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Reproductive Biology of the invasive plant *Elaeagnus umbellata*: breeding system, pollinators, and implications for invasive spread

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THESIS APPROVAL

REPRODUCTIVE BIOLOGY OF THE INVASIVE PLANT *Elaeagnus umbellata*:
BREEDING SYSTEM, POLLINATORS, AND IMPLICATIONS FOR INVASIVE
SPREAD

By

Nathan Soley

A Thesis Submitted in Partial
Fulfillment of the Requirements
for the Degree of
Master of Science
in the field of Plant Biology

Approved by:

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January 31, 2013

AN ABSTRACT OF THE THESIS OF

NATHAN SOLEY, for the Master of Science degree in Plant Biology, presented on *January 29, 2013 at Southern Illinois University Carbondale.

TITLE: Reproductive biology of the invasive plant *Elaeagnus umbellata*: breeding system, pollinators, and implications for invasive spread.

MAJOR PROFESSOR: Dr. Sedonia Sipes

Reproductive studies in invasive plants are necessary for an understanding of their potential to establish and spread in foreign environments. *Elaeagnus umbellata* Thunb. (autumn olive) is an invasive woody shrub that flowers early in the spring and is often noted for its abundant fruit set. This study examined the reproductive biology of *E. umbellata* in Illinois, where it is highly invasive. Hand-pollination experiments were performed to determine the breeding system of *E. umbellata*, and floral visitors were collected to determine its pollinators. Experiments showed that *E. umbellata* is a predominantly outcrossing species with a self-incompatible breeding system. However, individual variation was detected in several reproductive characteristics. Pollen tube analyses revealed that a small percentage of individuals allow successful self-pollen tube growth, and self-fruit set resulting from automatic self-pollination (autogamy) was relatively high in a few plants. Automatic self-pollination is possible because the male and female parts of flowers mature synchronously, but the likelihood of autogamy may vary among individuals due to variability in the spatial separation of male and female parts (herkogamy). Variability in the incompatibility system and the level of herkogamy may impact the outcrossing rates and reproductive success of individuals.

The majority of floral visitors to *E. umbellata* were generalist pollinators. Frequently visiting bees included small and large species such as native *Andrena* spp., *Augochlorella aurata*, *Bombus* spp., *Ceratina calcarata*, *Xylocopa virginica*, and the

introduced *Apis mellifera*. *Bombylius major* (large bee fly) and the moth *Mythimna unipuncta* (armyworm) were also frequent visitors. Most of the above insect taxa are pollinators of *E. umbellata* based on analysis of pollen on insect bodies. *E. umbellata* is likely to achieve its abundant fruit set where these common pollinators and other *E. umbellata* are present. However, in my study sites, many individuals experienced low fruit set on branches that were open to pollinator visitation, suggesting pollen limitation may be common in some years and at certain sites. The discovery of autogamous individuals demonstrates that some *E. umbellata* individuals may be able to establish and spread even when mates or pollinators are limiting.

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CHAPTER 1

INTRODUCTION

Invasive plants are an issue of great concern as they continue to become detrimental to the environment (Vitousek et al. 1996) and economically costly to manage (Hobbs & Humphries 1995; Pimentel et al. 2000). Invasiveness is the degree to which non-native species counteract the ecological stability or economic productivity of a native landscape. Plant invasiveness has been correlated with various abiotic and biotic factors (Thompson et al. 1995; Sutherland 2004; Lloret et al. 2005). In particular, the presence or performance of certain plant traits has been investigated extensively in invasive species and compiled in order to pinpoint which characters contribute to invasive ability (Pysek & Richardson 2007; van Kleunen et al. 2010). Reproductive traits are given frequent attention because of their influence on the persistence and spread of plant populations. For example, it is assumed that the rapid spread rate of some invasive plants is associated with a large production of seed. Identifying the reproductive traits influencing seed set and other invasive qualities may help predict potential pervasiveness (Lloret et al. 2005; Richardson & Pysek 2006; Hayes & Barry 2008) as well as inform efforts to manage invaded environments.

Breeding Systems in Invasive Plants

Plants achieve reproduction either asexually or sexually. Asexual reproduction occurs through the growth of vegetative parts or the setting of unfertilized seed (apomixis). Sexual reproduction is achieved through the setting of self-fertilized seed or cross-fertilized seed. Many flowering plants engage in more than one mode of

reproduction, and recent research has focused on deciphering the most prominent modes of reproduction among invasive plant species. The impetus for such research stemmed from Baker's prediction that self-compatible plants (plants capable of self-fertilization) are more successful weeds (Baker 1955). He based this prediction on observations of a high proportion of self-compatible plants on islands, which suggested that self-compatibility is advantageous to species colonizing a new environment. Unlike obligate outcrossing plants, self-compatible individuals do not require conspecifics to mate and generate offspring. Instead these species are able to establish a population from just one individual. Baker's prediction (known as Baker's Law) has been supported by the verification of self-compatibility in many invasive plants (Cavers et al. 1979; Crompton et al. 1988; Hao et al. 2011; Ward et al. 2012) and the finding that the advantages of self-compatibility go beyond just the colonization phase. For example, van Kleunen and Johnson (2007a) found that self-compatible invasives in the U.S. have a broader distribution range than self-incompatible invasive plants.

Self-compatible exotic plants that can set seed through automatic self-pollination (autogamy) may be at a particular advantage. Autogamous plants do not require external forces to transfer pollen from the anthers to the stigma, and hence can produce seeds without acquiring pollinators or mates. This ability may increase the chances of population establishment and subsequent spread. For example, when studying exotic Iridaceae plants of South African origin, van Kleunen and Johnson (2007b) found that those taxa that had become invasive outside of South Africa produced significantly more autogamous fruit than plants that had not become invasive. In a separate study, tests carried out by Rambuda and Johnson in South Africa (2004) found autogamy in all

13 of the woody invasive plants they analyzed. These results suggest that autogamy is important for plant invasions. These studies are also in contrast with conventional thought which has held that autogamy is not as important in colonizing perennial species because they have multiple years to achieve mating success and are not limited in mating opportunities by an annual or biennial lifespan (Lloyd & Schoen 1992; Bond 1994). The benefits of autogamy in some perennial invasive plants must outweigh any cost of selfing. One such cost of self-fertilization is increased homozygosity among progeny that may cause inbreeding depression (Charlesworth and Willis 2009). Although autogamy seems to be important among some invasive plants, more work is needed to determine if perennial invasive species possess strategies that lessen the chances of self-fertilization and its associated costs.

The chances of automatic self-fertilization in a self-compatible species can be largely determined by floral morphology and phenology. The male and female organs within a hermaphroditic flower can be close together or they can be separated spatially (herkogamy) and/or temporally (dichogamy). The degree of separation between stigma and pollen influences the ability to self-pollinate, the rate of outcrossing, and even pollinator behavior, so their examination is essential in predicting breeding habits. Also, adaptive changes in the floral morphology of hermaphroditic invasive populations may happen relatively quickly because of selection pressures during colonization. For example, the distance between stigma and anthers was significantly less in *Nicotiana glauca* that had recently colonized islands as compared to mainland invasive populations (Schueller 2004). Island populations self-pollinate more often as a result. Overlapping times in stigma receptivity and pollen viability allow self-pollination as well.

Studies of invasive plants can reveal whether traits promoting self-fertilization are favored by selection.

Depending upon the degree of herkogamy and dichogamy, plants that are self-compatible may or may not require pollen vectors to deposit self-pollen on stigmas in order to set fruit. For example, flowers of the invasive perennial *Senna didymobotrya* are self-compatible, but require manipulation by specific pollinators (van Kleunen and Johnson 2005). Wing vibrations from large insects cause the pollen from the anthers to be released directly onto the stigma, which is efficient enough that small populations of *S. didymobotrya* have just as great of fruit set as large populations (van Kleunen and Johnson 2005). Another woody invasive plant in the tropics, *Miconia calvescens*, was observed to set self seed despite the high degree of stigma and anther separation within flowers (Meyer 1998). The authors conclude that external processes (i.e. wind, rain, insects) could cause self-fertilization.

Plants that are obligate outcrossers cannot self-fertilize even with pollinator assistance, due to incompatibility systems. Rejection of pollen is governed at the genetic level where pollen having a similar genotype as the recipient carpel is prevented from causing self-fertilization as well as crosses between genetically similar individuals (Rea & Nasrallah 2008). Self-incompatible plants require both pollen vectors and mates and therefore may be limited in mating opportunities, especially after introduction to a foreign landscape.

Ecological theory predicts that obligate outcrossers are somewhat hindered as colonizers because of their requirements for cross pollen from different individuals (Barrett et al. 1986). Even if conspecifics are present in a founding population, the

transportation of cross pollen is dependent on the abundance and efficacy of pollen vectors (Aizen & Harder 2007). Inadequate pollen movement can occur because of a depressed amount of pollinators in less rewarding, small populations (Ågren 1996). Under these conditions, variation in the breeding system of obligate outcrossing species may allow some individuals to still produce propagules. For example, some plant species can show continuous variation in the strength of the self-incompatibility response, from individuals that strongly inhibit self-pollen tube growth to a minority that set occasional self-fertilized seed (Ferrer et al. 2009). Shifts from outcrossing to selfing via the breakdown of incompatibility systems is considered to be a common transition in plant evolution (Ilgic et al. 2008), but it has been rarely studied and found in few cases after invasion (Petanidou et al. 2012; Ward et al. 2012).

Brennan et al. (2005) suggested that variability in the self-incompatibility response could be retained and serve as an optimal situation for range expansion. When mates and pollinators are plentiful, outcrossing would still contribute a great majority to the propagule supply of partially self-compatible species. Outcrossing populations of plants are more genetically variable than selfing populations (Barrett 2011) and are better able to adapt to different environments – thereby increasing the possibility of invasive spread (Rice & Sax 2005). However, when opportunities for outcrossing are limited, self-compatibility may ensure an alternate method for reproduction. Indeed, the periphery of some species' range is dotted with self-compatible individuals that arose from primarily obligate outcrossing species (Levin 2012). This pattern is often due to more extreme environmental conditions at the edge of species' limits. Such environments may harbor few effective pollinators and mates.

Because these same challenges are faced by invasive species, genetic variations in incompatibility systems, herkogamy, or dichogamy may play a role in invasive spread (Prentis et al. 2008).

Despite the obstacles facing obligate outcrossers in a foreign landscape, there are notable examples of successful invasive plants being obligate outcrossers (Sun and Ritland 1998; Brennan et al. 2002; Hong et al. 2007; Lafuma & Maurice 2007). Hong et al. (2007) note that the invasive *Mikania micrantha* has spread rapidly to become one of the most invasive plants in the world despite being an obligate outcrosser that requires insect pollination. Also, contrary to studies suggesting a link between invasiveness and self-fertilization (van Kleunen and Johnson 2007a; Hao et al 2011; Ward et al 2012), an analysis of the U.S. flora by Sutherland (2004) showed that invasive exotics were more likely to be self-incompatible than non-invasive exotic plants and that woody perennial species are the majority among invasives. In order to better understand patterns in invasive plant breeding systems, it is necessary to examine their relationships with pollinators.

Insect Pollination in Invasive Plants

Invasive plants face the challenge of establishing important mutualisms after introduction into a foreign environment (Richardson et al. 2000). In instances where pollinators are required, only those plants that can attract pollinators have a chance of becoming invasive. Many successful invasive plants achieve widespread dominance because they are able to attract generalist pollinators (Chittka & Schürkens 2001; Brown & Mitchell 2002; Bjerknes et al. 2007; Bartomeus et al. 2008). For example,

Ward et al. (2012) found that pollinators are necessary for self-pollination in three invasive self-compatible milkweeds (Asclepiadaceae) in Australia, and that generalist lepidopteran and hymenopteran insects provide this service. An appeal to generalist pollinators allows even small populations of obligate outcrossing species the possibility of achieving colonization success (Lafuma & Maurice 2007). For example, successful reproduction seems to be attained in small populations of the obligate outcrossing *Gomphocarpus physocarpus* in its native range (Coombs et al. 2009). Efficient pollen transfer by generalist insects translated into high rates of fruit set, while the typical Allee effects associated with small populations (Ågren 1996) were not apparent.

Although there are cases demonstrating successful acquisition of pollinators by introduced plants (Lafuma and Maurice 2007; Coombs et al. 2009), there are still questions as to the degree to which dependency on pollinators is a hindrance to invasive success. Exotic plants in the introduced range often have different insect visitors from those in the native range, and the effectiveness of these visitors as pollinators may differ between regions. If pollinators are abundant and consistently carry invasive pollen, they contribute to invasiveness (Stout 2007). If not, invasive plants may be pollen limited (Parker 1997; Larson et al. 2002).

The complexity of plant-pollinator interactions requires direct experimental measures of pollinator efficacy or qualitative assessment of visitor behavior. Such measures are especially important for invasive species that attract numerous floral visitors, because the efficiency of pollination varies with each type of visitor (Inouye 1983; Stout 2000). For example, Stout et al. (2007) observed a wide range of bee and fly taxa visiting the invasive *Rhododendron ponticum*, but only bumblebee species

(*Bombus*) were deemed pollinators because they frequently come in contact with the stigma. In order to determine the pollinators of the invasive yellow star thistle (*Centaurea solstitialis*), Mciver et al. (2009) used the percentage of *C. solstitialis* pollen grains on insects collected from the plant's flowers. It is often found that the pollinators of invasive plants are also pollinators of native plants; therefore, invasive plants could affect the pollination of native plants and possibly disrupt a major ecosystem service (Traveset & Richardson 2006; Morales & Traveset 2009), so characterization of floral visitors is needed for proper invasive plant management.

Studies have shown that invasive plants can integrate into native pollination networks through interactions with generalist pollinators (Memmott & Waser 2002; Vila et al. 2009). Their presence can increase (Nielsen et al. 2008; McKinney 2010) or decrease (Chittka & Schürkens 2001; Brown et al. 2002) the number of floral visitors to native plants. A meta-analysis by Morales and Traveset (2009) revealed that invasives most frequently decrease visitation to native plants, but detriments to native plant reproduction were not as obvious. Indeed, although overall visitation rates may be higher to invasive plants, those insect taxa important to native plant pollination may still remain frequent visitors to native plants (Larson et al. 2006), or they may carry small amounts of invasive pollen (Bartomeus et al. 2008).

The amount of invasive plant pollen on flower-visiting insects can be used to predict impacts on native plant pollination and assess the degree to which insects utilize the floral resources of invasive plants. Invasive plants may be suitable sources of nectar and pollen and could bolster native pollinator populations. This in turn could

facilitate native plant reproduction (Tepedino et al. 2008), but more research is needed to support this idea.

An understanding of breeding systems and pollination within invasive plants benefits efforts to manage current invasions and prevent future ones. Not only do these studies help in predicting colonization success (Baker 1965) but they also inform estimates of naturalization rates (Barrett et al. 2008). A plant's mode of reproduction can provide insight into evolutionary studies as well. The degree of phenotypic plasticity, genetic recombination rates, and effective population size can more easily be inferred given prior knowledge of reproductive qualities (Sakai et al. 2001). All of these may factor into more effective management of a particular invasive plant species.

Study Species

This study investigates reproduction and pollination in the invasive plant *Elaeagnus umbellata* Thunberg (Elaeagnaceae). *E. umbellata* is a dense deciduous shrub that can grow 6-7 meters high (Church et al. 2004). It grows in moderately dry conditions in sandy or well-drained loamy soils but also does well on gravel beds of riparian zones. *E. umbellata* is invasive in 21 states in the eastern USA (USDA 2006) and continues to be problematic as control measures are costly and labor intensive (Byrd et al. 2012). It has the ability to spread rapidly and encroach into natural areas because of its prodigious fruit set (Ebinger & Lehnen 1981; Stark 2000).

Elaeagnus umbellata was introduced to North America in 1830 by Lake County Nursery, Ohio (Dirr 1998). Accounts vary as to the location from which seed was obtained for North American introduction: Korea (Redher 1940), Japan, Himalayas

(Eastman 2003), and Nepal (Dirr 1998) have been mentioned. The purpose of its initial introduction was for propagation as an ornamental (Rehder 1940), but it may have been introduced for reclamation use as well (Eastman 2003). It was most extensively planted throughout the eastern United States during the second half of the 20th century. *E. umbellata* was used as natural fencing in pasture at least as early as the 1940s, was popular in strip mine reclamation projects in the 1970s (Zarger 1980; Ashby et al. 1995), and was used in agroforestry practices during the 1980s because of its ability to fix nitrogen (Funk et al. 1979). Throughout the second half of the 20th century, it was planted as windbreaks along roads and was most ardently promoted for its benefits to wildlife (Henry 1980). The demand for *E. umbellata* was highest in the 1970s when it was the number one produced shrub among U.S. nurseries (Abbott and Fitch 1977). It was used in the restoration of mine spoils and other denuded landscapes because of its tolerance for poor soil conditions (Zarger 1980). These beneficial characteristics of *E. umbellata* resulted in its vigorous promotion up into the 1990s. Stark (2000) reported that *E. umbellata* was still being planted for wildlife cover by the Pennsylvania Game Commission in 2000.

Since becoming naturalized, it has had an extensive impact on the environment. The aggressive growth and spread rate of the species was documented by Ebinger and Lehnen (1981) while determining naturalization rates in eastern Illinois. From a distant patch planted in 1975, *E. umbellata* had rapidly spread a short distance away to weedy proportions – 1,359 plants (20% were over half a meter) within a 20x20 m area in 1981. Jones (1963) did not list *E. umbellata* as part of the Illinois flora and Mohlenbrock (1975)

stated that it rarely escapes cultivation; therefore, *E. umbellata* achieved invasive status in a very short period of time.

Elaeagnus umbellata easily forms dense monoculture stands that exclude native plant growth. Besides the impact of shading on native vegetation, *E. umbellata* may have a more widespread influence on ecosystem processes because of its ability to fix nitrogen. *E. umbellata* forms a symbiotic relationship with the nitrogen-fixing actinobacteria *Frankia*, allowing for the direct uptake of ammonia by the plant. Excess nitrogen can leach into the soil as a result. At one site in southern Illinois, soil nitrate levels were 16.7 times greater under *E. umbellata* than under grassland plots (Church et al. 2004). It has been suggested that the increased availability of nitrogen near *E. umbellata* may allow for the invasion of other weedy plants (Mostoller 2008). Also, there is a positive relationship between *E. umbellata* presence and stream nitrate levels (Goldstein et al. 2009), possibly decreasing stream biodiversity. The impact of this plant on nitrogen cycling (Baer et al. 2006) could rival other nitrogen-fixing invasive plants (e.g. *Myrica faya* in Hawaii) (Vitousek and Walker 1989) and may represent a more severe invasion than most noxious weeds.

The reproductive characters are known to some extent in *E. umbellata*. Vegetative reproduction is evident through the presence of root suckers (Kohri et al. 2002), but the extent to which *E. umbellata* spreads by this mode of reproduction is unknown. *E. umbellata* seems to invest a great deal in reproduction through flowering. Blooming occurs in early spring with the initiation of thousands of flowers. The blooming period lasts approximately two weeks for most plants (personal observation). The plant produces axillary inflorescences of 2-7 hermaphroditic flowers that span most

of the branch. Flowers are white at first and transition to yellow as the flowers reach 6-10 days old (personal observation). Their fragrant odor and significant nectar reserves could play a part in the attraction of many insect taxa (Sather & Eckardt 1987). The most pronounced chemical components of the floral scent are 4-methyl phenol, 4-methyl anisole, eugenol, and methyl esters that all combine to emit a heavily fragrant, dull vanilla odor (Potter 1995). The plant's generous nectar supply has been noted by bee keepers as ideal forage for honeybees (Hayes 1976). Approximately four months after the flowering season, fruits ripen to a scarlet red color. One *E. umbellata* can produce an abundant crop of up to 24,000 drupe-like fruits (Sather & Eckardt 1987). Fruits contain a single seed. Birds play a significant role in providing long-distance dispersal of the seed (Kohri et al. 2011). *E. umbellata* lacks prolonged seed dormancy and does not form seed banks (Kohri 2008; Carter & Ungar 2002). Many of the reproductive traits of *E. umbellata* are presumed to have a substantial influence on its invasiveness, but more data are needed to determine the reproductive biology of this species. Such data could be used to estimate the possible impact its current and future invasion will have on communities.

Objectives and Hypotheses

This study examines the reproductive biology and pollination ecology of the invasive plant *Elaeagnus umbellata* in Illinois, U.S.A. My first research objective was to assess whether the morphology and phenology of *E. umbellata* flowers allow for self-pollination. The morphology of *E. umbellata* flowers in relation to the position of stigma and anthers has only been addressed in a cluster analysis of *Elaeagnus* in China (Sun

and Lin 2010). That analysis categorized the stigma position of *E. umbellata* as “at or below the stamen,” which indicated no spatial separation. Although stigma position can change following introductions (Schueller 2004), my hypothesis was that *E. umbellata* would not show any signs of separation between male and female functions and that automatic self-pollination will be possible

My second research objective was to determine the breeding system of *E. umbellata*. A breeding system study of *Elaeagnus mollis* in China revealed that it is self-compatible but only 6.9% of flowers produced seed after self-pollinations (Wei et al. 2007). I predicted that *E. umbellata* would show some degree of self-compatibility as well. Reports of high fruit set in *E. umbellata* (Sather & Eckardt 1987) suggested that fruit set is not limited by the presence of mates or pollinators. I hypothesized that *E. umbellata* would have a mixed mating system.

My final research objective was to determine the floral visitors and pollinators of *E. umbellata*. In the native range, native bees were mentioned as primary floral visitors to *E. umbellata* (Wei et al. 2007). In the invasive range, the plant has been reported to be visited by a “variety of insects” (Sather & Eckardt 1987). The only specific floral visitor that has been recorded on invasive *E. umbellata* was the honeybee (*Apis mellifera*) (Hayes 1976). The floral traits of *E. umbellata* suggested the most probable pollinators to be moths. Moths are the most likely group to be attracted to the white-colored flowers and strong fragrance (Wyatt 1983). I hypothesized that *E. umbellata* would have a generalized pollination system and would be pollinated by bees and moths.

CHAPTER 2

MATERIALS AND METHODS

Study Sites and Sampling

Field studies were conducted during the spring and summer of 2011 and 2012 at multiple sites in central and southern Illinois, U.S.A. It is suspected that the Illinois region is inhabited by *E. umbellata* 'Cardinal' and 'Elsberry' (Ebinger & Lehen 1981; Allan Mickelson personal communication). Records from the Soil Conservation Service (NCRS) report that *E. umbellata* 'Cardinal' was first introduced in 1961. *E. umbellata* 'Elsberry' was introduced in 1979. Both cultivars were seed-propagated and selected for their abundant fruit production and fruit size.

The majority of data were collected at the following field sites (Table 1): Allenville, IL (ALL); Buffalo Trace Prairie, IL (BTP); Crab Orchard National Wildlife Refuge, IL (CONWR); Murphysboro, IL (MUR); Southern Illinois University Carbondale, IL (SIUC); Touch of Nature Environmental Center, IL (TONEC); Williams Hill, IL (WH). All of the sites had experienced past anthropogenic disturbance and many are closely adjacent to disturbed areas.

The ALL site is in central Illinois and is characterized by large fields of mostly *Festuca* species and some small herbaceous species with forested windbreaks bordering these fields. *E. umbellata* formed a continuous presence along the edge of forested patches.

The other central Illinois site (BTP) was purchased by the Champaign County Forest Preserve District (CCFPD) in 1976. The CCFPD initiated prescribed burns to

many parts in the 1990s and began officially restoring the site to prairie in 2000. It is a 107 hectare site consisting of restored prairie, savanna, successional, shrubland, and forested habitat. *E. umbellata* was common in the successional areas and sparsely populated the prairie.

TONEC is a southern IL site located within the purchase unit for Shawnee National Forest, approximately 13 kilometers south of Carbondale, IL, USA. Past disturbance caused by agricultural practices are evidenced by the presence of fragmented forest. Forest and prairie were opened to livestock pasture and crop production before being purchased by Southern Illinois University in 1949. TONEC was documented as being completely forested in land survey records from 1975. Ashman (1962) did not document *E. umbellata* as a secondary successional tree species at TONEC. The first records of *E. umbellata* within TONEC are in 1987 (Davis 1987). Yates et al. (2004) reported densities of 0.5 and 0.9 stems m⁻² for interior and edge patches of *E. umbellata* within TONEC. Experimental plants at TONEC were located in a power line cut, along the edge of primary successional forest, and within successional/shrubland sites containing patches that are mowed once a year.

CONWR is located approximately 26 kilometers east of Carbondale, IL. It is 17,762 hectares, incorporating various landscape types including lakes, agricultural land, successional old fields, restored prairie, and second-growth forest. The vegetation types of CONWR include upland forest, bottomland hardwood forest, fallow herbaceous old-fields, mixed upland shrubland, restored grassland, and forest regeneration sites (Battaglia 2005). Experimental plants were located in the northern portion of the refuge

in a successional/shrubland site dominated by other herbaceous vegetation, much of it being other invasive species. *E. umbellata* was the dominant shrub present.

WH is the second highest point in Illinois and is located within the southeast portion of the Shawnee National Forest. Communication towers occupy the summit, but the site mostly consists of primary successional forest containing *Cornus florida* (dogwood), *Cercis canadensis* (redbud), and a variety of hardwoods. *E. umbellata* is not as densely present on WH as the other study sites. Experimental plants were located near the communication towers and along a road leading to the top of WH.

Floral Morphology

Floral morphology was assessed at ALL and BTP in 2011 and TONEC and CONWR in 2012. At each site, three flowers were randomly selected from each of 14 plants and placed in Carnoy's fixative (6 cc. ethyl alcohol: 3 cc. chloroform: 1 cc. glacial acetic acid). Morphological measurements were made after approximately two months in fixative. Corolla length was measured from the base of the corolla tube to the opening of the corolla tube. Stigma and anther height was measured from the base of the corolla in order to calculate any degree of herkogamy. A mean distance between the stigma and anthers was obtained from measurements of the three flowers from each plant.

Floral Phenology

The timing of stigma receptivity and proportion of viable pollen was recorded at different flower stages. In 2011, flowers from 10 plants at BTP were sampled at the

following visually-assessed stages: bud, anthers newly dehisced, anthers old, petals yellow. An additional five plants were sampled exclusively for stigma receptivity at ALL. In 2012, flowers from 10 plants at TONEC were bagged before anthesis and sampled at 1, 2, 3, or 4 days following anthesis.

Four flowers – one for each stage – from each of the 10 experimental plants at BTP and 5 plants at ALL were sampled for stigma receptivity in 2011 ($n = 60$ flowers). In 2012, four flowers – one for each stage – from each of the 10 experimental plants were sampled for stigma receptivity ($n = 40$ flowers). Stigma receptivity was recorded for each flower using Peroxtesmo paper. A positive Peroxtesmo test confirms the presence of peroxidase and therefore is a good indicator that the stigma is receptive (Dafni 1998). A positive or negative result was recorded for each flower.

Pollen viability was tested using methods described by Peterson et al. (2010). From 10 experimental plants at BTP in 2011 and 10 experimental plants at TONEC in 2012, 12 flowers were sampled – three flowers for each stage – and placed in Carnoy's fixative ($n = 120$ flowers). After approximately two months in fixative, anthers from each stage were then placed on a slide, dissected, stained using a modification of Alexander's stain (Peterson et al. 2010), and examined under a light microscope. The stain differentially dyes viable pollen red and non-viable pollen blue. The percentage of viable pollen per flower was scored by totaling the number of viable pollen grains out of 500 grains examined. The mean percent of viable pollen was calculated for each flower stage.

Breeding System

A breeding system study was conducted in 2011 at TONEC ($n = 17$), and in 2012 at TONEC ($n = 14$ plants) and CONWR ($n = 20$ plants). On each plant, five small branches were randomly assigned to receive one of five treatments; therefore, plants were used as blocks and branches were used as the unit of replication in the study. The terminal 10 cm of the treatment branches were bagged with mesh bags before anthesis to exclude pollinators, except for controls, which were left open to pollinators. The 10 cm bagged sections of the branches generally had 100 or more flower buds. To allow marking and manipulation of individual flowers, flower buds were trimmed off, leaving only ~ 15 buds in the bagged section. To assess whether this trimming impacted the fruit set in the remaining flowers, an untrimmed control was included as one of the five treatments. The treatments administered were autogamy, self-pollination, outcrossed, trimmed control, and untrimmed control (Table 2). The treatment regime at TONEC in 2011 did not include untrimmed control, and so had only four treatments. The self-pollination and outcrossed treatments were pollinated by manually transferring either self or cross pollen from a recently collected donor flower to the recipient stigma using a clean insect pin. Flowers were too small to emasculate before anthesis without damage. Instead, self- and cross-pollination treatments were emasculated soon after anthesis in order to minimize self-pollen transfer. Emasculations were not performed on TONEC flowers during the 2012 flowering season. All hand pollinations were performed within 24-48 hours of anthesis.

In order to further survey for inter-populational variation in selfing ability, several additional abbreviated replicates of the breeding system study were conducted on 10 plants at BTP in 2011 and 9 plants at WH in 2012. These included only autogamy and

control treatments. At BTP, the autogamy, trimmed control, and untrimmed control tests were performed. At WH, the autogamy and untrimmed control tests were administered on plants.

Destruction of some plants and treatment branches by unknown causes resulted in a reduced sample size for breeding system studies. At 2011 TONEC, three individuals were destroyed between 4 weeks and 7 weeks after pollination treatments, which reduced the sample size to 14 plants. At WH, the destruction of some experimental branches resulted in the number of control replicates being 6 while the number of autogamy replicates remained at 9.

At the end of the flowering period bags were removed during the development of fruits. The presence or absence of developing fruit was recorded for each flower within a treatment at various times throughout the maturation process. Bags were placed back on treatment branches approximately one month before fruit was ripe to minimize frugivory. The mature fruit set per treatment branch was recorded when fruits were red. Percent fruit set was calculated as the number of fruits per branch divided by the number of treated flowers per branch.

Any fruits that matured on treatment branches were collected. Seeds were washed, dried for 24 hours, and weighed. Mean seed mass was recorded for each treatment within a plant. Seed viability was tested to determine if treatments differed in the proportion of viable seed. Using a 1.0% solution of 2,3,5-triphenyl tetrazolium chloride, viability was analyzed for all seeds gathered from each treatment. A tetrazolium test developed for *Elaeagnus umbellata* by Olson and Barbour (2008) was initially followed. This test gave ambiguous results; therefore, seeds from 2011 were

soaked for 72 h. In 2012, the test was further optimized by soaking seeds in gibberellic acid overnight, and then cut in half and immersed in 1.0% 2,3,5-triphenyl tetrazolium chloride for 24h at 37°C in the dark (Jill Barbour, personal communication).

Pollen Tube Growth

In order to corroborate results from the breeding system study, flowers from six plants at 2012 TONEC were used to assess pollen tube development in self- and cross-pollinated flowers. Two branches on each plant were bagged prior to anthesis and randomly assigned to be either self- or cross-pollinated. On each branch, 4-12 flowers were hand-pollinated using an insect pin. Bags were placed back on the branches following hand-pollinations. Flowers from each branch were then fixed in Formalin-Aceto-Alcohol (FAA) (90 cc. 50% ethyl alcohol: 5 cc. glacial acetic acid: 5 cc. formalin) 24-72 hours after pollination in order to halt pollen tube growth. These flowers were placed in 70% ethanol after a few weeks in FAA. Flowers were later dissected and soaked in a 2.5 g chloral hydrate: 1 ml 30% glycerol clearing agent for 48 hours. Carpels were then mounted in 0.1% decolorized aniline blue / 0.1 M K_3PO_4 buffer solution for 8-12 hours. Aniline blue contains a flouochrome that binds to the beta portion of the callose that is deposited by pollen tubes (Johnson-Brousseau & McCormick 2004), allowing the growth of pollen tubes to be viewed under a fluorescence microscope. The extent of pollen tube growth was assessed for each flower by recording whether pollen tubes reached the ovary or not. A similar procedure was carried out for control flowers except these flowers were mounted solely in the 0.1 M K_3PO_4 buffer solution.

Diurnal vs. Nocturnal Pollination

To test whether branches exposed only to diurnal or nocturnal pollinators would differ in fruit set, an experiment involving the bagging and unbagging of flowers at dawn or dusk was carried out at MUR in 2011 (n = 5 plants) and at SIUC in 2012 (n = 12 plants). Two branches on each plant were randomly assigned to two bagging treatments (diurnal and nocturnal pollination). Treatment branches testing for diurnal pollination success were bagged at dusk to exclude visitation during the night and then left open during the day starting at dawn. In the nocturnal pollination treatment, bags were removed at dusk and reapplied at dawn. This method of bag removal and reapplication continued until all treatment flowers had abscised (approximately two weeks).

In 2011, the number of flowers on a treatment branch was not counted and so percent fruit set could not be calculated; therefore, the total number of mature fruits within a treatment was the dependent variable. Percent fruit set was recorded 7 weeks after pollination at SIUC in 2012, but destruction of treatment branches on one plant reduced the sample size to 11.

Floral Visitors

Insect visitors to *Elaeagnus umbellata* flowers were collected opportunistically at all the field sites of 2011 and at all the field sites of 2012. *Bombus* spp. were not collected because *E. umbellata* flowering coincides with the time that new *Bombus* queens are foraging, and their collection might negatively impact *Bombus* populations.

All other floral visitors were collected with a mist net and placed in a cyanide kill jar. The number of insects and taxa within one kill jar was kept at a minimum in order to minimally disturb the pollen carried on each visitor's body. Insects were later identified, and pollen on the bodies of frequent visitors was characterized in terms of the amount of pollen present and the percentage that was *E. umbellata*.

Floral visitors were identified to the lowest taxonomic level possible using published keys (Michener et al. 1994) and the online key "Discover Life" (Ascher & Pickering 2011). Several difficult and diverse taxa were identified by systematists with appropriate expertise. *Lasioglossum* and *Andrena* species were identified by J. Gibbs (Cornell University) and J. Ascher (American Museum of Natural History), respectively. Syrphidae were identified by M. Hauser (California Department of Food and Agriculture). Noctuidae were identified by D. Lafontaine (Canadian National Collection of Insects, Arachnids and Nematodes).

The amount of total pollen coverage on the body was visually estimated by examining certain visitors under a stereomicroscope and recording a score of either 0 (0 pollen grains present), 1 (1-10 grains), 2 (10-50 grains), 3 (>100 grains, total pollen cover), or 4 (multiple layers of grains) on different regions of the body (based on methods by Beattie et al. 1973). The following regions of the body were given a score: (1) proboscis, (2) bottom half of head, (3) top half of head, (4) bottom and (5) top of thorax, (6) bottom and (7) top of abdomen, (8) front two legs, (9) upper back leg (scopae of many bees), (10) lower back leg. Scores for each region were averaged for each taxon for representation of the amount of pollen that each taxon carried in

particular regions of the body. *Bombylius major* and Noctuidae were only examined in the face region. The number of insects examined varied for each taxon.

Ten frequently collected floral visitor taxa were sampled for the percentage of *E. umbellata* grains in their body pollen. Insects were swabbed with fuschin-stained jelly on all ventral regions of the body (excluding the scopae of bees) and the entire head (Kearns & Inouye 1993). *Bombylius major* and Noctuid moths were just swabbed in the face region. These areas were thought to be most likely to come in contact with an *E. umbellata* stigma. The scopae of bees was analyzed separately, as pollen carried in scopae is not typically available for pollination, but does indicate what the bees are using as pollen hosts. The jelly was then mounted on microscope slides and pollen grains were identified under 100X and 400X magnification. A sample collection from the BTP field site was used as a reference for pollen identification. The percentage of *E. umbellata* grains in the body pollen of insects was calculated from counts of 500 total pollen grains in each swabbing sample. Some insects did not have 500 grains within their sample but were still included to calculate the percentage of *E. umbellata* grains in their body pollen.

Statistical Analysis

Descriptive statistics were performed to examine the level of variation among individuals and populations in floral morphology. A mixed model with plant nested within population was used to test for population differences in the degree of stigma and anther separation. Population was treated as a fixed effect and plant was treated as a random effect.

A chi-square test of independence was used to see if stigma receptivity differed among flowers of different ages. A mixed model analysis was used to see if the proportion of viable pollen differed among flower ages. Plants were treated as a randomized block with flower stage as a fixed effect and plant as a random effect. A *post hoc* Tukey's test was utilized to determine pair-wise differences between particular flower stages.

For the breeding system tests, percent fruit set per branch was arcsine transformed in order to better meet the assumption of normality of variance. Because some treatment flowers appeared to initiate fruit set but later aborted at various stages, a repeated measures analysis of variance was used to test for the effects of treatment type and time after pollination on *E. umbellata* fruit set at 2011 TONEC and 2012 TONEC, CONWR, and WH. Treatment type, time after pollination, and a treatment x time interaction were included as fixed effects. Time after pollination was the repeated effect. Plants were not treated as a separate random effect but were incorporated within the repeated statement as the subject measured. Independence was assumed across subjects, and nesting plant within treatment further specified that data are correlated on the same plant. The compound symmetry (CS) covariance structure or spatial power law (SP(POW)) covariance structure was used in repeated measures analysis of a population's fruit set. Both can be considered for analyzing unequally spaced measurements, but the SP(POW) covariance structure is often deemed more suitable for such data (Little et al. 2006). The CS structure assumes that measures have the same variance across all times. At first, each covariance structure was tested for each population in order to gauge which structure was the most appropriate. The

covariance structure that generated the lowest value according to the Akaike information criterion (AIC) was the structure deemed proper for analysis. The CS structure most accurately fit the data from the 2011 TNEC and 2012 COWR populations, and so this covariance structure was utilized in repeated measures analysis of fruit set. The SP(POW) structure was used in repeated measures analysis of the 2012 TNEC and WH populations. If there was a significant interaction between treatment and time, the interaction was included in repeated measures analysis and a *post hoc* comparison of differences of least squares means was utilized to determine pair-wise differences between treatment types at particular times.

Because the autogamy treatment fruit set was a measure of dependence on pollinators, differences in mature fruit set for the autogamy treatment among the 2012 populations were compared using a mixed model analysis. Because the open-pollinated controls were assessments of pollen limitation, differences in control mature fruit set among the 2012 populations were compared using a mixed model analysis. In both analyses, population was treated as a fixed effect and plant was treated as a random effect. A *post hoc* Tukey's test was performed to determine pair-wise differences between populations.

Any effect of yearly differences on open-pollinated mature fruit set was tested with a mixed model analysis comparing trimmed controls in 2011 to trimmed controls in 2012 at TONEC. Year was treated as a fixed effect and plant was treated as a random effect.

A mixed model analysis was used to determine whether treatment affected the seed mass or the proportion of viable seed among the fruits set. Treatment type was a

fixed effect and plant was a random effect. Data were arcsine transformed. A *post hoc* Tukey's test was performed to determine pair-wise differences between particular treatment types.

Results from the breeding system study at BTP and the diurnal vs. nocturnal tests at SIUC were analyzed using mixed models. Treatment type was a fixed effect and plant was a random effect. Data were arcsine transformed. A *post hoc* Tukey's test was performed to determine pair-wise differences between the three treatments at BTP.

The ten frequently collected insect taxa that were analyzed for the percentage of *E. umbellata* pollen carried on their bodies were the flies *Bombylius major* (n = 10) and large Syrphidae – *Eristalis dimidiata*, *Eristalis transversa*, *Helophilus fasciata*, *Syrphus ribesii* – (n = 10), Noctuid moths – *Mythimna unipuncta* and *Periodroma saucia* – (n = 10), and the following bees: small male *Andrena* spp. (n=10), female *Andrena illini* (n = 10), *Apis mellifera* workers (n = 10), female *Augochlorella aurata* (n = 10), male *Ceratina* spp. (n = 10), female *Lasioglossum* spp. (n = 10), and male *Xylocopa virginica* (n = 10). One-way ANOVA was used to test for differences among taxa with insect type treated as a fixed effect. A *post hoc* Tukey's test was utilized to determine pair-wise distinctions between particular taxa. All procedures were performed using SAS software (v.9.2; SAS Institute, Cary, NC, USA).

CHAPTER 3

RESULTS

Floral Morphology

The mean corolla tube length was 6.27 mm (SE = \pm 0.06 mm, n = 160 flowers). The mean length of the carpel from the base of the corolla tube to the stigma apex was 8.00 mm (SE = \pm 0.08 mm, n = 163 flowers). The degree of herkogamy was variable among *Elaeagnus umbellata* individuals (Figures 1, 2). There were individuals that showed some extension of the stigma beyond the anthers but they were rarely separated completely. Others showed no spatial separation within their flowers. The mean distance separating the stigma from the anthers was 0.51 mm (SE = \pm 0.08 mm, n = 56). There was no difference between populations in the degree of stigma and anther separation ($F_{(3, 52)} = 1.25$, $P = 0.30$, n = 56).

Floral Phenology

The timing of stigma receptivity was independent of visually assessed flower stage in 2011 at ALL and BTP ($\chi^2 = 5.66$, $P = 0.19$, n = 60); it was independent of flower age in 2012 at TONEC ($\chi^2 = 1.92$, $P = 1.0$, n = 46). Stigmas are receptive just prior to anthesis and remain receptive at least through the fourth day of flowering. In 2011, 27% of flowers that appeared yellow (approximate age of 6-10 days) were unreceptive (n = 15). Most stigmas that were moist were receptive. Visible necrosis of the stigmatic tissue often indicated that the flower was no longer receptive.

At BTP in 2011, pollen viability declined as the flowers aged (Fig. 3). Flowers at early stages had a higher percentage of viable pollen than flowers at later stages ($F_{(3, 27)}$

= 21.06, $P < 0.0001$, $n = 40$). At TONEC in 2012, pollen viability remained high as the flower progressed to 4 days old (Fig. 4), and there was no difference among 1, 2, 3, or 4 day-old flowers in the percentage of viable pollen ($F_{(3,27)} = 2.51$, $P = 0.08$, $n = 40$).

Breeding System

Among the breeding system treatments, outcrossed flowers had higher fruit set than all other treatments (Table 3; Figs. 5, 6, 7). This effect was significant for all pair-wise comparisons of mature fruit set at all three site/year replicates (differences of least square means, $P < 0.05$) except for the comparison between untrimmed controls and outcrossed treatments at CONWR (differences of least square means, $P = 0.06$). In 2011, there was no fruit set in the autogamy and self-pollination treatments. In 2012 at CONWR and TONEC, most plants set no fruit in the autogamy or self-pollination treatments (means = 2.07% and 1.08%, respectively) and among the few that did, most only produced one or two fruits per treatment branch. In 2012 at WH autogamous treatments resulted in significantly higher mature fruit set (mean = 21.3%, SE = \pm 11.6%, $n = 9$; Table 4; Fig. 8) than autogamous mature fruit set at TONEC (mean = 1.77%, SE = \pm 0.54%, $n = 14$; Table 4; Fig. 6) and CONWR (mean = 0.98%, SE = \pm 0.54%, $n = 20$; Table 4; Fig. 7).

Autogamous fruit set at WH was not significantly different than control fruit set at WH (Table 3). At BTP in 2012, both control and autogamy treatments had low fruit set; there was no significant difference among the treatments ($F_{(2, 18)} = 1.46$, $P = 0.26$, $n = 10$; Fig. 9).

Control treatments (branches that were open to pollinator visitation) had low fruit set in most populations (Figs. 5, 6, 7, 9). Control branches at WH had a significantly greater mature fruit set than CONWR and TONEC (Table 4; Fig. 8). At TONEC, there was no significant difference in mature fruit set between trimmed control branches in 2011 and trimmed control branches in 2012 ($F_{(1, 26)} = 0.06$, $P = 0.81$, $n = 12$). There was no difference between trimmed and untrimmed control branches in mature fruit set according to differences of least square means.

Seed viability tests revealed that most fruits contained a viable seed, even those from the autogamy and self-pollination treatments (Fig. 10). In 2011, only outcrossed treatments set a sufficient sample of seeds for viability testing. The mean percent viability of these was 83% (SE = $\pm 14.1\%$, $n = 7$). In 2012, the autogamy and self-pollination treatments were combined for seed viability analyses because of their relatively low seed output. The trimmed and untrimmed control groups were also combined. There was no significant difference between the selfed, outcrossed, and control treatments in the percentage of fruits containing a viable seed in 2012 (means = 92.8%, 78.5%, 84.1%, respectively; $F_{(2, 29.4)} = 1.07$, $P = 0.36$, $n = 29$), nor was there a difference among these treatments in seed mass ($F_{(2, 21.4)} = 1.92$, $P = 0.17$, $n = 29$).

Some fruits aborted at various stages, and repeated measures analysis indicated that this decline in fruit set over time was significant (Table 3). This influence of time is evident in the decline of fruit set as fruits mature (Figs. 5, 6, 7, 8). There was a significant time x treatment interaction in all site/year replicates except WH (Table 3).

Pollen Tube Growth

An analysis of 6 plants at the 2012 TONEC population revealed that four were strongly self-incompatible (Table 5; Figs. 11, 12) and two had a “leaky” self-incompatibility response (Table 5). In the four strongly self-incompatible individuals, 16 of 17 carpels examined exhibited no self pollen tube growth into the style, and one grew just to the top of the style. In the two “leaky” individuals, most carpels had self-pollen grow at least into the style, and 20% of self-pollinated flowers showed self-pollen tubes reaching the ovary ($n = 15$). 46% of cross-pollinated flowers showed cross-pollen tubes reaching the ovary ($n = 39$). The percentage of pollen tubes reaching the ovary may have been higher had more time been allowed for sufficient growth in some samples.

Diurnal vs. Nocturnal Pollination

At MUR in 2011, only the diurnal treatment resulted in fruit production. The total number of fruits instead of percent fruit set was the dependent variable at MUR in 2011, and two of five plants set 19 and 11 fruits within diurnal treatments. At SIUC in 2012, fruit set within diurnal treatments (mean = 6.99%, SE = $\pm 2.34\%$, $n = 11$) was slightly more than fruit set within nocturnal treatments (mean = 2.40%, SE = $\pm 0.84\%$, $n = 11$), although this difference was not statistically significant ($F_{(1, 20)} = 3.30$, $P = 0.08$, $n = 11$).

Floral Visitors

A total of 411 insect visitors were collected on *Elaeagnus umbellata* flowers during the 2011 and 2012 flowering season from all study sites (Table 6; Figs. 13, 14). Visitors included an abundant and diverse assemblage of bees (42% of visitors), dipterans (20.7%), lepidopterans (14.4%), and coleopterans (5%). Some visitors

collected in 2011 were not collected in 2012 and vice versa (Table 6). Most bees had pollen on their bodies. Lepidopterans had pollen on their faces. Few flies except for large syrphids and bombyliids carried pollen. Pollen was rarely seen on the bodies of coleopterans.

Bee visitors ranged in size from small *Ceratina* to one of the largest bees in North America, *Xylocopa virginica*. Long-tongued bees (Apidae and Megachilidae) accounted for 63% of the bees captured. This percentage would have been much higher had *Bombus* spp. been included in collections. Short-tongued bees accounted for 37% of the bees captured. *Andrena* was a particularly diverse group of visitors to *E. umbellata*.

Frequently visiting bee taxa, fly taxa, and Noctuidae were scored for the general amount of pollen carried in different regions of the insect body and/or face (Fig. 15). The specific bee taxa that underwent analysis were male *Andrena* spp. (n = 10), female *Andrena illini* (n = 10), *Apis mellifera* workers (n = 10), female *Augochlorella aurata* (n = 28), male *Ceratina* spp. (n = 14), female *Lasioglossum* spp. (n = 9), and male *Xylocopa virginica* (n = 10). The specific fly taxa examined were large Syrphidae flies – *Eristalis dimidiata*, *Eristalis transversa*, *Helophilus fasciata*, *Syrphus ribesii* – (n = 10), and *Bombylius major* (n = 9). The specific Noctuidae examined were *Mythimna unipuncta* and *Peridroma saucia* (n = 9). Many bee taxa carried pollen in regions important for the potential pollination of *E. umbellata*. The larger bees (*A. illini*, *A. mellifera*, *X. virginica*) had greater amounts of pollen than the smaller bees (*A. aurata*, *Lasioglossum* spp., *Ceratina* spp., male *Andrena* spp.). *Ceratina* spp. usually had only traces of pollen on their body. *Bombylius major* and Noctuid moths carried enough pollen to affect pollination.

The frequently collected insect taxa that were analyzed for the percentage of *E. umbellata* pollen carried on their bodies were the flies *Bombylius major* (n = 10) and large Syrphidae (n = 10), Noctuidae moths (n = 10), and the following bees: male *Andrena* spp. (n = 10), female *Andrena illini* (n = 10), *Apis mellifera* workers (n = 10), female *Augochlorella aurata* (n = 10), male *Ceratina* spp. (n = 10), female *Lasioglossum* spp. (n = 10), and male *Xylocopa virginica* (n = 10). There were significant differences between insect taxa in the percentage of *E. umbellata* within their body pollen ($F = 11.35$, $P < 0.0001$, $n = 100$; Fig. 16). Noctuid moths carried almost exclusively *E. umbellata* pollen on the proboscis. Another nectar-feeder, *Bombylius major*, had a relatively high percentage of *E. umbellata* pollen on the proboscis. The introduced *A. mellifera* carried a higher percentage of *E. umbellata* pollen than the native bees, although this finding was significant only when compared to *Andrena illini* females and male *Andrena*. Most native bees captured on *E. umbellata* carried a moderate percentage of *E. umbellata* pollen.

The body pollen of the male *Andrena*, female *Andrena illini*, and large Syrphidae flies contained a relatively low percentage of *E. umbellata* pollen. *Ceratina* spp. carried a higher percentage of *E. umbellata* pollen relative to the other native bees examined, but they carried very small amounts of pollen (Fig. 15).

Behavior of visitors was observed whenever possible. *Apis mellifera*, *Bombus* queens, and *Xylocopa virginica* visited many flowers per plant and often contacted the stigma and anthers. Other bees were not seen as frequently or did not remain on plants for very long and therefore it cannot be judged whether they foraged for nectar or

pollen. The fly *Bombylius major* and noctuid moths were other insects that seemed to forage for long bouts on one plant.

CHAPTER 4

DISCUSSION

Breeding System of *Elaeagnus umbellata*

Elaeagnus umbellata is a predominantly outcrossing species with a self-incompatible breeding system. Twenty percent of cross-pollinated flowers in 2012 developed a fruit, whereas self-pollinations resulted in significantly lower fruit set. Automatic deposition of self-pollen occurs because the male and female floral parts are not separated temporally and are usually in contact, but self-fertilization is prevented via an incompatibility response. All but one population demonstrated very low fruit set following autogamy and self-pollination treatments. Baker's law (1955) suggests that self-compatible plants have a distinct advantage during the invasion process because the trait provides reproductive assurance. Nevertheless, *E. umbellata* is a plant that successfully invades environments despite an inability to self-fertilize. Other studies have shown that primarily outcrossing species can become highly invasive and proliferate beyond source populations. One example is *Mahonia auifolium*, a shrub that was introduced to Germany for ornamental purposes and is now "among the most successful invasive shrubs in central and eastern Germany" despite being an obligate outcrosser (Auge & Brandl 1997). An incompatibility system may be beneficial for invasives for the same reason as other plants; it reduces the likelihood of inbreeding depression and increases population-level genetic variability (Charlesworth & Charlesworth 1995). Heightened genetic diversity within a population may allow for plants to more rapidly adapt to certain environments and therefore may positively affect

the rate of invasive spread (Barrett 2011). A diverse gene pool may sustain populations in times of environmental stress as well.

It is not surprising that *E. umbellata* has become invasive despite a general inability to self-fertilize. As a woody perennial, it has multiple years to achieve reproductive success after colonization of a mate-limited or pollinator-limited habitat. Also, seeds of *E. umbellata* from Japan have been shown to have a 99% germination rate, which is very high for a woody plant (Kohri 2008). Therefore, a population can establish quickly once seed production commences.

Although *Elaeagnus umbellata* has an incompatibility system that prevents self-fertilization, the male and female functions of flowers overlap temporally, and many individuals do not display complete separation of the stigma and anthers. Moreover, the opportunity for pollinator-mediated self-pollen transfer is large due to the very large number of flowers per plant. As a result, *E. umbellata* may suffer pre-zygotic costs associated with self-pollination including pollen discounting and clogging of the stigma with self-pollen (Galen et al. 1989; Harder & Barrett 1995).

An interesting observation I made was the variability among *E. umbellata* individuals with respect to the spatial separation of stigma and anthers. In some plants, the stigma surface is primarily beyond the anthers but not to an extent that it completely precludes automatic self-pollination. However, a large degree of physical separation was occasionally observed on plants in the field (> 2 mm), whereas other plants had stigmas just below the anthers (Fig. 2).

The ecological and evolutionary implications of this variation are not completely clear. Because *E. umbellata* has a strong pre-zygotic self-incompatibility response, the

risks of self-fertilization are low and may not maintain or drive adaptation for the separation of stigma and anthers. However, non-herkogamous *E. umbellata* may be more likely to experience stigma clogging and/or pollen discounting, both of which have been shown to negatively affect reproductive success (Harder & Barrett 1995). For example, stigma clogging by self pollen was shown to reduce fruit set in the self-incompatible *Ipomea wolcottiana* (Parra-Tabla & Bullock 2005), which has a relatively small degree of stigma-anther separation similar to *E. umbellata*, and the authors concluded that stigma position may be the product of a tradeoff between the risks of stigma clogging and the potential damage to long styles by pollinating insects.

The pollinator relationships of *E. umbellata* may affect its ability to evolve an optimal floral morphology for avoiding the detrimental effects of self-pollen deposition. Although pollinators of *E. umbellata* have not been surveyed in the native range, the diverse pollinator assemblage in the invasive range means it has a generalized pollination system. Studies have shown that self-incompatible hermaphroditic plants with generalized pollination systems receive optimal insect pollination when stigma and anthers are not separate (Conner et al. 1995). However, a diverse pollinator assemblage like that of *E. umbellata* probably does not exert much selection on floral morphology (Conner et al. 1995; Johnson & Steiner 2000).

It is also possible that variation in stigma and anther separation may be a product of environmental conditions during floral development rather than genetic variation. The degree of abiotic stress has been shown to be correlated with the degree of stigma and anther separation in *Arabidopsis thaliana* (Brock & Weinig 2007). Regardless of its underlying cause, this variability of floral morphology in *E. umbellata* is easily measured,

and future studies might examine whether such variability has any effect on reproductive success.

The low fruit set in the outcrossed treatments is not an uncommon finding in predominantly outcrossing plant species (Primack 1979; Jacobs et al. 2009), and has been found in several relatives of *E. umbellata*. The obligate outcrossing *Discaria toumatou* (Rhamnaceae), which is in a family phylogenetically close to Elaeagnaceae, had only 15% fruit set after artificial cross-pollination treatments (Primack 1979). Cross pollinations performed on *Elaeagnus angustifolia* (Pan et al. 2011) and *Elaeagnus mollis* (Wei et al. 2007) in China resulted in 14% and 25% fruit set respectively. In my study, the failure of some individuals to develop any fruit after supplemental outcrossing may have been due to the pollen donor carrying an identical incompatibility allele as the treatment plant. This could happen if the pollen donor happened to be a close relative of the pollen recipient, or if the pollen donor was part of the same genet as the pollen recipient. Such a scenario is possible given that *E. umbellata* is a clonal plant. The extent of genet size and dispersal ability is not fully known in *E. umbellata*, but there could have been relatedness among plants that caused low outcrossed fruit set, despite our 20 m mating distance. Anderson and Beare (1983) made “intrapatch” and “interpatch” (30-250m apart) crosses among individuals of the self-incompatible clonal plant *Trientalis borealis*. Interpatch crosses resulted in significantly higher fruit set, while some of the intrapatch crosses resulted in 0% fruit set.

Not all *E. umbellata* individuals set fruit exclusively through outcrossing. Some plants developed a small percentage of autogamous and self-pollinated fruit in the BTP, CONWR, and TONEC populations. This pattern of self fruit set was also evident in its

Chinese congener *Elaeagnus mollis* (Wei et al. 2007). Autogamy treatments resulted in 4.8 % fruit set, and self-pollination treatments resulted in 6.9% fruit set in *E. mollis*. In contrast, *Elaeagnus angustifolia* in its native range did not set any autogamous or self-pollinated fruit (Pan et al. 2011).

At WH, three out of nine plants had greater than 25% fruit set within autogamy treatments. Most of these seeds were viable and overall did not differ in percent viability from outcrossed seeds at other populations. Therefore, some populations of *E. umbellata* could be considered partially self-compatible (Brennan et al. 2005; Lafuma & Marice 2007), whereas others, such as the CONWR and TONEC populations in my study, are predominantly outcrossing. Recent studies have revealed that occasional self-seed set is possible in plants previously described as self-incompatible (e.g. Dipterocarps, Ghazoul & Satake 2009). In these partially self-compatible species, selfed seed set is still lower than outcrossed seed set, and self-pollen tubes grow slower than cross-pollen tubes (Levin 1996). Also, the strength of self-incompatibility varies continuously among individuals and may be due to both genetic variation and environmental circumstances. For example, in certain species, attenuation of the self-incompatibility response is evident in older flowers or at elevated temperatures (Good-Avila et al. 2008). For the *E. umbellata* in this study, temperature could have played a role in autogamous seed set in 2012, when temperatures were much warmer than the 2011 flowering season. Even if the environment can affect a plant's incompatibility response, there is generally an underlying genetic basis. The mutation of a particular S-allele could render the S-RNases responsible for self-pollen degradation dysfunctional (Tao et al. 2007). For example, a total of three S-alleles are found among *Prunus*

Persicus (peach) cultivars that are homologous to S-alleles in self-incompatible plum and almond cultivars, but mutations within the *P. persicus* alleles make it fully self-compatible (Tao et al. 2007). The mechanisms for partial self-compatibility are often less severe and sometimes involve changes to unlinked modifying genes impacting S-locus products (Good-Avila et al. 2008). Numerous unlinked modifying genes contribute to the proper function of S-RNases (McClure et al. 2011), and mutation of these genes could compromise the efficacy of S-RNases. Modifying genes of the S-locus have been quantified in *Campanula ranunculoides* and shown to segregate with varying degrees of self-fertility (Good-Avila & Stephenson 2002). In their study, Good-Avila and Stephenson found that a larger inheritance of these recessive modifying genes resulted in a greater capacity to self-fertilize. Their findings provided a possible explanation for continuous variation in *C. ranunculoides* self-seed set. The environmental and genetic influences on self-seed set in Elaeagnaceae are unknown, but the above scenarios are possible reasons for the “leaky” self-incompatible response recorded in two *E. umbellata* individuals at TONEC. In these two plants, self-pollen tubes grew into the ovary in 20% of carpels. One of these individuals formed autogamous and self-pollinated seed, and these seeds were viable. Although pollen tube analyses were not performed at WH, the discovery of autogamous individuals there suggests self-pollen tube growth resulted in self-fertilization. Therefore, continuous variation in self-seed set might exist across *E. umbellata*'s range, although this variation is highly skewed towards self-incompatibility.

There is the possibility that selfing is a legitimate alternative to outcrossing if inbreeding depression is weak in *E. umbellata*. My study's one proxy for inbreeding

depression (seed mass and viability) revealed no difference in the mass or viability of selfed and outcrossed seeds. However, the costs of inbreeding may instead be revealed in subsequent life stages of selfed offspring. Dudash and Fenster (2001) found that selfed and outcrossed progeny of *Silene virginica* did not significantly differ in mean seed mass or percent germination, but selfed progeny were inferior to outcrossed progeny in juvenile leaf number, adult biomass, and flower production. More research is needed to determine if similar costs are apparent in selfed *E. umbellata* offspring.

Decline of Developing Fruits in *Elaeagnus umbellata*

A notable outcome of all breeding system trials was the significant reduction in fruit set as fruits matured. *Elaeagnus umbellata* plants developed a large amount of fruits after pollination but did not sustain these fruits throughout the maturation stage. Indeed, statistical analysis revealed a highly significant effect of time after pollination on fruit set at 2011 TONEC and 2012 TONEC and CONWR. Fruit abscission occurred gradually as outcrossed fruits matured but was especially pronounced between 4 and 7 weeks after most treatments. The timing of fruit abscission has not been examined previously in Elaeagnaceae, but the period of fruit drop is similar to the phylogenetically related *Rhamnus alternus* (Bas & Pere Pons 2004). Reductions in developing fruit can be due to a number of factors including resource limitation (Stephenson 1981), weather conditions, early seed predation (Ghazoul & Satake 2009), disease, late-acting self-incompatibility (Seavey & Bawa 1986), inbreeding depression, genetic load (Wiens et al. 1987), and female choice (Korbecka et al. 2002).

Resource limitation is regarded as a very important factor in early fruit abscission (Stephenson 1981). Nutrient availability can vary within the plant body. Many *E. umbellata* can produce up to 10 flowers per cm of a branch, and adjacent flowers may compete for resources if they are pollinated. For example, Vaughton (1993) found that partial trimming of inflorescences greatly increased fruit set in *Banksia spinulosa*, a plant that, like *E. umbellata*, produces many flowers and one-seeded fruits. However, *E. umbellata* did not show any differences between trimmed control branches and untrimmed control branches in fruit set. Vaughton's (1993) method of trimming differed from this study in that more flowers were trimmed from a basal position on the stem. In my study, I trimmed flowers from apical portions of *E. umbellata* stems and it did not increase the proportion of fruit set of the remaining flowers. Trimming may increase fruit size though (Quinlan & Preston 1968). Other aspects of my experimental design may have implications for differential resource allocation. Flowers at the end of branches were treated, and growing fruits could have been competing with elongating shoots for resources (Quinlan & Preston 1971). Also, the bagging of branches could have negatively impacted fruit set, although bags were present for a small portion of the maturation stage. Company et al. (2005) found that bagging significantly reduces light intensity within mesh bags, which probably influenced their finding of substantially lower fruit set in bagged *Prunus amygdalus*. Mesh bags from treatment branches in this study were removed 3-4 weeks after pollination and then reapplied shortly before fruits showed signs of ripening. Therefore, only within the first four weeks following pollination could fruits have been limited in nutrient receipt from the bagging of leaves.

Beyond within-plant effects, competition between neighboring plants may have reduced net allocation to reproduction (Silander & Pacala 1985). No research has examined intraspecific competition in Elaeagnaceae, but many of the plants in this study grew in close proximity to other *E. umbellata* ramets. A recent study on tropical tree populations discovered that soil nutrients and tree size were the biggest factors influencing variability in the fruit set of self-incompatible trees (Jones & Comita 2008). Exact shrub size was not recorded for any of the *E. umbellata* in this study, but many of them did not approach the 6m height or 7m spread that they can achieve in southern Illinois regions (Church et al. 2004). Therefore, the size of the plants in this study may have limited reproductive output. Finally, the severe drought of 2012 could have exacerbated any issue of resource limitation in some plants. For example, two plants had lost all of their leaves 16 weeks after pollination, but one of these plants retained its fruit.

It is possible, but unlikely that pre-dispersal seed predators may have eaten the immature fruits after bags were removed. Developing green fruits were exposed to possible predation 3-4 weeks after pollination. Such activity was rarely seen on *E. umbellata*, and the closely related *E. angustifolia* has been recorded having low densities of insect herbivores in invasive populations (Katz & Shafroth 2003), but a study by Lind and Parker (2010) showed that secondary chemicals in invasive *E. umbellata* incite feeding behaviors in native caterpillars. There were a few rare instances in which seed predation was obvious because mesh bags contained frass, and this was often accompanied by significant foliar damage as well. However, most of

the treatment branches did not have any notable foliar damage or signs of insect frugivores.

The statistically significant interaction between treatment and time after pollination at 2011 TONEC and 2012 TONEC and CONWR was likely due to a substantial decline of outcrossed fruit development. The failure of many outcrossed fruits to fully develop is somewhat surprising and could be due to resource limitation, genetic load, or a combination of both. *E. umbellata* is predominantly outcrossing, and populations may be more likely to have a large genetic load if frequent outcrossing is assumed. In order to test whether genetic load explained prominent fruit abortion in the strongly self-incompatible perennial *Epilobium angustifolium*, Wiens et al. (1987) examined embryogenesis and also compared mature fruit set to the autogamous perennial *Epilobium ciliatum*. Most developing embryos survived in *E. ciliatum*, while *E. angustifolium* had significantly less embryo survivorship. Examination of *E. angustifolium* embryos revealed malfunctions occurring throughout embryogenesis, allowing the authors to conclude that developmental lethals caused abortion of seed across the seed development stage. Population genetic studies of *E. umbellata* in the native range have revealed a large amount of diversity within a small area (Ahmad et al. 2008). Therefore, it is not out of the question that genetic load could have played a role in the gradual abortion of outcrossed seeds in *E. umbellata*.

Autogamous and self-pollination treatments initiated some early fruit development on a few plants from the TONEC and CONWR populations, but most of these fruits were aborted between 4 and 7 weeks. *Discaria americana* (Rhamnaceae) showed similar tendencies to initiate a low self fruit set and then abort the fruits (Medan

1993). If self-pollination resulted in self-fertilization, as suggested by pollen tube analyses in this study, then inbreeding depression may have caused fruit abortion after four weeks of development. Another possibility is that a late-acting self-incompatibility system caused the abortion of fruits. Even though *E. umbellata* has an early self-incompatibility response, an early response can work in tandem with a late acting self-incompatibility response within one species (Ghazoul & Satake 2009). Such late-acting systems function either in the ovary, in the ovule before fertilization, or in the ovule after fertilization (Seavey & Bawa 1986). In pollen tube analyses of *E. umbellata*, one self-pollen tube was seen penetrating the ovule, which does not support late-acting self-incompatibility acting in the ovary. Instead, selfed fruits developed at least four weeks post-pollination, a time span that supports inbreeding depression as the more probable cause of abortion (Seavey & Bawa 1986; Vaughton et al. 2010). However, the reasons why a few plants aborted all of their high initial self fruit set, while other plants developed a very low amount of viable selfed seed, remain equivocal.

Evidence of Pollen Limitation in *Elaeagnus umbellata*

The low fruit set on open-pollinated control branches suggests that *E. umbellata* may be pollen limited in some populations. Outcrossing treatments resulted in at least 10% higher fruit set than control branches. In a review of pollen supplementation studies in flowering plants, Burd (1994) found that 62% of species are pollen limited, so pollen limitation is not a rarely recorded event. However, it is important that pollen supplementation experiments be judged cautiously when interpreting pollen limitation (Burd 2008). Pollen limitation may serve an adaptive purpose in perennials, whose

lifetime fitness is influenced by yearly costs to reproduction and stochastic factors like pollinator and frugivore abundance (Horvitz et al. 2010). This study did not test for any such stochastic influences, but a rough estimate of pollen limitation across two reproductive seasons was performed at TONEC. Although both seasons differed from each other with respect to weather, *E. umbellata* showed no difference in the percent fruit set on control branches, suggesting that pollen limitation may be a recurring hindrance to reproductive potential at least at the TONEC population.

E. umbellata is mostly self-incompatible and depends on insects to transfer genotypically distinct pollen to the stigma. Such a requirement is why many self-incompatible plants are more pollen limited than self-compatible plants (Burd 1994). A study by Larson et al. (2002) found that invasive populations of the mostly self-incompatible *Lonicera japonica* had a much higher fruit set when supplemented with cross-pollen (78.7%) than when exposed to pollinators (17.4%). The authors concluded that a lack of quality pollinators played a key role in the lower fruit set of open branches. Indeed, invasive plants leave behind mutualistic pollinators from the native range and colonize areas where floral visitors, mates, or both may be limiting. In a study by Parker (1997), hand-pollinations increased fruit set by 280-2620% in the invasive shrub *Cytisus scoparius*. Tests of resource limitation proved to not greatly affect fruit set, and measures of visitation rates allowed Parker to conclude that *C. scoparius* was pollinator limited. Visitation rates were not measured in this study, but infrequent sightings of pollinator abundance in the TONEC and CONWR populations suggest that lack of pollinators may have been a reason for low fruit set. I initially suspected that reduced pollinator activity associated with the cool and wet conditions of 2011 could have

resulted in the low fruit set on control branches at TONEC. However, control fruit set in 2012 was no different from 2011 despite the flowering season being much warmer and favorable to insect activity. 2011 weather conditions at BTP were optimal as well, but percent fruit set in the open pollinated controls was comparable to CONWR and TONEC populations.

In his study of pollinator limitation in *C. scoparius*, Parker (1997) also found that pollinator limitation was more pronounced in prairie populations than urban populations. He observed greater frequencies of *Bombus* spp. visits in urban populations, which probably resulted in higher fruit set on the control branches of urban plants. In my study, populations of *E. umbellata* differed in the amount of fruit set on control branches, but it is unclear whether differences in visitation are the cause. Control fruit set at WH was significantly greater than control fruit set at CONWR and TONEC in 2012. Also, autogamous fruit set was higher at WH, which could have contributed to the control fruit set. Visitation rates of insects to *E. umbellata* flowers were not recorded, but a diverse and relatively abundant collection of floral visitors was made at WH. One interesting aspect about the WH population is its location within the Shawnee National Forest, which offers a continuous tract of potential undisturbed habitat for pollinators. Such habitats have been shown to be correlated with pollinator diversity and abundance (Steffan-Dewenter & Tscharntke 1999; Aizen et al. 2002), while pollinator prevalence in disturbed habitat is more equivocal (Liu et al. 2006). For example, in central Florida the self-incompatible invasive vine *Paederia foetida* has a low fruit set in hedgerow (“highly disturbed”) and forested (“undisturbed”) sites (Liu et al. 2006). The species shows the highest fruit set in successional (“intermediately disturbed”) sites. Liu et al. concluded

that pollinator limitation likely caused the low fruit set at hedgerow and forested sites. Other studies besides Liu et al. (2006) and Parker (1997) have shown that habitats susceptible to plant invasion can differ in pollinator diversity and abundance (Morandin & Kremen 2012). Akin to Parker's finding of more frequent *Bombus* visitation in urban environments, the more urban MUR population in my study harbored an abundance of *Andrena illini*, *Apis mellifera*, and *Xylocopa virginica* (personal observation). Breeding system trials were not carried out in that population, but the large *E. umbellata* there were weighed down with fruit. Variation in fruit set and degree of pollen limitation in *E. umbellata* might ultimately be a product of variable pollinator abundances across different habitats.

Pollinators of *Elaeagnus umbellata*

Despite being pollen limited in some populations, *Elaeagnus umbellata* is capable of attracting numerous kinds of generalist insects for pollination. Generalized pollination is an ideal strategy for plants that leave behind pollinators from the native range. The habitats of introduction may pose challenges to successful pollination, like uncertain mate availability or pollinator activity. A predominantly outcrossing plant that is able to maintain reproductive success across different environments, coping with the presence or absence of particular pollinators, is one that is likely to persist on a large scale (Waser et al. 1996; Coombs et al. 2009). Reproduction in *E. umbellata* is highly dependent on local pollinators, and although some populations in Illinois reproduce at low output, it is apparent that a mix of pollinators provides this service. The level of diversity collected on *E. umbellata* is not surprising for a plant occurring in Illinois. A

thorough survey of all flowering plant species around Carlinville, IL revealed the mean number of effective pollinator species to be 33.5 per plant (Robertson 1929), although Robertson recorded more pollinator species for native plants than introduced plants (Robertson 1929; Memmott & Wasaer 2002). Some of the introduced plants may not have been as pervasive as they are today, and therefore may not have been as attractive to pollinators (Tepedino et al. 2008). *E. umbellata* must not have been present in the Carlinville, IL area at that time because Robertson does not mention any *Elaeagnus* species as a pollinator host. A more recent survey of insect visitation to the invasive *Alliaria petiolata* showed that at least 19 insect species act as pollinators (Cruden et al. 1996). Cruden et al. conclude that the commonality of the insect species assures pollination is likely in most areas of its range. Many of the bee, fly, and lepidopteran visitors to *E. umbellata* are common species as well, and my study provides evidence that many of the frequently visiting insects are pollinators.

Bees represented a diverse group of visitors that consisted of many long-tongued and short-tongued bees. The majority of bee taxa collected in this study are known to forage on multiple pollen hosts (i.e. they are polylectic). Many of the long-tongued bees seemed to be able to access nectar located at the bottom of the corolla tube. It is not clear whether short-tongued bees can do the same. *E. umbellata* flowers restrict even the smallest bee visitor (e.g. *Ceratina* spp.) from traveling into the corolla tube (personal observation).

The super-generalist *Apis mellifera* (honeybee) was the most common bee collected on *E. umbellata*. *Apis mellifera* is often associated with invasive entomophilous plants (Jesse et al. 2006; Lopezaraiza-Mikel et al. 2007; Jakobsson et

al. 2008; Bartomeus et al. 2008), and they enhance the spread of some invasive species (Stout et al. 2002). *Apis mellifera* that were collected on *E. umbellata* carried pollen on the proboscis and ventral regions, with a good amount of that pollen being *E. umbellata*, and thereby served as a reliable pollinator. *A. mellifera* workers are known for their tendency to forage consistently on one species over prolonged feeding bouts (Wells and Wells 1986), and invasive plants seem to be no exception. The pollen of *Carprobrotus affine acinaciformis*, an invasive plant in the Mediterranean, made up 90% of pollen present on *A. mellifera* visitors to the plant (Bartomeus et al. 2008). Although *A. mellifera* carried a relatively high percentage of *E. umbellata* pollen, it is slightly less than is normally found in studies of *A. mellifera*-invasive plant interactions (Lopezaraiza-Mikel et al. 2007; Bartomeus et al. 2008). This study represents a small sample, but the fidelity of *A. mellifera* on *E. umbellata* may not be as pronounced as it is on other invasive plants.

The only bees that consistently carried small amounts of pollen were small carpenter bee males (*Ceratina*; Apidae). The body pollen of *Ceratina* males was on average 50% *E. umbellata*, but this pollen was sparse, and therefore this bee may infrequently affect pollination in *E. umbellata*. *Ceratina* has been assessed for pollinator quality on *Lavandula latifolia* and was shown to deposit the least amount of pollen on stigmas among many bees analyzed (Herrera 1987). Interestingly, the majority of *Ceratina* specimens collected were male; it is likely that females had not yet emerged. Male bees can be effective pollinators even though they forage only for themselves and do not assist the females in nest provisioning. A survey of floral visitors to *Lonicera maackii* (bush honeysuckle), an invasive shrub that flowers at the same time as *E.*

umbellata, found that *Ceratina* accounted for 75% of visitation in Ohio (McKinney 2010). Pollinator quality was not the focus of McKinney's study, but perhaps the sheer number of visitors makes *Ceratina* a pollinator of *L. maackii*. *Ceratina* was a common visitor to *E. umbellata* and its frequent visitation may contribute to the pollination of *E. umbellata* despite its apparent low quality as a pollinator.

Other native bee species that were examined for pollinator quality were the sweat bees *Augochlorella aurata* and *Lasioglossum* (Halictidae), and the large carpenter bee (*Xylocopa virginica*, Apidae). The size, behavior, and placement of pollen suggest all of these species are likely pollinators of *E. umbellata*. Bumblebee (*Bombus*) queens are probable pollinators as well because they were frequently observed contacting the stigma during foraging (Fig. 14). The head of a large long-tongued bee like *Bombus* and *Xylocopa virginica* easily contact the stigma while they probe for nectar. Like honeybees, *Bombus* spp. are super-generalists, and are known to be major pollinators of invasive plants such as *Impatiens glandulifera* in Europe (Chittka & Schürkens 2001) and *Rhododendron ponticum* in Ireland (Stout 2007). *Xylocopa* is the primary pollinator of the invasive plants *Senna didymobotrya* in South Africa (van Kleunen & Johnson 2005) and *Opuntia stricta* in Australia (Bartomeus & Vilá 2009). The authors of these studies conclude that the size of *Bombus* and *Xylocopa* species ensures pollination (Stout 2007; Bartomeus & Vilá 2009). Because *E. umbellata* flowers are small, it is probable that smaller native bees also contact the stigma and anthers, though they may differ in pollination efficiency. The corolla tube and narrow opening of *E. umbellata* flowers might restrict smaller short-tongued bees like *Augochlorella aurata*, *Lasioglossum*, and some *Andrena* species from accessing

nectar, so their visits may simply be for pollen collection. *Lasioglossum* may prefer the co-flowering *Lonicera maackii* where both invasive plants co-exist. Goodell et al. (2010) found that *Lasioglossum* made up 9.4% of the visitor assemblage to *Lonicera maackii* along wooded edges in Ohio.

The only native bees that carried significantly lower percentages of *E. umbellata* pollen than *A. mellifera* were *Andrena* spp. Of the large *Andrena*, only females were examined. They tended to have a large amount of general pollen cover and so may occasionally pollinate *E. umbellata*. Only males made up the small *Andrena* examined, and they carried moderate amounts of pollen that was proportionally low in *E. umbellata* pollen. Therefore, small *Andrena* males rarely pollinate *E. umbellata*.

Diptera visited *E. umbellata* flowers, with hoverflies (Syrphidae) and the large bee fly (*Bombylius major*, Bombyliidae) being the most common. Syrphids and *B. major* are often significant components of generalized pollination systems in plants and are frequent visitors of invasives (Thompson 2001; Ghazoul 2006). Syrphids are the most frequent visitor to the invasive *Rosa multiflora* in Iowa (Jesse et al. 2006) and they pollinate the invasive *Lonicera japonica* in Arkansas (Larson et al. 2002). One generalist native plant in Elaeagnaceae, the dioecious *Shepherdia canadensis*, receives a significant amount of pollination from syrphids (Borkent & Harder 2007). The flowers of *S. canadensis* act as a “landing pad” for smaller insects on which nectar is exuded, whereas *E. umbellata* contains nectar at the bottom of a corolla tube. Nectar is likely inaccessible to syrphids in *E. umbellata* flowers, but pollen is available, and syrphids consume pollen as a nutritional resource (Faegri & van der Pijl 1979). However, not a great amount of *E. umbellata* pollen was deposited on the large Syrphidae sampled in

this study. *E. umbellata* pollen was present to a greater degree on the proboscis of the *B. major* sampled. These flies are able to reach nectar rewards through their long proboscis and may pollinate *E. umbellata* in the process.

Many aspects of *E. umbellata* flowering suggest past selection for some degree of moth pollination. The tubular flower shape, white coloration, and sweet scent are all common components of moth pollination syndromes (Faegri & van der Pijl 1979). Additionally, the floral volatiles found in *E. umbellata* flowers (Potter 1996) suggest moth pollination. These floral traits likely influenced abundant visitation and foraging by moths of Noctuidae at an invasive population (Fig. 14). All of the Noctuid moths were collected at BTP during three hours of sampling and consisted of *Mythimna unipuncta* (armyworm) and fewer *Peridroma saucia* (variegated cutworm). Given the high percentage of *E. umbellata* pollen on the proboscis and the frequent visitation, *M. unipuncta* and *P. saucia* are pollinators of *E. umbellata*. *M. unipuncta* has been known to visit *E. umbellata*. Wynne (1989) discovered that its prevalence was greater on *E. umbellata* than on 21 other co-flowering plants visited by the moth in northwest Missouri. 17 of those 21 plants had significantly lower visitation by *M. unipuncta*. Thirty *M. unipuncta* individuals were recorded on one *E. umbellata* plant at one time, and only *Tilia* plants had higher numbers of moths. *M. unipuncta* is a migratory species that travels from southern parts of North America to as far as Canada in the spring (Hendrix & Showers 1992). More importantly, pollen deposited on *M. unipuncta* is carried for great distances. For example, pollen from *Pithecellobium* and *Calliandra* plants in southern Texas was recovered off *M. unipuncta* specimens caught in Iowa (Hendrix & Showers 1992). There are no estimations for how rapidly *M. unipuncta* migrates

northward, but this species could be fulfilling relatively long-distance outcrossing in *E. umbellata*. Pollen on newly opened *E. umbellata* flowers is receptive for at least four days, making long distance transfers possible. However, a study by Richards et al. (2005) revealed that the percent of viable pollen from *Gossypium hirsutum* (cotton) and *Brassica napus* (canola) decreases rapidly when applied to a Noctuid proboscis. In *B. napus*, 81.6% of pollen grains were viable 36h after removal from the anthers whereas only 11-12% of pollen grains were viable just 8h after placement on a Noctuid proboscis. The findings by Richards et al. suggest that cross-pollination of *E. umbellata* over long-distances may be rare because pollen viability deteriorates rapidly on Noctuid mouthparts. Pollen may not lose viability as drastically on the Noctuid visitors to *E. umbellata* though, and pollen that happens to be placed on the face or legs may be preserved longer (Richards et al. 2005).

Fruit set due to nocturnal pollination did not differ statistically from fruit set by diurnal pollinators, although it seems diurnal pollination may account for a slightly higher fruit set. A larger sample size at the study sites or additional trials in other populations may have revealed significant differences between diurnal and nocturnal fruit set. Although many of the floral traits of *E. umbellata* appeal to moth visitation, it is obvious that diurnal pollinators are attracted to and maintain some degree of constancy on *E. umbellata* flowers. The floral design of *E. umbellata* may be an adaptation for both lepidopteran and long-tongued bee pollination. This “syndrome” has been suggested for *Elaeagnus rotundata* (Abe 2006), which has a similar floral display except for its slightly shorter corolla tubes. It cannot be concluded whether potential selection for nocturnal or diurnal pollination exists in the invasive range, but studies of *Lonicera*

japonica provide some insight. *Lonicera japonica* in its native Japan is pollinated by native bees - including *Lasioglossum* – and hawkmoths, but hawkmoths are more efficient pollinators, which may be the reason for the flower's timely dehiscence at dusk and sweet fragrance (Miyake & Yahara 1998). Invasive populations of *L. japonica* in Arkansas experienced very low fruit set early in the season when Syrphidae, Vespidae (wasps), and Xylocopinae (small and large carpenter bees) are visitors (Larson et al. 2002). Fruit set significantly increased as hawkmoths became more common later in the flowering term, so selection for hawkmoth pollination may be maintained in *L. japonica*. Unfortunately, I did not examine fruit set of *E. umbellata* at the BTP population in 2012, when many Noctuid visitors were witnessed. Fruit set at BTP in 2011 was low, but no observations of nocturnal visitors were attempted then. For now, it can only be concluded that diurnal and nocturnal visitors seem to contribute about equally to *E. umbellata* fruit set in some areas of southern Illinois.

Patterns of fruit set in *Elaeagnus umbellata* may vary from year to year because of differences in overall pollinator abundance. Collections in 2012 revealed a complete absence of some visitors that were collected in 2011. One reason for this is experimental. Time devoted to collections at each site was different between the 2011 and 2012 flowering season. Also, a couple of new sites were sampled for insects during 2012. Another likely reason was the difference in flowering time between years. Flowering occurred 3-4 weeks earlier in 2012 and some pollinators may not be common in early spring. For example, *Augochlorella aurata*, *Lasioglossum*, and *Toxomerus marginatus* were collected at BTP in 2011 but not in 2012. The butterfly *Vanessa atalanta* was not collected at BTP in 2011 but was one of the most frequently collected

visitors in 2012. The generalized pollination system of *E. umbellata* buffers against the absence of one pollinator species in a given year, but pollen limitation may be compounded by the absence of multiple pollinator species. *E. umbellata* flowers early in the spring and its phenology may be a primary reason for suspected pollinator limitation in this species. Parker (1997) speculated that early flowering in the invasive *Cytisus scoparius* contributed to severe pollen limitation of populations in prairie habitat. *C. scoparius* that flowered later received greater visitation and were not as severely pollen limited. The phenology of *E. umbellata* may hamper pollination in some years, and populations might experience selection for individuals that flower later in the season or for a longer period of time.

Ecological Implications of Pollinator Interactions with *Elaeagnus umbellata*

Plants that flower at the same time as *E. umbellata* and share its pollinators could be impacted in various ways. *E. umbellata* could have a competitive, facilitative, or neutral effect on the visitation of pollinators to native plants. Although abundant research has found evidence of invasive plants affecting native ones, relatively few studies have specifically addressed pollination relationships and impacts on native plant reproduction (see Chittka & Schürkens 2001; Brown & Mitchell 2002; Morales & Traveset 2009). Brown and Mitchell (2002) found that the invasive *Lythrum salicaria* (purple loosestrife) caused a 14-54% reduction in insect visitation rate to the co-flowering native congener *Lythrum alatum*. The reduced visitation rate and negative impacts of pollinator sharing (i.e. interspecific pollen transfer) likely caused the 22-34% reduction in *L. alatum* seed set, and also decreased *L. alatum* pollen dispersal.

Through meta-analysis, Morales and Traveset (2009) revealed that neighboring introduced plants more negatively impact native plant visitation and reproduction than native plant neighbors. Other studies show no effect of introduced plants on native plant reproduction even though the introduced plant concurrently causes a decrease in visitation to native plants (Totland et al. 2006; Bartomeus et al. 2010).

The degree to which native plant reproduction is affected by invasive presence may be highly dependent on spatial scale. The density of or distance between invasive and native plants likely determines whether native seed set is affected. Such factors have been shown to differentially affect pollinator behavior and native plant reproduction (Muñoz & Cavieres 2008; McKinney 2010). For example, Muñoz and Cavieres (2008) found that the presence of one nearby individual of the invasive *Taraxacum officinale* (dandelion) resulted in greater pollinator foraging times and seed set in native *Hypochaeris thrincooides*, while the presence of five nearby *T. officinale* individuals decreased visitation rates and seed set in *H. thrincooides*. For a large invasive plant like *E. umbellata*, the degree of shading imposed on neighboring plants may be an important factor in affecting pollinator visitation rates to neighboring plants (McKinney and Goodell 2010).

Although my study did not investigate interactions between invasive and native plants, it does provide information needed to conduct such studies in the future by identifying *E. umbellata* visitors from numerous sites in Illinois. Additionally, my analysis of pollen on the bodies of *E. umbellata* visitors revealed the proportion of *E. umbellata* pollen relative to that of other species. I found that some bee taxa collected in my study carry a low amount of *E. umbellata* pollen compared to amounts reported on pollinators

of other invasive plant pollinators (Lopezaraiza-Mikel et al. 2007; Bartomeus et al. 2008; Jakobsson et al. 2008). For example, in a study by Bartomeus et al. (2008), *Apis mellifera* and *Andrena* sp. that were captured on invasive *Carprobrotus affine acinaciformis* carried pollen that was 90% and 60% *Carprobrotus*, respectively. *Apis mellifera* and all *Andrena* examined in my study carried a comparatively lower percentage of *E. umbellata* pollen. *Apis mellifera*, *Bombylius major*, and *Mythimna unipuncta* were captured frequently on *E. umbellata*. Native plants might not receive as many visits from these pollinators, especially if their floral traits are similar to *E. umbellata*. For example, the meta-analysis by Morales and Traveset (2009) also revealed that those native plants most similar to introduced plants in floral morphology and color are most under threat of reduced visitation and reproduction. Any plant that flowers early alongside *E. umbellata* and produces similar flowers may be most at risk of reduced pollination, but a plant that has the same visitors could also be affected.

E. umbellata has the same visitors as other North American wildflowers so it is likely involved in the pollination network of co-flowering species. Attracting supergeneralists with long foraging seasons like *Apis mellifera* and *Bombus* increases the likelihood of connectivity with other plant species (Aizen et al. 2008). *Ceratina calcarata*, *Ceratina dupla*, and *Ceratina strenua* are also well-known generalist bees that visit a variety of plant taxa (Krombein et al. 1979). *C. calcarata* visits *Cornus florida* (flowering dogwood) and *Cercis canadensis* (redbud) (Krombein et al. 1979; Rhoades et al. 2011), two plants that were often observed co-flowering with *E. umbellata*. Other *E. umbellata* visitors previously recorded on *C. florida* include *Andrena cressoni*, *A. illini*, *A. imitatrix*, *A. sayi*, and *Lasioglossum mitchelli* (Rhoades et al. 2011). *Andrena carlini* is a

primary pollinator of many *Erythrium* species (Liliaceae) (Banks 1980; Harder et al. 1985) and *Sanguinari canadensis* (bloodroot, Papaveraceae) (Lyon 1992). *Augochlorella aurata* is a common generalist bee and is known to be important to some flowering species in prairie environments (Wagenius & Lyon 2010). Many of the aforementioned bee species are not only important to native plant pollination but also to crop pollination. For example, almost all of the bee species collected in my study were collected in an extensive survey of insect visitors to apple, blueberry, caneberry, and cucurbit crops in Virginia (Adamson 2011). As part of that study, Adamson listed 35 plant species flowering at the same time as apple (*Malus domestica*) and found that honeybees, bumblebees, “medium” bees, and “small” bees visited *E. umbellata* flowers. The only other co-flowering plant that had more “bee groups” was another invasive, *Taraxacum officinale*. Bees caught on apple flowers had small amounts of *T. officinale* in pollen loads, and Adamson makes no mention of *E. umbellata* pollen in pollen loads. Still, her results suggest apple and *E. umbellata* share some pollinators, so it is important to survey the impacts of invasives on both crop visitation and native plant visitation.

The possible level of pollinator sharing between native plants and *E. umbellata* is further broadened by the diverse pollinator types on *E. umbellata*. Dipteran and lepidopteran pollinators may also be shared with other plants. For example, *Tilia* co-flowers with *E. umbellata* and both plants are preferred by the moth *Mythimna unipuncta*, which has further been documented as a pollinator of *Cleome* (Cleomaceae) (Zhu et al. 1993) and *Tipularia discolor* (Orchidaceae) (Whigham & McWethy 1980). The syrphid fly *Toxomerus marginatus*, which was a frequent visitor to *E. umbellata* in

2011, was recently recorded as a floral visitor to 114 plant species in central Illinois (Tooker et al. 2006). Another fly, *Bombylius major*, is a frequent presence in woodland wildflower communities, often visiting *Claytonia virginica* (Portulacaceae), *Stellaria pubera* (Caryophyllaceae), and *Thalictrum thalictroides* (Ranunculaceae) (Campbell 1985; Motten 1986). *B. major* was common on the *E. umbellata* at the WH population, which is surrounded by a large tract of deciduous forest. The prevalence of *B. major* on *E. umbellata* in forested habitat demonstrates the potential for pollinator sharing between invasive and native woodland plants.

Whether pollinator sharing with *E. umbellata* is a detriment or benefit to native plant reproduction is likely dependent on scale. Obviously, dense stands of *E. umbellata* directly impact native plant reproduction through shading and other possible forms of direct competition. From observations at Illinois field sites, it can be concluded that this cost to native plant reproduction is unlikely to be offset by facilitated pollination to native plants that co-flower with *E. umbellata*.

The floral resources of *E. umbellata* could have lasting impacts on insects that are economically and ecologically important. The abundant nectar and pollen rewards of *E. umbellata* may increase the carrying capacity of some pollinator populations. The pollen of some invasive plants has been observed being collected by female generalist bees and possibly raises the carrying capacity of environments inhabited by native bees (Tepedino et al. 2008). One native bee that was observed gathering *E. umbellata* pollen and that had a substantial amount of *E. umbellata* pollen in scopal loads was the sweat bee *Augochlorella aurata*. *Augochlorella* species are ground-nesting bees that form primitively eusocial colonies (Mueller 1996). If *E. umbellata* pollen bolsters the

available food for larvae of *A. aurata* and other generalist bees, then this invasive plant may actually facilitate the pollination of other plants, even those that do not flower at the same time. For example, *A. aurata* is a primary pollinator of the widespread prairie plant *Echinacea angustifolia*, which blooms from late spring to mid-summer (Waginius & Lyon 2010). It is very difficult to predict the relationship between floral resource availability and bee abundance (Tepedino 1979; Kearns et al. 1998), and whether *E. umbellata* facilitates visitation to a later-flowering plant like *E. angustifolia* would depend on multiple factors. *E. umbellata* pollen would have to be nutritious, non-toxic, and more abundant than pollen in a non-invaded plant community. Also, *E. umbellata* presence could not significantly compromise nesting sites of pollinators; many ground-nesting bees need a dry, compacted substrate for nesting.

Bomblylius major may have an even more complicated relationship with *E. umbellata*, which it frequently used as a nectar source in my study sites. *B. major* is a parasitoid that deposits its eggs in the nests of ground-nesting bees and wasps (Stubbs & Drake 2001). The *B. major* larvae then feed on the bee larvae. Increases in the abundance of *B. major* have been shown to be correlated with decreases in the abundance of the ground-nesting bee *Andrena vaga* in Germany (Bischoff 2003). If *E. umbellata* is a plentiful and reliable food source for the parasitoid, then declines in native bee abundance may result.

Interactions between *E. umbellata* and other notable insects like the syrphid fly *Toxomerus marginatus* and the moth *Mythimna unipuncta* might have important implications for the agricultural industry. The larvae of *T. marginatus* and some other syrphid flies consume aphids, which are ubiquitous pests on numerous agricultural

crops (Colley & Luna 2000). The presence of *E. umbellata* along agricultural boundaries may be beneficial if they support a healthy population of aphidophagous syrphid flies such as *T. marginatus*.

Unfortunately, *E. umbellata* may also foster insects that are agricultural pests. For example, one of its nocturnal pollinators, the moth *M. unipuncta*, is a pest of grass species in North America, including corn, rice, and wheat. They have also been recorded causing damage to broad-leaved crops like alfalfa, clover, and tobacco (Wynne 1989). Outbreaks of *M. unipuncta* have been known to occur every 5-20 years and cause huge losses to the agricultural industry (Guppy 1961). Adult *M. unipuncta* likely need ample nectar sources to enact such outbreaks and future work should investigate whether the presence of *E. umbellata* is correlated with oviposition rates on crops.

Finally, the direct removal of invasive plants has been shown to increase native bee diversity and abundance (Hanula & Horn 2011; Fielder et al. 2012; Morandin & Kremen 2012). Hanula and Horn removed the invasive *Ligustrum sinense* (Chinese privet) from riparian forest habitat and found significant changes in the bee community a year after removal. The authors attribute the reestablishment of native herbaceous plants and increases in light intensity as possible reasons for a rapid increase in bees. *L. sinense* removal had a significant effect on increasing the prevalence of some of the insects collected in this study including *Andrena imitatrix*, *Augochlorella aurata*, *Ceratina calcarata*, *Ceratina dupla*, and *Lasioglossum mitchelli*. Removal of *E. umbellata* from areas heavily impacted by its invasion, especially dense stands, may have a similar effect.

CHAPTER 5

CONCLUSION

The establishment of *E. umbellata* across the eastern North American landscape has been a rapid process. Its success has been aided in part by the intentional propagation and planting of the species, but also because it is able to produce many high-quality seeds that are dispersed over relatively long-distances. The result is aggressive colonization of both disturbed and natural areas. This study revealed that *E. umbellata* is self-incompatible; therefore, seed production is limited by the presence of suitable mates and pollinators. These requirements may lessen the rate of spread, but if habitats harbor generalist pollinators and are sufficiently populated with *E. umbellata*, then the chances for seed production are high. Seed set was rarely absent on *E. umbellata* even though it is pollen limited in some habitats. In the situation that self-incompatible *E. umbellata* is isolated from conspecifics, their perennial nature affords them the luxury of “waiting it out” until the arrival of mates or pollinators makes cross-pollination possible. Successful reproduction among these predominantly outcrossing plants indicates that outcrossing will likely remain a dominant reproductive strategy in this species.

Predominant outcrossing is far from an optimal strategy according to Baker’s law (1955), but it is important to note that this is not the only means of reproduction for *E. umbellata*. Baker (1974) listed vegetative reproduction as an important trait of successful perennial weeds. *E. umbellata* is capable of clonal growth (Kohri et al. 2002) and this may contribute greatly to its invasiveness. Future work might examine the

extent of clonal growth in *E. umbellata* and whether it is the major reason for the monospecific stands that prevent native plant growth over large areas.

Self-compatible individuals do exist among invasive populations of *E. umbellata* and selective forces in certain environments could potentially favor this mating strategy over obligate outcrossing. As long as self-compatibility is heritable, then it is possible for populations of self-compatible *E. umbellata* to be established. In some species that are otherwise obligate outcrossers, self-compatible populations or individuals exist on the periphery of the species' range where mates may be more limiting (Levin 2012). This study found a few self-compatible individuals within a typical area of invasion, but it is possible that more exist at the edge of the invasive range. Range expansion evokes many colonization and local extinction events that could impose selection for self-compatibility, but theory predicts that selection for self-compatibility, in general, would be weak for a plant like *E. umbellata* (Pannell & Barrett 1998). As noted above, *E. umbellata* is perennial and has multiple years to fulfill its reproductive goals; therefore, there is less selective pressure for the quick and abundant seed output achieved through self-compatibility. Also, *E. umbellata* is generalized in its pollination strategy so that it has a good chance of being visited by pollinators once mate availability is adequate for reproduction. Reproduction does not always lead to population establishment though. Stochastic forces may cause local extinction and the lack of seed dormancy in *E. umbellata* (Kohri 2008; Carter and Ungar 2002) means that opportunities exist for self-compatible populations to establish. Partial self-compatibility is already apparent in *E. umbellata* populations and may persist if pollen limitation is a recurring hindrance to outcrossing and if inbreeding depression is weak.

Future work may focus further on the variable reproductive characters I found in *E. umbellata* and decipher whether particular characters promote invasiveness. For example, my study showed that a few individuals can reproduce autogamously, but it is unknown how common this trait is throughout the invasive range. My study also indicated that the degree of herkogamy varies among individuals. Possibly, this character may be correlated with self-compatibility. Because my study focused only on Illinois populations, it is still unknown whether there are geographic patterns in these reproductive characters. If so, then this may indicate past selective pressures during invasion and offers the unique opportunity for evolutionary studies on contemporary timescales.

This study demonstrated that *E. umbellata* has the ability to attract a wide array of floral visitors that are highly generalized in their choice of floral hosts. The major pollinators of *E. umbellata* in southern and central Illinois are also common throughout the invasive range. It is likely that most invasive populations will be assured at least some pollination given the commonality of some pollinators, and it is certain that native and introduced pollinators are significant players in the invasion of this plant. What is uncertain is whether *E. umbellata*'s interactions with these pollinators impact native plant pollination or affect other community processes.

In conclusion, this study demonstrates another case in which a predominantly outcrossing plant has become a successful invasive species, although self-compatibility is evident in a minority of *E. umbellata*. The rare findings of self-compatible individuals may be an important factor in the plant's invasiveness and future studies should investigate if partial self-compatibility is ubiquitous across the invasive

range. Despite there being instances of partially self-compatible individuals, predominant outcrossing will likely remain a successful strategy for the invasion of this species because many pollinator species in the invasive range fulfill cross-pollination. However, this study showed that heavy fruit set is not as common as previously thought, which can probably be attributed to pollinator limitation and post-zygotic influences on seed growth. This finding, along with the apparent inability of *E. umbellata* to establish a seed bank, offers some hope for future restoration of invaded areas. The removal of *E. umbellata* infestations will need to be carried out soon given the impact that *E. umbellata* may have on ecosystem-level processes like pollination and nitrogen cycling. Proper management might focus on targeting large *E. umbellata* individuals that are capable of high fruit production, so that the primary sources of invasive spread can be slowed. Further research is needed to determine whether high fruit production in some plants is due partly to autogamy or due strictly to insect pollination. Such studies are needed to more fully understand the invasiveness of *E. umbellata* and its impact on native pollination networks.

TABLES

Table 1. Location of study sites and experiments performed within each site.

Year	Study Site	Location	Experiment	N
2011	ALL	39.574N, 88.539W	Floral Morphology	14
		Moultrie Co., IL	Phenology (Stigma)	5
	BTP	40.203N, 88.398W	Breeding system	10
		Champaign Co., IL	Floral Morphology	14
			Phenology	10
	MUR	37.754N, 89.346W	D vs. N Pollination	5
		Jackson Co., IL		
	TONEC	37.478N, 89.158W	Breeding System	17
Jackson Co., IL				
2012	CONWR	37.736N, 89.071W	Breeding System	20
		Williams Co., IL	Floral Morphology	
	SIUC	37.713N, 89.235W	D vs. N Pollination	12
		Jackson Co, IL		
	TONEC	37.478N, 89.158W	Breeding System	14
		Jackson Co., IL	Floral Morphology	14
			Phenology	10
			Pollen Tube	6
	WH	37.575N, 88.454W	Breeding System	9
		Pope Co., IL		

Table 2. Treatment regime employed in breeding system study of *Elaeagnus umbellata*.

Treatment	Methods
Autogamy (tests for automatic self-pollination)	Bagged and unmanipulated
Self-pollination (tests for self-compatibility)	Bagged and hand pollinated with pollen from same plant.
Outcrossed	Bagged and hand pollinated with pollen from plant outside of genet (>20 m away)
Trimmed control	Unbagged and unmanipulated
Untrimmed control	Unbagged and unmanipulated (flowers not trimmed)

Table 3. Repeated measures ANOVA of the effects of treatment, time following pollination, and their interaction on *Elaeagnus umbellata* fruit set at one 2011 population and two 2012 populations.

	d.f.	<i>F</i>	<i>P</i>
2011 TONEC			
Treatment	3, 61.3	36.95	<0.0001
Time	2, 112	19.09	<0.0001
Treatment x time	6, 112	3.46	0.004
2012 COWR			
Treatment	4, 95	12.14	<0.0001
Time	3, 285	18.64	<0.0001
Treatment x time	12, 285	3.06	0.0005
2012 TONEC			
Treatment	4, 60.2	42.61	<0.0001
Time	3, 174	15.37	<0.0001
Treatment x time	12, 179	5.63	<0.0001
2012 WH			
Treatment	1, 15.1	0.42	0.53
Time	2, 27.1	3.64	0.04
Treatment x time	2, 27.1	0.72	0.49

Table 4. Mixed model analysis of the effects of population on autogamous and control fruit set in *Elaeagnus umbellata*, with mean mature fruit set displayed for each population. Different lettering indicates significant differences between populations in mature fruit set (Tukey's HSD, $P < 0.05$). *The autogamy treatment was replicated among 9 plants and the control treatment was replicated among 6 plants at WH.

Treatment	<i>F</i>	d.f.	<i>P</i>	Population mature fruit set % (\pm SE)		
				CONWR (n=20)	TONEC (n=14)	WH (n=*)
Autogamy				0.98 (\pm 0.54) ^a	1.77 (\pm 0.54) ^a	21.3 (\pm 11.6) ^b
Population	4.63	2, 40	0.016			
Control				3.16 (\pm 1.15) ^a	1.75 (\pm 0.80) ^a	11.1 (\pm 4.74) ^b
Population	5.03	2, 34	0.012			

Table 5. Extent of the furthest growing self-pollen tube (S) within a self-pollinated flower (n = 32) or cross-pollen tube (C) within a cross-pollinated flower (n = 39) from six *Elaeagnus umbellata* plants at 24, 48, and 72 hours after pollination. The number of letters within a plant column represents the number of flowers treated on the same plant. Out of six plants examined at TONEC in 2012, four plants (Plant 1, 2, 3, 4) showed strong inhibition of self-pollen tubes at the stigma, while two plants (Plant 5, Plant 6) showed significant self-pollen tube growth beyond the stigma.

Carpel Site	Plant 1			Plant 2			Plant 3			Plant 4			Plant 5			Plant 6			
	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	
Stigma	SS	S		SSS	SS	S	SS	SS				SSS					S		
Top-style				CCC	CCC	CCC		S					S	S	S				
Mid-style		C									C	C	SS	SS	S			SS	
End-style	C				C		C	C		C		C					S	C	
Ovary					C		CC	C	C	C	C		C	S	CCC	C		S	S
																	CCC	CCC	

Table 6. Insect visitors collected on *Elaeagnus umbellata* flowers during 2011 and 2012. *Bombus* spp. were not collected and no attempts were made to collect nocturnal visitors in 2011.

Insect Order	Family	Taxon	Amount Collected		
			2011	2012	Total
Coleoptera			21	0	21
Diptera	Bombyliidae	<i>Bombylius major</i> (Linnaeus)	1	15	16
	Syrphidae	<i>Allograpta obliqua</i> (Say)	2♀	0	2
		<i>Eristalis dimidiata</i> (Wiedemann)	0	1♀, 3♂	4
		<i>Eristalis transversa</i> (Wiedemann)	0	1♂	1
		<i>Eupeodes americanus</i> (Wiedemann)	3♀	2♂	5
		<i>Helophilus fasciata</i> (Walker)	0	4♀	4
		<i>Syrphus ribesii</i> (Linnaeus)	0	2♀	2
		<i>Syrphus vitripennis</i> (Meigen)	0	1♂	1
		<i>Toxomerus geminatus</i> (Say)	2♂	0	2
		<i>Toxomerus marginatus</i> (Say)	8♀	0	8
	Other				39

Table 6. Insect visitors collected on *Elaeagnus umbellata* flowers during 2011 and 2012, continued.

Insect Order	Family	Taxon	Amount Collected		
			2011	2012	Total
Hymenoptera	Apidae	<i>Apis mellifera</i> (Linnaeus)	30♀	45♀	75
		(long-tongued) <i>Bombus</i> spp.	-	-	N/A
		<i>Ceratina calcarata</i> (Robertson)	15♂	3♀, 11♂	29
		<i>Ceratina dupla</i> (Say)	1♂	1♂	2
		<i>Ceratina strenua</i> (Smith)	1♀, 4♂	1♂	6
		<i>Nomada</i> sp. 1	1	5	6
		<i>Nomada</i> sp. 2	0	3	3
		<i>Habropoda</i> sp.	0	3♂	3
		<i>Xylocopa virginica</i> (Linnaeus)	4♀, 9♂	2♀, 17♂	32
		Other	1	0	1
	Megachilidae	<i>Osmia</i> sp. 1	0	3♀	3
	(long-tongued)	<i>Osmia</i> sp. 2	0	1♀	1

Table 6. Insect visitors collected on *Elaeagnus umbellata* flowers during 2011 and 2012, continued

Insect Order	Family	Taxon	Amount Collected		
			2011	2012	Total
Hymenoptera	Andrenidae (short-tongued)	<i>Andrena carlini</i> (Cockerell)	2♀	3♀	5
		<i>Andrena c. cressonii</i> (Robertson)	2♀	1♀, 3♂	6
		<i>Andrena dunning</i> (Cockerell)	0	1♀	1
		<i>Andrena forbesii</i> (Robertson)	1♀	1♂	2
		<i>Andrena hippotes</i> (Robertson)	0	2♀, 1♂	3
		<i>Andrena illini</i> (Bouseman & LaBerge)	1♀	9♀, 1♂	11
		<i>Andrena illini/sayi</i>	1♂	0	1
		<i>Andrena imitatrix</i> (Cresson)	0	1♀, 3♂	4
		<i>Andrena macra</i> (Mitchell)	0	1♂	1
		<i>Andrena perplexa</i> (Smith)	0	1♂	1
		<i>Andrena sayi</i> (Robertson)	0	2♀	2
		Colletidae (short-tongued)	<i>Colletes banksi</i> (Swenk)	0	2♀
	<i>Colletes inaequalis</i> (Say)	0	2♀	2	

Table 6. Insect visitors collected on *Elaeagnus umbellata* flowers during 2011 and 2012, continued.

Insect Order	Family	Taxon	Amount Collected		
			2011	2012	Total
Hymenoptera	Halictidae (short-tongued)	<i>Augochlorella auarata</i> (Smith)	30♀	0	30
		<i>Augochlorella</i> sp.	0	1♀	1
		<i>Augochloropsis metallica</i> (Fabricius)	2♀	0	2
		<i>Augochloropsis</i> sp.	0	2♀	2
		<i>Halictus ligatus</i> (Say)	1♀	0	1
		<i>Halictus rubicundus</i> (Christ)	1♀	0	1
		<i>Lasioglossum anomalum</i> (Robertson)	3♀	0	3
		<i>Lasioglossum coeruleum</i> (Robertson)	1♀	0	1
		<i>Lasioglossum mitchelli</i> Gibbs	5♀	0	5
		<i>Lasioglossum viridatum</i> (Lovell)	1♀	0	1
	Other			16	

Table 6. Insect visitors collected on *Elaeagnus umbellata* flowers during 2011 and 2012, continued.

Insect Order	Family	Taxon	Amount Collected		
			2011	2012	Total
Lepidoptera	Geometridae		-	1	1
	Hesperiidae	<i>Erynnis</i> spp.	1	4	5
	Nymphalidae	<i>Vanessa atalanta</i> (Linnaeus)	0	14	14
	Papilionidae		1	5	6
	Sphingidae	<i>Hemaris thysbe</i> (Fabricius)	1	2	3

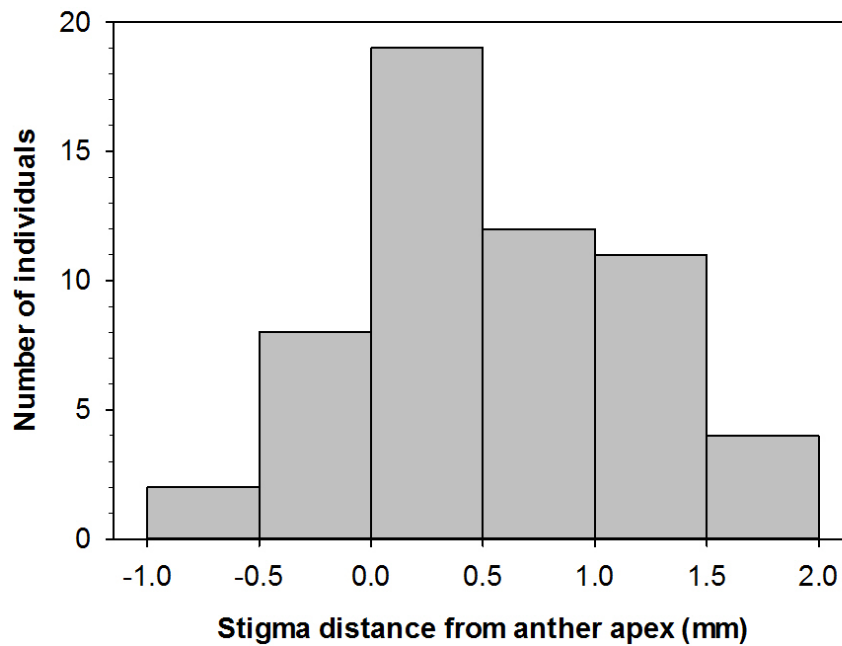


Figure 1. Frequency of *Elaeagnus umbellata* individuals with varying degrees of herkogamy. Measurements are the distance that the stigma apex was below (< 0 mm) or exerted beyond (> 0 mm) the anther apex (mean = 0.51 mm, SE = \pm 0.08 mm, n = 56 plants).



Figure 2. *Elaeagnus umbellata* flowers from a herkogamous individual (top) and a non-herkogamous individual (bottom) (mm scaling).

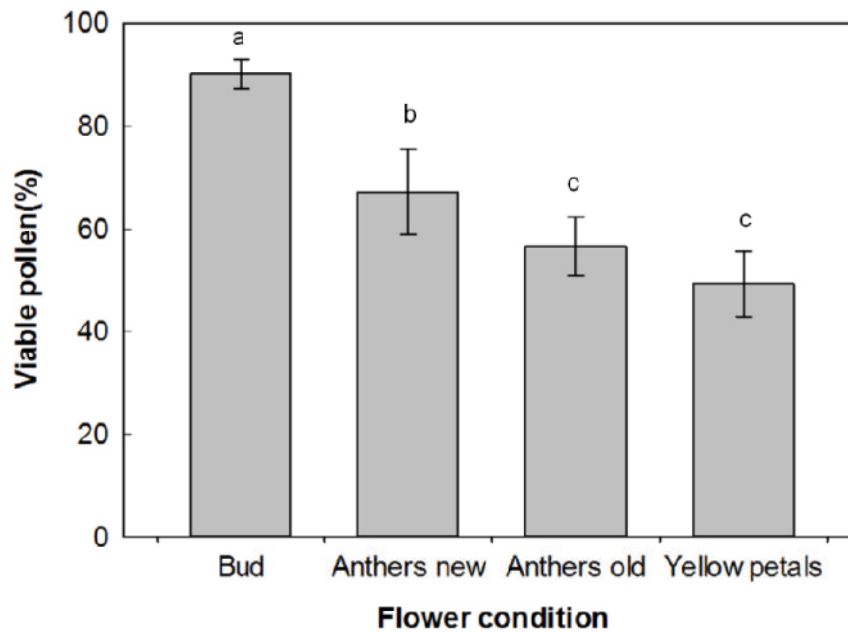


Figure 3. Percent pollen viability of *Elaeagnus umbellata* flowers of visually assessed ages (mean \pm 1 SE) at the BTP population in 2011. A higher percentage of viable pollen was present in flowers that appear to younger compared to flowers at later stages ($F_{(3, 27)} = 19.00$, $P < 0.0001$, $n = 40$). Different letters indicate significant differences according to Tukey's HSD test ($P < 0.05$).

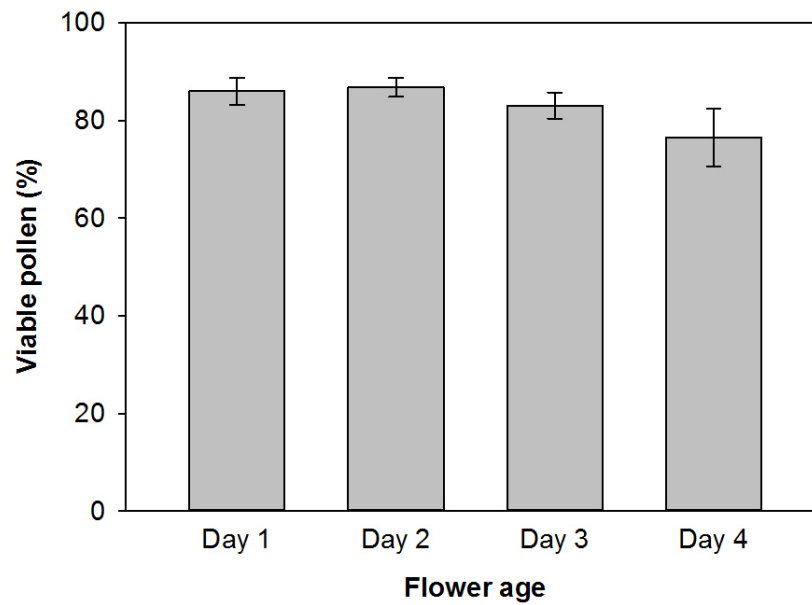


Figure 4. Percent pollen viability of *Elaeagnus umbellata* flowers (mean \pm 1 SE) at the TONEC population in 2012. A mixed model analysis indicated no significant differences in the percentage of viable pollen among different floral ages ($F_{(3, 27)} = 2.51$, $P = 0.08$, $n = 40$).

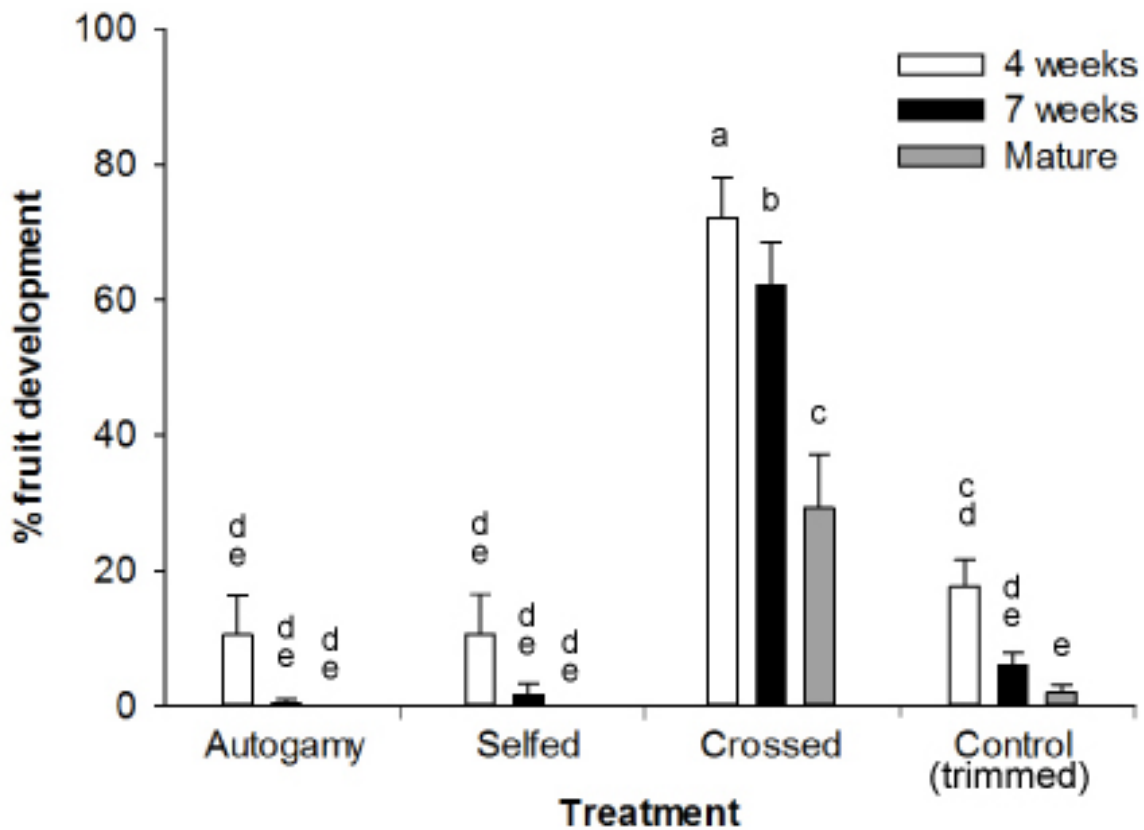


Figure 5. Mean (± 1 SE) percent fruit development of *Elaeagnus umbellata* at progressive stages of fruit development following four different pollination treatments at TONEC in 2011. Repeated measures ANOVA indicated a significant effect of treatment ($F_{(3, 61.3)} = 36.95, P < 0.0001$) and time after pollination ($F_{(2, 112)} = 19.09, P < 0.0001$) on fruit set. There was a significant interaction between treatment and time after pollination ($F_{(6, 112)} = 3.46, P < 0.01$). Different lettering indicates significant differences according to differences of least squares means ($P < 0.05$).

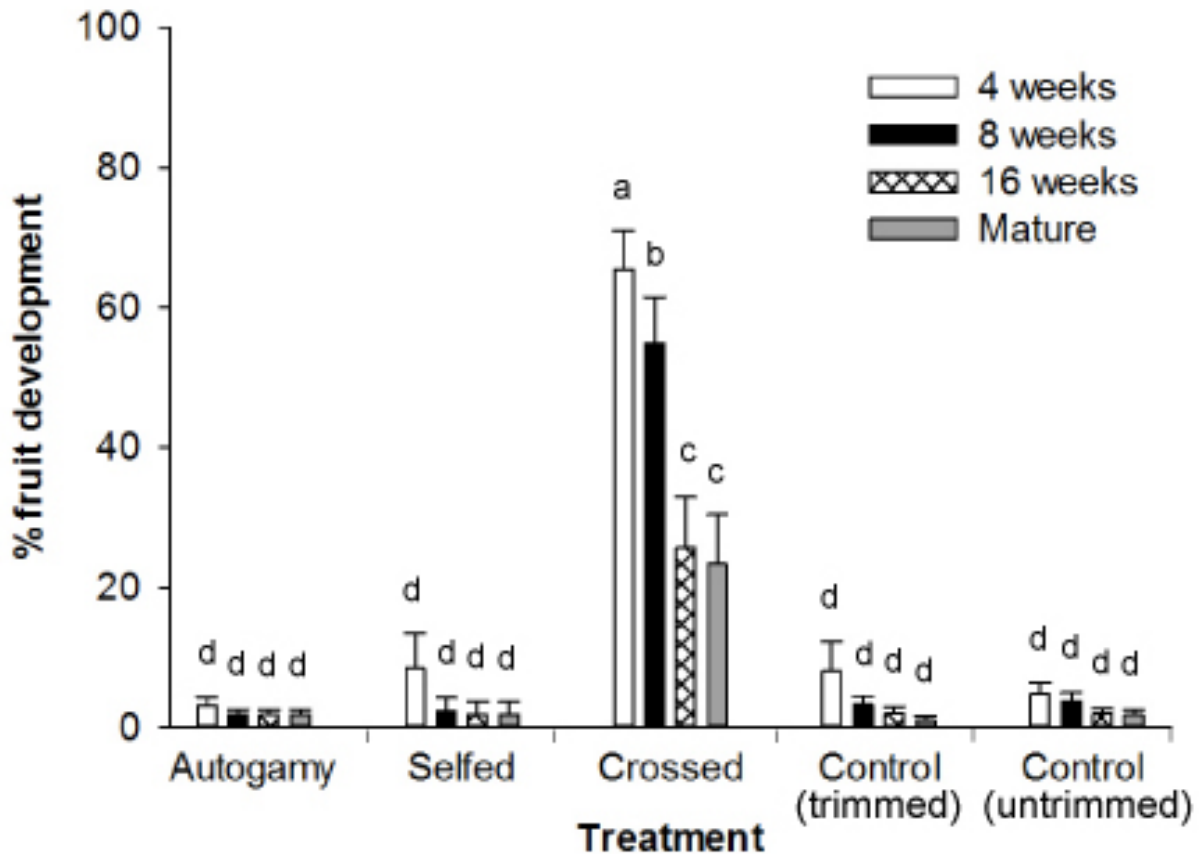


Figure 6. Mean (± 1 SE) percent fruit development of *Elaeagnus umbellata* at progressive stages of fruit development following five different pollination treatments at TONEC in 2012. Repeated measures ANOVA indicated a significant effect of treatment ($F_{(4, 60.2)} = 42.61$, $P < 0.0001$) and time after pollination ($F_{(3, 174)} = 15.37$, $P < 0.0001$) on fruit set. There was a significant interaction between treatment and time after pollination ($F_{(12, 179)} = 5.63$, $P < 0.0001$). Different lettering indicates significant differences according to differences of least squares means ($P < 0.01$).

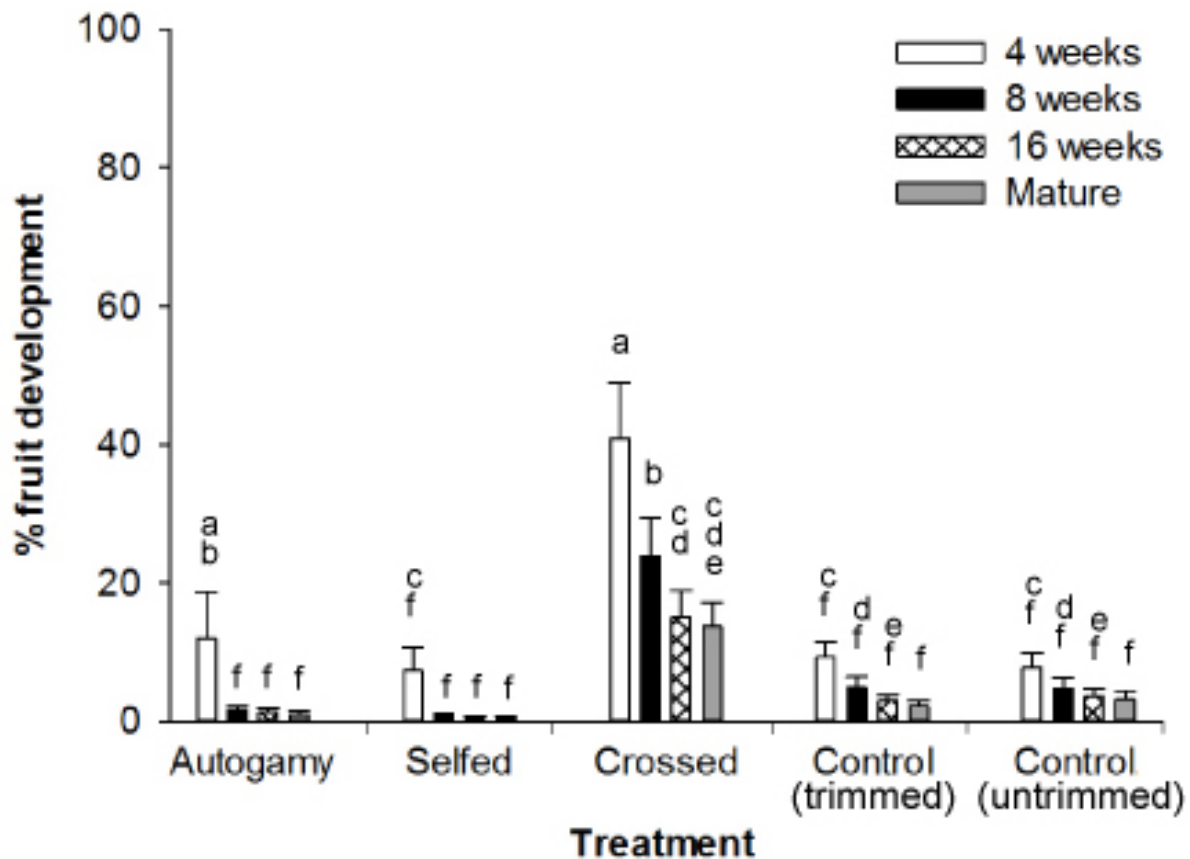


Figure 7. Mean (± 1 SE) percent fruit development of *Elaeagnus umbellata* at progressive stages of fruit development following five different pollination treatments at CONWR in 2012. Different lettering indicates significant differences according to differences of least squares means ($P < 0.05$). Repeated measures ANOVA indicated a significant effect of treatment ($F_{(4, 95)} = 12.14$, $P < 0.0001$) and time after pollination ($F_{(3, 285)} = 18.64$, $P < 0.0001$) on fruit set. There was a significant interaction between treatment and time after pollination ($F_{(12, 285)} = 3.06$, $P < 0.01$).

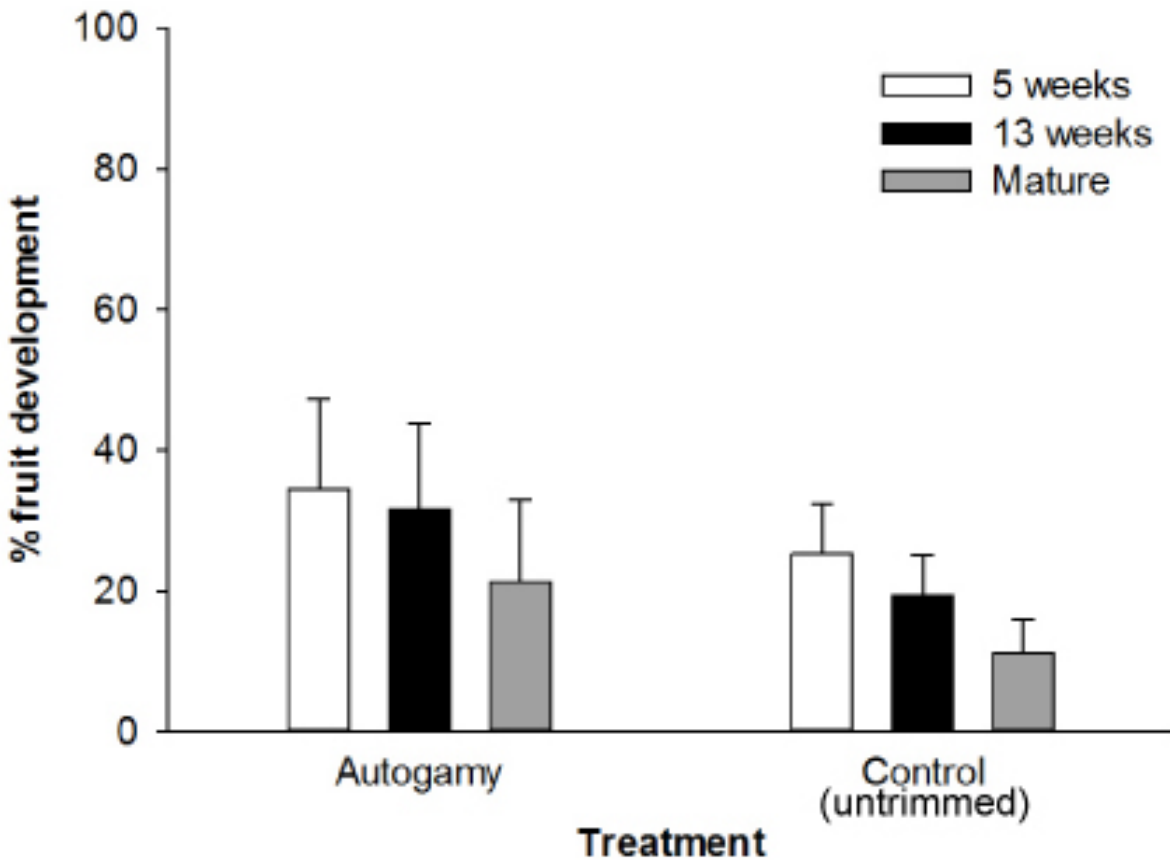


Figure 8. Mean percent fruit development (± 1 SE) of *Elaeagnus umbellata* at progressive stages of fruit development following two pollination treatments at WH in 2012. Repeated measures ANOVA indicated a significant effect of time after pollination on fruit set ($F_{(2, 27.1)} = 3.64$, $P < 0.05$, $n = 9$) but no significant effect of treatment on fruit set ($F_{(1, 15.1)} = 0.42$, $P = 0.53$, $n = 9$).

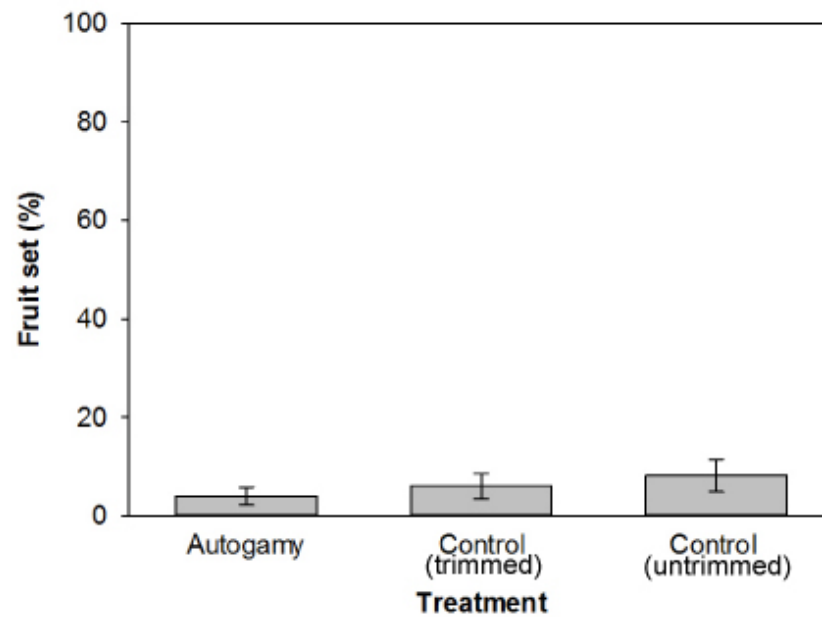


Figure 9. Mean (± 1 SE) mature fruit set of *Elaeagnus umbellata* at BTP in 2011. A mixed model analysis indicated no significant differences among treatments ($F_{(2, 18)} = 1.46$, $P = 0.26$, $n = 10$).

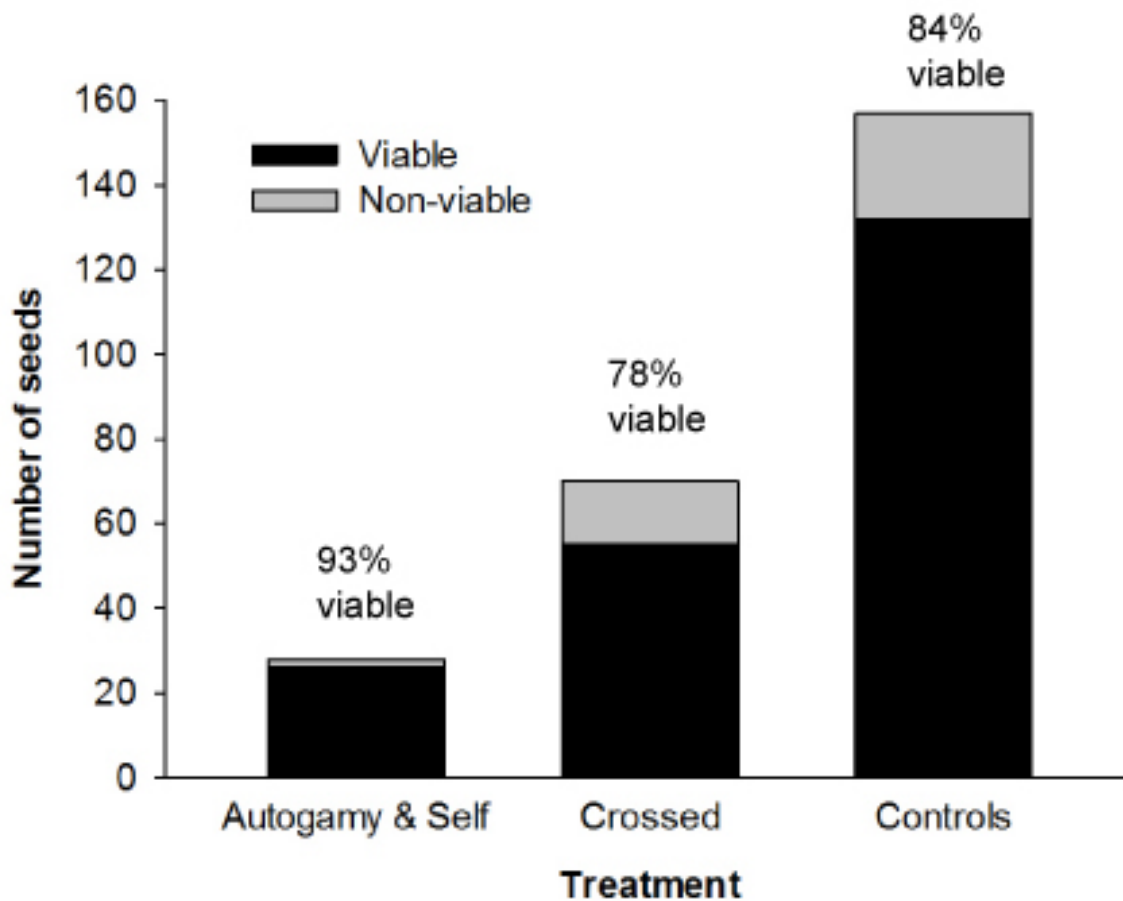


Figure 10. The number of viable and non-viable *Elaeagnus umbellata* seeds from selfed (autogamy and self-pollination), outcrossed, and control (trimmed and untrimmed) treatments in 2012. Mixed model analysis revealed no significant differences between treatments in the percent of fruits that contained a viable seed ($F_{(2, 29.4)} = 1.07, P = 0.36, n = 29$).

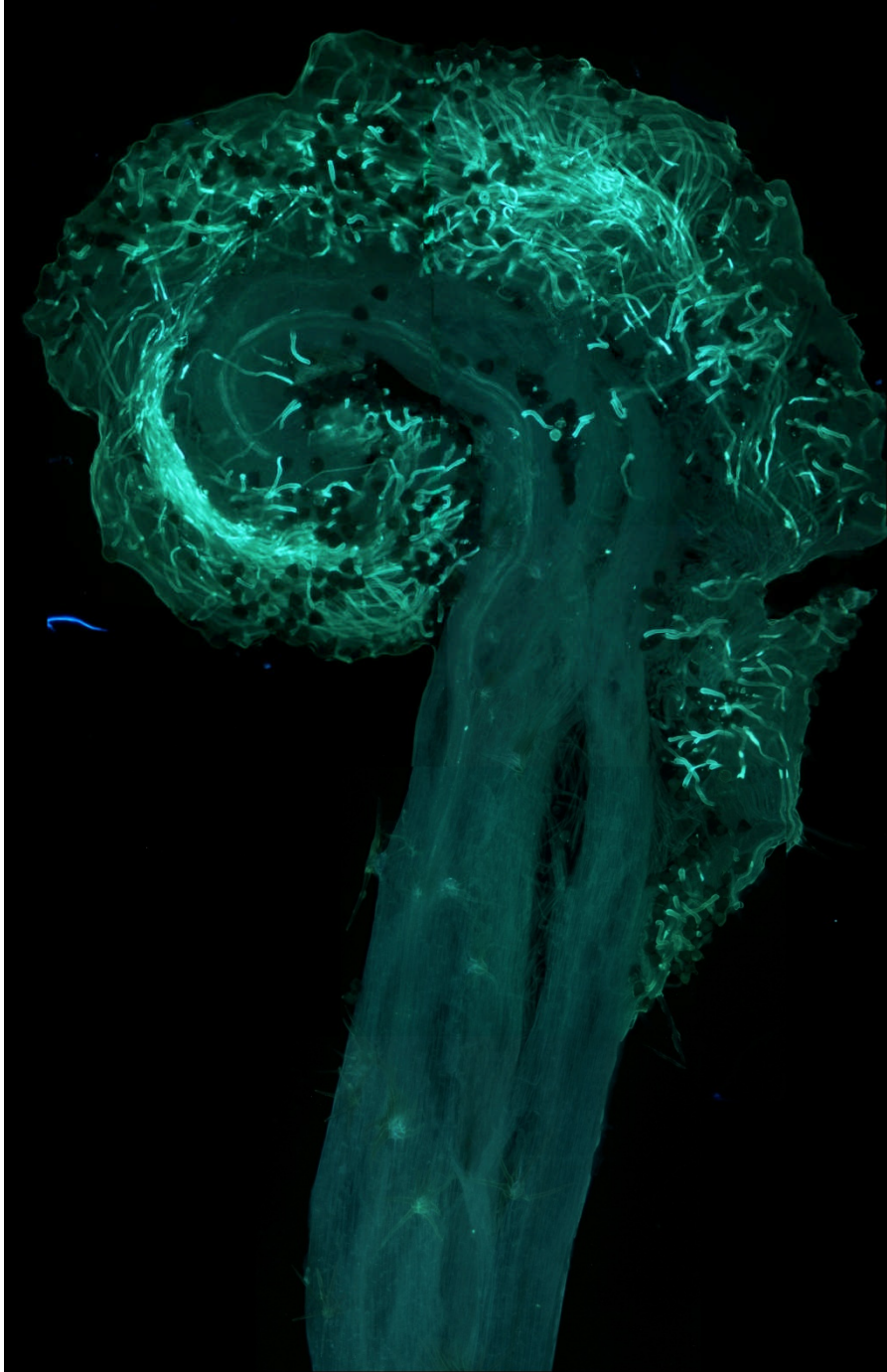


Figure 11. Pollen tube growth within the upper portion of a self-pollinated carpel from a strongly self-incompatible *Elaeagnus umbellata* plant. Fluorescing pollen tubes are concentrated on the stigma surface with no significant growth into the style. Compare to cross-pollinated flower from same plant (Fig. 12).

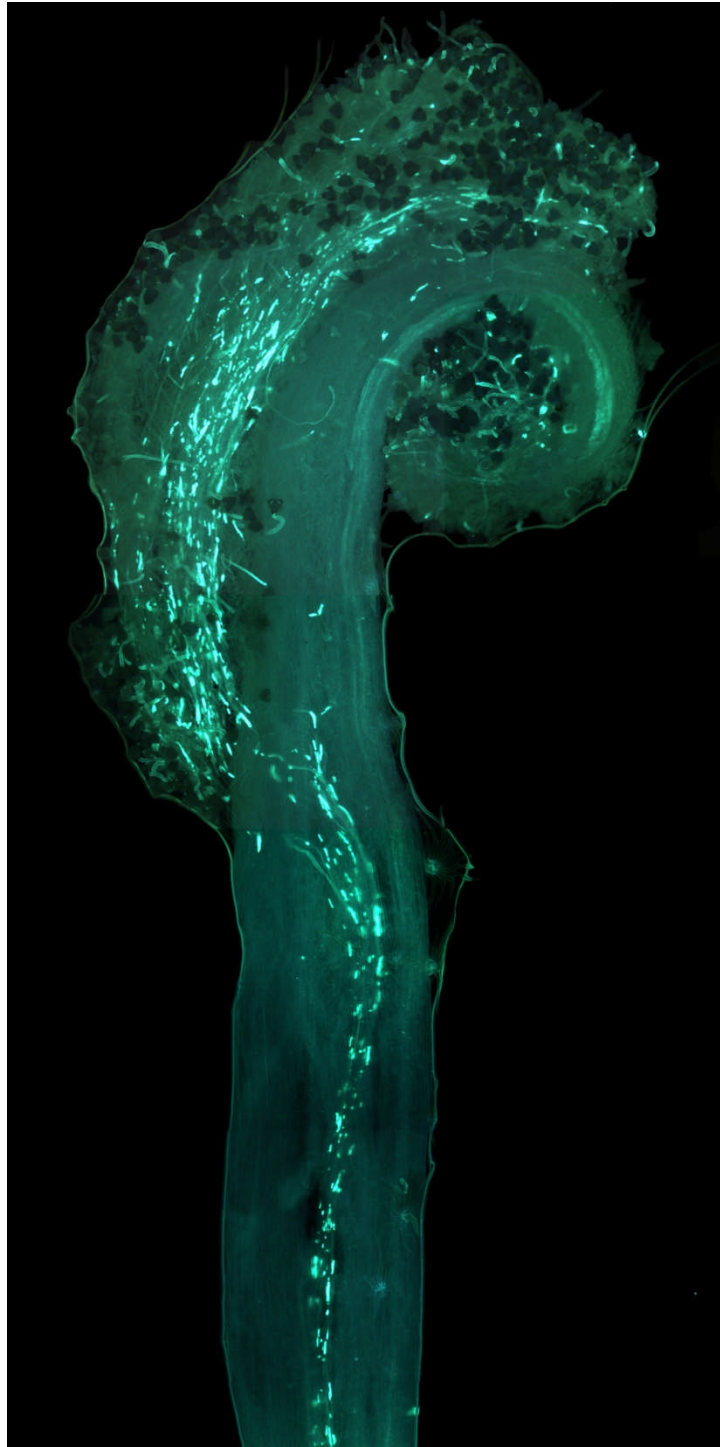


Figure 12. Pollen tube growth within the upper portion of a cross-pollinated carpel from a strongly self-incompatible *Elaeagnus umbellata* plant. Fluorescing pollen tubes grow down the style. Compare to self-pollinated flower from same plant (Fig. 11).



Figure 13. Insects collected on *Elaeagnus umbellata* flowers (mm scaling).
 Clockwise from top left: *Andrena illini* female, *Syrphus ribesii* female, *Augochlorellata aurata* female, *Lasioglossum mitchelli* female, large bee fly (*Bombylius major*), small carpenter bee (*Ceratina calcarata*) male, *Andrena immitatrix* male.



Figure 14. Insects foraging on *Elaeagnus umbellata* flowers. Clockwise from top left: Large bee fly (*Bombylius major*), Noctuid moth, honeybee (*Apis mellifera*), bumblebee (*Bombus* sp.).

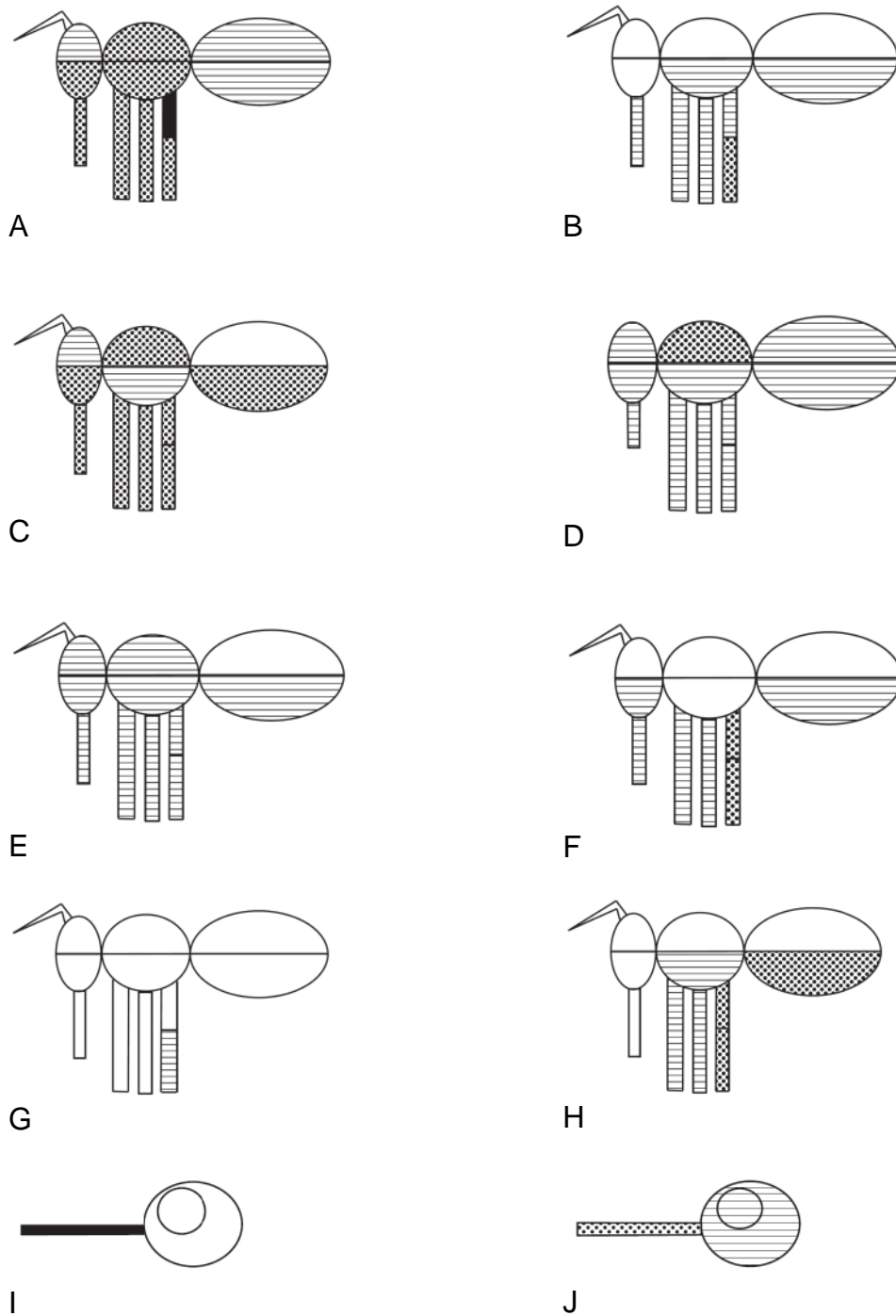





Figure 15. Average amount of pollen on different regions of the bodies of larger (A-F) and smaller (E-H) insect taxa and on different regions of the face of other insect taxa (I-J) that frequently visited *Elaeagnus umbellata* flowers. Different patterning indicates differences in the amount of pollen:  = 0-10 grains,  = 10-50 grains,  =

Figure 15. Continued

total pollen cover (>100 grains), ■ = > 1 pollen layer. The following insects were examined: (A) *Andrena illini* females (n = 10), (B) *Apis mellifera* workers (n = 10), (C) *Xylocopa virginica* males (n = 10), (D) Large Syrphidae flies (n = 10), (E) *Andrena* males (n = 10), (F) *Augochlorella aurata* females (n = 28), (G) *Ceratina* spp. males (n = 14), (H) *Lasioglossum* spp. females (n = 8), (I) Noctuidae moths (n = 9), (J) *Bombylius major* (n = 9).

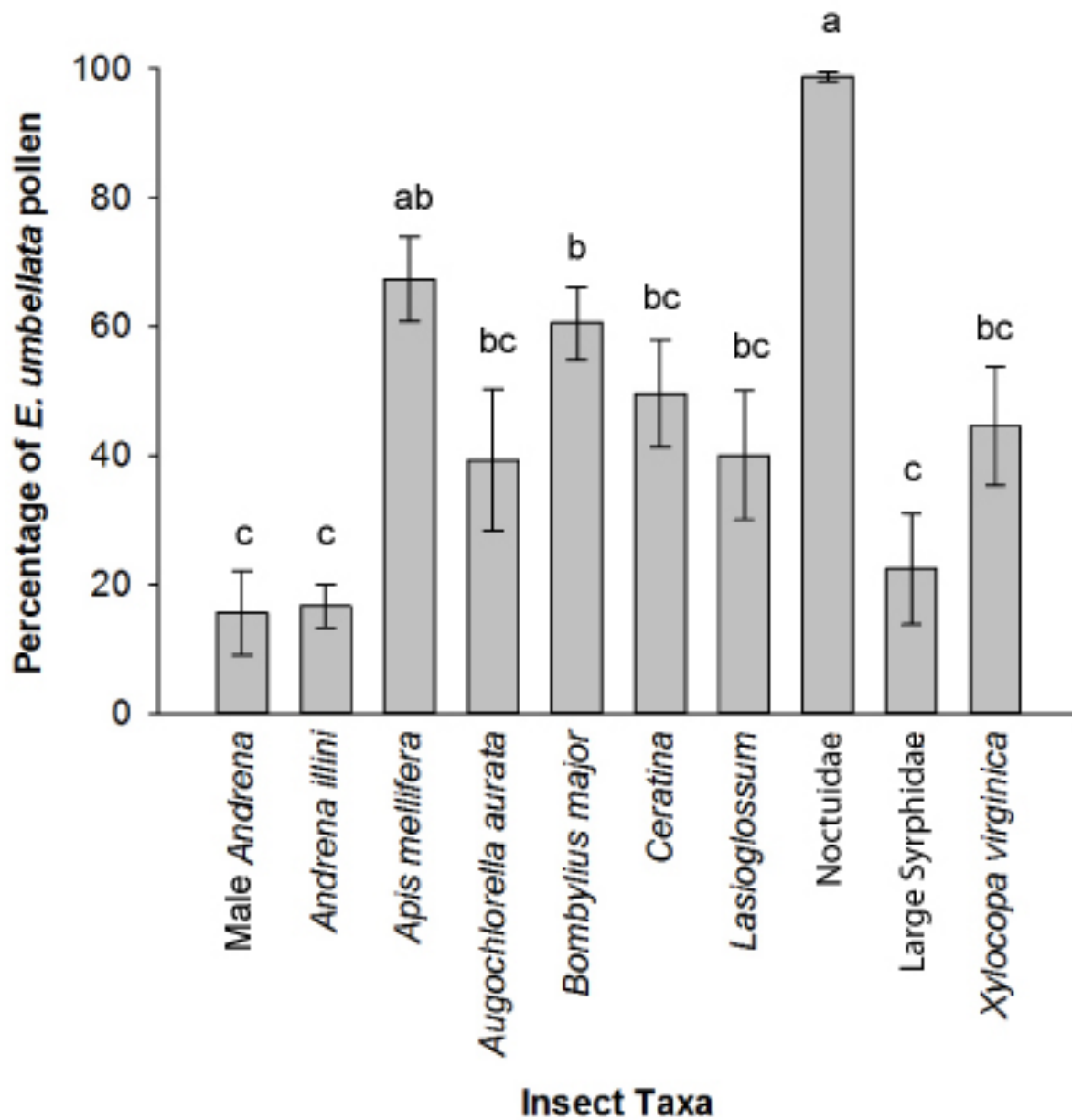


Figure 16. Percentage (mean \pm 1 SE) of *Elaeagnus umbellata* pollen on the bodies of ten frequently visiting insect taxa. Scopae of bees was not included. There was a significant difference among taxa in the percentage of *E. umbellata* carried ($F = 11.35$, $P < 0.0001$, $n = 100$). Different letters indicate significant differences between visitor taxa in the percentage of *E. umbellata* carried (Tukey's HSD, $P < 0.05$).

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APPENDICES

Appendix A. SAS output from least squares means analysis of the effect “Time after pollination x treatment” at TONEC in 2011. Treatment abbreviations: Auto, autogamy; ConTrim, trimmed control; Crossed, outcrossed; Self-poll, self-pollination. Arcsine-transformed least squares means (LS mean) are displayed. *P*-values from differences of least squares means and Tukey's HSD test are displayed.

Time after pollination	Treatment	LS mean	SE	Comparison treatment	Comparison time after pollination	d.f.	Pr > t	Tukey Adj p
4 weeks	Auto	0.139	0.083	Auto	7 weeks	114	0.0971	0.8765
4 weeks	Auto	0.144	0.083	Auto	Mature	114	0.0863	0.8503
4 weeks	Auto	-0.041	0.091	ConTrim	4 weeks	148	0.6509	1
4 weeks	Auto	0.086	0.096	ConTrim	7 weeks	152	0.367	0.999
4 weeks	Auto	0.126	0.096	ConTrim	Mature	152	0.1876	0.9743
4 weeks	Auto	-0.766	0.091	Crossed	4 weeks	148	<.0001	<.0001
4 weeks	Auto	-0.574	0.096	Crossed	7 weeks	152	<.0001	<.0001
4 weeks	Auto	-0.194	0.097	Crossed	Mature	154	0.0479	0.6964
4 weeks	Auto	-0.001	0.091	Self-poll	4 weeks	148	0.9911	1
4 weeks	Auto	0.126	0.096	Self-poll	7 weeks	152	0.1883	0.9746
4 weeks	Auto	0.142	0.096	Self-poll	Mature	152	0.1385	0.9406
4 weeks	ConTrim	0.180	0.096	Auto	7 weeks	152	0.0608	0.7636
4 weeks	ConTrim	0.185	0.096	Auto	Mature	152	0.0544	0.7326
4 weeks	ConTrim	0.128	0.083	ConTrim	7 weeks	114	0.1275	0.9273
4 weeks	ConTrim	0.168	0.083	ConTrim	Mature	114	0.0461	0.6813
4 weeks	ConTrim	-0.724	0.091	Crossed	4 weeks	148	<.0001	<.0001
4 weeks	ConTrim	-0.533	0.096	Crossed	7 weeks	152	<.0001	<.0001
4 weeks	ConTrim	-0.153	0.097	Crossed	Mature	154	0.1185	0.9161
4 weeks	ConTrim	0.040	0.091	Self-poll	4 weeks	148	0.659	1
4 weeks	ConTrim	0.168	0.096	Self-poll	7 weeks	152	0.0815	0.8388
4 weeks	ConTrim	0.184	0.096	Self-poll	Mature	152	0.0566	0.7435
4 weeks	Crossed	0.905	0.096	Auto	7 weeks	152	<.0001	<.0001
4 weeks	Crossed	0.909	0.096	Auto	Mature	152	<.0001	<.0001
4 weeks	Crossed	0.852	0.096	ConTrim	7 weeks	152	<.0001	<.0001
4 weeks	Crossed	0.892	0.096	ConTrim	Mature	152	<.0001	<.0001
4 weeks	Crossed	0.192	0.083	Crossed	7 weeks	114	0.023	0.4802
4 weeks	Crossed	0.571	0.085	Crossed	Mature	116	<.0001	<.0001
4 weeks	Crossed	0.764	0.091	Self-poll	4 weeks	148	<.0001	<.0001
4 weeks	Crossed	0.892	0.096	Self-poll	7 weeks	152	<.0001	<.0001
4 weeks	Crossed	0.908	0.096	Self-poll	Mature	152	<.0001	<.0001
4 weeks	Self-poll	0.140	0.096	Auto	7 weeks	152	0.1444	0.9463
4 weeks	Self-poll	0.145	0.096	Auto	Mature	152	0.1313	0.9328
4 weeks	Self-poll	0.087	0.096	ConTrim	7 weeks	152	0.3614	0.9988
4 weeks	Self-poll	0.127	0.096	ConTrim	Mature	152	0.1841	0.9727
4 weeks	Self-poll	-0.573	0.096	Crossed	7 weeks	152	<.0001	<.0001
4 weeks	Self-poll	-0.193	0.097	Crossed	Mature	154	0.0491	0.7034
4 weeks	Self-poll	0.127	0.083	Self-poll	7 weeks	114	0.1288	0.9289
4 weeks	Self-poll	0.143	0.083	Self-poll	Mature	114	0.0877	0.854

Appendix A. SAS output from 2011 TONEC, continued.

7 weeks	Auto	0.005	0.086	Auto	Mature	106	0.9559	1
7 weeks	Auto	-0.053	0.100	ConTrim	7 weeks	155	0.5982	1
7 weeks	Auto	-0.013	0.100	ConTrim	Mature	155	0.8989	1
7 weeks	Auto	-0.713	0.100	Crossed	7 weeks	155	<.0001	<.0001
7 weeks	Auto	-0.333	0.102	Crossed	Mature	156	0.0013	0.0587
7 weeks	Auto	-0.013	0.100	Self-poll	7 weeks	155	0.8973	1
7 weeks	Auto	0.003	0.100	Self-poll	Mature	155	0.9752	1
7 weeks	ConTrim	0.057	0.100	Auto	Mature	155	0.5655	1
7 weeks	ConTrim	0.040	0.086	ConTrim	Mature	106	0.6423	1
7 weeks	ConTrim	-0.660	0.100	Crossed	7 weeks	155	<.0001	<.0001
7 weeks	ConTrim	-0.281	0.102	Crossed	Mature	156	0.0064	0.211
7 weeks	ConTrim	0.040	0.100	Self-poll	7 weeks	155	0.6906	1
7 weeks	ConTrim	0.056	0.100	Self-poll	Mature	155	0.5768	1
7 weeks	Crossed	0.718	0.100	Auto	Mature	155	<.0001	<.0001
7 weeks	Crossed	0.700	0.100	ConTrim	Mature	155	<.0001	<.0001
7 weeks	Crossed	0.380	0.088	Crossed	Mature	108	<.0001	0.0019
7 weeks	Crossed	0.700	0.100	Self-poll	7 weeks	155	<.0001	<.0001
7 weeks	Crossed	0.716	0.100	Self-poll	Mature	155	<.0001	<.0001
7 weeks	Self-poll	0.018	0.100	Auto	Mature	155	0.8597	1
7 weeks	Self-poll	0.000	0.100	ConTrim	Mature	155	0.9983	1
7 weeks	Self-poll	-0.320	0.102	Crossed	Mature	156	0.0019	0.0828
7 weeks	Self-poll	0.016	0.086	Self-poll	Mature	106	0.8525	1
Mature	Auto	-0.017	0.100	ConTrim	Mature	155	0.8613	1
Mature	Auto	-0.338	0.102	Crossed	Mature	156	0.0011	0.0515
Mature	Auto	-0.002	0.100	Self-poll	Mature	155	0.9867	1
Mature	ConTrim	-0.321	0.102	Crossed	Mature	156	0.0019	0.0823
Mature	ConTrim	0.016	0.100	Self-poll	Mature	155	0.8744	1
Mature	Crossed	0.336	0.102	Self-poll	Mature	156	0.0012	0.0539

Appendix B. SAS output from least squares means analysis of the effect “Time after pollination x treatment” at TONEC in 2012. Treatment abbreviations: Auto, autogamy; ConTrim, trimmed control; ConUntr, untrimmed control; Crossed, outcrossed; Self-poll, self-pollination. Arcsine-transformed least squares means (LS mean) are displayed. *P*-values from differences of least squares means and Tukey’s HSD test are displayed.

Measurement time	Treatment	Mean	SE	Comparison treatment	Comparison time	d.f.	LSD	Tukey
4 weeks	Auto	0.014	0.028	Auto	8 weeks	151	0.6139	1
4 weeks	Auto	0.014	0.042	Auto	16 weeks	231	0.7383	1
4 weeks	Auto	0.014	0.046	Auto	Mature	250	0.7614	1
4 weeks	Auto	-0.051	0.054	ConTrim	4 weeks	127	0.3397	1
4 weeks	Auto	0.004	0.054	ConTrim	8 weeks	127	0.9466	1
4 weeks	Auto	0.013	0.054	ConTrim	16 weeks	127	0.8102	1
4 weeks	Auto	0.016	0.054	ConTrim	Mature	131	0.7716	1
4 weeks	Auto	-0.016	0.054	ConUntr	4 weeks	127	0.7666	1
4 weeks	Auto	-0.005	0.054	ConUntr	8 weeks	127	0.9255	1
4 weeks	Auto	0.013	0.054	ConUntr	16 weeks	127	0.8037	1
4 weeks	Auto	0.014	0.054	ConUntr	Mature	127	0.7934	1
4 weeks	Auto	-0.716	0.055	Crossed	4 weeks	127	<.0001	<.0001
4 weeks	Auto	-0.573	0.055	Crossed	8 weeks	131	<.0001	<.0001
4 weeks	Auto	-0.288	0.057	Crossed	16 weeks	135	<.0001	0.0002
4 weeks	Auto	-0.261	0.057	Crossed	Mature	133	<.0001	0.0014
4 weeks	Auto	-0.058	0.054	Self-poll	4 weeks	127	0.285	0.9999
4 weeks	Auto	0.009	0.054	Self-poll	8 weeks	127	0.8738	1
4 weeks	Auto	0.014	0.054	Self-poll	16 weeks	127	0.7996	1
4 weeks	Auto	0.014	0.054	Self-poll	Mature	127	0.7996	1
4 weeks	ConTrim	0.065	0.054	Auto	8 weeks	127	0.2249	0.9996
4 weeks	ConTrim	0.065	0.054	Auto	16 weeks	127	0.2248	0.9996
4 weeks	ConTrim	0.065	0.054	Auto	Mature	127	0.2248	0.9996
4 weeks	ConTrim	0.055	0.028	ConTrim	8 weeks	151	0.0491	0.9047
4 weeks	ConTrim	0.064	0.042	ConTrim	16 weeks	231	0.1263	0.9926
4 weeks	ConTrim	0.067	0.047	ConTrim	Mature	250	0.1522	0.9966
4 weeks	ConTrim	0.035	0.054	ConUntr	4 weeks	127	0.5099	1
4 weeks	ConTrim	0.046	0.054	ConUntr	8 weeks	127	0.3888	1
4 weeks	ConTrim	0.065	0.054	ConUntr	16 weeks	127	0.2295	0.9997
4 weeks	ConTrim	0.065	0.054	ConUntr	Mature	127	0.2244	0.9996
4 weeks	ConTrim	-0.664	0.055	Crossed	4 weeks	127	<.0001	<.0001
4 weeks	ConTrim	-0.521	0.055	Crossed	8 weeks	131	<.0001	<.0001
4 weeks	ConTrim	-0.237	0.057	Crossed	16 weeks	135	<.0001	0.0069

Appendix B. SAS output from 2012 TONEC, continued

4 weeks	ConTrim	-0.210	0.057	Crossed	Mature	133	0.0003	0.039
4 weeks	ConTrim	-0.006	0.054	Self-poll	4 weeks	127	0.9083	1
4 weeks	ConTrim	0.060	0.054	Self-poll	8 weeks	127	0.2659	0.9999
4 weeks	ConTrim	0.065	0.054	Self-poll	16 weeks	127	0.2275	0.9996
4 weeks	ConTrim	0.065	0.054	Self-poll	Mature	127	0.2275	0.9996
4 weeks	ConUntr	0.030	0.054	Auto	8 weeks	127	0.5773	1
4 weeks	ConUntr	0.030	0.054	Auto	16 weeks	127	0.5772	1
4 weeks	ConUntr	0.030	0.054	Auto	Mature	127	0.5772	1
4 weeks	ConUntr	0.020	0.054	ConTrim	8 weeks	127	0.716	1
4 weeks	ConUntr	0.029	0.054	ConTrim	16 weeks	127	0.5914	1
4 weeks	ConUntr	0.032	0.054	ConTrim	Mature	131	0.5593	1
4 weeks	ConUntr	0.011	0.028	ConUntr	8 weeks	151	0.6938	1
4 weeks	ConUntr	0.029	0.042	ConUntr	16 weeks	231	0.4848	1
4 weeks	ConUntr	0.030	0.046	ConUntr	Mature	250	0.5155	1
4 weeks	ConUntr	-0.700	0.055	Crossed	4 weeks	127	<.0001	<.0001
4 weeks	ConUntr	-0.557	0.055	Crossed	8 weeks	131	<.0001	<.0001
4 weeks	ConUntr	-0.272	0.057	Crossed	16 weeks	135	<.0001	0.0006
4 weeks	ConUntr	-0.245	0.057	Crossed	Mature	133	<.0001	0.0044
4 weeks	ConUntr	-0.042	0.054	Self-poll	4 weeks	127	0.439	1
4 weeks	ConUntr	0.025	0.054	Self-poll	8 weeks	127	0.6487	1
4 weeks	ConUntr	0.030	0.054	Self-poll	16 weeks	127	0.582	1
4 weeks	ConUntr	0.030	0.054	Self-poll	Mature	127	0.582	1
4 weeks	Crossed	0.730	0.055	Auto	8 weeks	127	<.0001	<.0001
4 weeks	Crossed	0.730	0.055	Auto	16 weeks	127	<.0001	<.0001
4 weeks	Crossed	0.730	0.055	Auto	Mature	127	<.0001	<.0001
4 weeks	Crossed	0.720	0.055	ConTrim	8 weeks	127	<.0001	<.0001
4 weeks	Crossed	0.729	0.055	ConTrim	16 weeks	127	<.0001	<.0001
4 weeks	Crossed	0.732	0.055	ConTrim	Mature	131	<.0001	<.0001
4 weeks	Crossed	0.711	0.055	ConUntr	8 weeks	127	<.0001	<.0001
4 weeks	Crossed	0.729	0.055	ConUntr	16 weeks	127	<.0001	<.0001
4 weeks	Crossed	0.730	0.055	ConUntr	Mature	127	<.0001	<.0001
4 weeks	Crossed	0.143	0.030	Crossed	8 weeks	154	<.0001	0.0006
4 weeks	Crossed	0.428	0.046	Crossed	16 weeks	236	<.0001	<.0001
4 weeks	Crossed	0.455	0.050	Crossed	Mature	250	<.0001	<.0001
4 weeks	Crossed	0.658	0.055	Self-poll	4 weeks	127	<.0001	<.0001
4 weeks	Crossed	0.724	0.055	Self-poll	8 weeks	127	<.0001	<.0001
4 weeks	Crossed	0.730	0.055	Self-poll	16 weeks	127	<.0001	<.0001
4 weeks	Crossed	0.730	0.055	Self-poll	Mature	127	<.0001	<.0001
4 weeks	Self-poll	0.072	0.054	Auto	8 weeks	127	0.1842	0.9987
4 weeks	Self-poll	0.072	0.054	Auto	16 weeks	127	0.1842	0.9987
4 weeks	Self-poll	0.072	0.054	Auto	Mature	127	0.1842	0.9987
4 weeks	Self-poll	0.061	0.054	ConTrim	8 weeks	127	0.2561	0.9998
4 weeks	Self-poll	0.071	0.054	ConTrim	16 weeks	127	0.1911	0.9989
4 weeks	Self-poll	0.073	0.054	ConTrim	Mature	131	0.1782	0.9984

Appendix B. SAS output from 2012 TONEC, continued

4 weeks	Self-poll	0.053	0.054	ConUntr	8 weeks	127	0.3289	1
4 weeks	Self-poll	0.071	0.054	ConUntr	16 weeks	127	0.1882	0.9988
4 weeks	Self-poll	0.072	0.054	ConUntr	Mature	127	0.1839	0.9987
4 weeks	Self-poll	-0.515	0.055	Crossed	8 weeks	131	<.0001	<.0001
4 weeks	Self-poll	-0.231	0.057	Crossed	16 weeks	135	<.0001	0.0102
4 weeks	Self-poll	-0.203	0.057	Crossed	Mature	133	0.0005	0.0546
4 weeks	Self-poll	0.066	0.028	Self-poll	8 weeks	151	0.0183	0.6713
4 weeks	Self-poll	0.071	0.042	Self-poll	16 weeks	231	0.0905	0.9772
4 weeks	Self-poll	0.071	0.046	Self-poll	Mature	250	0.1237	0.992
8 weeks	Auto	0.000	0.037	Auto	16 weeks	195	0.9999	1
8 weeks	Auto	0.000	0.043	Auto	Mature	237	0.9999	1
8 weeks	Auto	-0.010	0.054	ConTrim	8 weeks	127	0.8464	1
8 weeks	Auto	-0.001	0.054	ConTrim	16 weeks	127	0.9835	1
8 weeks	Auto	0.002	0.054	ConTrim	Mature	131	0.9742	1
8 weeks	Auto	-0.019	0.054	ConUntr	8 weeks	127	0.7232	1
8 weeks	Auto	-0.001	0.054	ConUntr	16 weeks	127	0.9903	1
8 weeks	Auto	0.000	0.054	ConUntr	Mature	127	0.9991	1
8 weeks	Auto	-0.587	0.055	Crossed	8 weeks	131	<.0001	<.0001
8 weeks	Auto	-0.302	0.057	Crossed	16 weeks	135	<.0001	<.0001
8 weeks	Auto	-0.275	0.057	Crossed	Mature	133	<.0001	0.0005
8 weeks	Auto	-0.005	0.054	Self-poll	8 weeks	127	0.9188	1
8 weeks	Auto	0.000	0.054	Self-poll	16 weeks	127	0.9945	1
8 weeks	Auto	0.000	0.054	Self-poll	Mature	127	0.9945	1
8 weeks	ConTrim	0.010	0.054	Auto	16 weeks	127	0.8463	1
8 weeks	ConTrim	0.010	0.054	Auto	Mature	127	0.8463	1
8 weeks	ConTrim	0.009	0.037	ConTrim	16 weeks	195	0.7994	1
8 weeks	ConTrim	0.012	0.044	ConTrim	Mature	237	0.7805	1
8 weeks	ConTrim	-0.009	0.054	ConUntr	8 weeks	127	0.8725	1
8 weeks	ConTrim	0.010	0.054	ConUntr	16 weeks	127	0.8559	1
8 weeks	ConTrim	0.010	0.054	ConUntr	Mature	127	0.8454	1
8 weeks	ConTrim	-0.576	0.055	Crossed	8 weeks	131	<.0001	<.0001
8 weeks	ConTrim	-0.292	0.057	Crossed	16 weeks	135	<.0001	0.0001
8 weeks	ConTrim	-0.265	0.057	Crossed	Mature	133	<.0001	0.0011
8 weeks	ConTrim	0.005	0.054	Self-poll	8 weeks	127	0.9268	1
8 weeks	ConTrim	0.010	0.054	Self-poll	16 weeks	127	0.8518	1
8 weeks	ConTrim	0.010	0.054	Self-poll	Mature	127	0.8518	1
8 weeks	ConUntr	0.019	0.054	Auto	16 weeks	127	0.7232	1
8 weeks	ConUntr	0.019	0.054	Auto	Mature	127	0.7232	1
8 weeks	ConUntr	0.018	0.054	ConTrim	16 weeks	127	0.7387	1
8 weeks	ConUntr	0.021	0.054	ConTrim	Mature	131	0.7019	1
8 weeks	ConUntr	0.018	0.037	ConUntr	16 weeks	195	0.6157	1
8 weeks	ConUntr	0.019	0.043	ConUntr	Mature	237	0.6567	1
8 weeks	ConUntr	-0.568	0.055	Crossed	8 weeks	131	<.0001	<.0001
8 weeks	ConUntr	-0.283	0.057	Crossed	16 weeks	135	<.0001	0.0002

Appendix B. SAS output from 2012 TONEC, continued.

8 weeks	ConUntr	-0.256	0.057	Crossed	Mature	133	<.0001	0.002
8 weeks	ConUntr	0.014	0.054	Self-poll	8 weeks	127	0.8008	1
8 weeks	ConUntr	0.019	0.054	Self-poll	16 weeks	127	0.7284	1
8 weeks	ConUntr	0.019	0.054	Self-poll	Mature	127	0.7284	1
8 weeks	Crossed	0.587	0.055	Auto	16 weeks	131	<.0001	<.0001
8 weeks	Crossed	0.587	0.055	Auto	Mature	131	<.0001	<.0001
8 weeks	Crossed	0.586	0.055	ConTrim	16 weeks	131	<.0001	<.0001
8 weeks	Crossed	0.588	0.056	ConTrim	Mature	135	<.0001	<.0001
8 weeks	Crossed	0.586	0.055	ConUntr	16 weeks	131	<.0001	<.0001
8 weeks	Crossed	0.587	0.055	ConUntr	Mature	131	<.0001	<.0001
8 weeks	Crossed	0.284	0.041	Crossed	16 weeks	200	<.0001	<.0001
8 weeks	Crossed	0.312	0.048	Crossed	Mature	241	<.0001	<.0001
8 weeks	Crossed	0.581	0.055	Self-poll	8 weeks	131	<.0001	<.0001
8 weeks	Crossed	0.586	0.055	Self-poll	16 weeks	131	<.0001	<.0001
8 weeks	Crossed	0.586	0.055	Self-poll	Mature	131	<.0001	<.0001
8 weeks	Self-poll	0.005	0.054	Auto	16 weeks	127	0.9188	1
8 weeks	Self-poll	0.005	0.054	Auto	Mature	127	0.9188	1
8 weeks	Self-poll	0.004	0.054	ConTrim	16 weeks	127	0.9352	1
8 weeks	Self-poll	0.007	0.054	ConTrim	Mature	131	0.8941	1
8 weeks	Self-poll	0.005	0.054	ConUntr	16 weeks	127	0.9285	1
8 weeks	Self-poll	0.006	0.054	ConUntr	Mature	127	0.9179	1
8 weeks	Self-poll	-0.297	0.057	Crossed	16 weeks	135	<.0001	<.0001
8 weeks	Self-poll	-0.269	0.057	Crossed	Mature	133	<.0001	0.0008
8 weeks	Self-poll	0.005	0.037	Self-poll	16 weeks	195	0.8891	1
8 weeks	Self-poll	0.005	0.043	Self-poll	Mature	237	0.9055	1
16 weeks	Auto	0.000	0.030	Auto	Mature	162	1	1
16 weeks	Auto	-0.001	0.054	ConTrim	16 weeks	127	0.9835	1
16 weeks	Auto	0.002	0.054	ConTrim	Mature	131	0.9743	1
16 weeks	Auto	-0.001	0.054	ConUntr	16 weeks	127	0.9902	1
16 weeks	Auto	0.000	0.054	ConUntr	Mature	127	0.9991	1
16 weeks	Auto	-0.302	0.057	Crossed	16 weeks	135	<.0001	<.0001
16 weeks	Auto	-0.275	0.057	Crossed	Mature	133	<.0001	0.0005
16 weeks	Auto	0.000	0.054	Self-poll	16 weeks	127	0.9944	1
16 weeks	Auto	0.000	0.054	Self-poll	Mature	127	0.9944	1
16 weeks	ConTrim	0.001	0.054	Auto	Mature	127	0.9835	1
16 weeks	ConTrim	0.003	0.031	ConTrim	Mature	165	0.9275	1
16 weeks	ConTrim	0.000	0.054	ConUntr	16 weeks	127	0.9932	1
16 weeks	ConTrim	0.001	0.054	ConUntr	Mature	127	0.9826	1
16 weeks	ConTrim	-0.301	0.057	Crossed	16 weeks	135	<.0001	<.0001
16 weeks	ConTrim	-0.274	0.057	Crossed	Mature	133	<.0001	0.0006
16 weeks	ConTrim	0.001	0.054	Self-poll	16 weeks	127	0.9891	1
16 weeks	ConTrim	0.001	0.054	Self-poll	Mature	127	0.9891	1
16 weeks	ConUntr	0.001	0.054	Auto	Mature	127	0.9902	1
16 weeks	ConUntr	0.002	0.054	ConTrim	Mature	131	0.9647	1

Appendix B. SAS output from 2012 TONEC, continued.

16 weeks	ConUntr	0.001	0.030	ConUntr	Mature	162	0.9813	1
16 weeks	ConUntr	-0.302	0.057	Crossed	16 weeks	135	<.0001	<.0001
16 weeks	ConUntr	-0.274	0.057	Crossed	Mature	133	<.0001	0.0005
16 weeks	ConUntr	0.000	0.054	Self-poll	16 weeks	127	0.9959	1
16 weeks	ConUntr	0.000	0.054	Self-poll	Mature	127	0.9959	1
16 weeks	Crossed	0.302	0.057	Auto	Mature	135	<.0001	<.0001
16 weeks	Crossed	0.304	0.057	ConTrim	Mature	139	<.0001	<.0001
16 weeks	Crossed	0.302	0.057	ConUntr	Mature	135	<.0001	<.0001
16 weeks	Crossed	0.027	0.034	Crossed	Mature	161	0.4261	1
16 weeks	Crossed	0.302	0.057	Self-poll	16 weeks	135	<.0001	<.0001
16 weeks	Crossed	0.302	0.057	Self-poll	Mature	135	<.0001	<.0001
16 weeks	Self-poll	0.000	0.054	Auto	Mature	127	0.9944	1
16 weeks	Self-poll	0.002	0.054	ConTrim	Mature	131	0.9688	1
16 weeks	Self-poll	0.000	0.054	ConUntr	Mature	127	0.9935	1
16 weeks	Self-poll	-0.275	0.057	Crossed	Mature	133	<.0001	0.0005
16 weeks	Self-poll	0.000	0.030	Self-poll	Mature	162	1	1
Mature	Auto	0.002	0.054	ConTrim	Mature	131	0.9743	1
Mature	Auto	0.000	0.054	ConUntr	Mature	127	0.9991	1
Mature	Auto	-0.275	0.057	Crossed	Mature	133	<.0001	0.0005
Mature	Auto	0.000	0.054	Self-poll	Mature	127	0.9944	1
Mature	ConTrim	-0.002	0.054	ConUntr	Mature	131	0.9752	1
Mature	ConTrim	-0.277	0.057	Crossed	Mature	136	<.0001	0.0005
Mature	ConTrim	-0.002	0.054	Self-poll	Mature	131	0.9688	1
Mature	ConUntr	-0.275	0.057	Crossed	Mature	133	<.0001	0.0005
Mature	ConUntr	0.000	0.054	Self-poll	Mature	127	0.9935	1
Mature	Crossed	0.275	0.057	Self-poll	Mature	133	<.0001	0.0005

Appendix C. SAS output from least squares means analysis of the effect “Time after pollination x treatment” at CONWR. Treatment abbreviations: Auto, autogamy; ConTrim, trimmed control; ConUntr, untrimmed control; Crossed, outcrossed; Self-poll, self-pollination. Arcsine-transformed least squares means (LS mean) are displayed. *P*-values from differences of least squares means and Tukey’s HSD test are displayed.

Time after pollination	Treatment	LS mean	SE	Comparison treatment	Comparison time after pollination	d.f.	Pr > t	Tukey Adj p
4 weeks	Auto	0.160	0.049	Auto	8 weeks	285	0.0011	0.1148
4 weeks	Auto	0.163	0.049	Auto	16 weeks	285	0.0009	0.096
4 weeks	Auto	0.167	0.049	Auto	Mature	285	0.0007	0.0798
4 weeks	Auto	0.082	0.058	ConTrim	4 weeks	302	0.1584	0.9974
4 weeks	Auto	0.128	0.058	ConTrim	8 weeks	302	0.0283	0.796
4 weeks	Auto	0.147	0.058	ConTrim	16 weeks	302	0.0117	0.5558
4 weeks	Auto	0.154	0.058	ConUntr	Mature	302	0.0084	0.4673
4 weeks	Auto	0.098	0.058	ConUntr	4 weeks	302	0.0899	0.9778
4 weeks	Auto	0.128	0.058	ConUntr	8 weeks	302	0.0281	0.7938
4 weeks	Auto	0.141	0.058	ConUntr	16 weeks	302	0.0156	0.6375
4 weeks	Auto	0.145	0.058	ConUntr	Mature	302	0.0129	0.5844
4 weeks	Auto	-0.319	0.058	Crossed	4 weeks	302	<.0001	<.0001
4 weeks	Auto	-0.076	0.058	Crossed	8 weeks	302	0.1887	0.999
4 weeks	Auto	0.023	0.058	Crossed	16 weeks	302	0.6963	1
4 weeks	Auto	0.037	0.058	Crossed	Mature	302	0.5233	1
4 weeks	Auto	0.100	0.058	Self-poll	4 weeks	302	0.084	0.973
4 weeks	Auto	0.169	0.058	Self-poll	8 weeks	302	0.0037	0.2795
4 weeks	Auto	0.173	0.058	Self-poll	16 weeks	302	0.0031	0.2467
4 weeks	Auto	0.173	0.058	Self-poll	Mature	302	0.0031	0.2467
4 weeks	ConTrim	0.078	0.058	Auto	8 weeks	302	0.1762	0.9985
4 weeks	ConTrim	0.082	0.058	Auto	16 weeks	302	0.1596	0.9975
4 weeks	ConTrim	0.085	0.058	Auto	Mature	302	0.1443	0.996
4 weeks	ConTrim	0.046	0.049	ConTrim	8 weeks	285	0.3486	1
4 weeks	ConTrim	0.065	0.049	ConTrim	16 weeks	285	0.1825	0.9987
4 weeks	ConTrim	0.072	0.049	ConTrim	Mature	285	0.1416	0.9956
4 weeks	ConTrim	0.046	0.058	ConUntr	8 weeks	302	0.4284	1
4 weeks	ConTrim	0.059	0.058	ConUntr	16 weeks	302	0.3099	1
4 weeks	ConTrim	0.063	0.058	ConUntr	Mature	302	0.2781	0.9999
4 weeks	ConTrim	-0.401	0.058	Crossed	4 weeks	302	<.0001	<.0001
4 weeks	ConTrim	-0.158	0.058	Crossed	8 weeks	302	0.0067	0.4096
4 weeks	ConTrim	-0.059	0.058	Crossed	16 weeks	302	0.3069	1
4 weeks	ConTrim	-0.045	0.058	Crossed	Mature	302	0.4388	1
4 weeks	ConTrim	0.019	0.058	Self-poll	4 weeks	302	0.7494	1

Appendix C. SAS output from CONWR, continued.

4 weeks	ConTrim	0.088	0.058	Self-poll	8 weeks	302	0.131	0.994
4 weeks	ConTrim	0.091	0.058	Self-poll	16 weeks	302	0.117	0.9907
4 weeks	ConTrim	0.091	0.058	Self-poll	Mature	302	0.117	0.9907
4 weeks	ConUntr	0.062	0.058	Auto	8 weeks	302	0.2861	0.9999
4 weeks	ConUntr	0.065	0.058	Auto	16 weeks	302	0.2625	0.9999
4 weeks	ConUntr	0.068	0.058	Auto	Mature	302	0.2402	0.9998
4 weeks	ConUntr	-0.017	0.058	ConTrim	4 weeks	302	0.7742	1
4 weeks	ConUntr	0.029	0.058	ConTrim	8 weeks	302	0.616	1
4 weeks	ConUntr	0.048	0.058	ConTrim	16 weeks	302	0.4036	1
4 weeks	ConUntr	0.055	0.058	ConTrim	Mature	302	0.3418	1
4 weeks	ConUntr	0.029	0.049	ConUntr	8 weeks	285	0.5479	1
4 weeks	ConUntr	0.042	0.049	ConUntr	16 weeks	285	0.386	1
4 weeks	ConUntr	0.046	0.049	ConUntr	Mature	285	0.3425	1
4 weeks	ConUntr	-0.417	0.058	Crossed	4 weeks	302	<.0001	<.0001
4 weeks	ConUntr	-0.175	0.058	Crossed	8 weeks	302	0.0028	0.2292
4 weeks	ConUntr	-0.076	0.058	Crossed	16 weeks	302	0.191	0.999
4 weeks	ConUntr	-0.061	0.058	Crossed	Mature	302	0.2889	1
4 weeks	ConUntr	0.002	0.058	Self-poll	4 weeks	302	0.974	1
4 weeks	ConUntr	0.071	0.058	Self-poll	8 weeks	302	0.2207	0.9996
4 weeks	ConUntr	0.074	0.058	Self-poll	16 weeks	302	0.1998	0.9993
4 weeks	ConUntr	0.074	0.058	Self-poll	Mature	302	0.1998	0.9993
4 weeks	Cross	0.479	0.058	Auto	8 weeks	302	<.0001	<.0001
4 weeks	Cross	0.482	0.058	Auto	16 weeks	302	<.0001	<.0001
4 weeks	Cross	0.485	0.058	Auto	Mature	302	<.0001	<.0001
4 weeks	Cross	0.446	0.058	ConTrim	8 weeks	302	<.0001	<.0001
4 weeks	Cross	0.466	0.058	ConTrim	16 weeks	302	<.0001	<.0001
4 weeks	Cross	0.472	0.058	ConTrim	Mature	302	<.0001	<.0001
4 weeks	Cross	0.446	0.058	ConUntr	8 weeks	302	<.0001	<.0001
4 weeks	Cross	0.459	0.058	ConUntr	16 weeks	302	<.0001	<.0001
4 weeks	Cross	0.463	0.058	ConUntr	Mature	302	<.0001	<.0001
4 weeks	Cross	0.243	0.049	Crossed	8 weeks	285	<.0001	0.0002
4 weeks	Cross	0.341	0.049	Crossed	16 weeks	285	<.0001	<.0001
4 weeks	Cross	0.356	0.049	Crossed	Mature	285	<.0001	<.0001
4 weeks	Cross	0.488	0.058	Self-poll	8 weeks	302	<.0001	<.0001
4 weeks	Cross	0.492	0.058	Self-poll	16 weeks	302	<.0001	<.0001
4 weeks	Cross	0.492	0.058	Self-poll	Mature	302	<.0001	<.0001
4 weeks	Self-poll	0.060	0.058	Auto	8 weeks	302	0.301	1
4 weeks	Self-poll	0.063	0.058	Auto	16 weeks	302	0.2766	0.9999
4 weeks	Self-poll	0.066	0.058	Auto	Mature	302	0.2535	0.9999
4 weeks	Self-poll	0.027	0.058	ConTrim	8 weeks	302	0.6391	1
4 weeks	Self-poll	0.047	0.058	ConTrim	16 weeks	302	0.4221	1
4 weeks	Self-poll	0.053	0.058	ConTrim	Mature	302	0.3586	1
4 weeks	Self-poll	0.027	0.058	ConUntr	8 weeks	302	0.6364	1
4 weeks	Self-poll	0.040	0.058	ConUntr	16 weeks	302	0.4861	1

Appendix C. SAS output from CONWR, continued.

4 weeks	Self-poll	0.044	0.058	ConUntr	Mature	302	0.4439	1
4 weeks	Self-poll	-0.419	0.058	Crossed	4 weeks	302	<.0001	<.0001
4 weeks	Self-poll	-0.177	0.058	Crossed	8 weeks	302	0.0025	0.2127
4 weeks	Self-poll	-0.078	0.058	Crossed	16 weeks	302	0.1802	0.9987
4 weeks	Self-poll	-0.063	0.058	Crossed	Mature	302	0.2744	0.9999
4 weeks	Self-poll	0.069	0.049	Self-poll	8 weeks	285	0.1564	0.9972
4 weeks	Self-poll	0.072	0.049	Self-poll	16 weeks	285	0.1375	0.995
4 weeks	Self-poll	0.072	0.049	Self-poll	Mature	285	0.1375	0.995
8 weeks	Auto	0.003	0.049	Auto	16 weeks	285	0.9488	1
8 weeks	Auto	0.006	0.049	Auto	Mature	285	0.8978	1
8 weeks	Auto	-0.033	0.058	ConTrim	8 weeks	302	0.5715	1
8 weeks	Auto	-0.013	0.058	ConTrim	16 weeks	302	0.8166	1
8 weeks	Auto	-0.007	0.058	ConTrim	Mature	302	0.9073	1
8 weeks	Auto	-0.033	0.058	ConUntr	8 weeks	302	0.574	1
8 weeks	Auto	-0.020	0.058	ConUntr	16 weeks	302	0.7351	1
8 weeks	Auto	-0.016	0.058	ConUntr	Mature	302	0.7879	1
8 weeks	Auto	-0.236	0.058	Crossed	8 weeks	302	<.0001	0.0085
8 weeks	Auto	-0.138	0.058	Crossed	16 weeks	302	0.018	0.6764
8 weeks	Auto	-0.123	0.058	Crossed	Mature	302	0.0339	0.8379
8 weeks	Auto	0.009	0.058	Self-poll	8 weeks	302	0.8741	1
8 weeks	Auto	0.013	0.058	Self-poll	16 weeks	302	0.829	1
8 weeks	Auto	0.013	0.058	Self-poll	Mature	302	0.829	1
8 weeks	ConTrim	0.036	0.058	Auto	16 weeks	302	0.5354	1
8 weeks	ConTrim	0.039	0.058	Auto	Mature	302	0.5004	1
8 weeks	ConTrim	0.019	0.049	ConTrim	16 weeks	285	0.6912	1
8 weeks	ConTrim	0.026	0.049	ConTrim	Mature	285	0.5929	1
8 weeks	ConTrim	0.013	0.058	ConUntr	16 weeks	302	0.8199	1
8 weeks	ConTrim	0.017	0.058	ConUntr	Mature	302	0.7665	1
8 weeks	ConTrim	-0.204	0.058	Crossed	8 weeks	302	0.0005	0.0597
8 weeks	ConTrim	-0.105	0.058	Crossed	16 weeks	302	0.0709	0.9582
8 weeks	ConTrim	-0.090	0.058	Crossed	Mature	302	0.1188	0.9912
8 weeks	ConTrim	0.042	0.058	Self-poll	8 weeks	302	0.469	1
8 weeks	ConTrim	0.045	0.058	Self-poll	16 weeks	302	0.4344	1
8 weeks	ConTrim	0.045	0.058	Self-poll	Mature	302	0.4344	1
8 weeks	ConUntr	0.036	0.058	Auto	16 weeks	302	0.5378	1
8 weeks	ConUntr	0.039	0.058	Auto	Mature	302	0.5028	1
8 weeks	ConUntr	0.000	0.058	ConTrim	8 weeks	302	0.9971	1
8 weeks	ConUntr	0.019	0.058	ConTrim	16 weeks	302	0.7411	1
8 weeks	ConUntr	0.026	0.058	ConTrim	Mature	302	0.6557	1
8 weeks	ConUntr	0.013	0.049	ConUntr	16 weeks	285	0.7899	1
8 weeks	ConUntr	0.017	0.049	ConUntr	Mature	285	0.7272	1
8 weeks	ConUntr	-0.204	0.058	Crossed	8 weeks	302	0.0005	0.059
8 weeks	ConUntr	-0.105	0.058	Crossed	16 weeks	302	0.0703	0.9573
8 weeks	ConUntr	-0.091	0.058	Crossed	Mature	302	0.1179	0.9909

Appendix C. SAS output from CONWR, continued.

8 weeks	ConUntr	0.042	0.058	Self-poll	8 weeks	302	0.4712	1
8 weeks	ConUntr	0.045	0.058	Self-poll	16 weeks	302	0.4365	1
8 weeks	ConUntr	0.045	0.058	Self-poll	Mature	302	0.4365	1
8 weeks	Cross	0.240	0.058	Auto	16 weeks	302	<.0001	0.007
8 weeks	Cross	0.243	0.058	Auto	Mature	302	<.0001	0.0056
8 weeks	Cross	0.223	0.058	ConTrim	16 weeks	302	0.0001	0.0199
8 weeks	Cross	0.230	0.058	ConTrim	Mature	302	<.0001	0.0132
8 weeks	Cross	0.217	0.058	ConUntr	16 weeks	302	0.0002	0.0287
8 weeks	Cross	0.221	0.058	ConUntr	Mature	302	0.0002	0.0226
8 weeks	Cross	0.099	0.049	Crossed	16 weeks	285	0.0431	0.8867
8 weeks	Cross	0.113	0.049	Crossed	Mature	285	0.0207	0.7141
8 weeks	Cross	0.249	0.058	Self-poll	16 weeks	302	<.0001	0.0037
8 weeks	Cross	0.249	0.058	Self-poll	Mature	302	<.0001	0.0037
8 weeks	Self-poll	-0.006	0.058	Auto	16 weeks	302	0.9168	1
8 weeks	Self-poll	-0.003	0.058	Auto	Mature	302	0.9598	1
8 weeks	Self-poll	-0.023	0.058	ConTrim	16 weeks	302	0.6963	1
8 weeks	Self-poll	-0.016	0.058	ConTrim	Mature	302	0.7834	1
8 weeks	Self-poll	-0.029	0.058	ConUntr	16 weeks	302	0.6194	1
8 weeks	Self-poll	-0.025	0.058	ConUntr	Mature	302	0.6691	1
8 weeks	Self-poll	-0.246	0.058	Crossed	8 weeks	302	<.0001	0.0046
8 weeks	Self-poll	-0.147	0.058	Crossed	16 weeks	302	0.0117	0.5558
8 weeks	Self-poll	-0.132	0.058	Crossed	Mature	302	0.0227	0.7401
8 weeks	Self-poll	0.003	0.049	Self-poll	16 weeks	285	0.9454	1
8 weeks	Self-poll	0.003	0.049	Self-poll	Mature	285	0.9454	1
16 weeks	Auto	0.003	0.049	Auto	Mature	285	0.9488	1
16 weeks	Auto	-0.017	0.058	ConTrim	16 weeks	302	0.7749	1
16 weeks	Auto	-0.010	0.058	ConTrim	Mature	302	0.8646	1
16 weeks	Auto	-0.023	0.058	ConUntr	16 weeks	302	0.6948	1
16 weeks	Auto	-0.019	0.058	ConUntr	Mature	302	0.7467	1
16 weeks	Auto	-0.141	0.058	Crossed	16 weeks	302	0.0155	0.636
16 weeks	Auto	-0.126	0.058	Crossed	Mature	302	0.0297	0.8072
16 weeks	Auto	0.009	0.058	Self-poll	16 weeks	302	0.8713	1
16 weeks	Auto	0.009	0.058	Self-poll	Mature	302	0.8713	1
16 weeks	ConTrim	0.020	0.058	Auto	Mature	302	0.7339	1
16 weeks	ConTrim	0.007	0.049	ConTrim	Mature	285	0.8907	1
16 weeks	ConTrim	-0.002	0.058	ConUntr	Mature	302	0.9704	1
16 weeks	ConTrim	-0.124	0.058	Crossed	16 weeks	302	0.0326	0.8291
16 weeks	ConTrim	-0.110	0.058	Crossed	Mature	302	0.0585	0.9358
16 weeks	ConTrim	0.026	0.058	Self-poll	16 weeks	302	0.6542	1
16 weeks	ConTrim	0.026	0.058	Self-poll	Mature	302	0.6542	1
16 weeks	ConUntr	0.026	0.058	Auto	Mature	302	0.6554	1
16 weeks	ConUntr	0.006	0.058	ConTrim	16 weeks	302	0.9152	1
16 weeks	ConUntr	0.013	0.058	ConTrim	Mature	302	0.8244	1
16 weeks	ConUntr	0.004	0.049	ConUntr	Mature	285	0.9343	1

Appendix C. SAS output from CONWR, continued.

16 weeks	ConUntr	-0.118	0.058	Crossed	16 weeks	302	0.0422	0.8828
16 weeks	ConUntr	-0.104	0.058	Crossed	Mature	302	0.0741	0.9625
16 weeks	ConUntr	0.032	0.058	Self-poll	16 weeks	302	0.5794	1
16 weeks	ConUntr	0.032	0.058	Self-poll	Mature	302	0.5794	1
16 weeks	Cross	0.144	0.058	Auto	Mature	302	0.0134	0.5947
16 weeks	Cross	0.131	0.058	ConTrim	Mature	302	0.0244	0.7582
16 weeks	Cross	0.122	0.058	ConUntr	Mature	302	0.0357	0.8492
16 weeks	Cross	0.014	0.049	Crossed	Mature	285	0.768	1
16 weeks	Cross	0.150	0.058	Self-poll	Mature	302	0.0099	0.5113
16 weeks	Self-poll	-0.006	0.058	Auto	Mature	302	0.914	1
16 weeks	Self-poll	-0.019	0.058	ConTrim	Mature	302	0.7395	1
16 weeks	Self-poll	-0.028	0.058	ConUntr	Mature	302	0.6277	1
16 weeks	Self-poll	-0.150	0.058	Crossed	16 weeks	302	0.0099	0.5113
16 weeks	Self-poll	-0.136	0.058	Crossed	Mature	302	0.0196	0.6997
16 weeks	Self-poll	0.000	0.049	Self-poll	Mature	285	1	1
Mature	Auto	-0.013	0.058	ConTrim	Mature	302	0.8224	1
Mature	Auto	-0.022	0.058	ConUntr	Mature	302	0.7061	1
Mature	Auto	-0.130	0.058	Crossed	Mature	302	0.0259	0.7736
Mature	Auto	0.006	0.058	Self-poll	Mature	302	0.914	1
Mature	ConTrim	-0.117	0.058	Crossed	Mature	302	0.0449	0.8942
Mature	ConTrim	0.019	0.058	Self-poll	Mature	302	0.7395	1
Mature	ConUntr	0.009	0.058	ConTrim	Mature	302	0.8787	1
Mature	ConUntr	-0.108	0.058	Crossed	Mature	302	0.0636	0.9463
Mature	ConUntr	0.028	0.058	Self-poll	Mature	302	0.6277	1
Mature	Self-poll	-0.136	0.058	Crossed	Mature	302	0.0196	0.6997

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