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*Old Dominion University*

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**FORAGING BEHAVIOR OF SPRING BEES AND THEIR AGRICULTURAL IMPLICATIONS**

by

Michael D. Gregory  
B.S. May 2014, Old Dominion University

A Thesis Submitted to the Faculty of  
Old Dominion University in Partial Fulfillment of the  
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## **ABSTRACT**

### **FORAGING BEHAVIOR OF SPRING BEES AND THEIR AGRICULTURAL IMPLICATIONS**

Michael D. Gregory

Old Dominion University, 2017

Director: Dr. Lisa Horth

Colony Collapse Disorder has greatly reduced honey bee populations in the last decade. Native bees have been largely neglected in research, and their floral preferences may have agricultural implications because native bees ameliorate the demand to rely solely on non-native bees. In this work, pollinator visitation to flowers with manipulated ultraviolet floral patterns was monitored. Populations of wild showy golden-eye in Colorado, plus cultivar populations of black-eyed Susan and lawn populations of buttercups in Virginia were monitored for pollinator landings to compare preference for unmanipulated ultraviolet-absorptive floral guide preferences. Anther number, a proxy for pollen load, was manipulated on strawberry flowers to determine whether this treatment affected mason bee visitation frequency. Three mason bee densities (three, six and nine bees in  $\approx 2.33 \text{ m}^2$ ) were compared in three greenhouses, each stocked with 10 strawberry plants. Bee visitations were monitored and berry size and symmetry were measured. Mason bee emergence rates were compared in two environments (natural conditions and temperature-controlled conditions) for cocoons. Field surveys was conducted to evaluate which native pollinators naturally recruited to field blackberries. Higher visitation rates were found for flowers with larger ultraviolet-absorptive

floral guides and stamen manipulations. Mason bees did visit flowers but did not demonstrate any preference for flowers with more stamen. Bee density did not affect berry weight or berry symmetry. High emergence rates were observed in both natural and controlled environments and more bees emerged in the controlled environment. Sweat bees and bumble bees were the major visitors to blackberry flowers but honey bees were not present. This work builds upon earlier findings that pollinators prefer larger ultraviolet patterns and supports a more generalizable phenomenon. This study further suggests that mason bees may be commercially valuable given their peak of in mid-summer, coinciding with the pollination period of fruiting crops. Native pollinators may play an important role in blackberry pollination because of their relatively high abundance relative to honey bees.

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## CHAPTER 1

### NATIVE MASON BEES (*OSMIA LIGNARIA SAY*) ARE EFFECTIVE BERRY POLLINATORS IN THE FIELD AND IN GREENHOUSES

#### INTRODUCTION

Native bee pollination of crops has become the focus of many applied ecological research studies since Colony Collapse Disorder (CCD) was discovered in honey bees (*Apis mellifera* Say) (Patten, Shanks & Mayer 1993; Pisanty & Mandelik 2015). Apiculture, or *A. mellifera* husbandry, increased in economic value in the USA between the years 2000 and 2009 from 14 billion USD to more than 15.12 billion USD (Morse & Calderone 2000; Calderone 2012). *Apis mellifera* is responsible for nearly one-third of the food supply in the United States (USA; Buchman & Nabhan 1996) and 35% of the food supply worldwide (Klein *et al.* 2007). Globally, approximately 90% of commercial pollination results from *A. mellifera* colonies, but the agricultural demands are swiftly exceeding the pollination capacity of this species (Aizen & Harder 2009). The annual overwinter mortality of *A. mellifera* colonies in the USA was estimated at ~30% for the period from 2006 until 2015 (vanEngelsdorp *et al.*, 2007, 2008, 2010, 2011, 2012; Spleen *et al.* 2013; Steinhauer *et al.* 2014; Lee *et al.* 2015; Seitz *et al.* 2015). The most recent estimate for annual colony losses in 2015 was even greater, at ~44.1% (Steinhauer *et al.* 2016). Prior to 1985, *A. mellifera* colony losses did not show any specific pattern of decline across European countries. However, between 1985 and 2005, there was a net decrease of ~16% of *A. mellifera* colonies across Europe (Potts *et al.* 2010). Colony Collapse Disorder drives these drastic declines, and involves the mass disappearance of the worker bee population, with only the immature brood and the queen remaining at the hive (vanEngelsdorp



*et al.* 2009). Such grave colony losses create a dire need for rapid assessment of native pollinators and the ability of alternative species' to successfully pollinate commercial crops. Reduced reliance on *A. mellifera* release the agricultural industry from the catastrophic events associated with the sudden loss of large numbers of pollinators at unpredictable times.

*Apis mellifera* is traditionally considered the primary pollinators of many fruits, including strawberries (*Fragaria X ananassa* Duch.) and blackberries (*Rubus fruticosus* L.) (Free 1968; Nye & Anderson 1974; Goodman & Oldroyd 1988). *Fragaria X ananassa* naturally produce blooms from late-March through early-May in temperate climates (Hancock & Simpson 1995), and *Rubus* spp. naturally produce blooms from April through August in temperate climates (Taylor 2005). Both crops have blooming periods that overlap with the emergence times of blue orchard mason bees (*Osmia lignara* Say), which naturally emerge when their cocoons are warmed to 13.89°C (Bosch & Kemp 2000). Neither of these crops have had *O. lignaria* considered as a potential pollinator, and, in this study, *O. lignaria* is investigated for its use in the pollination of strawberry (*F. X ananassa*) flowers in greenhouses and blackberry (*R. fruticosus*) crops. With the advent of CCD, expanding the scope of native bee pollinator services has become of increasing interest (Watanabe 1994; Steffan-Dewenter, Potts & Packer 2005).

The first attempt to determine whether Megachlidae could be used for commercial pollination in orchards occurred with hornfaced bees (*Osmia cornifrons* Radoszkowski) in Japan (Maeta & Kitamura 1964), demonstrating that *Osmia* spp. can successfully pollinate apples (*Pyrus malus* Mill.) and may even provide better quality fruit than pollination by *A. mellifera*

(Maeta & Kitamura 1964). Since then, *Osmia* spp. have been widely used throughout Japan and the USA for pollination services in apple orchards (Kuhn & Ambrose 1984; Sekita & Yamada 1993; Sekita 2001; Adamson *et al.* 2012). Interest in *Osmia* spp. has also grown in other countries and successful pollination services have now been provided in almond [*Prunus dulcis* (Mill.) D. A. Webb] groves in Spain (Bosch 1994) and the USA (Bosch & Kemp 2000), as well as on blueberry (*Vaccinium* spp.) farms, (Stubbs, Drummond & Osgood 1994), plum orchards (*Prunus domestica* L.; Torchio 1976), and alfalfa fields (*Medicago sativa* L.) in the USA (Bosch & Kemp 2001). Despite several native European species of *Osmia*, the genus has not been adequately managed in most of Europe (Kronic & Stanisavljevic 2006). Native pollinators have shown strong promise with respect to their economic value (Parker 1981, 1982; Bosch & Kemp 2001), in field pollination studies, but native pollinator use in greenhouse agriculture is still very limited.

Prior to, and during the 1980's, most pollination of commercial produce in greenhouses was performed either by hand or machine a practice that proved time consuming and costly (Rasmussen 1985). However, the standard species of choice for commercial pollination, *A. mellifera*, demonstrated foraging patterns that rendered them ineffective in greenhouses (Visscher & Seeley, 1982). In contrast, another largely social genus, *Bombus*, in the same Family (Apidae) as honeybees, has been used successfully in greenhouse pollination for a few decades (Fisher & Pomeroy 1989; Shipp *et al.* 1994; van der Eijnde 1990). The services provided by the buff-tailed bumble bee (*Bombus terrestris* L.) and the common eastern bumble bee (*Bombus impatiens* Cresson) were shown to increase the quality of several fruits and vegetables, including musk melons (*Cucumis melo* L.; Fisher & Pomeroy 1989), sweet peppers (*Capsicum*

*anuum* L.; Shipp *et al.* 1994) and tomatoes (*Solanum lycopersicum* L.; van der Eijnde 1990). Presently, there are some concerns regarding the escape of greenhouse *Bombus* (Dafni *et al.* 2010) and transmission of parasites, such as mites (Goka, Okabe, & Yoneda, 2006) and fungi (*Nosema* spp.; Winter *et al.* 2006), from them to wild bees.

Leafcutter bees (Megachilidae) are solitary bees and they can be used as native pollinators in many different regions. The alfalfa leafcutter bee (*Megachile rotundata* F.) is native to Eurasia, but has also been introduced outside of its native range and proven valuable for agriculture. For example, *M. rotundata* increase seed production in alfalfa grown in fields (Pitts-Singer & Cane, 2011). *M. rotundata* has also been studied for its effectiveness in greenhouse pollination and produces better seed set for sweet peppers than three other pollination methods, including manual, machine, and crop self-pollination (Rasmussen 1985). Mason bees (*Osmia* sp.) are also members of the same family (Megachilidae) of bees, so the study of their value in greenhouses is clearly warranted. Indeed, it has been proposed that the red mason bee (*Osmia rufa* L.) would thrive in greenhouses because of its readiness to settle in artificial nests and forage on available plants (Holm 1974). In China, *Osmia cornifrons* has been shown to successfully pollinate watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai) and nectarines (*Prunus persica* (L.) Batsch) in greenhouses. In fact, watermelon flowers pollinated by *O. cornifrons* yielded more marketable fruit than artificially pollinated, or selfed flowers (Ma *et al.* 2012).

*Osmia lignaria* is a broadly distributed, native North American bee, that has not been assessed for effectiveness in greenhouse pollination. Like apples, *Fragaria* and *Rubus* are

members of the Rosaceae. Because *O. lignaria* successfully pollinates apples, by extension, it may be capable of providing pollination services to related crops, as well. One benefit of the commercial use of a native over non-native species (in the USA, *O. lignaria* are native but *O. cornifrons* and *O. rufa*, also called *O. bicornis* L., are not) is that if escape occurs, at least the species is not invasive. Solitary bees may also be less likely to spread devastating diseases that kill entire colonies of honeybees or other native bees, because, for example, they are not susceptible to CCD. Moreover, *O. lignaria* are immune to *Varroa* mites (*Varroa destructor*; Anderson & Truman 2000), which are currently considered possible contributors to CCD, but they do harbor parasitic mites of their own (*Chaetodactylus nipponicus* Kurosa) that cause minimal problems to *O. lignaria* health (Kuwahara *et al.* 1995). *Osmia* spp. have shown promise in greenhouse agriculture, but identifying an effective foraging density of these bees to produce commercial fruit has not been determined. To my knowledge, there is are no published data addressing the ability of any Megachilidae to pollinate strawberries in greenhouses.

In nature, *O. lignaria* pupae metamorphose into adults in Late-Summer. To ensure bee survival, cold stratification must occur (Bosch & Kemp 2000). Increasing temperatures following a wintering period leads to bee emergence within about five days (Bosch & Kemp 2003). A substantive amount of research has been conducted on rearing *O. lignaria* cocoons so that adult bees will emerge from them earlier than is natural, for apple and almond pollination (Torchio 1976, 1979, 1985; Bosch & Kemp 1999; Bosch & Kemp 2003). There is, however, a lack of published data addressing the ability to delay emergence of bees, which could increase their ability to pollinate summer crops, including some berries.

There is global supply and demand for *F. X ananassa* fruit with Egypt, Korea, Mexico, Poland, Spain, Turkey, and the USA utilizing *F. X ananassa* production for substantial revenue (Wu, Guan & Whidden 2012). The USA is the global leader in *F. X ananassa* production, where this fruit commodity is valued at over \$2.8 billion USD, annually [United States Department of Agriculture (USDA) 2015]. Many small farms, which are often family owned, rely heavily on the economic value of *F. X ananassa* (USDA 2015). These fruits are now commonly grown in tunnels and greenhouses in the USA during the natural strawberry off-season. Greenhouse production of strawberries has a global value of \$231 million USD annually (Jensen 1999; Takeda 1999). Commercial *F. X ananassa* and *R. fruticosus* can self-pollinate and produce fruit from selfing, but if self-pollinated fruits develop, they are usually more malformed and smaller than insect-pollinated fruits. These traits make selfed berries less preferable to consumers (Knight & Winters 1963; Nye & Anderson 1974; Klatt *et al.* 2014).

Blackberries (*Rubus fruticosus*) are worth \$50.1 million USD annually (USDA 2015). Blackberries, however, require insect pollination to produce larger and more marketable fruit (Free 1993). *Apis mellifera* are conventionally rented for *R. fruticosus* pollination, but alternative pollinators for this crop are presently limited to rented *Bombus* and wild sweat bees (Halictidae) (Cane 2005). Berry bees (*Osmia aglaia* Sandhouse) have been investigated for their ability to pollinate *R. fruticosus* and proved more successful than *A. mellifera*. In fact, fewer *O. aglaia* individuals were needed to produce bigger, more marketable berries than *A. mellifera* (Cane 2005). Determining which native pollinators visit and collect pollen from *R. fruticosus* could improve our ability to provide berry crop pollination services.

Angiosperms compete intra- and interspecifically for pollinators to transport pollen and increase the likelihood of effective reproduction (Waser 1983, 1986; Caruso 2000). To attract pollinators, flowers have many pigments and display colorful patterns, usually indicating a reward (Waser 1983). Ultraviolet (UV) absorptive “bulls-eye” patterns, often found on asters (Horovitz & Cohen 1972; Guldberg & Atsatt 1975; Lunau 1992) result from the production of flavonol glucosides (Thompson *et al.* 1972; Gronquist *et al.* 2001; Schlangen *et al.* 2009). UV “bulls-eye” patterns, more prevalent near the equator, are posited to have evolved because they lessen UV-radiation damage to pollen after dehiscence; this beneficial trait increases pollen viability (Zhang, Yang & Duan 2014; Koski & Ashman 2015). Evidence indicates that UV patterns, sometimes called nectar or floral guides, are used by pollinators in recruitment and resource orientation (Manning 1956; Johnson & Dafni 1998; Dinkel & Lunau 2001; Horth, Campbell & Bray 2014).

The function and value of UV floral signals has been a longstanding interest in pollination biology (McCrea & Levy 1983; Koski & Ashman 2014; Koski & Ashman 2015). Silverweed flowers (*Argentina anserina* L.) were manipulated to evaluate if bees would preferentially visit the completely UV-reflective “bulls-eye” pattern, normal “bulls-eye” pattern, or inverse “bulls-eye” pattern flowers in nature, and results indicated that “bulls-eye” patterns enhanced the distance perception of the flowers by bees (Koski & Ashman 2014). Black-eyed susan (*Rudbeckia hirta* L.) flower manipulations, which increased and decreased the sizes of the “bulls-eye” patterns on the flowers, led to more pollinators on the enhanced “bulls-eye” pattern (Horth, Campbell & Bray 2014). These UV patterns aid in pollinator recruitment. However, the association between UV floral pattern and floral reward is currently unknown.

However, pollen is believed to be the most ancient form of insect attractant in flowers (van der Pijl 1960) and bee pollinated flowers often emit an attractive aroma from the oily coating of pollen grains, to signal when pollen is available (Willmer 2011). These UV patterns are found in North American and European native flora, and they can be used to gauge pollinator preferences (Horth, Campbell & Bray 2014).

Many angiosperms have UV absorbent “bulls-eye” patterns. Three species, the showy goldeneye (*Heliomeris multiflora* Nutt.; Asteraceae), bulbous buttercup (*Ranunculus bulbosus* L.; Ranunculaceae), and black eyed susan (*Rudbeckia hirta*; Asteraceae) were selected as study systems for this work because they could be empirically manipulated to evaluate pollinator preferences. Asteraceae produce composite flower heads, which are composed of multiple ligulate flowers on the periphery of the head and disk flowers in the center of the head (Weakley, Ludwig & Townsend 2012). *Rudbeckia hirta* and *H. multiflora* ligules (petals) have a longitudinal surface with a UV absorbance: reflectance ratio (Fig 1) that varies somewhat (Horth, Campbell & Bray 2014). Artificially manipulating this ratio, biasing toward more UV absorbance, encourages a higher rate of pollinator visitation (Horth, Campbell & Bray 2014). Ranunculaceae also exhibit a UV pattern, which is strongly influenced by stamens (Fig 2). If similar pollination patterns occur between these two families (Asteraceae and Ranunculaceae) of angiosperms, a generalizable phenomenon will be uncovered in this work. The cosmopolitan distribution and ease of manipulation make these three taxa ideal for UV pattern research.

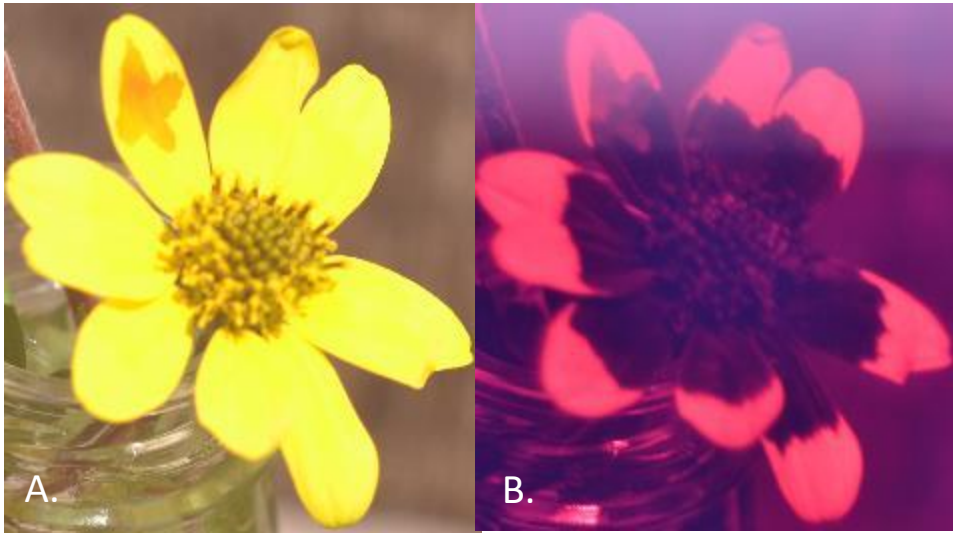


Figure 1: Photographs of *Heliomeris multiflora* in color and UV: A: The color image of *H. multiflora* is what humans see. B: The UV image of *H. multiflora* is what bees see, This pattern varies across individual flowers, and a linkage between this pattern and reward are unknown. *R. hirta* have similar UV-absorptive patterns to *H. multiflora*; both UV-absorptive patterns create a “bulls-eye” pattern.

Many insects see these UV patterns quite well. Based on the spectral sensitivities of the compound eyes of 43 hymenopteran species, three major absorptive peaks occur in the compound eyes of these species at the light wavelengths  $\sim 340$  nm, 430 nm, and 535 nm (Peitsch *et al.* 1992). These three peaks vary somewhat in specific absorptive maxima across species, but largely coincide with three receptor type cone-cells (UV, blue, and green) found in the compound eyes of hymenoptera (Menzel & Blakers 1976). UV light ranges from  $\sim 200$  nm to 400 nm wavelengths in the electromagnetic spectrum, blue light ranges from 450 nm to 495 nm wavelengths, and green, from 495 nm to 570 nm. The UV receptor is found in combination with one of the other two receptors (Kevan 1978, 1979). These receptors combine to allow bees to perceive bee-specific colors when bees see certain pigments in flowers, which affects pollinator recruitment, but resources, such as pollen, are the reason why bees visit flowers.





Figure 2: Photographs of *Ranunculus bulbosus* in Color and UV: A: The color image is what humans see when looking at this flower. It has oils on its petals that make UV photography difficult. B: The UV image shows that the stamens make up most the UV absorbent portion of the flower. The stamens play a large part in the UV absorptive pattern for this flower, unlike *H. multiflora* and *R. hirta*.

*Halictus* spp. predominantly use pollen to provision larvae (Linsley 1958; Stephen, Bohart & Torchio 1969). Some solitary bees depend on pollen for protein, which is essential to their diet (Stephen *et al.* 1969), important for development (Stephen, Bohart & Torchio 1969; Dodson 1987) and may influence foraging choices (Dodson 1987). Sweat bees (e.g. *Halictus farinosus* Smith) and mason bees (*Osmia* spp.) carry more pollen between flowers (e.g. sunflower, *Helianthus annuus* L.) and pollinate more flowers more evenly, relative to *A. mellifera* (Parker 1981). *Halictus farinosus* are better pollinators of onion (*Allium cepa* L.) flowers than *A. mellifera*, which results in a higher seed set (Parker 1982). *Halictus* spp. may have agricultural applications because of their uses in *H. annuus* and *A. cepa* production (Parker 1981; Parker 1982) and high abundance in agricultural systems; they have also been observed pollinating native flora, such as *R. hirta* (Horth, Campbell & Bray 2014). The pollination preferences of Halictidae and Megachilidae, which may differ among different native bee families, are currently unknown.

The goal of this work was to determine whether 1) *O. lignaria* could successfully be used as greenhouse pollinators of strawberry plants (*F. X ananassa*), 2) the density of these bees would affect berry size, 3) *O. lignaria* pollination resulted in larger, more symmetrical berries than selfing of berry flowers, 4) *O. lignaria* would preferentially alight on flowers with greater pollen load, 5) *O. lignaria* could be used as potential pollinators of mid-summer crops (not just early spring crops), 6) native pollinator taxa pollinate *R. fruticosus*, and 7) UV patterns of flowers similarly affect pollinator visitations across plant families.

## **MATERIALS AND METHODS**

### **Bee emergence station**

A station was created to allow adult bees to emerge from cocoons. A small plastic box (11.50 cm x 8 cm x 6 cm) was placed inside a ≈38 L aquarium. The top of the aquarium was covered with a mesh-screen lid and sealed around the edges with tape to prevent escape of emerging bees. A layer of sand was provided on the bottom of the tank and it was stocked with damp paper towels to maintain a relatively constant humidity for the bees. Whenever a bee emerged from a cocoon (~24 hrs after being placed in the aquarium), it remained in this emergence station until used in a trial. Three fresh strawberry and clover (*Trifolium repens* L.) flowers were placed inside the station as bee forage every three days. The station was monitored every 24 hrs for emergent bees, which were relocated to an experimental greenhouse, as needed. Bees that expired in the station were discarded within a 24 hr timeframe.

**Pollen load preference experimental study:** This experiment took place from 16April2016 to 23April2016 between the hours of ~10:00 am and 2:00 pm (EST) to allow for consistent light exposure across all trials. All trials were performed at the Oceanography and Physics building fisheries lab at Old Dominion University (Lat 36.884544/Long -76.307507). To initiate this study, 30 *O. lignaria* cocoons were placed in the emergence station. A large, hard-plastic greenhouse (Palram Nature Series Hybrid Hobby Greenhouse, PALRAM Applications Ltd, Kutztown, PA), 254 cm x 249 cm x 260 cm in size, was used for all trials. Two metal shelving racks (78 cm x 39 cm x 3 cm), ~115 cm above the ground, were placed in the greenhouse. Six total *F. X ananassa* plants, each with one single bloom, and contained in individual, small plastic pots (5 cm x 5 cm x 8.5 cm) were placed on two parallel shelves, one shelf per rack (three plants per shelf).

As a proxy for pollen load, flower stamen number was manipulated with clean metal forceps such that three flowers each had five stamen and the other three flowers had 15 stamen (to create low and high pollen load, respectively). The plants were arranged such that no two plants of the same stamen density were adjacent to one another. Each experimental flower was given a unique identification number and used in the experimental greenhouse on only the second or third day after flower opening to ensure that nectar content was similar across flowers based upon stage of development (Gottsberger, Arnold & Linskins 1990).

At the inception of each trial, three *O. lignaria* were placed in the greenhouse and allowed the opportunity to pollinate flowers for a 10 min period. Each bee landing, along with the total time spent on the flower, was recorded. Activity was low in most trials, so it was

possible to monitor visually individual landings relatively easily (a camera was available for back up). All three bees and six plants were removed from the greenhouse after 10 min, when the trial ended. Six total trials were conducted with a total of 30 *F. X ananassa* flowers. Noseum mesh (10.16 cm x 12.07 cm) jewelry pouches (Mudderonline, Seattle, WA) were very gently placed over each experimental flower at the end of each trial so that no further pollination events could occur. Each bag was labeled using a black marker with either a three, six or nine, which corresponded to a greenhouse bee density and the flower number. All experimental bagged flowers were placed in a second greenhouse to allow them to produce fruit without further disruption or movement. After 26 days (standard time to ripen), all fruit from experimental plants were weighed and assessed for symmetry (Nye & Anderson 1974). Both greenhouses were contained inside a bright, spacious aquatics facility at Old Dominion University with floor to ceiling plexiglass windows that allowed natural light in, while also allowing for a controlled habitat (the absence of wind and insect pollinators).

**Control plants for pollen load study:** During the experimental time-frame, mesh bags were also placed on 25 additional flowers (not used in trials) and grown under the same conditions as experimental plants. These control plants each had one flower in bloom and were at the same stage of development as the experimental flowers. Control flowers were not pollinated by insects and were assumed not to be self-pollinated by wind or other elements because they were also held in the second greenhouse with the used experimental plants. After 26 days, fruit from the control plants were weighed and assessed for symmetry, and these data were compared to experimental flower data (below).

**Symmetry methodology:** Comparing left and right side-symmetry is a proxy for analyzing a three-dimensional (3-d) object's actual true 3-d symmetry measurement. To evaluate left-right side symmetry, a photograph (Samsung Galaxy Note7/12-megapixel rear-facing camera) of each berry was taken in a standardized format: berries were placed on their side on a weighing scale and a ruler (mm) was placed in each image as a size scale. The camera was rested on a glass plate 20 cm above the berry to establish uniform distance from berry to camera across all images. After each photograph was taken, subsequent analysis for symmetry was conducted. Individual image files were opened in ImageJ software (1.47v, National Institutes of Health, USA) and then the berry perimeter was traced using the "Freehand Selections" tool. The berry centroid coordinates were then calculated using the "Centroid" measurement found under "Set Measurements". After ImageJ identified the x- and y-coordinates for the centroid, the centroid was marked manually with a point on the image using these coordinates. A line was then drawn from the center of the stem of the berry through the berry centroid using the "Straight, segmented or freehand line" tool. This separated the berry into two halves. Each half of the berry was traced completely using the "Freehand Selections" tool (Fig 3). 'Side-symmetry' was assessed quantitatively by calculating the absolute value of the difference in total pixel number for the two berry halves. Perfect symmetry would be reflected as a zero measurement and increasing asymmetry would be reflected as a greater absolute value difference.

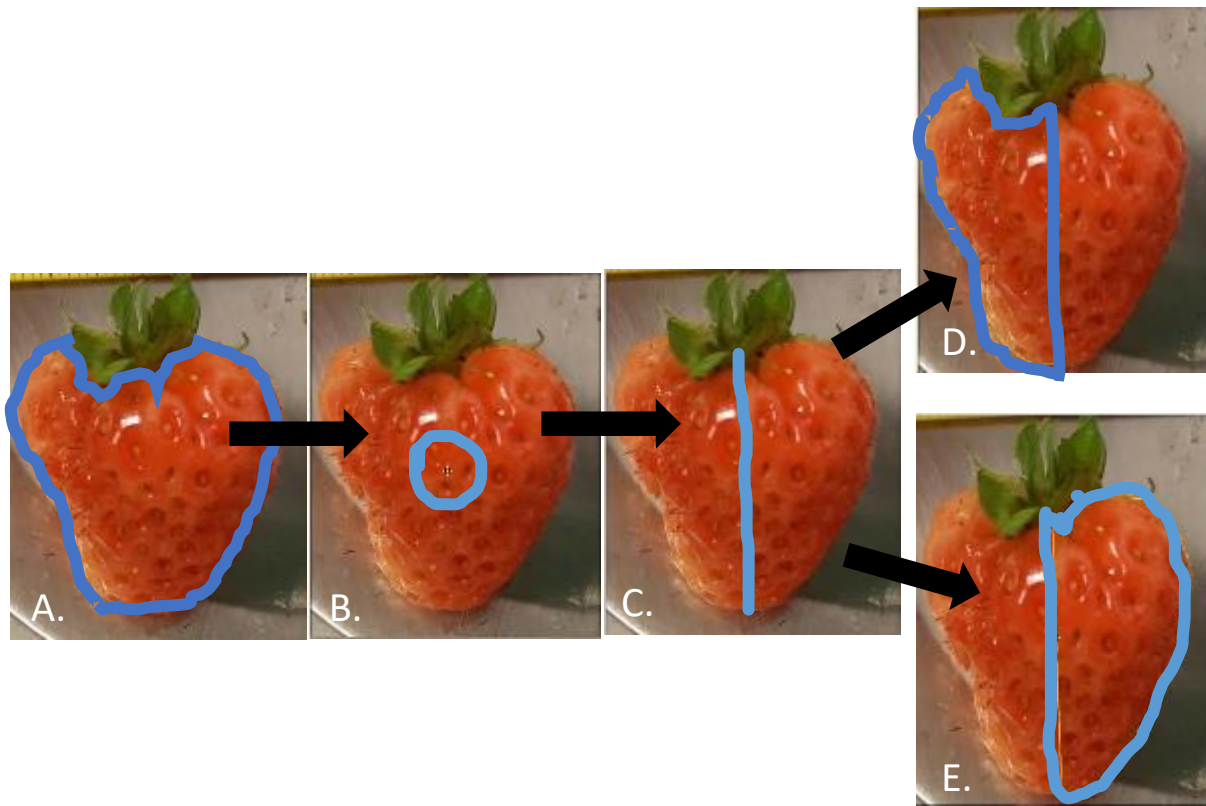


Figure 3: ImageJ protocol: A. A strawberry picture, before any screenshots, with its entire area encircled. B. Strawberry image with a centroid marked. A blue circle encloses the centroid point to enable better viewing. C. The cropped screenshot of a strawberry with a separating line. The line runs from the stem, through the centroid, and to bottom of the berry. D. The left half of the berry is traced and its area is quantified. E. The right half of the berry is traced and its area is quantified.

**Pollen load preference experimental study statistics:** A Pearson chi-square test was used to compare whether there was a difference in total number of bee landings ( $N=18$  bees) for larger ( $N=15$ ) versus smaller ( $N=15$ ) stamen load (IBM SPSS Statistics software, Version 21.0, 2012, Chicago, IL). A Pearson chi-square test was used in place of a paired t-test because of violated assumptions. The dependent variable was pollinator visitations, and the independent variable was the stamen number. Control plants were not considered in this analysis because

they received no pollinator visitations. For each trial, the mean visitation number for plants in each treatment group (i.e. five stamen and 15 stamen) was compared. The null hypothesis was that there would be no difference in pollinator visitation rate for the low (five stamen) versus high (15 stamen) stamen number.

A Wilcoxon signed rank test was used to compare the weights of 18 berries (i.e. dependent variable) from the two treatments (low stamen,  $N=8$  berries and high stamen,  $N=10$  berries). The null hypothesis was that there would be no difference in berry weights between treatments. A Wilcoxon signed rank test was also used to compare the symmetry of these same 18 berries across two treatments. The null hypothesis was that there would be no difference in berry symmetry between treatments.

### **Effect of bee density on berry size in greenhouse pollination study**

This experiment took place from 21April2016 to 10May2016 between 9:00 am and 12:00 pm (EST) for a total of 18 observation trials. All trials were performed at the Oceanography and Physics building fisheries lab at Old Dominion University. Three greenhouses (Palram Nature Series Hybrid 6 x 4 Green Greenhouse, PALRAM Applications Ltd, Kutztown, PA), L 126 cm x W 185 cm x H 209 cm in size, were established. Each greenhouse contained a treatment: one of three bees (*O. lignaria*) densities: three, six or nine bees. An upside down  $\approx 19$  L aquarium was placed in each greenhouse, which had a shallow lip allowing for a small pool of water to be held for bee hydration. Additionally, a single bee nest home, made from non-treated pine lumber, with 10 drilled holes, 7.94 mm in size, was placed in each greenhouse for possible use by adult bees laying eggs. A small plastic box (12 cm x 8.5 cm x 5

cm) with clay-based soil collected from Henley's Farm in Pungo, VA, USA (Lat 36.699986/Long - 75.993002) was also provided to allow bees to cap off nests, if constructed.

Daily, 30 min monitoring trials occurred to record pollinator visitation events and duration of visits for each treatment (video cameras were available for back up recording of observations). Thirty *F. X ananassa* plants were distributed equally among the three greenhouses (10 plants per house). Plants were placed in two rows of five individuals, on metal shelves (78 cm x 39 cm x 3 cm) ~ 20 cm above the ground. Shelves were positioned on top of four cinderblocks. Plants were placed equidistant from each other to standardize light availability. One flower was present on each plant throughout the experiment. After six days of behavior observations, all experimental plants were moved to a second greenhouse and held, until berry formation, in the manner described previously. After 26 days, each berry was removed and all berry weights (mg) were recorded and symmetry (mm) was assessed as described above. Some berries aborted, so the number of aborted berries for each treatment was also recorded. The aborted berry data were used to determine if bee densities influenced fruit set. Bee densities remained constant for the duration of the experiment: if a bee died, it was replaced with a new bee from the stock.

**Effect of bee density on berry size in greenhouse pollination study statistics:** Berry data from the different treatments (i.e. bee density) could not be statistically analyzed due to confounding factors. Berry data is strictly observational.

A Kruskal-Wallis test was used to compare the effect of bee density (i.e. independent variable) on visitation frequencies (i.e. dependent variable). The null hypothesis was that there



would be no difference in the number of pollinator visitations on flowers for any of the differing bee densities. A Kruskal-Wallis test was used in place of an ANOVA because data could not be transformed to meet the ANOVA assumptions.

A Kruskal-Wallis test was used to compare the effect of bee density on (i.e. independent variable) on pollination duration (i.e. dependent variable). The null hypothesis was that there would be no difference in the number of pollinator visitations on flowers for any of the differing bee densities. Pollination durations were summed for each greenhouse for every trial.

### **Mid-Summer Emergence of *O. lignaria* in Controlled and Natural Environments**

A comparison of bee emergence rates was conducted for greenhouse and farm emergence of adults from cocoons. This work occurred from 20 June 2016 to 04 July 2016 between the hours of 9:00 am and 12:00 pm (EST), and cocoons were counted once every seven days to determine bee emergence rates. An indoor treatment group and a farm treatment group were observed concurrently.

**Indoor Treatment Group:** One-hundred cocoons were placed in a plastic holding container (15 cm x 11 cm x 10 cm) with a plastic lid with venting holes, and held in the temperature-controlled facility (Oceanography and Physics building at ODU) at 18.33°C. Damp paper towels were placed in the experimental container with the cocoons to ensure proper humidity.

One week after placing cocoons in the holding container, all bees were counted as emerged or not emerged. Paper towels were re-moistened after counting, and un-emerged

cocoons were maintained in the holding container until the next counting period. The second counting period occurred one week after the first counting period. After the second counting period, the cocoons were retained in the experimental container. Observations ceased after the second observation period because cocoons began to shrivel, which is indicative of bee death. On average, *O. lignaria* take two to three days to emerge at temperatures above 14.2°C, and emergence probability was drastically decreased after eight days of the same temperature (Bosch & Kemp 2000).

**Farm Treatment Group:** Four bee homes, which were constructed of pine blocks, were placed on a commercial blackberry patch at Henley Farm in Pungo, VA, USA on 20 June 2016 (EST). Each bee house was composed of three blocks of untreated pine with 10 holes that were 15.24 cm deep and 9.52 mm in diameter. Foam was attached to the bottom of each house to prevent water saturation during heavy rains. One-hundred cocoons were placed in the homes: three bee houses held one cocoon per hole and 30 total cocoons, and the last house held the remaining 10 cocoons. Houses were placed along the soil mounds where a row of *R. fruticosus* plants were planted. During the first field counting period, empty cocoons, from which adult bees had departed, were tallied as “emerged”. Cocoons that had not been chewed through by an emerging bee were tallied as “non-emerged.” If the cocoons were not in the drilled holes, the grass was searched below the berry bushes for them. Any non-emerged cocoons in the grass were placed in a bee house hole, and any emerged cocoons were tallied as emerged. Bee houses were removed after the second observation period. All the bees did not emerge from their cocoons, however the remaining cocoons were beginning to shrivel and crumble, which suggested non-viability.

### **Mid-Summer Emergence of *O. lignaria* in Controlled and Natural Environments**

**statistics:** A 2x2 contingency table was used to compare whether there was a difference in the bee emergence rate (i.e. dependent variable) in a controlled or natural environment (i.e. independent variable). The null hypothesis was that there would be no difference in bee emergence between controlled and natural environments.

### **Assessment of the Native Pollinators of *R. fruticosus***

This experiment took place from 08June2016 to 24June2016 between the hours of 9:00 am and 12:00 pm (EST). Five trips were taken to Henley Farm on 08June2016, 10June2016, 14June2016, 20June2016, and 24June2016, and bees were collected directly from *R. fruticosus* flowers. Aspirators and butterfly nets were used throughout the collection period to capture all bees observed on the flowers. Bees were stored in 50 mL date-labelled aspirator vials that were frozen after bee collection, and netted bees were placed in individual aspirator vials. Bees were identified using Discover Life ([discoverlife.org](http://discoverlife.org)). Discover Life is an interactive identification guide that was compiled and maintained by experts of native bee identification, and it is the most reliable identification guide to date (Pickering 2010).

## **RESULTS**

### **Effect of bee behavior on berry size in greenhouse pollination study**

*Osmia lignaria* landed on *F. X ananassa* flowers and some of these flowers produced fruit, demonstrating that *O. lignaria* can be used successfully for greenhouse berry pollination. Bees did not prefer to pollinate flowers with smaller or larger stamen loads ( $\bar{X}$  pollinator visits:

five stamen plants =3.62 (standard deviation +/- 1.07), 15 stamen=3.2 (+/- 1.99); Table 1;  $\chi^2_{(1)} = 0.148, P= 0.701$ ).

Table 1: Stamen manipulated berry data, showing mean +/- standard deviation.

	Number of Viable Berries	Mean Berry Weight (g)	Mean Berry Symmetry (mm)	Mean Number of Landings	Mean Pollination Times (sec)	Mean Number of Aborted Berries
5 stamen	8	1.39 (+/- 1.07)	0.89 (+/- 1.00)	3.62 (+/- 1.07)	86.06 (+/- 68.49)	8
15 stamen	10	3.23 (+/- 1.99)	0.80 (+/- 0.53)	3.2 (+/- 1.99)	176.38 (+/- 240.16)	6
Mean or Totals	18	2.41 (+/- 1.86)	0.95 (+/- 0.73)	1.91 (+/- 1.35)	129.22 (+/- 180.20)	14

Stamen number, a proxy for pollen load, had no significant effect on berry weight ( $\bar{X}$  berry weight (g): five stamen= 1.39 (+/-1.07), 15 stamen= 3.23 (+/-1.99); Table 1;  $W_{(1,05,8)} = 7.000, P > 0.05$ ).

Stamen load did not influence berry symmetry ( $\bar{X}$  berry symmetry (mm) five stamen= 0.89 (+/-1.00), 15 stamen= 0.80 (+/-0.53); Table 1;  $W_{(1,05,6)} = 9.000, P > 0.05$ ).

### Effect of bee density on berry size in the greenhouse pollination study

Berry data from the different treatments (i.e. bee density) is strictly observational due to confounding factors. Observationally, the berries were larger in greenhouses with higher bee densities, and berries became less symmetrical with increasing bee densities (Table 2).

Table 2: Average berry data for bee density greenhouses, showing mean +/- standard deviation.

Bee Density	0	3	6	9
Number of Viable Berries	13	13	19	17
Mean Berry Weight (g)	0.81 (+/-0.57)	0.604 (+/- 0.447)	2.34 (+/- 3.08)	1.98 (+/- 1.71)
Mean Berry Symmetry (cm)	0.78 (+/-0.85)	0.95 (+/-0.74)	1.09 (+/-0.95)	1.13 (+/-1.19)
Mean Number of Landings	0	1.24 (+/- 1.82)	1.74 (+/- 2.15)	3.65 (+/- 3.26)
Mean Pollination Times (sec)	0	64.50 (+/- 105.24)	103.47 (+/- 140.74)	135.12 (+/- 167.45)
Number of Aborted Berries	12	17	11	13

Bee density had a significant effect on pollinator visitations ( $\bar{X}$  visitations: three bee= 1.24 +/- 1.82, six bee= 1.74 +/- 2.15, nine bee= 3.65 +/-3.26; Table 2;  $\chi^2_{(05,2)}=8.302$ ,  $p=0.016$ ).

Larger bee densities led to more pollination events.

*Osmia lignaria* were observed vectoring pollen between strawberry blooms inside greenhouses and pollen was clearly visible adhering to the underside, amongst the scopal hairs (Fig 4). Because these bees were exposed to *F. X ananassa* pollen through the entirety of their adult life, this pollen is not from any other plant species.



Figure 4: *Osmia lignaria* with *Fragaria X ananassa* pollen: *O. lignaria* were found actively collecting and transporting *F. X ananassa* pollen between flowers. This is *F X ananassa* pollen because these bees were only given access to *F X ananassa* flowers. A. *Fragaria X ananassa* with an abundance of pollen. B. *Osmia lignaria* foraging on *F. X ananassa* flowers. C. *Osmia lignaria* with pollen adhering to scopal hairs. D. Magnification of pollen on scopal hairs.

#### Mid-Summer Emergence of *O. lignaria* in Controlled and Natural Environments

Significantly more bees emerged in a controlled environment than a natural environment ( $\chi^2_{(05, 1)} = 6.125, p = 0.0208$ ).

Seventy-three percent of the *O. lignaria* held in natural, mid-summer conditions on a commercial blackberry farm emerged within the first seven days. Eighty-seven percent of the *O. lignaria* held in a controlled environment emerged within the first seven days. Overall, more bees emerged in the controlled environment over the natural environment.

*Osmia lignaria* were observed visiting *R. fruticosus* flowers near the deployed nest boxes (Fig 5). *Rubus fruticosus* flowers did have an abundance of pollen, and it is possible that *O. lignaria* could have been transporting pollen between flowers.



Figure 5: *Osmia lignaria* visiting *Rubus fruticosus* flowers.

#### **Assessment of the Native Pollinators of *Rubus fruticosus***

The dominant genus of commercial *R. fruticosus* pollinators identified on Henley Farm was *Halictus*. There were 88 *Halictus* sp. individuals collected on *R. fruticosus* flowers, with *Halictus confusus* Smith the most abundant taxon (82 of 88 individuals). *Halictus confusus* was found with an abundance of pollen on its entire body (Fig. 6). *Bombus* was the second most abundant genus on *R. fruticosus* with nine of 12 individuals identified as *B. impatiens*. Other *Bombus* individuals that were found were the two-spotted bumble bee (*Bombus bimaculatus* Cresson) ( $N=2$ ) and the brown-belted bumble bee (*Bombus griseocolus* De Geer) ( $N=1$ ). A single carpenter mimic leafcutter bee (*Megachile xylocopoides* Smith) was collected from the *R. fruticosus* flowers, and this bee had an abundance of pollen found on its scopal hairs (Fig. 6).

One Maine blueberry bee (*Osmia atriventris* Cresson), which is a relative of *O. lignaria*, was also found on the *R. fruticosus* flowers (Table 3).

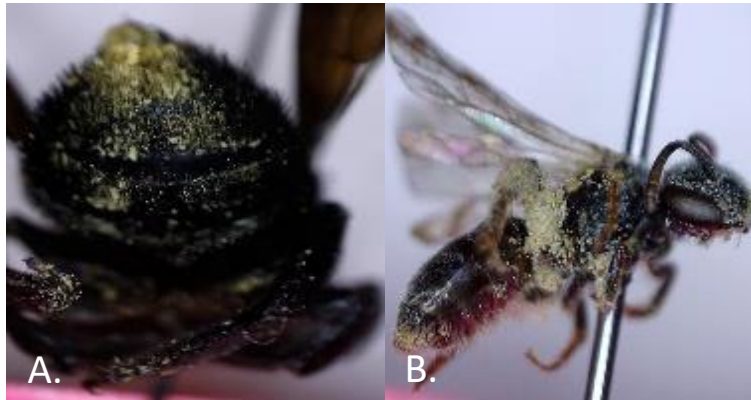


Figure 6: Pollen adherence on native bees: Pollen on the scopae of *M. xylocopoides* is depicted in the image on the left. Pollen on the body of *H. confusus* is depicted on the image on the right.

Table 3: Suite of native pollinators collected from *R. fruticosus*.

Genus	Species	Number of Individuals
<i>Bombus</i>	<i>B. bimaculatus</i>	2
	<i>B. impatiens</i>	9
	<i>B. griseocolus</i>	1
<i>Osmia</i>	<i>O. atriventris</i>	1
<i>Halictus</i>	<i>H. ligatus</i>	6
	<i>H. confusus</i>	82
<i>Megachile</i>	<i>M. xylocopoides</i>	1



## DISCUSSION

Overall, this study demonstrated that native *O. lignaria* individuals can serve as greenhouse pollinators for *F. X ananassa* crops. This work has important agricultural applications because these native bees do not experience CCD.

*Osmia lignaria* did not pollinate *F. X ananassa* flowers with large stamen loads preferentially over smaller ones, which suggesting that differences in stamen load alone may not present any detectable cue for *O. lignaria*. Other floral cues, such as nectar load, scent or color, may be detectable signals that *O. lignaria* use to preferentially pollinate flowers. More research needs to be conducted to determine which signals, if any, serve as an honest indicator of reward, or if *O. lignaria* have any pollinator preferences. The berry weight (power=0.536) and symmetry (power=0.116) for this study should be interpreted with caution because of low power.

As pollinator density increases, the total number of visitations to flowers increased, and thus possibility for cross-fertilization increased. One factor to consider for future work includes artificial lighting. Cloudy weather, which occurred for the duration of this experiment, may have affected final berry weights, so artificial lighting may have resulted in better berry growth. A previous study using grapes (*Vitis vinifera* L.) demonstrated that berries in no light in their early developmental stages delayed ripening and resulted in a reduction of berry size, and when lighting was increased in later developmental stages, the normal development of the berry was not resumed (Dookozlian & Kliewer 1996). Fruit set may have been affected by the weather in my study, and, even though this work suggests that bees did not play a major role in fruit set in

*F. X ananassa*, future studies with supplemental lighting may yield different results. This work demonstrates that *O. lignaria* can play an important role in greenhouse production, and even though the six bee treatments produced the largest berries, the greenhouse used for this study were only a fraction of the size of commercial greenhouses. It is likely that larger plant densities would require larger bee densities to produce similar effects. And, likewise, it would be useful to see if greater bee densities result in the production of bigger berries with higher experimental replication with a larger number of plants. Results should be interpreted with caution for the bee density experiment berry weight (power=0.556) and symmetry (power=0.091) because of low power.

The high emergence rates of *O. lignaria* both indoors and outdoors in mid-summer suggests that these pollinators may have valuable commercial applications for mid- and late-summer crops, such as *C. melo* (Fisher & Pomeroy 1989) and *C. anuum* (Shipp, Whitfield & Papadopoulos 1994). In Virginia, local farmers grow *R. fruticosus* after *F. X ananassa*, and *O. lignaria* could be a potential pollinator of *R. fruticosus* when *O. lignaria* emergence is delayed. More *O. lignaria* emerged from cocoons in controlled conditions than in natural conditions, so allowing bees to emerge indoors would be a fruitful technique for farmers wishing to establish *O. lignaria* populations. The experiment in April 2016 had excessively high mortality rates among emerging bees, which may have been the result of sudden cold weather, but this later emergence yielded much higher *O. lignaria* emergence. Farmers using *O. lignaria* as pollinators could, therefore, deploy bees in increments throughout spring and summer to enable a consistent pollinator population across many flowering crops' blooming periods. *Osmia lignaria* have had their emergence successfully delayed, and *O. lignaria* was observed on *R. fruticosus*

flowers. *Rubus fruticosus* flowers did have an abundance of pollen, and it is possible that *O. lignaria* could have been transporting pollen between flowers.

*Rubus fruticosus* has traditionally been pollinated by *A. mellifera* (Cane 2005), and native pollinators are thought to only provide supplementary pollination. Interestingly, no *A. mellifera* were observed on the *R. fruticosus* flowers in this study even though other native pollinators such as *H. confusus* were abundant. This paradox suggests that native bees may have more of an effect on *R. fruticosus* fruit formation than previously thought. Moreover, native solitary bees are not thought to be adequate pollinators of *R. fruticosus* because of their low abundance (MacKenzie & Winston 1984), but my study contradicts. Pollen was found in abundance on several bees in this study, but it is unknown whether such pollen came from *R. fruticosus*. Native bees were clearly vectoring *R. fruticosus* pollen between flowers, which is precisely how cross-fertilization and better berry formation occur. A native mason bee species, *Osmia atriventris*, was observed pollinating *R. fruticosus* flowers, which suggests that a congener such as *O. lignaria* may pollinate *R. fruticosus* as well.

## CHAPTER 2

### ASSESSING THE EFFECT OF ULTRAVIOLET ABSORPTIVE FLORAL PATTERN SIZE ON POLLINATOR VISITATION RATE

#### MATERIALS AND METHODS

##### UV floral pattern influence on native pollinators in a natural landscape

This experiment took place in July 2012 between the hours of 10 am and 2 pm (MST) in Colorado (Lat. 38.808688/ Long. -106.884531). Twenty-eight pairs of *Heliomeris multiflora* were used as a sample. All data was collected by Dr. Lisa Horth and analyzed by Michael Gregory.

A field of hundreds of *H. multiflora* flowers, which was far from major human settlements, was observed for pollinator visitations. Once a landing occurred, the flower that was visited was marked, using a marker, with a (+) for landing, and the nearest neighbor to the pollinated flower was marked with a (-) for no landing. This pair of flowers were near each other, and nothing about their UV patterns was known at the time of selection. Color and UV images were taken with a Baader U-filter, that transmits UV wavelengths 325 to 369 nm (Savazzi 2011; Horth, Campbell & Bray 2014), and the filter was used in conjunction with an AF Micro Nikkor 60 mm lens, several mounts and a Nikon D70 DSLR digital camera (Horth, Campbell & Bray 2014).

The UV photographs were analyzed using ImageJ software (1.47v, National Institutes of Health, USA). The flower image was uploaded to ImageJ, and three petals were haphazardly chosen on each flower. Each petal was traced fully with the “Freehand Selections” tool to

obtain its area, and then the UV absorbent portion of the petal was traced in the same manner. The percentage of UV absorbent area on the flower, which corresponds to the size of the “bulls-eye” pattern, was calculated for each flower using the three petal-area measurements.

**UV floral pattern influence on native pollinators in a wild landscape statistics:** A Pearson chi-square test was used to compare if native pollinators landed on *H. multiflora* flowers with larger or smaller UV absorptive patterns. The null hypothesis stated that there would be no difference in native pollinator landings between flowers with larger or smaller UV absorptive patterns.

**Pollinator preferences in a managed, urban landscape with unknown UV pattern size**

The experiment took place on 31 March 2012 between the hours of 10 am and 2 pm (EST) and was conducted on the grounds of the Virginia Zoo (Lat 36.876307/Long -76.278286). Sixty wild *R. bulbosus* plants were collected from grounds surrounding Old Dominion University (Lat 36.885552/ Long -76.307768). A pair of flowers from the same plant was placed in a single clear glass bottle (GT Kombucha 16 oz, Beverly Hills, CA) with 473 mL of water. There was a total of 30 pairs monitored for pollinator landings. UV images were taken of each flower for UV pattern measurement using the previously mentioned setup. Flower pairs were arranged in a line after UV images were taken. No knowledge of the UV pattern was known prior to placement of the flowers. After being arranged in the line, each flower pair was about 1.5m from the next. All insects were captured with mesh nets upon landing so no single bee could pollinate more than once. Multiple landings occurred on some flowers, and all landings were tallied for each flower. Five volunteers aided in monitoring the flowers and capturing the

pollinators. The experiment ended after four hours of observing pollinator behavior. UV patterns were measured using ImageJ software as previously described.

#### **Pollinator preferences in a managed, urban landscape with unknown UV pattern size**

**statistics:** A Pearson correlation was used to determine if the variance in pollinator visitations of *R. bulbosus* was attributed to UV pattern size. Assumptions were checked and met prior to performing the test. The null hypothesis was that there would be no relationship between UV cue size and pollinator visitation rate.

#### **Pollinator preferences in a managed, urban landscape with known UV pattern size**

The experiment took place on 12May2012 between the hours of 10 am and 2 pm (EST). Sixty wild *R. bulbosus* flowers were collected from the grounds surrounding Old Dominion University. UV images were taken, and analyzed visually before flower placement. Thirty pairs of flowers, one with a relatively small UV absorbent cue and one with a larger cue, were paired and observed for pollinator visitation at the Virginia Zoo. Bottles were placed in one row about 1.5m apart and about 3m in front of a various asters, such as *R. hirta*, and lavender (*Lavandula spica* L.). The number of visitations were tallied for each pair of flowers, with multiple landings on individual flowers possible. Pollinators were captured after each landing. Five volunteers aided in monitoring the flowers and capturing the pollinators. UV patterns were quantified using ImageJ software, as previously mentioned.

#### **Pollinator preferences in a managed, urban landscape with known UV pattern size**

**statistics:** A Pearson chi-square test was used to compare whether pollinators preferred larger or smaller UV absorptive patterns among paired *R. bulbosus* in an urban, managed landscape.

The null hypothesis stated that there would be no difference in pollination events between flowers with larger or smaller UV absorptive patterns among *R. bulbosus* flowers.

### **Pollen reduction influence on native pollinator in an urban landscape type**

The experiment took place on 15 April 2012. Thirty *R. bulbosus* flowers were divided into two sets. The first 15 natural, unmanipulated flowers were placed in clear bottles, ~1.5m apart at the Virginia Zoo, were arranged in a straight line and monitored for pollinator landings. The second set of 15 flowers had all anthers removed with forceps, which decreased the size of the UV absorbent pattern in these flowers, were arranged in a straight line and monitored for pollinator landings. One week later, a second trial was conducted. UV images were taken, and the UV photos were analyzed as described previously.

### **Pollen reduction influence on native pollinator in an urban landscape type statistics: A**

Pearson chi-square test was used to compare whether pollinators preferred pollen deficient flowers with smaller UV absorptive patterns or pollen retaining flowers with larger UV absorptive patterns. The null hypothesis stated that the number of pollination events would not differ between pollen deficient flowers with smaller UV absorptive patterns or flowers with pollen and larger UV absorptive patterns.

### **Manipulated UV patterns with standardized pollen loads**

The experiment took place from 09 August 2016 through 24 August 2016 between the hours of 10 am and 2 pm (EST) at Old Dominion University's Kaplan Orchid Conservatory (Lat 36.884921/ Long -76.30667) in Norfolk, VA. *Rudbeckia hirta* were selected for this experiment because its pollen does not have a noticeable effect on their UV absorptive patterns. All

flowers were collected from Old Dominion University's Kaufman Hall (Lat 35.885796/Long - 76.304963). Sixty experimental flowers had manipulated UV-absorptive patterns using the "cut-and-paste" method (Andersson 1982; Horth, Campbell & Bray 2014).

Many flowers were collected to supply petals for cutting and pasting. The UV absorptive area of the ligulate flowers were glued over the UV reflective area of the experimental ligulate flowers to create an exaggerated UV pattern. The UV reflective area of the ligulate flowers were glued over the UV absorptive area of the experimental ligulate flowers to create the diminished UV pattern. Thirty flowers had UV-absorptive patterns manipulated to be ~60% of their total petal area, and another thirty flowers to ~ 20% (Fig 7).

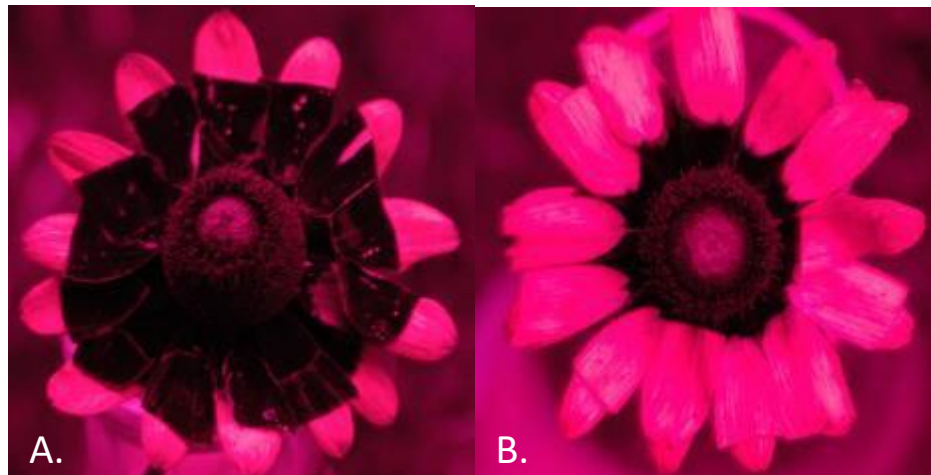


Figure 7: "Cut-and-Paste" Methods for *Rudbeckia hirta* Manipulations: The "cut-and-paste" method was used to adhere reflective and absorptive sections of the petals of other *Rudbeckia* flowers to the experimental flowers; this process was used to exaggerate UV-absorptive patterns of some flowers and diminish the UV-absorptive patterns of others. A: The manipulated flower with the 60% UV-absorptive pattern B: The manipulated flower with the 20% UV-absorptive pattern.

Polyvinyl acetate-based glue (Elmer's School Glue, High Point, NC) was used as an adhesive to attach the snipped petals to the flowers; it has been shown to have no effect on



pollinator visitations (Horth, Campbell & Bray 2014). Each manipulated flower had either five, 10 or 15 dehisced stamens, which varied based on the experiment being conducted (Fig 8).

Extra stamens were removed with forceps.

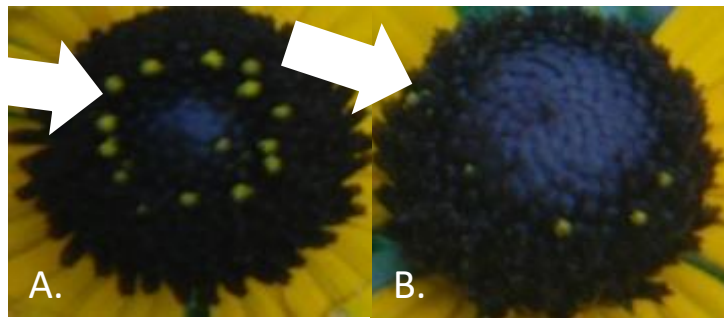


Figure 8: *Rudbeckia hirta* Pollen Manipulations: A: An example of a flower with 15 fully-dehisced stamens. B: An example of a flower with five fully-dehisced stamens. Stamens for this flower were UV-absorbent, which makes them undiscernible in UV images. The stamens are small yellow dots found on the flower head. The dots are marked with a large white arrow.

Prior to the “cut-and-paste” method, *Rudbeckia hirta* flowers were chosen based on similarity in size and paired. A pair of manipulated *R. hirta* flowers, one flower having 60% UV-absorptive pattern with 10 stamen and the other flower having a 20% UV-absorptive pattern with 10 stamen. The experimental flowers were in glass bottles (Perrier Sparkling Natural Mineral Water, 25.3 oz, Greenwich, CT) filled with 719 mL of water, bottles were approximately five cm apart. The bottles were placed in grass outside the Kaplan Orchid Conservatory approximately 15 cm from the background flora, which consisted of several native asters, conspecifics and lavender.

After a single landing, the bee was captured in a vial with an aspirator. A sample size of 30 native bees was collected for the experiment. Each vial was labeled (date, flower treatment)

and stored in -80°C freezer until bees were identified to species. Each trial was defined as a pair of manipulated flowers being observed for pollinator landings. Each trial ceased once a landing occurred to prevent displacement of pollen. After each trial, the pair of flowers was marked with a unique number using a sharpie, removed from the green bottle filled with water, and set in a beaker filled with water so that other flowers could be used in the green bottles. Color and UV photographs were taken of the flowers after the trial using methods described previously. After completion of field work, bees were removed from the -80°C freezer, pinned and identified using Discover Life ([discoverlife.org](http://discoverlife.org)).

**Manipulated UV patterns with standardized pollen loads statistics:** A chi-square test was used to compare whether pollinators preferred larger UV absorptive patterns or smaller UV absorptive patterns when pollen was standardized. The null hypothesis was that there would be no difference in pollinator landings between treatment flower types (larger or smaller UV absorptive patterns with pollen present).

#### **Manipulated UV patterns with manipulated pollen loads**

The experiment took place from 25July2016 through 04August2016. The same experimental design from the previous experiment was used for this experiment. Here, the flower with the 60% UV-absorptive pattern had five fully-dehisced stamens and the flower with 20% UV-absorptive patterns had 15 fully-dehisced stamens. A total of 30 native bees were collected for this experiments sample size.

**Manipulated UV patterns with manipulated pollen loads statistics:** A chi-square test was used to compare whether pollinators preferred larger UV absorptive patterns with less

pollen, or smaller UV absorptive patterns with more pollen. The null hypothesis was that there would be no difference in pollinator landings between treatment flowers (larger UV absorptive patterns and smaller stamen loads versus smaller UV absorptive patterns and larger stamen loads).

## RESULTS

### UV floral pattern influence on native pollinators in a wild landscape

Pollinators preferred to pollinate flowers with the larger UV patterns in a natural environment ( $\chi^2_{(.05,1)} = 4.481, P=0.034$ ). A total of 19 landings were on flowers with naturally larger UV patterns ( $\bar{X} = 54.35\% \pm 11.77\%$ ) and a total of eight landings were on flowers with naturally smaller UV patterns ( $\bar{X} = 48.41\% \pm 8.57\%$ ) (Table 1). All pollinators were *Bombus* spp.

Table 4: *Heliomeris multiflora* landings and UV pattern percentage of flowers: A (+) sign designates a pollinator landing and a (-) sign designates no landing.

Mean UV Percentage	(+)	(-)
	54.35 +/- 11.77	48.41 +/- 8.57
Pollinator Landings	20	8
Pearson chi-square test ( $\chi^2_{(.05,1)} = 4.481, P=0.034$ ).		

### Pollinator preferences in a managed, urban landscape with unknown UV pattern size

The variance in the number of visitations was likely explained by UV cue size in these *R. bulbosus* ( $r = 0.141, df=58, P < 0.05$ ). Out of the 70 total landings, more pollinators visited flowers with the larger UV patterns. All visitors were Halictidae.

### **Pollinator preferences in a managed, urban landscape with known UV pattern size**

Pollinators preferentially chose the flowers with the larger UV absorptive pattern, and pollen load, when given a choice between larger and smaller UV absorptive patterns and pollen loads ( $\chi^2_{(.05,1)}=28.471$ ,  $P< 0.05$ ). The mean number of visits to the larger cues of the pairs was 1.86 (+/- = 0.265) and of the smaller cues, 0.41 (+/- = 0.105). Out of the 68 total landings, visitors included Halictidae (66) and Megachilidae (2).

### **Pollen reduction influence on native pollinator in a managed, urban landscape type**

Pollen may influence the likelihood of a bee choosing certain flowers ( $\chi^2_{(.05,1)}=11.333$ ,  $P< 0.05$ ). Anther removal in treatment flowers decreased their UV absorbent cue size by about 6% from their original size, so pollen and UV absorptive patterns in this species of plant may affect one another. Out of the 102 total pollinator visits, fewer bees ( $\bar{X} = 2.26$ ) visited the pollen-deficient plants with smaller UV absorbing cues than the untreated plants ( $\bar{X} = 4.33$ ). Visitors included Halictidae (98) and Apidae (3). The mean UV absorbent cues size for plants with anthers was composed of 25% of the petal surface, and for plants without anthers, 17%.

### **Determining the effect of UV patterns and pollen loads on pollination in *Rudbeckia hirta***

Pollen, which does not affect the visual floral display for these flowers, presence does not seem to influence the bees' choices to visit the flowers with the larger UV absorptive patterns ( $\chi^2_{(.05,1)} = 8.700$ ,  $P=0.0082$ ). A total of 22 pollinators chose the 60% UV pattern, and a

total of 8 pollinators chose the 20% UV pattern. Native pollinators preferred larger UV patterns over smaller UV patterns when pollen was standardized (Table 5).

Table 5: Landing data for the *Rudbeckia hirta* experiments.

	60% UV Pattern with 10 Stamen	20% UV Pattern with 10 Stamen
Total Landings	22	8
$\chi^2_{(.05,1)} = 7.000, P < 0.01$		
	60% UV Pattern with 15 Stamen	20% UV Pattern with 5 Stamen
Total Landings	20	10
$\chi^2_{(.05,1)} = 3.452, P = 0.06$		

Large differences in pollen load may have an influence on the bees' choices to visit the flowers with larger UV patterns ( $\chi^2_{(.05,1)} = 3.452, P = 0.0632$ ). The null hypothesis is accepted; large differences in stamen loads may influence hymenopteran visitations. A total of 20 landings were on the flowers with the larger UV patterns, and a total of 10 landings were on the flowers with the smaller UV patterns (Table 5).

Overall, Apidae ( $N=21$ ) and Halictidae ( $N=28$ ) were the most abundant families landing on the experimental flowers. Apidae preferentially chose the flowers with the 60% UV absorptive pattern ( $N=16$ ), which is approximately 76% of the total Apidae individuals collected, over the 20% UV absorptive pattern ( $N=5$ ), regardless of stamen load. Halictidae preferentially chose the flowers with the 60% UV absorptive pattern ( $N=20$ ), which is approximately 77% of the total Halictidae individuals collected, over the 20% UV absorptive pattern ( $N=6$ ), regardless of stamen loads. Colletidae ( $N=2$ ) and Megachilidae ( $N=6$ ) were the other two families of bees

that landed on the experimental flowers. No clear pattern could be discerned for the pollination preferences of Colletidae or Megachilidae.

## DISCUSSION

Whether in a natural environment or an urban, manicured one, pollinators preferentially chose flowers with the larger, natural UV-absorptive patterns across plant families, but for what reason? These UV absorptive patterns, which are nectar guides, help orient pollinators towards floral rewards in Fabaceae flowers (Jones & Buchmann 1974), and bees that land on flowers with UV absorptive patterns spend considerably less time on the flowers (Leonard, Dornhaus & Papaj 2011), which could lessen the amount of pollen wasted during pollination (Harder & Thompson 1989; Leonard, Dornhaus & Papaj 2011).

Like the word nectar guide suggests, these guides aid in the pollinators' detection of nectar, but floral rewards, such as nectar and pollen, can vary between conspecific flowers. Some flowers have anther, or pollen, mimicking patterns in their nectar guides, that may increase pollinator visitations while providing pollinators with adequate reward (Lunau 2000). In artificial flowers, the presence of yellow dots, which mimic pollen, caused *B. terrestris* to not be able to distinguish between flowers with or without reward (Pohl, Watolla & Lunau 2008). In the absence of pollen, *R. hirta* with larger UV-absorptive patterns are preferentially pollinated by pollinators (Horth, Campbell & Bray 2014), and even though pollen was used in this experiment, the pollinator preference for visiting flowers with larger UV absorptive patterns was reinforced. In *R. bulbosus*, the larger UV absorptive pattern was an honest indicator of more pollen reward, but *R. hirta* does not share this phenomenon. Pollinators

preferentially pollinated the *R. hirta* flowers with the larger UV pattern, and pollen load did not seem to influence bee behavior nor floral display. Nectar is UV absorbent when irradiated with UV light, and bees can use the absorbance of nectar to determine nectar quantities (Thorp *et al.* 1975). In *R. hirta*, pollinators are posited to be using the increased UV absorptive “bull’s-eye” pattern as an indicator of increased nectar reward, but the relationship between UV absorptive pattern size and nectar quantity has not been evaluated in this plant species. The larger UV absorptive patterns could be an honest indicator of reward, but this preferential pollination could also be the result of learned preferences (Makino & Sakai 2007).

Research on the ability of non-*Apis* bees to learn is limited, but bees can learn which flowers yield the most reward. Bumble bees have been shown to initially pollinate larger floral displays in artificial flowers, but, through experience, learn to pollinate flowers with the most nectar (Makino & Sakai 2007). Half-black bumble bees (*Bombus vagans* Smith) also show a tendency to undergo floral constancy based on their sampling of the “reward spectrum.” These bees learn which flowers yield the most nectar and preferentially pollinate them until those rewards become depleted, at which point the bees shift their pollination to the next highest nectar reward available (Heinrich 1979). Halictidae (sweat bees), which are poorly studied, cosmopolitan pollinators frequently found in urban ecosystems (Dikmen 2007), preferentially chose flowers with larger UV absorptive patterns, regardless of pollen load. These bees have been shown to assess large differences in nectar load (Ashman *et al.* 2000), like bumble bees (Thorp *et al.* 1975), but aside from their ability to remotely determine nectar quantities, knowledge of Halictidae preferences to UV absorptive patterns is minimal. My study suggested that Halictidae do not pollinate flowers based on pollen loads, but they may have an interest in

larger UV absorptive patterns, which could be a learned preference from finding flowers with larger nectar loads.

The preference of native bees (Apidae and Halictidae) to pollinate flowers with larger UV absorptive patterns has been shown across flowering plant families (Asteraceae and Ranunculaceae), which is highly suggestive of a generalizable phenomenon. The preferences of Apidae and Halictidae are similar, but preferences of other native bee families (Colletidae and Megachilidae) are still unknown. Determining if these floral preferences hold true among Colletidae and Megachilidae could suggest a generalizable phenomenon among all major hymenopteran families.

## **CONCLUSIONS**

*Osmia lignaria* have the potential to be used in the *F. X ananassa* greenhouse industry, and may have further applications in the *R. fruticosus* industry. *Osmia lignaria* may be a versatile and fruitful pollinator in agricultural systems. Native pollinators in this study chose the flowers with the larger UV pattern, regardless of pollen load, and this pollination preference appears to be a generalizable phenomenon across hymenopteran families. The tendency for angiosperms to have varying UV pattern sizes, with the larger sizes being more preferentially pollinated, suggests a generalizable phenomenon across plant families.



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**DEGREES**

B.S., BIOLOGICAL SCIENCES, OLD DOMINION UNIVERSITY, MAY 2014

**PUBLICATIONS AND PRESENTATIONS**

Gregory, M.D. & Horth, L. Assessing the effectiveness of *Osmia lignaria* as a greenhouse pollinator of *Fragaria x ananassa*. Poster presented at: Novel Ecosystems in the Anthropocene. 101<sup>st</sup> ESA Annual Meeting; 2016 Aug 7-12; Fort Lauderdale, FL.

**ACADEMIC AWARDS AND ACHIEVEMENTS**

Fall 2010-May 2014: Academic Dean's List

Fall 2010-May 2014: Monarch Pride Scholarship

**MEMBERSHIPS AND ASSOCIATIONS**

Member, Botanical Society of America

Member, Conservation Biology Club

Member, Biology Graduate Student Organization