijl 1971, Peakall et al. 1991). Additionally, their bodies are commonly hard and smooth and are inefficient for transporting pollen. Furthermore, ants now have been found to produce antibiotic secretions from their metapleural glands that, while killing pathogenic microorganisms, also may disrupt normal pollen function (Iwanami & Iwadare 1978, Beattie et al. 1984, 1985, 1986, Hull & Beattie 1988). This effect is widespread, having been found in 6 of the 11 subfamilies of ants (Beattie et al. 1984, Hull & Beattie 1988). While studies of ant pollinated systems do exist, the evidence for ant pollination leading to viable seed set is small (Peakall et al. 1991).

In this study, the ant *Prenolepis imparis* Say was found at the Ruckersville site in both years (19% in 2005 and 17% in 2006). This ant is the single North American species in the genus and ranges across the country. *P. imparis* nests in soil and the workers have a generalist diet, feeding on honeydew, exudates from galls, earthworms, other arthropods, fruit, and the secretions of floral and extrafloral nectaries. They are known to prefer cooler temperatures for foraging, remaining active during winter months (Talbot 1943). This may be why these ants were only found in Ruckersville as it was the first site sampled both years. The other ants collected (*Formica* sp., *Camponotus* sp., and *Crematogaster* sp.) have a number of species present in the eastern United States and

feeding habits similar to *P. imparis*. *Crematogaster* species are native arboreal ants that nest in branches and tree cavities. They are known to tend aphids and feed on other arthropods and are abundant and ecologically diverse. The *Formica* species are also diverse with over 70 North American species. The *Camponotus* genus, containing the carpenter ants, is omnivorous feeding on honeydew, sap, and arthropods. All of these ant species could have been attracted to the copious nectar produced by *Ailanthus*. Whether or not they act effectively as pollinators is unknown at this point.

No apparent diurnal pattern was observed in the visitation of *Ailanthus* except at the Newport site in 2006. The reason for the difference in sampling times at only the Newport location and only in that year is unclear. The lack of significance between times of day in the rest of the study was in contrast to other flowering plants with various peaks in visitation (Fell 1986, Dupont et al. 2004). This may occur due to *Ailanthus* producing a steady supply of floral rewards that can be collected throughout the day by a variety of visitors, further supported by its primitive, easily accessible blossom. Differences between male and female trees were significant at the Ruckersville site and at the Newport site in 2006. Surprisingly this difference was opposite between the sites. Counts were taken off only 1 female tree in Newport and 2 female trees in Ruckersville compared to 3 male trees in Newport and 2 male trees in Ruckersville. This particular female Newport tree may have been especially attractive to visitors, or the insect assemblage of visitors at the two sites may have had different preferences for female and male trees. The difference in genders at the Ruckersville site may also involve male trees producing more flowers per panicle than female trees, and having a stronger scent, either because of the numbers or in addition to it. Shykoff and Bucheli (1995) found a gender preference by insect visitors for male plants due to higher sugar concentration in nectar and we may find this to be the case with *Ailanthus* as well. It is interesting to note that despite the differences in gender between the Ruckersville and Newport sites, in both cases the total number of insect visitor counts for male trees was decreased considerably more than for female trees by removing *C. marginatus* beetles from the analysis. This is most likely related to these beetles' attraction to pollen as a food source. Removal from the analysis also caused the tree genders to become not significantly different at the

Ruckersville site, likely due to this decrease. A comparison of these results with a detailed description of *Ailanthus*'s nectar availability throughout the day and sugar profile will aid in making conclusions regarding its sexual reproductive process.

Table 2.1. Number and percent of dominant floral visitors of *Ailanthus altissima* collected in 2005 and 2006 by location.

| 2005 | # | % | 2006 | # | % |
|--|----|-------|--|----|-------|
| Ruckersville, VA | | | | | |
| <i>Chauliognathus marginatus</i> (Cantharidae) | 63 | 38.89 | <i>Chauliognathus marginatus</i> (Cantharidae) | 59 | 34.50 |
| <i>Camponotus</i> (Formicidae) | 22 | 13.58 | <i>Prenolepis imparis</i> (Formicidae) | 17 | 9.94 |
| <i>Prenolepis imparis</i> (Formicidae) | 19 | 11.73 | <i>Formica</i> (Formicidae) | 11 | 6.43 |
| <i>Formica</i> (Formicidae) | 11 | 6.79 | <i>Melanotus</i> (Elateridae) | 8 | 4.68 |
| <i>Melanotus</i> (Elateridae) | 6 | 3.70 | Sarcophagidae | 5 | 2.92 |
| Dolichopodidae | 6 | 3.70 | Muscidae | 5 | 2.92 |
| Coccinellidae | 5 | 3.09 | Syrphidae | 4 | 2.34 |
| | | | Coccinellidae | 4 | 2.34 |
| | | | <i>Crematogaster</i> (Formicidae) | 4 | 2.34 |
| | | | | | |
| 23 total taxa | | | 43 total taxa | | |
| | | | | | |
| Harpers Ferry, WV | | | | | |
| <i>Chauliognathus marginatus</i> (Cantharidae) | 67 | 44.67 | <i>Chauliognathus marginatus</i> (Cantharidae) | 22 | 25.58 |
| <i>Formica</i> (Formicidae) | 39 | 26.00 | <i>Formica</i> (Formicidae) | 9 | 10.47 |
| Coccinellidae | 3 | 2.00 | | | |
| <i>Agrilus</i> (Buprestidae) | 3 | 2.00 | | | |
| <i>Bombus</i> (Apidae) | 2 | 1.33 | | | |
| Syrphidae | 2 | 1.33 | | | |
| | | | | | |
| 30 total taxa | | | 37 total taxa | | |
| | | | | | |
| Newport, VA | | | | | |
| <i>Chauliognathus marginatus</i> (Cantharidae) | 22 | 25.00 | <i>Formica</i> (Formicidae) | 9 | 15.25 |
| <i>Formica</i> (Formicidae) | 14 | 15.91 | <i>Chauliognathus marginatus</i> (Cantharidae) | 4 | 6.78 |
| <i>Bombus</i> (Apidae) | 12 | 13.64 | <i>Apis mellifera</i> (Apidae) | 4 | 6.78 |
| <i>Camponotus</i> (Formicidae) | 8 | 9.09 | <i>Bombus</i> (Apidae) | 4 | 6.78 |
| <i>Apis mellifera</i> (Apidae) | 4 | 4.55 | Halictidae | 4 | 6.78 |
| | | | <i>Camponotus</i> (Formicidae) | 3 | 5.08 |
| | | | | | |
| 30 total taxa | | | 33 total taxa | | |

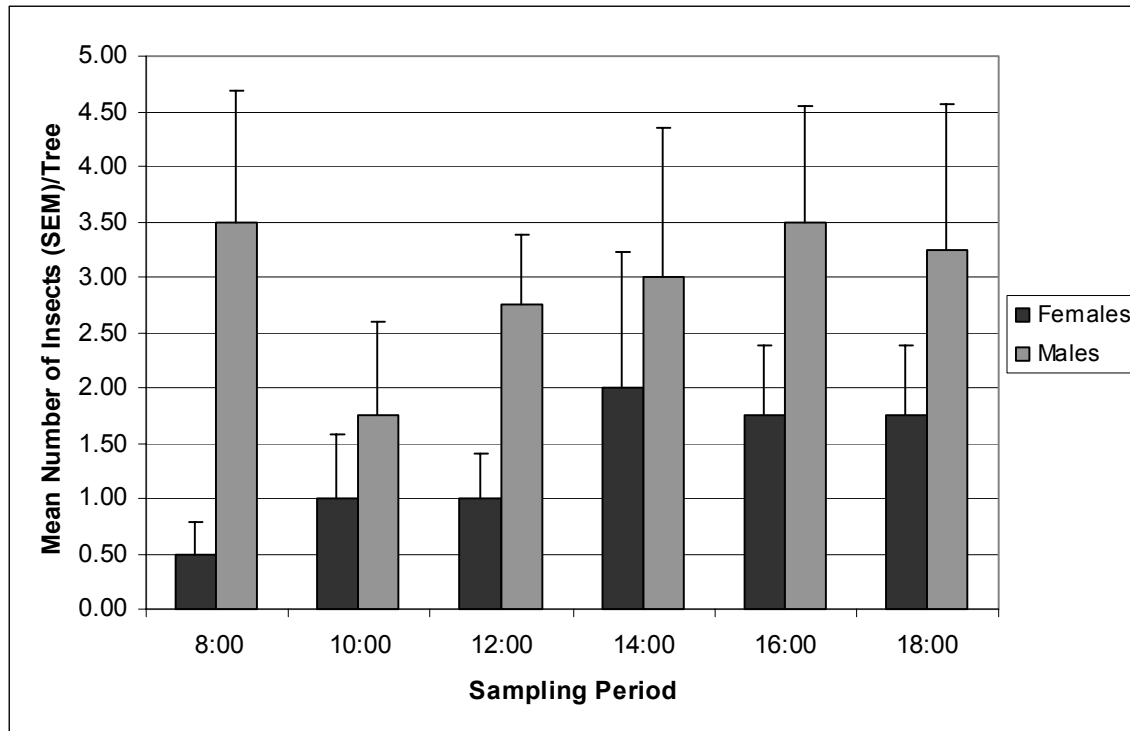


Fig. 2.1. Mean insect visitor instantaneous counts (\pm SEM) for 10 flower panicles for Ruckersville, VA in 2006. No significant difference was found in counts between sampling periods ($F = 0.575$, d.f. = 5, 36, $P = 0.719$). Male trees had significantly higher numbers of insect floral visitors compared to female trees ($F = 10.094$, d.f. = 1, 36, $P = 0.003$).

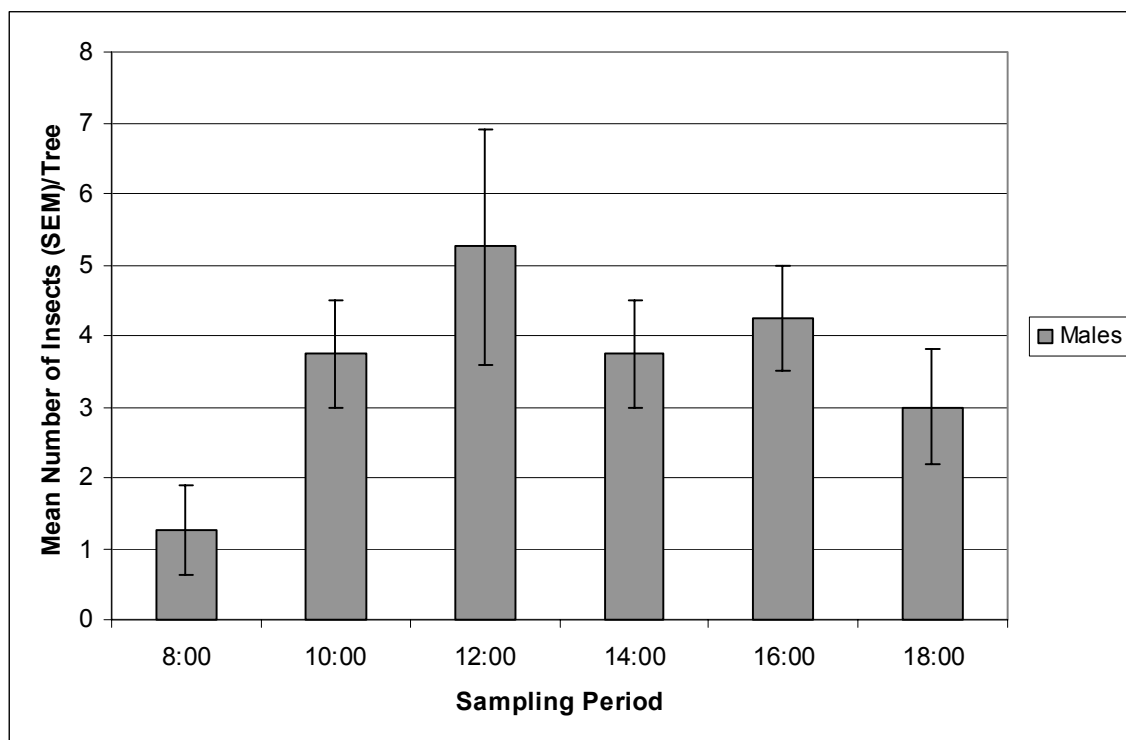


Fig. 2.2. Mean insect visitor instantaneous counts (\pm SEM) for 10 flower panicles for Harpers Ferry, WV in 2006. Only male trees were sampled at this site. No significant difference was found in counts between sampling periods ($F = 2.41$, d.f. = 5, 18, $P = 0.077$).

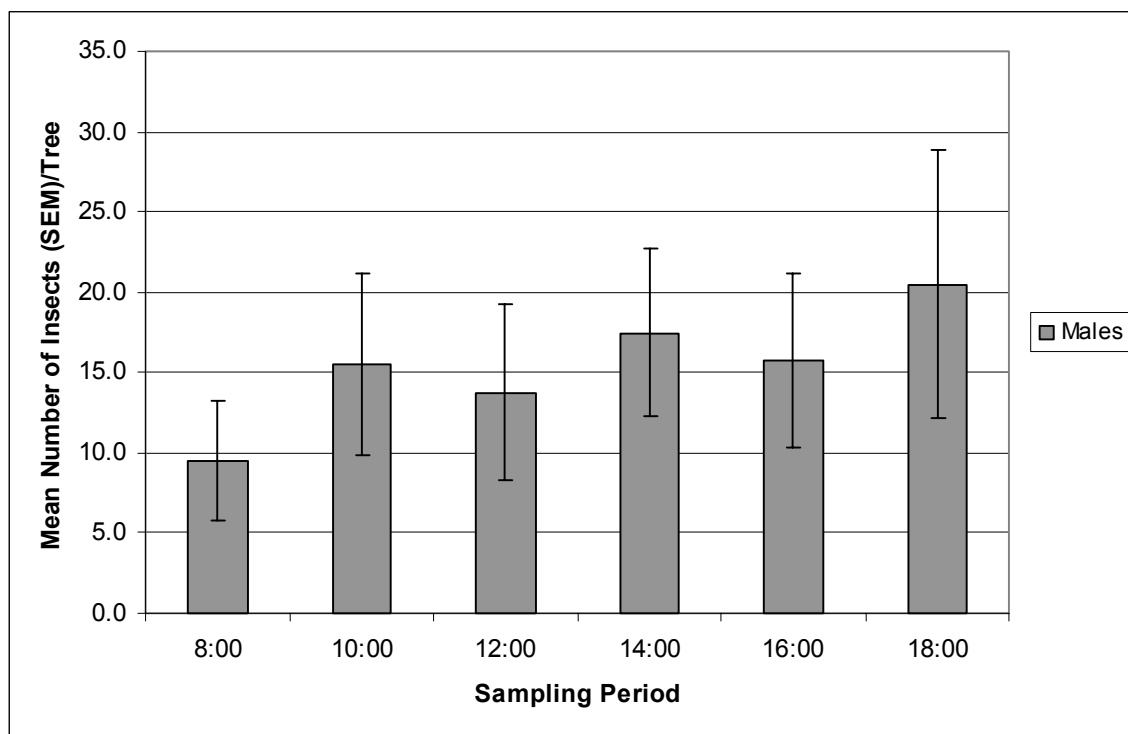


Fig. 2.3. Mean insect visitor instantaneous counts (\pm SEM) for 10 flower panicles for Newport, VA in 2005. Only male trees were sampled at this site. No significant differences were found between sampling times ($F = 0.405$, d.f. = 5, 18, $P = 0.839$).

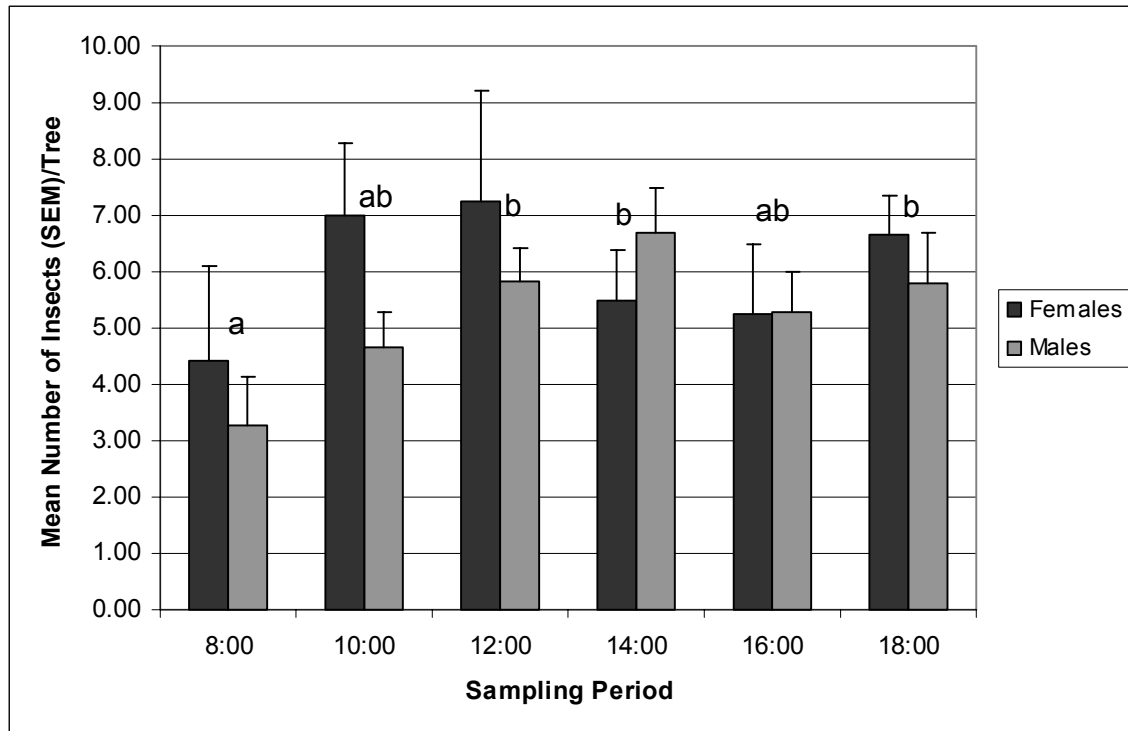


Fig. 2.4. Mean insect visitor instantaneous counts (\pm SEM) for 10 flower panicles for Newport, VA in 2006. Sampling period ($F = 3.72$, d.f. = 5, 102, $P = 0.004$) and gender ($F = 833.30$, d.f. = 2, 102, $P \ll 0.05$) were found to have significant differences. Means for the sampling period followed by the same letter are not significantly different ($P = 0.05$, Tukey's HSD test).

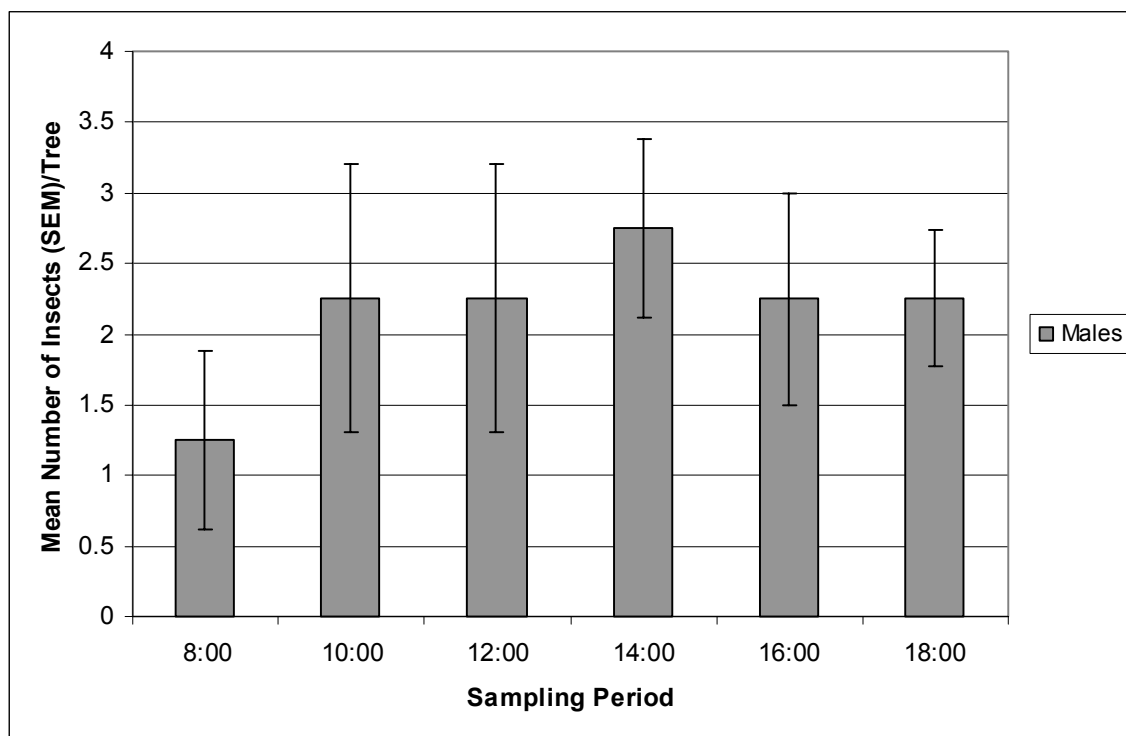


Fig. 2.5. Mean number of insect visitors (\pm SEM) for 10 flower panicles with removal of the instantaneous counts of *Chauliognathus marginatus* for Harpers Ferry, WV in 2006. Only male trees were sampled at this site. No significant difference was found in counts between sampling periods ($F = 0.531$, d.f. = 5, 18, $P > 0.05$).

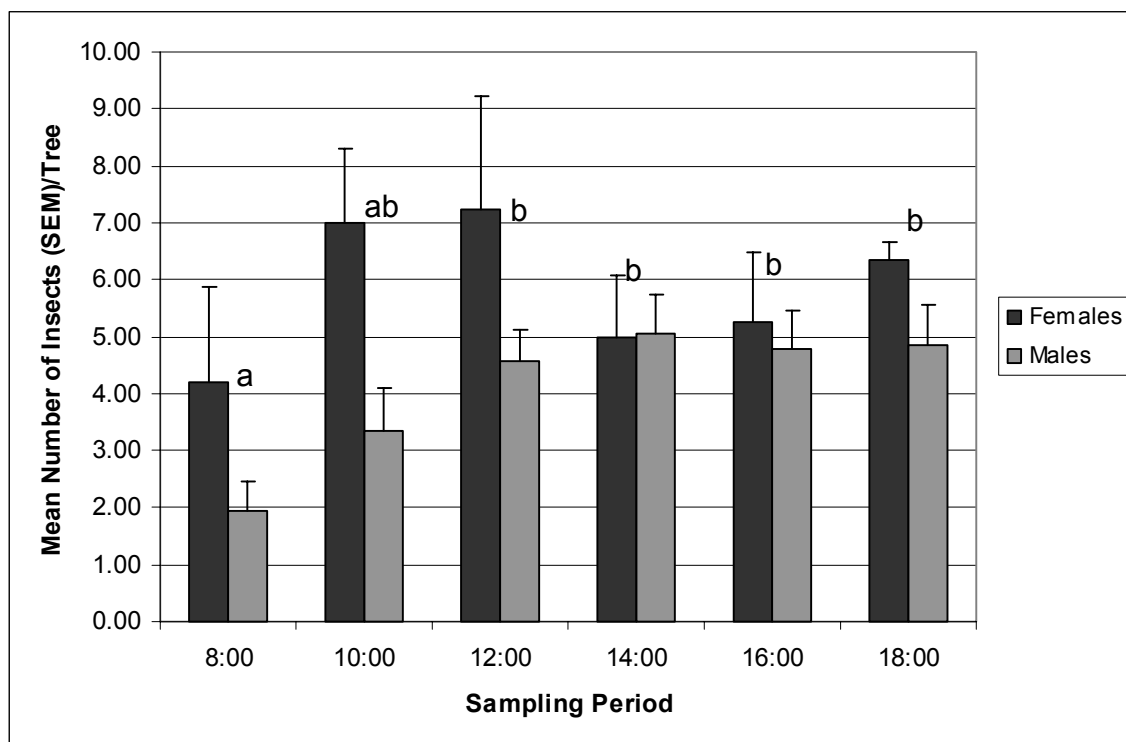


Fig. 2.6. Mean number of insect visitors (\pm SEM) for 10 flower panicles with removal of the instantaneous counts of *Chauliognathus marginatus* for Newport in 2006. Sampling periods ($F = 4.53$, d.f. = 5, 102, $P = 0.001$) and genders ($F = 633.66$, d.f. = 2, 502, $P \ll 0.05$) were found to have significant differences. Means for the sampling period followed by the same letter are not significantly different ($P = 0.05$, Tukey's HSD test).

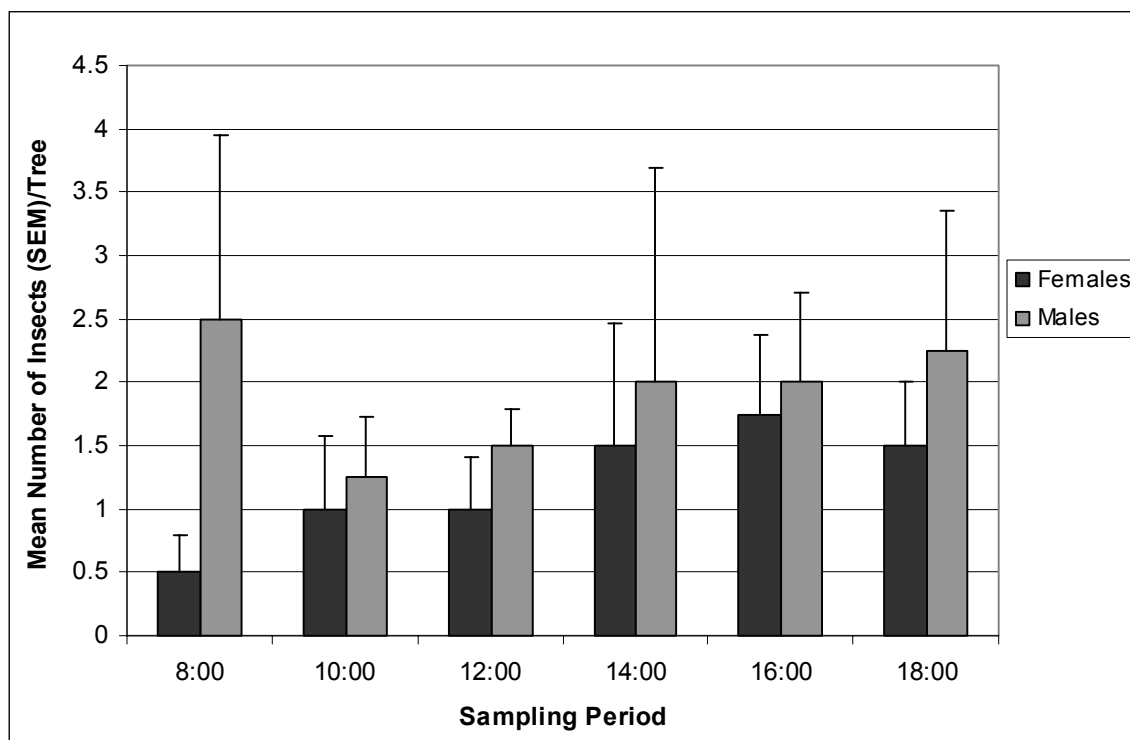


Fig. 2.7. Mean number of insect visitors (\pm SEM) for 10 flower panicles with removal of the instantaneous counts of *Chauliognathus marginatus* for Ruckersville, VA in 2006. Differing from the analysis where all insect counts were used, the tree genders were not found to be significantly different with the removal of the soldier beetles ($F = 1.490$, d.f. = 1, 36, $P > 0.05$). Sampling periods were not significantly different ($F = .295$, d.f. = 5, 36, $P > 0.05$).

CHAPTER 3. Nectar Analysis of *Ailanthus altissima*.

Introduction

The sugar in nectar is an important reward for floral visitors seeking an energy providing carbohydrate source. Each visit may provide a chance of pollination and a plant often will expend considerable energy producing such rewards to that end. While a greater amount of sugar in nectar may be more attractive to potential pollinators, the gains per energy expenditure of the plant will reach a level where its overall fitness is reduced. This balance drives a coevolutionary relationship between nectar characteristics and type of pollinator (Percival 1974, Baker 1975, Pyke & Waser 1981, Galetto & Bernardello 2004).

In addition to total amounts of sugar, the type of sugars produced by a plant may have an impact on potential pollinators. Most floral nectars studied have been found to contain some ratio of three primary sugar molecules: sucrose, fructose, and glucose (Baker & Baker 1983, Freeman et al. 1991). Other sugars such as raffinose, melibiose, and maltose, may be present, but generally are found only in much lower amounts (Percival 1961). The proportions of the three main sugars are commonly used to describe the type of floral nectar (Baker & Baker 1983). Nectar can be “balanced”, having equal quantities by weight of sucrose, fructose, and glucose. The nectar is described as sucrose-rich or dominant if the amount by weight of the disaccharide sucrose is as much as that of the combined hexoses, fructose and glucose. If the combined amount of the hexoses is more than that of sucrose, the nectar is considered hexose-rich or dominant. There is some evidence that different floral visitors are associated with different mixture ratios of the three major sugars, although other factors such as flower shape and nectar accessibility can not be discounted (Baker & Baker 1983, Stiles & Freeman 1993). This mixture ratio can thus be used as a possible predictive factor as to what animals are likely to be pollinators of a specific plant species.

Attractiveness of a plant's floral nectar can be affected by multiple factors. Nectar, being a liquid form of reward, is subject to changes with diurnal variations in temperature and humidity. While the total amount by weight of sugar may not change throughout the day, it is possible for the concentration of sugar in nectar to increase with a change in ambient relative humidity. This change in concentration can make nectar more attractive at particular times of the day than at others. In addition, in dioecious plant species one gender may produce higher or lower amounts of sugar than the other (Bell et al. 1984, McDade 1986, Jordano 1993). This strategy may have evolved to save resources by "tricking" pollinators into visiting female flowers with low levels of sugar after they have already visited male flowers with higher sugar concentrations. There could also exist a difference in the sugar mixture ratios between genders, although this seems less likely. A plant will benefit most from a constancy of pollinators within plant species and a varying sugar ratio might affect nectar preference and flower constancy. Finally, nectar removal by floral visitors throughout the day has been shown to affect the total nectar sugar amount (Gupta et al. 1990, Bernardello et al. 1994, Galetto & Bernardello 2004).

Ailanthus altissima (Miller) Swingle (Simaroubaceae) is an exotic invasive introduced from China to the U.S. in the late 1700s. It has since become an economically important weed species as an aggressive colonizer of disturbed habitats such as old fields, forest edges, and roadsides, suppressing growth of surrounding plants through release of allelopathic compounds (Brizicky 1962, Hoshovsky 2005). The process of asexual reproduction through root sprouting in *Ailanthus* has received some attention (Davies 1943/1944, Feret 1985, Burch & Zedaker 2003, Hoshovsky 2005); however, its sexual reproductive cycle is relatively unstudied. Little to nothing is known about the pollination of *Ailanthus*. Most reports in the literature are anecdotal and deal with the apiculturists' problems with foul flavored *Ailanthus* honey (Melville 1944, Dalby 2000). Detailed information on the pollination biology of *Ailanthus* may help in the control this plant species.

I conducted a study on nectar availability and composition for *Ailanthus* at three locations in the mid-Atlantic region of the United States. We sought to answer the following

questions: 1) What total concentrations of sugar are found in *Ailanthus* flowers? 2) What proportions of different sugars are present in the floral nectar? 3) Does the total amount of sugar in flowers vary over time of day and do total sugar amounts, concentration, and proportion vary between male and female trees? And finally, 4) how does removal of nectar by floral visitors affect the total sugar amount in flowers?

Materials and Methods

Studies were conducted at three sites along highway right-of-ways. The sites were located in Ruckersville, VA on Rt. 29 (38.22N -78.39W), in Harpers Ferry, WV on Rt. 340 (39.32N -77.76W), and in Newport, VA on Rt. 460 (37.29N -80.48W). At the Ruckersville site, sampling was conducted from June 11-13 in 2005 with 11 trees and from June 1-6 in 2006 with 18 trees. The Harpers Ferry site was sampled from June 16-18 in 2005 with 9 trees and from June 8-10 in 2006 with 8 trees. At the Newport site sampling was conducted from June 22-24 in 2005 with 11 trees and from June 15-17 in 2006 with 13 trees. Relative humidity and temperature readings were taken at each sampling with a sling psychrometer.

Nectar Composition

To determine nectar composition, nectar was collected from the flower disc using 1 μ L microcapillary tubes. Samples were collected between 8:00 a.m. and 1:00 p.m. Eastern Daylight Savings Time from three to eight similar aged flowers to account for intra-tree variation (Kearns & Inouye 1993). Each microcap was filled with as much nectar as possible, but if the entire microcap tube could not be filled, the distance from the end of the tube to the fill line was measured with a ruler. Nectar volume collected was determined by dividing the length of this distance by the total length of the microcap (32 mm) and multiplying by 1 μ L. This nectar was deposited into centrifuge tubes containing 50 μ L of 70% ethanol. Samples were frozen until immediately before analysis.

The microcap samples were analyzed using high performance thin layer chromatography (HPTLC) to determine sugars in the nectar and their relative concentrations (Kearns &

Inouye 1993). Reagents were prepared and the analysis was performed similarly to the procedure described by Fell (1990). Pre-coated silica gel 60 plates (Merck, Darmstadt, Germany) were treated and activated. 1 μ L of both mixed standards of glucose, fructose, and sucrose (Sigma Chemical Company, St. Louis, MO) at 2.0, 1.0, and 0.5 mg/mL, and sample solutions were applied in duplicate to plates with a TLC sample applicator (Nanomat II, CAMAG Scientific, Wilmington, NC) and microcaps (Drummond Scientific Co., Broomall, Pa). Plates were dried for 90 s at each drying step with a hairdryer as this was found to produce better spot separation. Dried plates were developed with acetonitrile: double-distilled H₂O (85:15) three times, drying after each development. Plates were then dipped into a ceric sulfate/H₂SO₄ solution made from a dilution of 0.100 N ceric sulfate in 2 N sulfuric acid into 15% sulfuric acid (1:10). The dipped plates were heated in an oven at 110°C for 15 min to char the sugars for visualization. The plates were allowed to cool in a desiccator, and then quantitative measurements were made by absorbance scanning using a CAMAG TLC Scanner II (CAMAG Scientific) at a wavelength of 440 nm connected to a Spectra Physics SP 4270 integrator (CAMAG Scientific) (Fell 1990).

Quantification of Sugars

To determine total amount of sugar produced, the same trees used for the nectar composition analysis were used. Samples from each tree were collected at three different times of day: morning (8-10 a.m.), midday (11-1 p.m.), and afternoon (3-5 p.m., EDST). In 2005, 1 flower was collected from each tree at all sites. In 2006, 3 flowers of approximately the same age were collected from each tree at all sites to better account for intra-tree variability. These were individually placed in vials containing 2 mL of distilled water to rinse out nectar sugars. The flowers were removed at the end of the day. These samples were frozen until immediately before analysis.

The nectar samples were analyzed using an anthrone analysis for determining total amounts of sugar (Trevelyan & Harrison 1952, McKenna & Thomson 1988, Stein & Fell 1994). The analysis was conducted similar to the methods described by Trevelyan & Harrison (1952). Glucose (Sigma Chemical Company, St. Louis, MO) standards were

used at 10, 25, 50, 75, and 100 µg/mL for each analysis. In addition, standards were run at 150, 200, 250, 300, 350, and 400 µg/mL to account for higher concentrations found in samples and were also found to produce a linear standard curve. The reagent was prepared by adding 0.2 g of anthrone (Alfa Aesar, Ward Hill, MA) into 100 mL of a dilution of concentrated H₂SO₄ and distilled water (5:2) and shaking vigorously. Tubes were prepared in an ice water bath with 5 mL of the anthrone reagent layered under 1 mL of the nectar or standard sugar solution, then covered and mixed with a vortex mixer. Tubes were heated in a boiling water bath for exactly 13 min., then immediately placed in an ice bath for 3 min. to halt the reaction. The resulting colored solutions were read using a spectrophotometer (Lambda 20, Perkin-Elmer, Norwalk, CT) at a wavelength of 620nm. Sugar amount (µg) was calculated by comparing spectrophotometer measurements to the standard curve.

Nectar Removal

Bagging of panicles to determine nectar removal by pollinators was conducted at the Newport site only in 2005 from June 24-30 on 3 trees. In 2006, bagging was conducted in Ruckersville from June 4-5 on 4 trees, in Harpers Ferry from June 10-11 on 2 trees, and in Newport from June 18-23 on 4 trees. Each tree was sampled during the same times of day as with the nectar quantification study: morning, midday, and afternoon. One panicle per tree was covered with a mesh fabric bag and one uncovered panicle was marked with flagging tape in the evening before sampling was to begin (similar to Dupont et al. 2004). In both cases, all open flowers on marked panicles were removed so that only flower buds remain. This provided certainty that flowers sampled were all of the same age. Two-three whole flowers were collected, depending on availability, and placed in vials of water as previously described for the determination of total sugars. The temperature and relative humidity were recorded at the experimental sites. Whole flower nectar sugars were analyzed from the bagged and non-bagged flowers using an anthrone analysis for total sugars as described above.

Data Analysis

The Shapiro-Wilk test for normality was performed on all data sets. Data for the total μg of sugar in the general study and in the nectar removal study were cube root transformed.

Sugar amounts (μg) for the nectar quantification study were analyzed with a two-factor analysis of variance (ANOVA), blocking for location and year which were found to be significantly different ($F = 33.62$, d.f. = 2, 746, $P < 0.0001$, and $F = 153.06$, d.f. = 1, 746, $P < 0.0001$, respectively) (SAS version 9.1, SAS Institute Inc., Cary, NC). In the nectar removal study, significant differences were found between years ($F = 4.48$, d.f. = 1, 729, $P = 0.0347$) and location ($F = 43.34$, d.f. = 2, 730, $P < 0.0001$) and were blocked to account for these factors in a three-factor ANOVA.

Sampling location was found to be significantly different in all of the nectar composition analyses (total sugar concentration: $F = 9.48$, d.f. = 2, 34, $P = 0.0005$; % sucrose: $F = 11.98$, d.f. = 2, 29, $P = 0.0002$; % fructose: $F = 8.05$, d.f. = 2, 29, $P = 0.0017$; % glucose: $F = 13.66$, d.f. = 2, 29, $P < 0.0001$). Year was not found to be significantly different in all nectar composition analyses (total sugar concentration: $F = 1.17$, d.f. = 1, 36, $P = 0.2864$; % sucrose: $F = 0.00$, d.f. = 1, 30, $P = 0.9466$; % fructose: $F = 0.00$, d.f. = 1, 30, $P = 0.9921$; % glucose: $F = 0.01$, d.f. = 1, 30, $P = 0.9057$). Location was blocked in four separate one-factor ANOVAs. Differences were considered significant when $P \leq 0.05$.

Results

Nectar Composition

Nectar analyzed by HPTLC was found to contain the sugars sucrose, fructose, and glucose. If other sugars were present, they were at undetectable levels through this analysis (detection level ~ 100 ng, Fell 1990). Differences in gender were compared for both total sugar concentration in nectar and for relative percents of the individual sugars. Mean values across gender and sugar type are shown in Table 3.1. For all trees, sucrose was the most abundant nectar sugar, averaging slightly over half of the total sugar amount with approximately similar amounts of fructose and glucose (at about 25% each).

For the individual sugars, expressed as a percent of the total sugar, no significance was found between tree genders for sucrose ($F = 0.44$, d.f. = 1, 28, $P = 0.5145$), fructose ($F = 0.90$, d.f. = 1, 28, $P = 0.3504$), or glucose ($F = 0.05$, d.f. = 1, 28, $P = 0.8248$) (Fig. 3.1). Male trees had a significantly higher mean total sugar concentration in nectar than did female trees ($F = 4.66$, d.f. = 1, 33, $P = 0.0382$). A regression analysis on the relative humidity (%) readings taken at each nectar sampling showed no significant effect on sugar concentration in nectar ($R^2 = 0.0202$, $F = 0.78$, d.f. = 1, 38, $P = 0.3815$).

Quantification of Sugars

The analysis of total flower sugars from male and female trees were compared at three different times of the day: morning, midday, and afternoon. Results are shown in Fig. 3.2. No significant differences were found between the overall means for male and female trees ($F = 0.44$, d.f. = 1, 746, $P = 0.5061$) or between the times of day ($F = 0.53$, d.f. = 2, 746, $P = 0.589$).

Nectar Removal

Flowers covered with bags were found to have significantly different mean total amounts of sugar compared to those left uncovered and open for insect visitation ($F = 6.07$, d.f. = 1, 729, $P = 0.014$). These results were not even across all the treatments, however. The highest amounts of sugar averaged over all times of day were found in bagged male panicles ($284.28 \pm \text{S.E. } 10.05 \mu\text{g}$) followed by non-bagged female panicles ($236.35 \pm 17.96 \mu\text{g}$). Female bagged ($180.17 \pm 17.78 \mu\text{g}$) and male non-bagged ($160.52 \pm 9.72 \mu\text{g}$) panicles had no statistical differences. A significant interaction effect was found to occur between bagging, gender, and time ($F = 6.99$, d.f. = 2, 729, $P = 0.001$). When compared within the morning time period, male bagged flowers were significantly different from female bagged and male non-bagged flowers, but not from female non-bagged flowers. In the midday time period, no significant differences were found between female non-bagged and male bagged flowers, but both were different from female bagged and male non-bagged flowers. Within the afternoon period, male bagged flowers were not significantly different from female bagged flowers, but were different from female and male non-bagged flowers. Results are shown in figure 3.3. When total sugar was

compared for overall time and gender however, no significant differences were found to occur (time: $F = 1.22$, d.f. = 2, 729, $P = 0.2967$; gender: $F = 3.23$, d.f. = 1, 729, $P = 0.0726$).

Discussion

It was not unexpected to see differences in the nectar samples from different locations and years in which this study was performed. Qualitatively, the *Ailanthus* trees at the Ruckersville and Newport, VA sites were generally healthier, more productive, and more mature than those at the Harpers Ferry, WV site. Also, many trees sampled in the first year at Harpers Ferry were lost for the second year due to highway maintenance.

Some differences were found in the nectar sugar rewards of *Ailanthus* between male and female trees. However, the relative percents of glucose, sucrose, and fructose for the genders were statistically similar. In both sexes, the nectar is sucrose-dominant, having over 50% of the total sugar amount with the hexoses fructose and glucose present at levels between 18-25%. This is a reasonable finding as the mixture ratio may substantially affect or may be affected by the pollinator assemblage. Different insects may have a preference for different ratios of the three sugar molecules (Baker & Baker 1983). Pollination would be significantly decreased if different types of pollinators were visiting male and female plants, as an insect must visit both sexes in order to transfer pollen.

Male and female flowers were found to produce similar total amounts of sugar; however, the concentration of sugars in the nectar of male flowers was higher than that in female flowers. This implies that female trees produce a more dilute nectar with the same amount of sugar. Why this would occur is somewhat unclear. The energy required for seed and fruit development can be a restricting force for the female plant. If female trees are producing a higher volume of nectar despite the 5-6% sugar solution difference from male trees, insects may remain on the flower longer to feed and increase the chance of successful pollination. However, additional studies are needed to verify this. Differences

in the sampling method for concentration versus that of total sugar amount could be a potential confounding factor in this study. Nectar for the concentration analysis was collected directly from the nectary disc using a microcapillary tube, much as an insect might, while the nectar for the total sugar analysis was collected by washing the whole flower in distilled water. Attempting to determine the total amount of sugar, or any nectar constituent, from a flower can be challenging as it requires the collection of *all* the available nectar from that flower. The whole flower washing method is effective at achieving this end, but if the flowers are allowed to sit in water for an extended period, breakdown of sugar chemicals can occur, particularly at elevated temperatures (Baker & Baker 1983). Every effort was made to prevent this, but sample collection and transport from remote field sites could have affected samples.

The difference in concentration between genders would seem to be problematic in encouraging flower constancy and outcrossing. Pollinators that require high levels of energy may utilize male flowers, but may not find female flowers worth their time and energy if the reward is too poor (Price 1997). If pollinators are unable to distinguish between male and female trees, however, pollination may not be affected. Female *Ailanthus* trees appear similar to male trees with the most noticeable difference being that male trees have many more flowers than female trees. From a distance, male and female flowers are also very similar in appearance. Although the concept of a search image has traditionally been used to describe cryptic prey, it may have an application to foraging pollinators as well (Goulson 2000). If pollinators are using a search image to locate *Ailanthus* flowers, the difference between males and females may not become apparent until closely inspected, at which point even low rewards may be worth taking.

No significant difference was found in the overall time of day for either of the studies measuring total amount of sugar per flower. Many plants exhibit peaks of production in nectar (Faegri & van der Pijl 1971), but a lack of change in sugar amount over time tends to imply a constant production throughout the day. The results in this study were similar to those of Bernardello et al. (1994) who found no significant differences between two times of day with the standing crop of nectar in the liana *Combretum fruticosum*

(Combretaceae). The flowering period of *Ailanthus* occurs during May and June in the mid-Atlantic region of the United States. This is a warm time of year, averaging 80°F (26.4°C) at midday. At these temperatures, many potential pollinators can be active from early morning through evening. *Ailanthus* is an unspecialized plant in terms of pollination syndrome and attracts a wide variety of floral visitors which may be active at differing times of day. It may be most beneficial to be producing a steady amount of nectar rewards throughout the day to accommodate this variety of insects.

Bagging flowers is a useful way to determine the effect of nectar foraging on production by comparing what is present in flowers that are exposed to visitors with those where visitation is blocked. The overall total amount of sugar was higher in the closed samples. An interaction effect between gender, time of day, and bagging complicated the results somewhat. Male flowers enclosed in bags were consistently higher in total sugar than those left open. In female flowers, however, the open samples were actually higher in sugar than the closed in the morning and midday sampling periods, though the opposite was true in the afternoon. Higher sugar amount in the female open flowers may be evidence of a stimulatory effect from nectar feeding. Manual removal of nectar throughout the day at specific intervals has been shown to be capable of inhibiting sugar production, stimulating an increase, or having no effect at all, depending on the plant species (Bernardello et al. 1994, Galetto & Bernardello 2004). Bagging itself may also have had an effect on sugar production. While this is a possibility in *Ailanthus*, it seems somewhat unlikely that differences in this physiological response would be present between males and females and over time. The discrepancies in the female samples more likely may have resulted from a low sampling size of female trees. Whether there is actually a lower sex ratio of females to males is unknown, but at these study sites it was difficult to find mature female trees in safe areas for sampling and only three total female trees were used for the bagging study.

Sugar in nectar is a vital energy source for many floral visitors; however, other nectar constituents, such as amino acids, resins, and gums, also may play a role in rewarding potential pollinators (Simpson & Neff 1983, Price 1997). In addition, pollen is an

important protein source for many insects including brood managers such as honey and bumble bees as well as for the Cantharid beetles that were so prevalent as *Ailanthus* visitors (Faegri & van der Pijl 1971). The pollen dehiscence schedule could well be an equally important predictor of pollinator visitation. As this study appears to be the first of its kind on *Ailanthus*, many more questions are in need of answering before its reproductive biology is fully understood.

Table 3.1. Mean percent of total sugar for sucrose, glucose and fructose and mean total concentration ($\mu\text{g}/\mu\text{L}$) of sugar found in nectar of *Ailanthus* for all locations. Male trees had a significantly higher mean total concentration of sugar in nectar than did female trees ($F = 4.66$, $d.f. = 1, 33$, $P = 0.0382$).

| | Mean (\pm SEM) | | | Total sugar Concentration ($\mu\text{g}/\mu\text{L}$) |
|-----------------------|------------------------|----------------|----------------|---|
| | Percent of Total Sugar | | | |
| | Sucrose | Glucose | Fructose | |
| Female (n = 8) | 60.1 \pm 4.9 | 18.4 \pm 2.4 | 21.6 \pm 2.8 | 353.5 \pm 48.0 |
| Male (n = 12) | 52.0 \pm 2.6 | 22.1 \pm 1.8 | 25.9 \pm 1.5 | 406.7 \pm 38.4 * |
| Total (n = 20) | 56.6 \pm 2.6 | 23.3 \pm 1.5 | 25.6 \pm 1.5 | 386.5 \pm 36.8 |

* within column indicates a significant difference

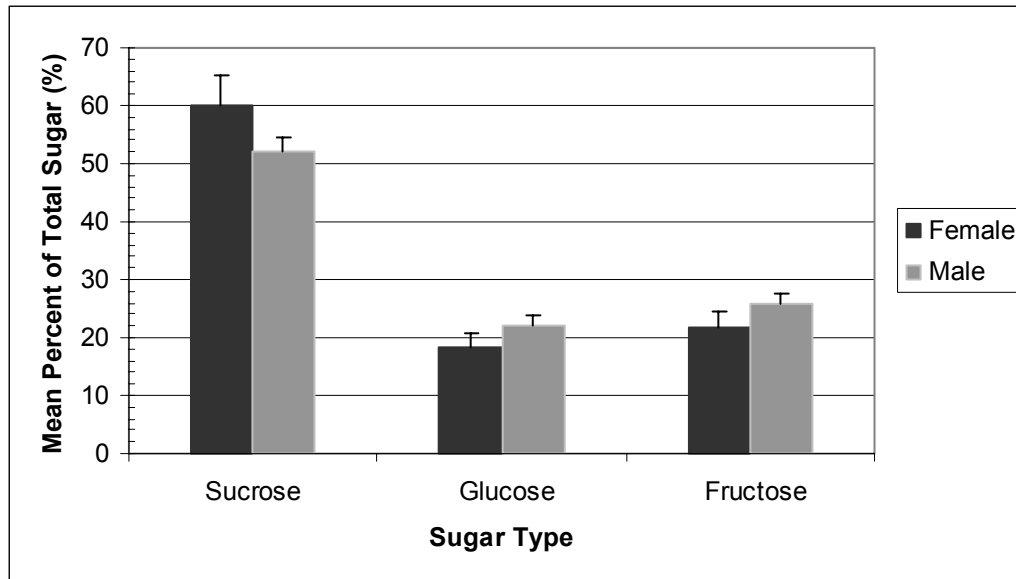


Fig. 3.1. Mean relative percent of total sugar + SEM for sucrose, glucose, and fructose in nectar samples in male and female *Ailanthus* trees. Differences in percent were not significant for any of the three sugar types (sucrose: $F = 0.44$, d.f. = 1, 28, $P = 0.5145$; fructose: $F = 0.90$, d.f. = 1, 28, $P = 0.3504$; glucose: $F = 0.05$, d.f. = 1, 28, $P = 0.8248$).

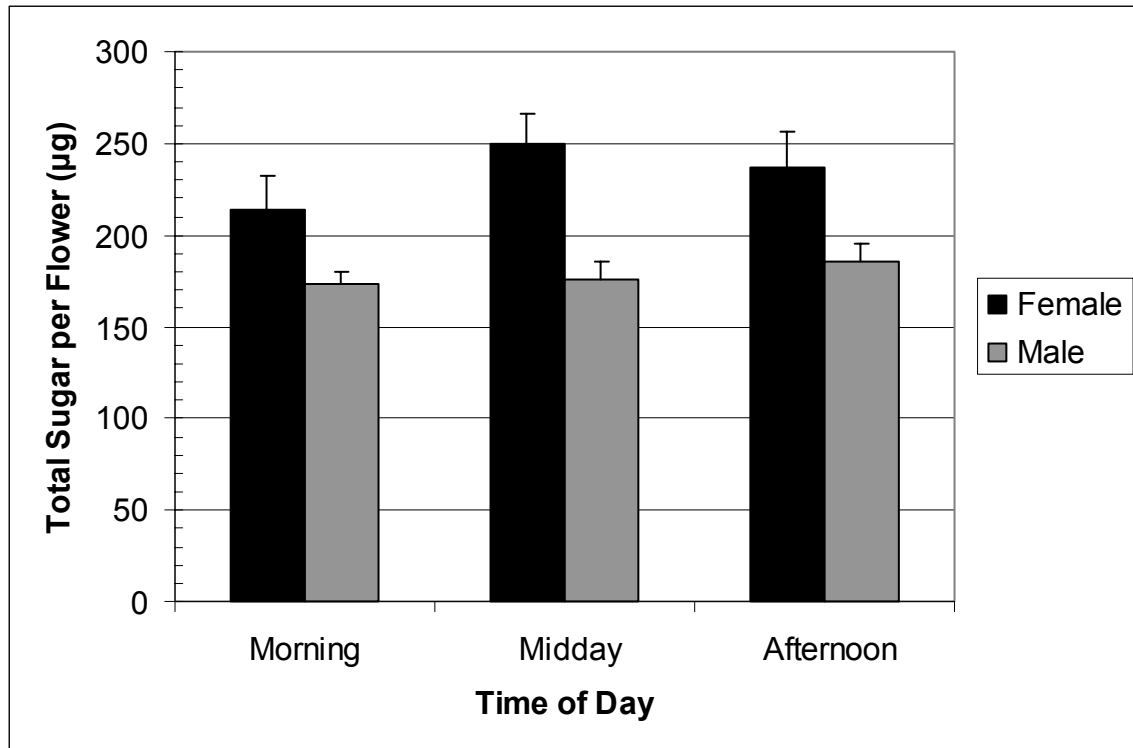


Fig. 3.2. Effect of time of day on mean amount of total sugar per flower (μg) + SEM collected for male and female trees in 2005 and 2006. No significant differences were found for either tree genders ($F = 0.44$, d.f. = 1, 746, $P = 0.5061$) or time of day ($F = 0.53$, d.f. = 2, 746, $P = 0.589$).

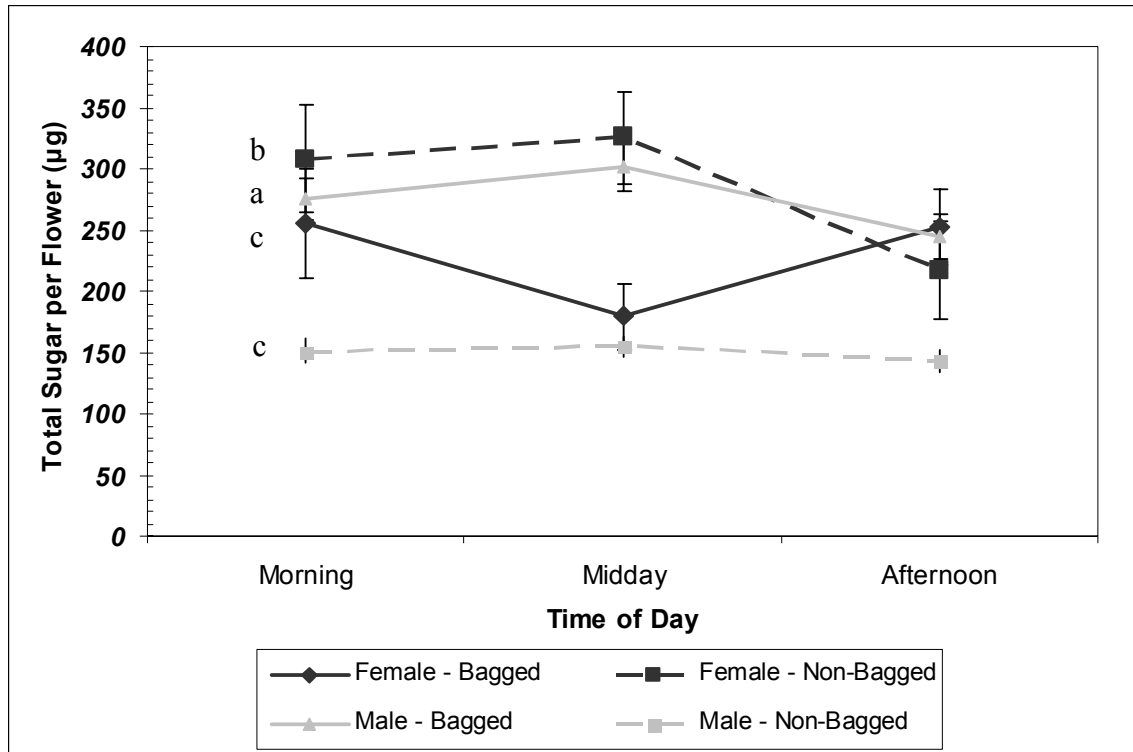


Fig. 3.3. Mean total amount of sugar \pm SEM per flower comparing panicles left open to nectar removal throughout the day by floral visitors to those with visitation prevented by covering with mesh bags for male and female trees. Overall, bagging treatments were significantly different ($F = 6.07$, d.f. = 1,729, $P = 0.014$), but time and gender were not (time: $F = 1.22$, d.f. = 2, 729, $P = 0.2967$; gender: $F = 3.23$, d.f. = 1, 729, $P = 0.0726$); however, a significant interaction effect was found to occur between bagging, gender, and time ($F = 6.99$, d.f. = 2, 729, $P = 0.001$). Lines accompanied by letters not in common indicate significant difference ($P < 0.05$) from other treatments over all time periods.

CHAPTER 4. Summary and Conclusions.

This study utilized several different techniques to examine the pollination biology of *Ailanthus altissima*, focusing on floral visiting species, visitation times, and the nectar profile. This is the first such study on *Ailanthus* and can provide a first step toward understanding its sexual method of reproduction.

It is evident from the data on insect visitation that there are a number of native pollinators visiting *Ailanthus* in the mid-Atlantic region and they are not likely to be a limiting factor in its propagation. We found that the floral visitors of *Ailanthus* were dominated by the soldier beetle *Chauliognathus marginatus*. Ants made up a large majority of the species assemblage as well, with the prevalent genera being *Formica*, *Prenolepis*, and *Camponotus*. Smaller numbers of a variety of other species were found, including halictid bees, syrphid and muscid flies, and several other beetles. In regard to the apiculturists' concern for poor quality *Ailanthus* honey, we found a relatively low percent of *Apis mellifera* visiting *Ailanthus* at our sites (though up to 13.6% for *Bombus* spp. in the second year at Newport). This does not necessarily mean that *Ailanthus* should not be a major concern for beekeepers. If concentrated amounts of *Ailanthus* are located near a hive, visitations could be much higher as nectar is easily accessible and flowers on male trees are numerous. Also, it may only take a small amount of *Ailanthus* nectar to change the flavor of a large amount of honey.

No major diurnal pattern was found in the visitation count survey. A significant difference was seen at the Newport site in 2006, however, no one sampling period was significantly different from all others. Gender was only a factor in the second year at Ruckersville and Newport. Differences were found between the male and female trees in visitation rates; however these differences opposed each other. Males had higher visitation rates in Ruckersville while females had higher rates in Newport. These results may have been due to a small sample size for female trees in Newport. When the soldier beetle *C. marginatus* was removed from the count survey analysis at the Ruckersville site in 2006, male and female trees were not found to be significantly different. For both

Ruckersville and Newport, overall numbers of visitors to male trees were considerably decreased with the removal of these beetles, which are known to eat pollen. The removal of *C. marginatus* did not affect significance for any other factors in the analysis.

In the nectar profile study, time of day was not found to be a significant factor overall. Sugar amounts and concentrations remained constant throughout the day, suggesting a continuous production of nectar by *Ailanthus*. Male and female flowers produced similar amounts of total sugar, but male flowers were found to have a higher concentration of total sugar in nectar: 40.7% sugar solution for males versus 35.3% for females. Nectar was found to be sucrose dominant with similar amounts of fructose and glucose. When flowers were denied insect visitation by covering with mesh bags, overall higher amounts of sugar were collected from flowers, however, these differences were not even across all treatments. Male bagged flowers had the highest amount of sugar, followed by female non-bagged flowers, female bagged, and male non-bagged (though the last two were not significantly different).

The lack of an overall diurnal pattern in insect visitation is consistent with the constant availability of nectar in *Ailanthus* flowers. This constant supply of rewards has likely led to a constant supply of visitors throughout the day, or perhaps vice versa. While *Ailanthus* flowers are small in size, limiting their reward size accordingly, the constant source of nectar could be a strong attraction to larger visitors. A presumably low handling time and travel distance between flowers might also increase attractiveness to more skilled pollinators. However, competition tends to be higher for the rewards from easily manipulated flowers and because of this the profit from more complex flowers can be substantial by comparison (Heinrich 1983). This may explain the relatively low number of honey bees collected. Competition between plant species for pollinators may also have had an effect on the visitor numbers and species for *Ailanthus*. Other plants in close proximity to the *Ailanthus* trees studied may have had more attractive floral rewards for certain insect species. Many species flower along roadsides during the month of June in the mid-Atlantic region.

Pollination syndromes are generally described relative to a specific dominant pollinator. While cantharid beetles were the dominant species collected, *Ailanthus* was visited by a number of different groups of insects. Furthermore, cantharophily tends to be rather uncharacteristic and lacking in any specialization. The simple dish type of blossom with floral rewards open and easily accessible that is seen in *Ailanthus* tend to be attractive to beetles, flies, some wasps, short-tongued bees, and ants (which are often attracted to any sugar source) (Faegri & van der Pijl 1971). However, as was seen in this study, this type of flower may be visited by any insect able to handle equal or higher complexity in floral structure (Leppik 1957). The sucrose/hexose ratio was 1.16 overall (1.08 for males and 1.51 for females), making it sucrose-dominant according to the terminology described by Baker and Baker (1983). This sugar ratio seems contrary to what is normally expected for fly and short-tongued bee pollinators as they tend to be associated with a higher proportion of hexose to sucrose in nectar. While these were present, they were not the most common floral visitors. The sugar mixture ratio preference for beetles has been studied in too few flower species to make accurate predictions on associations (Baker and Baker 1983). Beetles' floral associations also may be confounded by their attraction to and the availability of pollen (Faegri & van der Pijl 1971).

As inevitably happens with any research, certain factors are limiting in data collection. A number of challenges arose during this study that may be useful for consideration in future work. Time constraints in blooming period of *Ailanthus* resulted in smaller sample sizes than may have been preferable for the visitation pattern and caging studies. We thought it important to space out trees studied to avoid sampling the same genetic clone produced through root suckers, and this involved greater driving time. The use of several different locations with slightly different climates to extend the blooming time available for sampling from two weeks to around a month, however, was found to be an effective strategy and is recommended for future studies on *Ailanthus*.

At the three locations used for this study, there was a dearth in female trees that made even treatment sizes difficult to impossible. Whether this is a peculiarity of these locations or if it is evident of a low female to male sex ratio is unknown. Male trees do

tend to be more conspicuous than female trees with around 3 times as many flowers. Greater visibility of male trees may have had an effect on visitation rates as well. Another issue of the study sites was the exclusive use of highway right-of-ways for sampling. Right-of-ways were advantageous for their ease of access, lack of landowner permission needed, and abundance of *Ailanthus*, but additional survey work should be done before the trees there can be conclusively declared as representative as a whole of *Ailanthus* and its associated pollinators.

An initial component of this research was to survey *Ailanthus* for any evidence of decline in the region, as there has recently been some interest in the use of a *Fusarium* fungus as a biological control of the invasive tree. We did not find a great enough occurrence of this decline at any of the three sites studied to report it in the main findings, however one patch at the Harpers Ferry, WV site did appear to be in decline. Symptoms in this patch ranged from chlorotic leaves to total loss of canopy and death. The cause of the decline there is currently unknown. Dr. Jay Stipes of Virginia Tech is currently conducting additional research into *Fusarium* infection of *Ailanthus*.

When examining an ecosystem, particularly for the first time, the complexity of interactions can limit the examination of every aspect at once. Work on pollen composition and dehiscence will greatly add to the understanding of *Ailanthus*'s floral rewards and pollinator visitation, as will determining other possible nectar constituents such as amino acids. Further investigation into the reason and outcome of floral visitation by specific insects will also be helpful. Future research on these interactions is needed for developing effective control measures for the invasive *Ailanthus*.

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