

ROLE OF COLOR AND ODOR ON THE ATTRACTION OF INSECT VISITORS
TO SPRING BLOOMING TRILLIUM

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By

Natasha Marie Shipman

Director: Dr. Laura DeWald
Professor of Biology
Biology Department

Committee Members: Dr. Beverly Collins, Biology
Dr. Amy Boyd, Biology

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TABLE OF CONTENTS

	Page
List of Tables.....	iv
List of Figures.....	v
Abstract.....	vi
Chapter 1: Introduction.....	1
Chapter 2: Literature Review.....	3
Floral Cues and Insect Response.....	3
Plant-Pollinator Interactions: Specializations - Generalizations Continuum.....	10
Trillium.....	14
Chapter 3: Manuscript.....	18
Introduction.....	18
Methods and Materials.....	21
Study Species.....	21
Study Site.....	23
Insect Visitors on Real Plants.....	24
Insect Visitors on Artificial Flowers.....	26
Identification of Insects.....	31
Data Analysis: Insect Visitor on Real Plants.....	32
Data Analysis: Insect Visitor on Artificial Flowers.....	32
Results.....	34
Insect Visitors on Real Plants.....	34
Insect Visitors on Artificial Flowers.....	35
Order Level Results.....	35
Family Level Results.....	38
Discussion.....	46
Insect Visitors on Real Plants.....	46
Insect Visitors on Artificial Flowers.....	56
Summary.....	64
Chapter 4: Literature Cited.....	66

LIST OF TABLES

Table	Page
1. Flowering period for plant species at the Botanical Gardens.....	25
2. Abundance of insects collected on <i>T. cuneatum</i> and <i>T. grandiflorum</i>	36
3. Abundance of insect orders collected on artificial flowers.....	39
4. Orders of insects analyzed among the six color by odor treatment combinations.....	41
5. Abundance of insect families collected on artificial flowers.....	42
6. Distribution of families of insects analyzed for color and odor treatments.....	45
7. Families of insects analyzed among the six color by odor treatment combinations.....	48
8. Comparison of patterns of attraction for color and odor between families within orders.....	50
9. Pairwise comparisons of color and odor treatments using ANOSIM.....	52

LIST OF FIGURES

Figure	Page
1. Native range of <i>Trillium cuneatum</i> and <i>Trillium grandiflorum</i>	22
2. Bottle traps constructed for insect collection.....	27
3. Example of experimental design.....	29
4. Example of scented artificial flowers.....	30
5. Average of percent individuals on artificial flower color and odor for orders of insects not equally distributed among colors or odors.....	40
6. Average of percent of individuals on artificial flower color and odor for families of insects not equally distributed among colors or odors....	47
7. MDS ordination of color and odor treatments.....	51

ABSTRACT

ROLE OF COLOR AND ODOR ON THE ATTRACTION OF INSECT VISITORS TO
SPRING BLOOMING TRILLIUM

Natasha Shipman, M.S. Biology

Western Carolina University (July 2011)

Director: Dr. Laura DeWald

Plants relying on insects to pollinate flowers attract pollinators through varying floral cues such as unique colors and scents. Pollinators rely on these cues to identify flowers for sources of food such as nectar, pollen, and oils. The goals of this study were to investigate color and odor cues associated with pollinator attraction in populations of *Trillium* at the Botanical Gardens, Asheville, NC. Insect visitors to the red-scented *T. cuneatum* and white-non-scented *T. grandiflorum* were collected using tangle-trap, bottle-traps, transect walk methods. Floral color and odor cues also were investigated using artificial flowers placed among a spring blooming plant community. Artificial flowers colored wine-red, white, or yellow and scented or unscented were covered with transparency film and sprayed with tangle-trap to capture insect visitors. Insects were identified to the level of order and family. Insect visitors to *T. cuneatum* and *T. grandiflorum* did not differ and primarily consisted of individuals belonging to the order Diptera, Hymenoptera, and Coleoptera. Diptera were the most abundant visitors consisting of weakly flying dipteran such as Sciaridae and Mycetophilidae. Similar to the real flowers the main visitors to artificial flowers were Diptera, Hymenoptera, and Coleoptera but also included 13 additional orders representing 106 families. Some orders and families collected showed low abundances that could reflect they were being repelled by the floral cues or might not be abundant in the study area. Overall there

was a difference in color ($p < 0.05$) but not odor ($p > 0.05$). However, when looking at each individual order or family separately, some orders and families were equally distributed for color and odor while others were not, indicating that the importance of floral cues depends on the insect family investigated. For those that were not equally distributed for color, insects were found in greater average percent of individuals on yellow flowers but there was no difference between red and white. For those that were not equally distributed for odor, a greater average percent of individuals were found on scented flowers. Some insects were generalists; visiting all artificial flower treatments randomly, while other insects were more specialized visiting certain color and/or odor treatments in greater numbers indicating a continuum along a gradient of generalized to specialized insect visitors. The ability for plants to attract generalist insect visitors and the ability for insects to visit multiple floral cues might be important where visitation is affected by varying weather conditions and advantageous in the event of environmental change and human altered ecosystems.

CHAPTER 1

INTRODUCTION

There are many reasons why we should care about the longevity and diversity of plant and insect species that inhabit our world. Have you ever enjoyed a flower for its beauty, scent or maybe even its taste? Do you prefer certain floral colors, smells or tastes? Have you observed the large diversity of insects visiting flowers? Like us, insects use multisensory cues in plant-pollinator interactions. Cues such as flower color, shape and odor help direct insects to potential sources of food (Faergj and van der Pijl 1979, Dieringer *et al.* 1999) while these pollinators aid in sexual reproduction (Mauseth 1998). Without insects, many species of flowering plants might not exist and without flowering plants many insect species might not exist. These interactions can be mutualistic, antagonistic or neutral (Elberling and Olesen 1999) and are linked to humans through the food, aesthetics, and other products they provide.

Plants, because of their immobility, rely on external vectors such as wind or animals to achieve mating through pollination. The most common color in the plant world is green. Because pollination is necessary for sexual reproduction, plants must stand out among the sea of green. Floral cues such as color, odor, shape and arrangement are important cues that help attract pollinators (e.g., Robertson 1928, Strauss 1997, Berjano *et al.* 2009). Yet pollination dynamics of many plant species, such as *Trillium* species, are not well understood.

My study focuses on the sensory cues produced by two species of *Trillium* and the insects these cues attract. The goals of this study were to investigate color and odor cues involved in insect attraction in populations of *Trillium cuneatum* and *Trillium grandiflorum* at the Botanical Gardens, Asheville, NC. From this study I hoped to

answer the following questions: 1) Is there a difference in the insect community attracted to the wine-red *Trillium cuneatum* versus the white *Trillium grandiflorum*? 2) What difference does flower color and scent have on the attraction of insect visitors?

I embarked on this journey as a learning process. The challenges of research development and implementation were not always easy. In addition to learning much about pollination dynamics, I have learned greater patience and perseverance. Having looked at over 20,000 insects under a microscope I have developed an affinity for these little "bugs", finding them beautiful beyond my imagination. I have immensely enjoyed this process and by furthering my education through a MS Thesis project, my desire to be a life-long learner and to always be curious and always ask why has become firmly entrenched. In the following pages I present what I have learned about the complex associations between flowers and insects. Chapter Two of this thesis is a Literature Review that synthesizes our knowledge of pollination dynamics involved in plant-animal interactions. Chapter Three is the Manuscript I hope to submit to the journal *Castanea*. Chapter Four lists the literature cited in the thesis.

CHAPTER 2

LITERATURE REVIEW

There are two historic starting points for the discipline of plant-pollinator interactions. The first began in 1733 with Kölreuter's detailed observations of floral mechanisms that expanded to studies by Sprengel in 1793 of the natural history of ecological relationships between plants and pollinators (Waser 2006, Mitchell *et al.* 2009). The second starting point focused on evolutionary processes that might affect and be affected by pollination, beginning with Darwin in the mid 1800's (Mitchell *et al.* 2009). Today there is continued interest in of pollination biology with studies focusing on topics such as functional ecology of floral traits, dynamics of pollen transport, competition for pollinator services, niche relationships, and community ecology of pollination (Mitchell *et al.* 2009). Our understanding of plant-pollinator interactions comes from both field observations (*e.g.*, Schemske *et al.* 1978, Kearns and Inouye 1994) and experiments using artificial floral arrays (*e.g.*, Chittka and Thomson 1997, Roy and Raguso 1997, Chittka and Raine 2006, Kudo *et al.* 2007). These studies include the investigation of individual plant species (*e.g.*, Thien *et al.* 1983, Knight 2003) and communities of plants (*e.g.*, Totland 1994, Junker *et al.* 2010).

Floral Cues and Insect Response

Plants, because of their immobility, rely on external vectors such as wind or animals to achieve mating through pollination (Knight 2003). Renner (2006) reports that of the 13,500 genera of angiosperms, 874 are wind or water pollinated, 250 are bat pollinated, 500 are bird pollinated, and the remainder are mostly insect pollinated. The study of these plant-pollinator interactions can help answer questions which improve our

understanding of plant breeding systems, floral evolution, foraging theory, animal behavior, developmental biology and community ecology (McCall and Primack 1992, Mitchell *et al.* 2009).

Flowers pollinated by wind and fruits distributed by wind are almost never brightly colored (Mauseth 1998). This is in contrast to plants that rely on insects to pollinate flowers and distribute fruit. Effective pollination by insects requires plants to stand out among the background of green and brown; thus plants attract insects with a variety of floral cues such as color, shape, size, arrangement and scent (Robertson 1928, Faegri and van der Pijl 1979, Menzel and Shmida 1993, Kevan *et al.* 1996, Salzman *et al.* 2007, Wright and Schiestl 2009, Miller *et al.* 2011). Floral cues might also discriminate among insect visitors, making the flowers less conspicuous to inefficient pollinators. For example, red, tubular flowers that are pollinated by birds may discriminate against bees as pollinators (Wilson *et al.* 2004). In addition, floral odor can be used to deter herbivorous insects (Willmer and Stone 1997, Schiestl 2010). Flowers provide pollinators with floral rewards (i.e., sources of food), such as nectar, pollen, and oils (Dobson 1994, Wright and Schiestl 2009). Foraging insects exert energy traveling among flowers and extracting the floral rewards, and the amount of energy exerted could be a cost if more energy is expended than gained (Mitchell 1989). Therefore, floral cues are important signals for insects to use to identify sources of food (Menzel and Shmida 1993).

The ability to recognize floral cues can be innate (Henning *et al.* 1992) or learned (Heinrich *et al.* 1977, Wells and Wells 1985). Recognition of cues is important because it allows food to be rapidly located and allows insects to distinguish between food types (Schemske *et al.* 1978, Chittka and Menzel 1992). Color acts as a long distance cue and location signal for insect visitors (Faegri and van der Pijl 1979). The ability of

insects to recognize flowers based on color is generally described at the level of order (Waser *et al.* 1996). For example, Hymenoptera tend to visit violet, blue, pink and yellow but rarely red flowers (Menzel and Shmida 1993, Miller *et al.* 2011). Coleoptera tend to visit white or dull colored flowers (Miller *et al.* 2011) while Diptera tend to visit dull red or brown colored flowers (Miller *et al.* 2011). Even though insects have innate preferences in floral color, they are often observed foraging on multiple species of plants with different floral colors (McCall and Primack 1992, Waser *et al.* 1996, Miller *et al.* 2011). Diptera, Hymenoptera, some Lepidoptera, and Coleoptera can recognize color, extending from near UV (320 nm) to near red (600-650 nm) wavelengths and thus can identify many floral color cues (Menzel and Backhaus 1991 in Waser *et al.* 1996). Therefore, despite innate preferences, insects can learn to associate additional floral color with rewards. In addition, Schaefer *et al.* (2004) found pollinators often pick new floral colors that are similar to those that have previously been learned. Although insects can visit multiple floral colors and the frequency insects visit flowers can be influenced by color, the relationships of insect visitors and floral color can vary among plant communities (McCall and Primack 1992).

Our previous understanding of sensory cues was generally based on studies of visual cues such as flower color (Menzel and Shmida 1993, Chittka and Waser 1997). Newer floral cue studies have examined chemical components involved with scent (e.g., Goodrich *et al.* 2006, Raguso 2008, Goodrich and Raguso 2009, Wright and Schiestl 2009, Schiestl 2010). Floral scents consist of low-molecular weight compounds and although different flower species share many scent components, the combination and concentration of components is unique to each plant species and can also vary in concentration within a species (Galizia and Menzel 2000, Raguso 2001, Cunningham *et al.* 2006). In addition to visual acuity, insects have antennae with several types of

sensilla that can detect and differentiate chemical compounds (Chapman 1998). Similar to those for color, preferences for floral odor have been described at the level of order. For example, Hymenoptera prefer “sweet” scented flowers (Galen and Kevan 1980), while Diptera prefer unscented flowers (Miller *et al.* 2011). Flower odor attracts pollinators (Dobson 1994, Laloi *et al.* 2000, Junker and Blüthgen 2010), deters herbivores from consuming reproductive structures (Willmer and Stone 1997, Theis *et al.* 2007, Schiestl 2010), or repels insects that take nectar but do not pollinate flowers (Junker and Blüthgen 2008). Therefore, odor can elicit either a positive or negative response from the insect (Junker *et al.* 2010).

Associative learning occurs when insects learn from trial and error that specific scents indicate the type or quantity of floral reward present (Theis and Raguso 2005). Studies show that pollinators often learn to prefer scented to non-scented flowers with increased landing or approaches occurring on scented flowers (Knudsen *et al.* 1999, Kunze and Gumbert 2001, Majetic *et al.* 2009). In addition, olfactory cues are often remembered longer than visual cues (Menzel 1985 in Wright and Schiestle 2009, Kunze and Gumbert 2001). However, just as insects do not solely visit a single floral color, insects also do not visit a single olfactory cue. For example, Raguso and Willis (2005) reported *Manduca sexta* L. visited flowers with different odor cues.

Floral cues can vary within plant genera and within species for color, odor, and sometimes both (Gegear and Lavery 2001). In the genus *Aquilegia*, flowers are white or red (Grant 1952). The genus *Asimina* varies in color and odor with some species having red yeasty scented flowers and other having white sweet scented flowers (Goodrich and Raguso 2009). In the genus *Trillium*, flowers can be wine-red, white, or yellow. They can have a sweet, fermented or rotten odor (Zomlefer 1996, Case and Case 1997). Within species, variations of color and odor also occur. For example

T. cuneatum has several color morphs including wine-red, brown-purple, green-purple, clear green, yellow-green, pale lemon-yellow, or bicolored (wine-red and yellow combination) (Case and Case 1997). *Polemonium viscosum* Nutt. has morphs for both color and scent with flowers varying from light blue, blue purple, to purple flowers and scent varying from “sweet” to “skunky” scent (Galen and Kevan 1980). Pollinators can react to these color and scent polymorphisms. Some pollinators are indifferent to the variation (e.g., Waser 1983, Goulson and Wright 1998, Gegear and Lavery 2001), while others show preferences for color (e.g., Kay 1976, Waser and Price 1981, Gegear and Lavery 2001) or scent morphs (Galen and Kevan 1980).

Floral signals associated with floral reward can lead to flower constancy where for short periods of time, insects visit multiple flowers of one species before visiting flowers of another species (Schemske *et al.* 1978, Waser 1986, Salzman *et al.* 2007, Mitchell *et al.* 2009). Schemske *et al.* (1978) and Motten (1986) found that foragers in wildflower communities show similar tendencies in their steady progression from flower to flower. Raguso (2004b) noted that *Apis mellifera* L. and *Bombus* spp. demonstrate this type of flower constancy, especially when floral signals such as color and scent are multisensory.

Many studies show that floral cues are multisensory (e.g., Roy and Raguso 1997, Raguso 2004b, Hegland and Totland 2005) and can be synergistic where combined signals of color and odor are more effective than when alone, complementary where one cue attracts while the other guides visitation, or redundant where environmental conditions or prior experience determines the floral cue used by visitors (Raguso 2004a). Roy and Raguso (1997) created artificial pseudoflowers and found that yellow-scented flowers had the most approaches and landings, white non-scented flowers were ignored, and white scented flowers were approached but had no landings. They concluded odor

was acting as an orientation cue while color was acting as a landing cue. In the order Lepidoptera, Raguso and Willis (2002) found the hawkmoth *Manduca sexta* prioritized odor over color and would not visit a flower if the visual cue was correct but lacked the olfactory cue. In contrast, Omura and Honda (2005) found the butterfly *Vanessa indica* Herbst appeared to prioritize color over odor but the addition of an odor cue increased the probability of floral visits.

Within a community, different plant species with overlapping flowering periods are interacting with the same pollinator community. These interactions can be positive and lead to facilitation, be neutral and have no effect, or be negative and cause competition (Brody 1997, Berjano *et al.* 2009). Facilitation occurs when the insects visiting early blooming species remain in the area and visit species that bloom later, or when synchronous blooming species attract more visitors than if either bloomed alone. Neutral interactions occur when the insects visiting the flowers of a community do not affect each other and negative interactions occur when flowers compete for insect visitors (Brody 1997). When floral rewards between species are similar, insects with fixed preferences are at a disadvantage because they expend more energy searching for specific plant species (Chittka and Menzel 1992). The ability of insects to recognize and visit multiple flower species is especially advantageous to the plant and insect when visitation is affected by varying weather conditions (McCall and Primack 1992, Lundemo and Totland 2007), in the event of environmental change (Hingston and McQuillan 2000), or in human altered ecosystems (Jules 1998, Johnson and Steiner 2000).

The potential pool of pollinators varies between and within habitats and these insect assemblages are largely determined by the vegetation composition (Jonas and Joern 2007, Kato *et al.* 2008). It has also been found that insects may adjust their life cycle to correlate with the flowering phenology of the plants in the area (Kato *et al.*

2008). Visitation rates have been documented to vary between and within sites (Davis 1981, McCall and Primack 1992, Lundemo and Totland 2007) and this variation is attributed to changes in weather within a season and from year to year, as well as to changes in floral density and variety of plant species in a community (Kearns 2001). Visitation does not always result in pollination because some insects visit flowers incidentally while others take floral rewards but do not pollinate the flower because the insect does not come into contact or properly come into contact with the plants reproductive features (Waser *et al.* 1996, Berjano *et al.* 2009). Higher visitation also does not always mean greater seedset because higher rates of visitation could increase pollen loss or the transfer of heterospecific pollen (Roy and Raguso 1997).

In conclusion, floral cues are often multisensory and the relative importance of these cues depends on the insect visitor investigated (Roy and Raguso 1997, Wright and Schiestl 2009), the plant community examined (McCall and Primack 1992), and the assemblage of insect visitors present in the area (Pettersson 1991, Hingston and McQuillan 2000). Although insects show both innate (Kugler 1934 in Salzman *et al.* 2007, Henning *et al.* 1992) and learned (Heinrich *et al.* 1977, Wells and Wells 1985, Salzman *et al.* 2007) preferences for floral cues, these preferences are not always consistent (Faegri and van der Pijl 1979, Menzel 1985 in Waser *et al.* 1996, Dobson 1987) especially when rewards between plant species are similar (McCall and Primack 1992, Waser *et al.* 1996). However, even though insects may visit multiple plant species with varying floral cues, most insects exhibit some floral constancy (Roberstion 1928, Schemske *et al.* 1978, Chittka and Menzel 1992, Chittka and Raine 2006, Salzman *et al.* 2007). Flower constancy may be more important to plant species than to visitor specialization because of differences in insect assemblages found across the range of plants' habitats (Jonas and Joern 2007, Kato *et al.* 2008), and because insect pollinators

are subject to diverse and changing environmental conditions (Jules 1998, Hingston and McQuillan 2000, Johnson and Steiner 2000).

Plant-Pollinator Interactions: Specialization - Generalization Continuum

Interactions of plants and pollinators have been investigated for many years. Sprengel (1793) described pollination of plants by animals as a mutualistic interaction (Sprengel 1793 in Thomson 2003). Darwin (1862) concluded that animals were agents of natural selection on floral characters and Müller and Delpino (1869) described patterns of visitation (Darwin 1862 and Müller and Delpino 1869 in Thomson 2003). These early works established the idea that evolution of plant-pollinator interactions occurs as a tight co-adaptive process where plants evolve traits to certain pollinators, pollinators evolve traits to better exploit floral resources of particular plants, and these interactions are mutualistic (Baker 1963, Stebbins 1970, Faegri and van der Pijl 1979, Aigner 2001, Fenster *et al.* 2004). This co-evolution has resulted in specializations summarized as “pollination syndromes” where floral traits reflect adaptations to pollinator type at roughly the level of order (*e.g.*, beetles versus butterflies versus bees) or family or above (*e.g.*, birds versus beetles). Because floral traits often taxonomically differentiate plant species, pollinator specialization is considered critical to plant speciation and evolutionary radiations (Grant 1949, Stebbins 1970, Crepet 1983). Work on figs (*Ficus spp.*) and fig wasps (*Pegoscapus spp.*) (Janzen 1979), and yuccas (*Yucca whipplei* Torr) and yucca moths (*Tegiticula maculata* Riley) (Aker and Udovic 1981) illustrate these highly co-evolved and mutualistic interactions. However, other plant-pollinator specializations occur when no floral reward is present, and pollinators are attracted by deception, or when a reward other than pollen or nectar is available (Minckley and Roulston 2006, Berjano *et al.* 2009).

Pollination syndromes are described at length by Faegri and van der Pijl (1979), Wyatt (1983), and Pellmyr (2002). For example, bat (Chiroptera) pollinated flowers may be white, have strong odors, and produce large amounts of nectar (Sahley 1996, Winter and Helverson 2001). Hummingbird (Trochilidae) pollinated flowers often are red, have narrow tubes, lack landing platforms, and produce dilute nectar (Grant 1966 in Healy and Hurlly 2001); and bee (Hymenoptera) pollinated flowers tend to be blue or yellow, have vestibules to crawl into, have landing platforms, and produce concentrated nectar (Wilson *et al.* 2004). General descriptions of floral syndromes for dipteran pollinators vary in the literature. In reviews by Hingston and McQuillan (2000) and Miller *et al.* (2011), flowers pollinated by dipterans are described as dull red or brown in color and odorless, while a review by Menzel and Shmida (1993) said that flowers visited by dipterans were primarily yellow. This discrepancy might be because the syndrome was described at the order level but for Diptera at the family level such as syrphids, calliphorids, tephritids, and anthomyiids seem to have preferences for yellow flowers (references in Weiss 2001), while bombyliids have preferences for pink, blue, or violet flowers (Proctor *et al.* 1996, references in Weiss 2001).

In contrast to specialized plant-pollinator interactions, empirical studies by McCall and Primack (1992), Waser *et al.* (1996), Herrera (1996), and Gómez and Zamora (2006) have shown these interactions to be more generalized than previously thought. Generalized plant-pollinator interactions are when many insect species pollinate a specific species or when many plant species are pollinated by specific insect species (Waser *et al.* 1996). From an evolutionary view, specialization allows the plant to respond to selection by abundant or efficient pollinators allowing for microevolution or speciation (Eriksson and Bremer 1992, Johnson and Steiner 2000). From an ecological view, generalization may confer the plants' or insects' competitive ability, colonization

capacity, or invasion ability (Richardson *et al.* 2000). In generalized systems a single reproductive season does represent an episode of selection and selective pressures may change over a lifetime (Brody 1997).

Frequent generalization in plant-pollinator interactions can result in intermediate levels of pollinator diversity that are considered optimal in plant communities (Gómez *et al.* 2007). When pollinator diversity is too high the quality of pollen transfer decreases because of the transfer of geitonogamous pollen, or low flower constancy of pollinators that can lead to heterospecific pollen transfer (Gómez *et al.* 2007). Generalizations can be explained by the learning capability of insects (Chittka and Menzel 1992 and references therein, Chittka and Raine 2006, Cunningham *et al.* 2006 and references therein) and composition of floral species (Kato *et al.* 2008) because patterns of floral resources are too unpredictable within a few insect generations for a fixed insect behavior pattern, but not so unpredictable that the pollinator is unable to track the changes (Weiss 2001, Chittka and Raine 2006). The ability of insects to associate and learn what floral rewards are present based on floral cues can overwhelm preferences (Menzel 1985 in Waser *et al.* 1996, Dobson 1987). Another explanation for generalized plant-pollinator interaction is that plant species in different habitats or environments exploit animal pollinators differently because pollinator assemblages differ based on the composition of the regional biota present (McCall and Primack 1992, Hingston and McQuillan 2000, Kato *et al.* 2008), and the abundance of insects varies in both space and time (Bosch *et al.* 1997, Hingston and MCQuillan 2000).

Generalization by both plants and insect pollinators are an advantage when the risk of dependence on another species is high due to unpredictability, especially in the event of habitat loss, a changing environment, and anthropogenic changes to the

ecosystem (Jules 1998, Hingston and McQuillan 2000, Kremen and Ricketts 2000, Kearns 2001, Gonzales *et al.* 2006, Mitchell *et al.* 2009).

Descriptions of generalized plant-pollinator interactions have resulted in re-examination of the pollination syndrome concept (Waser *et al.* 1996). The discussion of specialization versus generalization revolves around the following contradictory observations: (a) the diversity in morphology and scent and reward in plants is recognized as being clustered around the some classic 'syndrome'; and (b) the wide array of potential pollinators that visit flowers do not fit the traditional 'syndromes' (Mitchell *et al.* 2009). Works that promote specialization recognize there are limitations (Waser *et al.* 1996), and Faegri and van der Pijl (1979) stressed that pollination syndromes were actually only generalizations because animals other than those associated with the pollination syndrome may visit the flower (Faegri and van der Pijl 1979, Hingston and McQuillan 2000). These generalizations of pollination syndromes are made using the Most Effective Pollinator Principle (MEPP), where floral phenotype is described as evolving in response to the pollinator that is most frequent and most effective in transferring pollen (Stebbins 1970). The most effective pollinator principle tends to favor Hymenoptera as effective pollinators because they carry large amounts of pollen and move quickly between flowering plants (Galen and Kevan 1980, Kearns and Inouye 1994). The effectiveness of Diptera, Coleoptera, and Lepidoptera as pollinators is not well known (Kearns 2001, Weiss 2001, references in Lundemo and Totland 2007). Although most of the research has been on pollination by and sensory learning of Hymenoptera (Chittka and Thomson 2001 and references therein, Weiss 2001 and references therein, Chittka and Raine 2006, Kudo *et al.* 2007), it has been found that Diptera, Coleoptera, and Lepidoptera are also key pollinators of many plants (Kevan and Baker 1983, McCall and Primack 1992, Proctor *et al.* 1996, Raguso and Willis 2005). In

some cases the most effective pollinator is not the most abundant (Horvitz and Schemske 1990), therefore, specialization may be hindered by the presence of other insects (Aigner 2001).

The universality of the pollination syndrome concept is reflected in the scarcity of narrow plant-insect associations in community studies with most plant species being visited by a diverse array of insects representing two to three orders (Motten 1986, McCall and Primack 1992, Bosch *et al.* 1997 and references therein). Instead of describing general floral syndromes, an understanding of plant-pollinator interactions at the temporal (diurnal, seasonal, annual) and spatial (neighborhood, landscape, geographic) scales will be important to best further the understanding of pollination biology (McCall and Primack 1992, Totland 1994, Waser *et al.* 1996 and references therein, Brody 1997, Johnson and Steiner 2000).

In conclusion, pollination systems found in nature can be summarized by Sprengel's conclusion about pollination in 1793, "It is certain that many flowers are fertilized by multiple species of insects...It also is certain that many flowers are fertilized solely by one species of insect, and this in a very distinct fashion", and "in the first case, the fertilization of the ovary and production of fruit must progress more easily" (Sprengel 1793 in Waser 2006, p.7).

Trillium

Trillium is an example of a genus where floral cues vary. In addition to variation in colors, yellow, white, and wine-red, trilliums vary in odor from lemon-like, sweet rose-like, spicy, and fetid (Zomlefer 1996). However, it is thought that position of flower organs and color, coupled with odor helps determine pollination visitors (Case and Case 1997). These variations are attributed to differences in pollinators (Case and Case

1997, Irwin 2000). This color and odor variation is dispersed among the more than 54 species in the genus that are found in eastern Asia (11 *spp.*), western North America (8 *spp.*), and eastern North America (35 *spp.*), with the greatest species diversity occurring in the Southern Appalachian Mountains (Serota and Smith 1967, Zomlefer 1996, Case and Case 1997, Hill 2005). There are two subgenera of *Trillium*: *Trillium*, the pedicellate flowered species, distributed in Asia and North America, and *Phyllantherum*, the sessile flowered species, which are limited to North America and mostly the eastern United States (Zomlefer 1996). *Trillium* species develop from short, stocky, tuber-like rhizomes that produce a single stem (rarely two) between 15-45 cm high and a whorl of three, net-veined, green or mottled leaves (Case and Case 1997, Kalisz *et al.* 1999, Hill 2005). Flowers are single (rarely two), perfect, petiolate or sessile, and consist of three sepals alternating with three leaves (Case and Case 1997, Kalisz *et al.* 1999, Hill 2005). Seeds of *Trillium* have double dormancy, where they must be exposed to two winter seasons before above ground growth occurs (Gonzales *et al.* 2006). The first cold period stimulates root emergence and development, and a second cold period stimulates shoot development (Zomlefer 1996). The life history stages of *Trillium* consist of a cotyledon stage, one-leaf vegetative stage (several years), three-leaf vegetative stage (several years), and a three-leaf reproductive stage (Zomlefer 1996, Jules 1998). After 7-10 years *Trillium* plants reach maturity (Davis 1981, Case and Case 1997) and produce one flower per year until the plant dies. Plant longevity is estimated to be as much as 30+ years, based on cataphyll scar counts (Zomlefer 1996). The long time frame required for plant maturity indicates that young seedlings may have lower survivorship than older plants (Davis 1981). Gonzales *et al.* (2006) reported a high percentage of *T. cuneatum* plants flowered, which is typical of herbaceous species that occupy a variety of habitats. Even though many plants flowered, the fruit yield was low (less than 1%). However,

Motten (1986) found only a small increase (~ 9%) in fruit set when he hand pollinated *Trillium cuneatum* and concluded that pollination success of spring wildflowers was related to their accessibility to different kinds of effective pollinators.

Trillium species occupy many habitats (Zomlefer 1996, Case and Case 1997, Gonzales *et al.* 2006), and insect community assemblages vary between habitats and in space and time (McCall and Primack 1992, Bosch *et al.* 1997, Hingston and McQuillan 2000, Kato *et al.* 2008). The wide differences in community structure of plants and insects across the range of trillium indicates that specialization of *Trillium* for specific insect pollinators might not be clear and a degree of generalization in insect pollinators might exist within or between habitats.

Although few studies have investigated the pollination dynamics of *Trillium*, descriptions of pollinators of *Trillium* exist. *T. erectum* is described as pollinated primarily by dipteran species and to lesser extents coleopterans (Davis 1981), while *T. grandiflorum* is pollinated primarily by hymenopterans (Carter 1892 and Robertson 1896 in Irwin 2000) and to lesser extents dipterans (Irwin 2000). Insect visitors to trillium flowers are generally reported as nonexistent to infrequent and some question if insects affect pollination. However, the attraction of ants that act as seed dispersers to the arils as a food source is well documented (Zomlefer 1996, Kalisz *et al.* 1999, Gonzales *et al.* 2006, Junker *et al.* 2007). Case and Case (1997) report that pedicellate trilliums tend to rely on bees and flying insects while the fetid or putrid odors of sessile trilliums rely on beetles, crawling insects, carrion fly, and such for pollination. Research of a spring wildflower community by Motten (1986) observed very few insects visiting *T. cuneatum* flowers. Gonzales *et al.* (2006; 2008) reported insects visiting *T. cuneatum* consisted of weakly flying Diptera species such as *Drosophila* and sometimes *Califoridae*, and these visits occurred on warm, moist evenings when plants emitted scent.

Trillium species vary, at the level of population, in clonality, self-compatibility and numbers of insects pollinating flowers (Davis 1981, Kalisz *et al.* 1999, Knight 2003, Gonzales *et al.* 2006, Gonzales *et al.* 2008). Proportion of vegetative spread and sexual reproduction can vary depending on population and habitat type (Serota and Smith 1967, Gonzales *et al.* 2008). Some view clonal reproduction as a way to prolong population survival in the absence of sexual reproduction (Honnay and Bossuyt 2005, Gonzales *et al.* 2008). This co-reproductive strategy could be due to fluctuations in effective pollinator species present each year because of varying environmental conditions (McCall and Primack 1992), or due to anthropogenic disturbance (Jules 1998, Gonzales *et al.* 2008). Gonzales *et al.* (2008) found clonal populations of *Trillium cuneatum* maintained high levels of genet richness indicating continued sexual reproduction.

In conclusion, there are many species of *Trillium* that occur in eastern Asia and North America (Zomlefer 1996, Case and Case 1997, Hill 2005) and species vary in floral color and odor cues. These differences have been attributed to their specific pollinators (Case and Case 1997, Irwin 2000) however, little is known about the pollination dynamics of *Trillium* and few studies have investigated plant-pollinator interactions (Davis 1981, Case and Case 1997, Irwin 2000, Knight 2003). Although floral cues are attributed to specific pollinators, pollinator assemblages vary widely among habitats and in space and time (McCall and Primack 1992, Jonas and Joern 2007, Kato *et al.* 2008). *Trillium* species occupy many habitats over a large range and this might indicate attraction of a generalist pollinator assemblage with insects that are present in the area during the flowering season being the primary visitors.

CHAPTER THREE

MANUSCRIPT

ROLE OF COLOR AND ODOR ON THE ATTRACTION OF INSECT VISITORS
TO SPRING BLOOMING TRILLIUM

Introduction:

Plants rely on external vectors such as wind or animals to achieve pollination. The study of these plant-pollinator interactions can help improve our understanding of plant breeding systems, floral evolution, foraging theory, and animal behavior (McCall and Primack 1992). Pollinator interactions can occur within species, can be mutualistic, antagonistic or neutral; and can vary in space, time, intensity, and specificity (McCall and Primack 1992, Bosch *et al.* 1997, Elberling and Olesen 1999). Flowers relying on wind for pollination and fruit distribution by are almost never brightly colored (Mauseth 1998). However, plants relying on animals for the functions attract pollinators through varying floral cues such as unique colors, shapes, arrangements, and scents (Mauseth 1998). Pollinators rely on these cues to identify flowers for sources of food such as nectar, pollen, and oils (Dobson 1994), and the attraction to these cues can be instinctive (Henning *et al.* 1992, Salzmänn *et al.* 2007) or learned (Heinrich *et al.* 1977, Wells and Wells 1985, Salzmänn *et al.* 2007).

Flower color has been described as a long distance cue and initial location signal for many insect visitors (Faegri and van der Pijl 1979) because color makes the flowers stand out against the green vegetative background (Kevan *et al.* 1996, Glover and Whitney 2010). The ability of insects to associate color with floral rewards is important because food can be rapidly located and insects can distinguish between food types (e.g. pollen, nectar, oils) found in different colored flowers (Schemske *et al.* 1978,

Chittka and Menzel 1992). Insects with fixed preferences can be at a disadvantage because floral food sources change seasonally and annually (Chittka and Menzel 1992). Therefore, although some insects have innate preferences in floral color, they are often found foraging on species of plants with different floral colors (Waser *et al.* 1996, Miller *et al.* 2011). McCall and Primack (1992) found coloration influenced frequency of insect visitors but the patterns of insect visitors on specific colors was not consistent in three floral communities they examined. Insects visited all flower colors but visited yellow and mixed colored flowers at a greater rate. These differences in visitation rates could be related to temperature and light variation between the sites, or because the abundance of insects or flowers varied between the sites (McCall and Primack 1992).

Similar to color, olfactory cues produced by plants are complex and vary among and within taxa (Raguso 2001, Schiestl 2010). As indicated by increased landings or approaches floral visitors often prefer scented to non-scented flowers (Knudsen *et al.* 1999, Kunze and Gumbert 2001, Majetic *et al.* 2009). These odor cues can increase floral constancy (Schemske *et al.* 1978, Waser 1986, Dobson 1994, Mitchell *et al.* 2009). Odor cues can also deter herbivores from consuming reproductive structures (Willmer and Stone 1997, Theis *et al.* 2007, Schiestl 2010).

Different types of floral cues can work together at attracting insects. Raguso (2004b) notes *Apis mellifera* L. and *Bombus* spp. showed greater constancy when floral signals were multisensory (e.g., color and scent). However, the relative influence of color and scent varies among insects (e.g., Roy and Raguso 1997, Weiss 2001 and references therein, Wright and Schiestl 2009). For example, Roy and Raguso (1997) found both visual and olfactory cues attracted insects to flowers infected with the fungus *Puccinia minoica* Arth. pseudoflowers. In their study, although more insects landed on the scented flowers, yellow flowers (scented or non-scented) had a higher number of

visitors than white scented flowers, and white scented flowers had more visitors than white unscented flowers. It has also been reported that although bees learn to associate floral rewards with floral cues, they still make quick inaccurate choices between species with flowers of similar color (Chittka and Raine 2006). These quick decisions allow more pollen and nectar to be gathered, indicating there is no incentive for bees to make an accurate decision when floral rewards are similar (Burns 2005, Chittka and Raine 2006).

Plant-pollinator interactions have been examined for many plant taxa (*e.g.*, Grant 1950, Mesler *et al.* 1980, Davis 1981) and within plant communities (*e.g.*, Schemske *et al.* 1978, Hegland and Totland 2005). Some studies indicate that floral cues occur in non-random combinations called syndromes associated with distinctive communities of insect visitors (Stebbins 1970, Faegri and van der Pijl 1979, Proctor *et al.* 1996). However, other studies suggest plants are visited and pollinated more generally by a diverse range of animals (*e.g.*, Waser *et al.* 1996, Bosch *et al.* 1997, Gomez and Zamora 2006). In addition to the question of syndromes versus more generalist interactions, plant-pollinator interactions vary temporally (diurnal, seasonal, annual) and spatially (neighborhood, landscape, geographic), and the relationships between floral cues and visitation patterns are unclear (McCall and Primack 1992, Totland 1994, Waser *et al.* 1996 and references therein, Brody 1997, Johnson and Steiner 2000, Kearns 2001).

Floral cues can vary among and within plant taxa (*e.g.*, Case and Case 1997, Goodrich and Raguso 2009). For example, flowers of different *Trillium* species vary in color, odor, size, shape, and arrangement (Case and Case 1997). Flowers can be wine-red, white, pink, or yellow with a sweet, fermented, or rotten odor or no scent. This variation in floral cues has been related to specific pollinators (Galen 1985, Irwin 2000, Salzmann and Schiestl 2007), to pollinator composition differences in relation to bloom

time (Galen and Kevan 1980, Salzman and Schiestl 2007), and might be particularly important where trillium species grow among each other (Case and Case 1997). For example, the wine-red fermenting smelling *T. cuneatum* Rafinesque can grow in close proximity or intermixed with the white unscented *T. grandiflorum* (Serota and Smith 1967, personal observation). The different color and odor of *Trillium cuneatum* flowers might attract a unique set of pollinator insects (Zjhra *et al.* 2007). For example, Gonzales *et al.* (2006) reported weakly flying insects such as *Drosophila* spp. and occasionally *Calliforidae* visiting *T. cuneatum*. While Irwin (2000) and Kalisz *et al.* (1999) reported that flowers of *T. grandiflorum* was visited primarily by hymenopterans (including Apidae) and to a lesser extent dipterans. Despite these trends in *Trillium* and studies of other spring blooming species (e.g., Grant 1950, Willson and Schemske 1980), little is known about the relationships between color and odor floral cues and insect attraction in early spring blooming species such as *Trillium*.

The purpose of this study was to improve our understanding of floral cues involved in attracting pollinators to spring blooming flowers using *Trillium* as an example species. A field study was conducted to compare insect visitors to the wine-red scented flowers of *T. cuneatum* and the white non-scented flowers of *T. grandiflorum*? Artificial flowers were constructed to examine the separate and combined effects of color (wine-red, white, yellow) and odor (scented and non-scented) on insect visitors.

Materials and Methods:

Study Species

Trillium cuneatum and *T. grandiflorum* are spring woodland herbs in eastern deciduous forests (Figures 1a and 1b) (Zomlefer 1996, Case and Case 1997, Knight 2003, Gonzales *et al.* 2008, USDA 2009). Although there is a large area of overlap in their

(a)



(b)



Figure 1: Native range of *Trillium cuneatum* (a) *Trillium grandiflorum* (b)
(<http://plants.usda.gov>)

ranges, the primary location both species are found together is in the mountains of Southern Appalachia in Georgia and North Carolina (Case and Case 1997, Kalisz *et al.* 1999). The flowering phenology of *T. cuneatum* and *T. grandiflorum* overlaps only for the later half of the season (Orbosen and Ruiz 1999). *T. cuneatum* is the largest of the eastern sessile trilliums and has the earliest and longest bloom time that begins in early March and lasts through May (Case and Case 1997). *T. grandiflorum* has a large flower with a shorter bloom time that begins in early April and lasts through early May (Case and Case 1997, Orbosen and Ruiz 1999, Knight 2003). Throughout its range, *T. cuneatum* has many forms but is characterized by erect, twisted petals with maroon claws and with a scent of fermenting apple or pear that can be pleasant to harsh (Case and Case 1997, personal observation). *T. grandiflorum* also shows variation, primarily in size, and is characterized by pedicellate unscented white flowers that fade to a dullish pink-purple with age (Case and Case 1997).

Study Site

The Botanical Gardens of Asheville, NC (Latitude N 35° 36' 47.47", Longitude W 82° 34' 0.50") was the site of my study. This site was selected because it contained both *T. cuneatum* and *T. grandiflorum* plants and represents the native ecosystem of mixed deciduous forest of these species. The gardens are located in the diverse temperate Southern Appalachian mountains (Hill 2005) and reflect the large diversity of both plants and insect species in this ecosystem. This 10-acre native plant refuge was established in 1964 and now has more than 700 plant species (Orbosen and Ruiz 1999). The plots of *T. cuneatum* and *T. grandiflorum* are shared with other early spring blooming plants including: *Claytonia virginica* L., *Sanguinaria canadensis* L., *Erythronium americanum* Ker. Gawl., *T. grandiflorum* (less than ten), *T. leuteum* (Muhl.) Harbison (less than ten),

Asimina triloba, *Calycanthus floridus* L., *Uvularia grandiflora* Sm., *Uvularia perfoliata* L., and *Uvularia sessilifolia* L. Although the start and ending bloom time of these species varies, they all overlap with each other (Table 1). The colors represented by these flowers include wine-red, white, white-pink, and yellow. While all of these plant species inhabit the same location, the species are found in distinct clumps rather than being evenly mixed among each other.

A parcel of mixed deciduous forest containing *Trillium* was located within the gardens and two adjacent populations of *Trillium cuneatum* and *T. grandiflorum* were studied. The *T. cuneatum* population was in a 0.18 hectare area at 618 m elevation with a south facing, 42.5% slope. The area consisted of a relatively large population of over 600 individuals. The population of *T. grandiflorum*, was in a 0.1 hectare area at an elevation of 613 m with a west facing, 45% slope. The area consisted of a population of 30 individuals.

Insect Visitors on Real Plants

Insects were collected from blooming and non-blooming plants of both *T. cuneatum* (wine-red and scented) and its white congener *T. grandiflorum* (white and non-scented) to quantify visitors to these related species with different floral cues but over-lapping geographic ranges and bloom time. Insects were collected from non-blooming plants to account for insects visiting vegetation and not just flowers. Because visitation rates to early spring blooming flowers are often infrequent (Willson and Schemske 1980, Thien *et al.* 1983, Irwin 2000) three methods were used to capture as many insect visitors as possible. Tangle-Trap[®] (The Tanglefoot Company [Grand Rapids MI], see Davis 1981, Irwin 2000), bottle traps (see Woolley *et al.* 2007), and transect walks (see Zjagar *et al.* 2007) were used concurrently from May 2-8, 2010.

Table 1: Flowering period for plant species in the study site area of *Trillium cuneatum* and *T. grandiflorum* flowers.

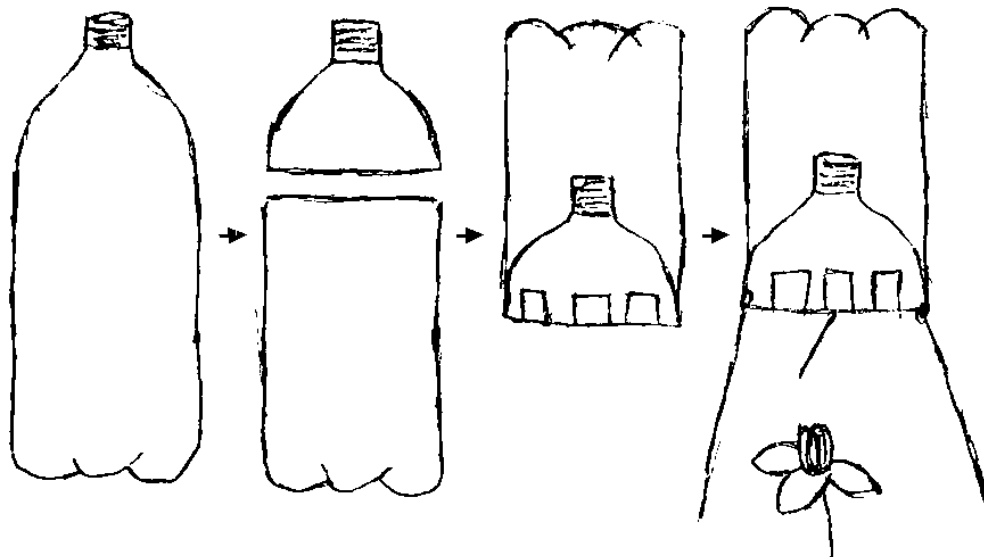
Plant species	Color ¹	Odor	Flowering Period				
			March	April	May	June	
<i>Asimina triloba</i> (L.) Dunal	wine-red	yeasty		—			
<i>Calycanthus floridus</i> L.	wine-red	sweet		—	—	—	
<i>Claytonia virginica</i> L.	white-pink	sweet		—			
<i>Erythronium americanum</i> Ker Gawl.	yellow	no-scent		—			
<i>Sanguinaria canadensis</i> L.	white	no-scent	—				
<i>Trillium cuneatum</i> Raf.	wine-red	spicy	—	—			
<i>Trillium discolor</i> Wray ex Hook.	pale yellow	faint fragrance		—			
<i>Trillium grandiflorum</i> (Michx.) Salisb.	white	no-scent		—	—		
<i>Trillium leuteum</i> (Muhl.) Harbison	yellow	citrus		—			
<i>Uvularia grandiflora</i> Sm.	yellow	no-scent		—	—		
<i>Uvularia perfoliata</i> L.	yellow	no-scent		—	—		
<i>Uvularia sessilifolia</i> L.	yellow	no-scent		—	—		

¹color, odor, and flowering period descriptions are based on Willson and Schemske 1980, Grant 1950, Schemske et al. 1978, Case and Case 1997, USDA 2009, and personal observation.

Because of the small population size of *T. grandiflorum* only Tangle-Trap[®] was used to collect insects from non-blooming plants of both *Trillium* species. Tangle-Trap[®] (The Tanglefoot Company [Grand Rapids MI]) aerosol adhesive was applied to ten blooming plants of *T. cuneatum*, 10 non-blooming plants of *T. cuneatum*, three blooming plants of *T. grandiflorum*, and 3 non-blooming plants of *T. grandiflorum* on May 2 and the above ground vegetation was collected on May 8. Insects stuck to the vegetation were identified immediately to family, or saved for later identification. Only three blooming plants of *T. grandiflorum* were used to protect the small population size due to removal of above ground vegetation from the field. Bottle traps (Figure 2) were made of 2L soda bottles. Tops were cut off and inverted into the base then taped back together. Bottle traps were suspended directly above ten blooming *T. cuneatum* plants and seven *T. grandiflorum* plants by wire to capture insects flying up off the plant. Bottle traps remained in the field for one week (May 2-8, 2010). Insects were collected by placing a piece of crumpled plastic wrap in the opening to prevent insects from falling out of the bottle. Bottles were placed in the freezer to kill the insects and traps were taken apart and insects identified immediately to family, or saved for later identification. For the transect walk, 20 blooming *T. cuneatum* plants and seven *T. grandiflorum* plants within the site were randomly marked with a pin flag and observed on 1 May 2010. Any insects observed on or in the flower were either identified immediately or collected by positioning a wide mouth jar near the flower and using fingers or tweezers to push the insect into the jar to identify later.

Insect visitors on Artificial Flowers

To determine the role of color and scent on the attraction of insect visitors, plots of artificial flowers were set up within the natural population of *T. cuneatum* and insects



2L soda bottle

Top 1/3 cut and
inverted into the
bottom and taped
together

Metal wires
suspended
bottle trap over
flower

Figure 2: Bottle traps constructed for insect collection.

were collected from March 26 through May 2, 2010. The artificial flowers were positioned among the natural population to sample the same pollinator assemblages in the *Trillium* habitat. The experiment was designed to compare differences in insect visitors among three different colors, among scented and non-scented flowers and among the combinations of color and odor. There were two plot odor treatments; scent and no scent with 10 replicates of each plot odor treatment. Each plot consisted of twelve scented or unscented artificial flowers with four flowers of each color tested (wine-red, white, yellow). These colors were used because they are seen in other spring blooming flowers in the area and are seen in different *Trillium spp.* (Case and Case 1997). Colors were clustered in the plot (Figure 3) because that is how natural populations of *Trillium* exist (Case and Case 1997).

The artificial flower design was modified from the methods of Roy and Raguso (1997), Chittka and Raine (2006), Kudo *et al.* (2007), and Van den Berg *et al.* (2008). Flowers were constructed of painted PVC pipe attached to a thin wire rod to hold the flower off the ground and to hold scintillation vials of the scent component in place. Artificial flowers were designed to mimic real flowers, with flowers placed in a clumped distribution and at the proper height and proper scent mimic to ensure olfactory and color cues were in the proper context (Majetic *et al.* 2009, Wright and Schiestl 2009). These criteria indicate insect visitors to artificial flowers represent potential pollinators of *T. cuneatum*. The artificial flowers in my study were covered with transparency film sprayed with Tangle-Trap® (The Tanglefoot Company [Grand Rapids MI]) to collect any insects that visited the flower (Figure 4). Fleishman's yeast in sugar water was used to replicate *T. cuneatum* scent following the recommendations of Guerenstein *et al.* (1995) and Landolt *et al.* (2001). Other brands of yeast give off a different scent component and cannot be used (Zjhra *et al.* 2007). Scent was replaced every other day to maintain

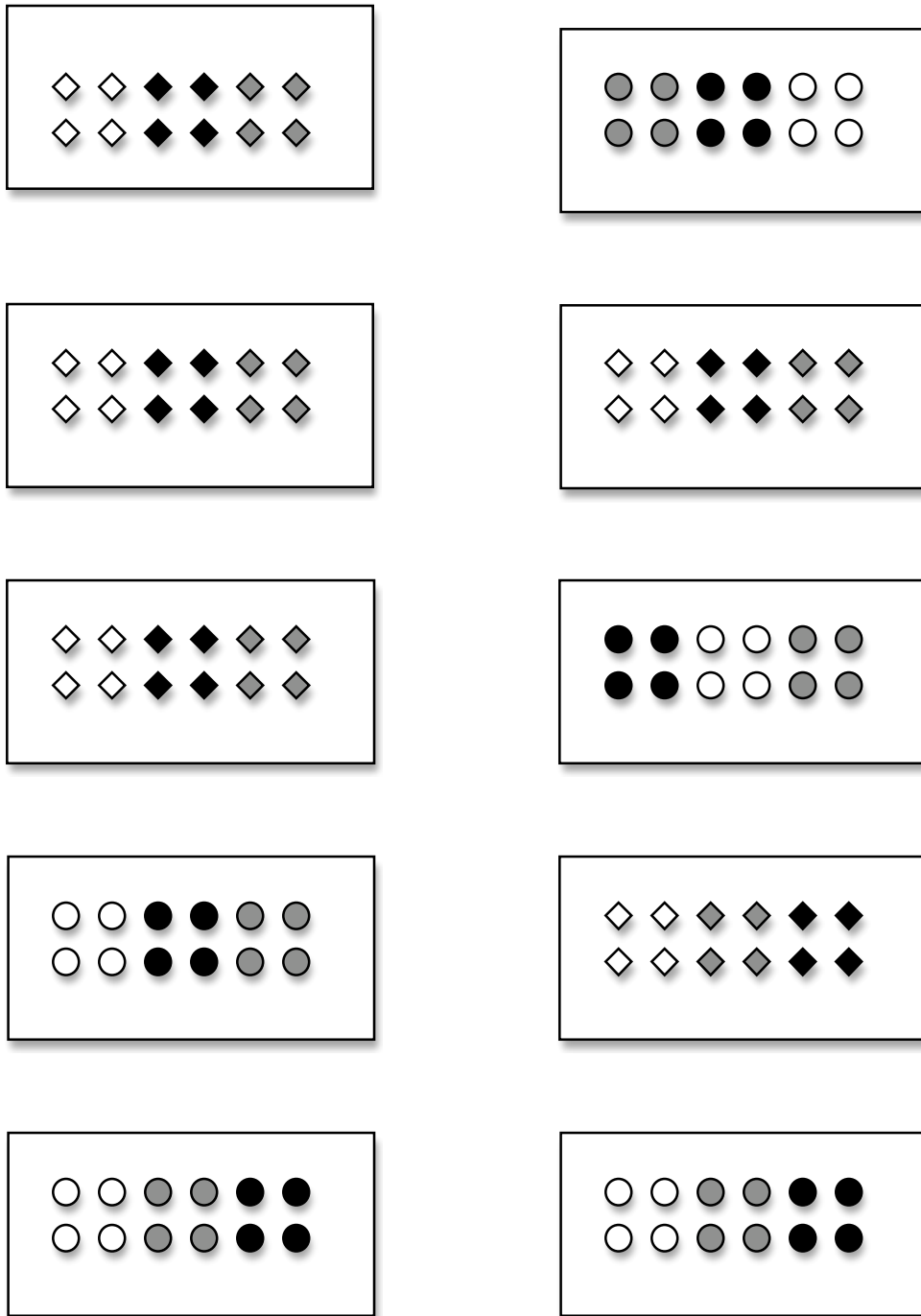


Figure 3: Example of experimental design. Plot treatments were scented (circles) or non-scented (diamonds) and consisted of 12 artificial flowers; 4 of each color (wine-red, white, yellow). Plot treatments were randomly placed among real flowers of various color and scents listed in Table 1.



Figure 4: Example of scented artificial flowers. Scintillation vials in center of PVC pipe were used to hold sugar water and yeast.

a fresh smell. The transparency film was replaced on a weekly basis and Tangle-Trap® was reapplied. Collected pieces of transparency film were marked with treatment specifics and stored in boxes in a refrigerator until identification of insects could be made.

Identification of Insects

Insects stuck to the tangle-trap were identified to order following convention in pollinator syndromes (van der Pijl 1961, Baker and Hurd 1968, Waser *et al.* 1996) and how many previous studies describe insect visitors (e.g., Elberling and Olesen 1999, Hegland and Totland 2005, Lundemo and Totland 2007). Insects were identified further to family to compare to other studies that described visitors to that level (e.g., Bosch *et al.* 1997, Gomez *et al.* 2007, Berjano *et al.* 2009). Insects were identified using Triplehorn and Johnson (2005) and Dr. David Alsop (Prof., retired, Department of Biology, Queens College, The City University of New York) assisted with insect identification.

To identify insects, each collected plant or piece of transparency film was examined under a dissection microscope. Insects that could be identified immediately were tallied and removed from the transparency film. Many insects had to be removed from the transparency film for identification to be possible. In these cases the insects were removed by cutting out the section of transparency film that held them in place and extracting them from the transparency film following the methods of Miller *et al.* (1993). Individual insects were placed in a finger bowl and completely covered with Goo-Gone® (Magic American Product, Inc. [Cleveland, OH]) for 24 hours, then transferred to tetrahydrofuran (THF) for 3 hours to remove oils collected from Goo-Gone®. Insects

were rinsed with pure alcohol, 90% alcohol, and stored in 70% alcohol in scintillation vials until they could be identified.

Data Analysis: Insect Visitors to Real Plants

Insects collected were combined from the tangle-trap, bottle-traps, and transect walk methods for *Trillium cuneatum* (40 total plants) and *Trillium grandiflorum* (17 total plants). A Chi-square test using GraphPad Software QuickCalcs (GraphPad Software, Inc., 2005) was used to determine if the abundances of orders and families of insects visiting flowers were randomly distributed between *Trillium* species. A Chi-square was also used to determine if the abundance of insects within each order and family were randomly distributed between *Trillium* species. Chi-square was also used to determine if the abundance of insects were randomly distributed among blooming and non-blooming plants of the same species. Note that relative abundance was used to compare between the two *Trillium* species to account for differences in the smaller sample size of *Trillium grandiflorum*.

Data Analysis: Insect Visitors to Artificial Flowers

A Chi-square test using GraphPad Software QuickCalcs (GraphPad Software Inc., 2005) was used to determine if the abundance of orders and families of insect visitors were randomly distributed among color and odor treatments. Because Chi-square requires a minimum expected value in each category tested (Preacher 2001), only orders and families with an abundance of 15 or more individuals were analyzed for the two treatment categories.

A Cochran-Mantel-Haenszel Chi-square test using SAS (Statistical Analysis System 2008) was used to test for distribution among the six color and odor treatment

combinations. Only the eight orders and 37 families with a total abundance of at least 35 individuals were analyzed to meet the minimum abundance required of this Chi-square test. A general association statistic was used to determine if some treatment combinations differed from others (McCall and Primack 1992, Menzel and Schmida 1993, Cunningham *et al.* 2006, Statistical Analysis System 2008).

Data collected at the family level were large enough to examine community composition among color and odor treatments using replicate count data with multivariate routines (MDS, ANOSIM) using PRIMER-E Software (Clark and Gorley 2006, Blum *et al.* 2006). Data were transformed (square root) to allow immediately and most abundant species to contribute to the analysis and preventing the most abundant species from dominating the similarity measure (Clarke and Warwick 2001). A Bray-Curtis dissimilarity matrix was produced using the square root transformed data. Ordination of the Bray-Curtis matrix was performed by non-metric multidimensional scaling (nMDS) where the data points are in the same rank order of the dissimilarities. A MDS 2-dimensional plot was generated that summarized the relationships of the samples where points that are closer together represent samples that are similar, while points that are farther apart represent samples that are different. Stress values of < 0.1 from the MDS analysis correspond to a good ordination while a stress value of < 0.2 indicates potential relationships between sample points (Clarke and Warwick 2001). An analysis of similarities (ANOSIM) tested for differences among treatment groups to gain a better understanding of the MDS analysis results. ANOSIM is a permutation test that produces a R statistic which is an absolute measure of the separation between treatment groups. The R statistic generally ranges between 0 and 1, with 0 indicating complete overlap and 1 indicating complete separation (Clarke and Gorley 2006). A

two-way crossed ANOSIM was used to make pairwise comparisons between the three colors in the color treatment and between the two scents in the odor treatment.

Results:

Insect Visitors on Real Plants

The Tangle-Trap[®] method (110 individuals) resulted in the greatest number of insect visitors collected, with few individual collected using Bottle traps (12 individuals) or during the transect walk (8 individuals). The 40 blooming *Trillium cuneatum* plants were visited by 94 insects distributed among six orders and 25 families, while the 17 blooming *T. grandiflorum* plants were visited by 36 insects distributed among six orders and 17 families (Table 2). Members of the order Homoptera visited *T. grandiflorum* but not *T. cuneatum* while members of the order Collembola visited *T. cuneatum* and not *T. grandiflorum*. However, the Chi-square analysis indicated that the overall relative abundance of individuals found were equally distributed among *T. cuneatum* and *T. grandiflorum* ($p = 0.2768$) and for each order and family of insect collected, the individuals were equally distributed between floral species ($p > 0.05$).

In contrast, the numbers of individuals collected within trillium species were not equally distributed among the orders for *T. cuneatum* ($p < 0.0001$, Chi-square) or *T. grandiflorum* ($p < 0.0001$, Chi-square). Diptera contained the greatest number of individuals for both floral species and was primarily represented by small flies, such as *Sciaridae* and *Chironomidae*. Few individuals (14 or less) of Araneae, Coleoptera, Collembola, Hemiptera and Hymenoptera visited either *Trillium* species. Within the order Hymenoptera, the family Formicidae that visited is associated with seed dispersal of trillium (Kalisz *et al.* 1999) while the other families were mostly very small parasitoid wasps (e.g., *Cynipidae*, *Platygastridae*) that prey on larvae of Coleoptera, Diptera,

Lepidoptera and other Hymenoptera (Borror and White 1970; Triplehorn and Johnson 2005).

The 10 non-blooming plants of *T. cuneatum* were visited by 42 individuals distributed among five orders and 17 families, while the three non-blooming plants of *T. grandiflorum* were visited by three individuals distributed among 1 order and 2 families. The abundance of insects was not randomly distributed among blooming and non-blooming plants of *T. cuneatum* with more individuals visiting non-blooming plants ($p = 0.0026$). There was a similar composition of orders and families that visited both blooming and non-blooming plants of *T. cuneatum* (Table 2). However, the abundance of insects was randomly distributed among blooming and non-blooming plants of *T. grandiflorum* ($p = 0.2012$). Although the insect abundance was randomly distributed, the composition of insects found on non-blooming plants of *T. grandiflorum* is different from that found on blooming plants (Table 2).

Insect Visitors on Artificial Flowers

During the course of this study, 24,549 individual insects were counted on artificial flowers with 6,669 individuals on wine-red flowers, 7,010 individuals on white flowers, 10,870 individuals on yellow flowers. Among the odor treatment 12,448 individuals were counted on non-scented flowers and 12,101 individuals were counted on scented flowers. Multiple orders and multiple families were found on each experimental treat combination.

Order-level Results

Insects collected on artificial flowers were represented by 16 orders. The greatest number of visitors belonged to the order Diptera, followed by Hymenoptera,

Table 2: Abundance of insects found on blooming and non-blooming *Trillium cuneatum* and *Trillium grandiflorum* plants from three collection methods: Tangle-Trap[®], bottle traps, transect walks. Chi-square analysis shows that orders and families of insects collected on blooming plants were equally distributed between species ($p > 0.05$), but orders were not equally distributed within each floral species ($p < 0.0001$). Chi-square analysis shows that abundance of insects were not equally distributed among blooming and non-blooming plants of both species ($p < 0.001$) with more individuals on blooming plants. Potential pollinators are represented by a P or P?.

Order/Family	Potential Pollinator	<i>Trillium cuneatum</i>				<i>Trillium grandiflorum</i>			
		tangle-foot	bottle trap	transect walk	no flower	tangle-foot	bottle trap	transect walk	no flower
Araneae									
Pholcidae		0	0	0	1	1	0	0	0
Salticidae		0	0	2	1	0	0	0	0
unknown		0	1	0	0	0	0	0	0
Coleoptera									
Coccinellidae	P	0	2	0	0	0	0	0	0
Curculionidae	P?	1	0	0	0	0	0	0	0
Elatерidae	P	4	0	0	1	1	0	0	0
Erotylidae	P	0	0	1	0	0	0	0	0
Collembola									
Entomybridae		0	1	0	0	0	0	0	0
Diptera									
Califoridae	P	3	0	0	0	1	0	0	0
Cecidomyiidae		6	3	0	0	3	0	0	0
Chironomidae	P	12	0	0	5	6	0	0	2
Drosophilidae	P	0	0	0	0	1	0	0	0
Fanniidae	P	0	1	0	0	0	0	0	0
Mycetophilidae	P	4	1	0	2	3	0	0	0
Phoridae	P	11	0	0	3	1	0	0	1
Psychodidae		2	0	0	2	1	0	0	0

Table 2: Continued

Order/Family	Potential Pollinator	<i>Trillium cuneatum</i>				<i>Trillium grandiflorum</i>			
		tangle-foot	bottle trap	transect walk	no flower	tangle-foot	bottle trap	transect walk	no flower
Diptera									
Scatopsidae	P?	3	0	0	0	0	0	0	0
Sciariidae	P	16	0	0	9	7	0	0	0
Tipulidae		1	0	0	3	0	0	0	0
Hemiptera									
Aphidae		1	0	0	3	1	0	1	0
Cicadellidae		1	1	0	0	1	0	0	0
Homoptera									
Psyllidae		0	0	0	0	2	0	0	0
Hymenoptera									
Braconidae		0	0	0	1	0	0	0	0
Ceraphronidae		0	0	0	0	1	0	0	0
Chalcididae		1	0	0	1	0	0	0	0
Cynipidae		3	0	0	1	3	0	0	0
Eulophidae		0	0	0	1	0	0	0	0
Eupelmidae		1	0	0	0	0	0	0	0
Formicidae		2	1	4	4	1	0	0	0
Ichneumonidae		2	0	0	1	0	0	0	0
Platygastridae		1	1	0	2	0	0	0	0
Tenthredinidae		0	0	0	0	1	0	0	0
Total Insects		75	12	7	41	35	0	1	3

Homoptera, Collembola, Coleoptera, and Araneae (Table 3). Of the nine orders with sufficient numbers to test for random distributions among the color and odor treatments three were not equally distributed for color and odor ($p < 0.01$), one was not equally distributed for color only ($p < 0.01$), and one was not equally distributed for odor only ($p < 0.01$) (Table 3). For the orders not equally distributed across colors, the greatest average numbers of individuals within orders were found on yellow flowers (42%). The average numbers of individuals within orders found on red and white flowers were similar at 30 and 28%, respectively (Figure 5a). For the orders not equally distributed across odors, the overall average numbers of individuals within order were found on scented flowers at 56% (Figure 5b). Of the eight orders analyzed using the Cochran-Mantel-Haenszel Chi-square test, 50% of the orders were equally distributed among the color by odor treatment combinations (Table 4). Patterns among orders for the color by scent combinations were not obvious however, Hymenoptera landed more frequently on scented flowers of all colors ($p < 0.05$).

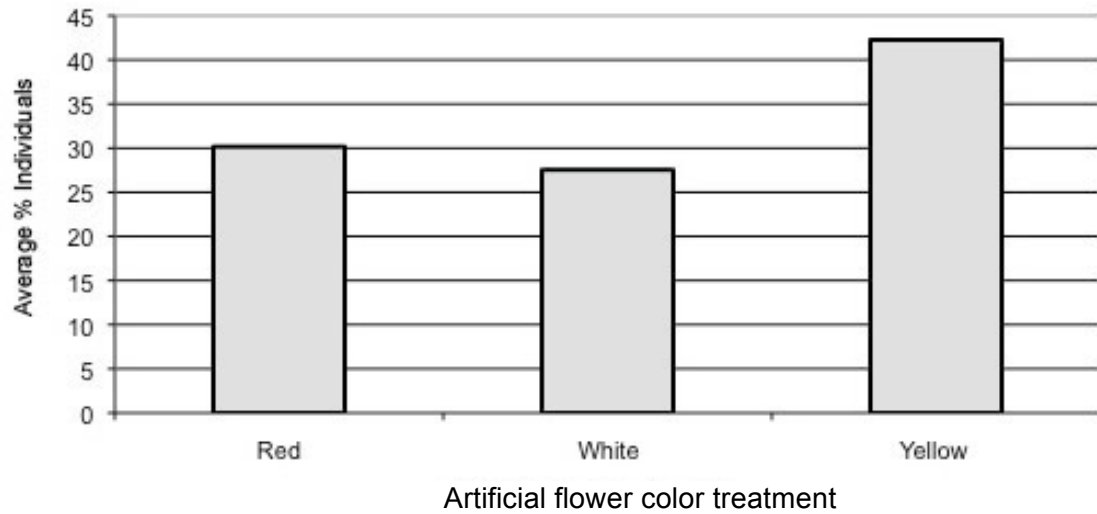
Family-level Results

Insect visitors collected represented 106 families. Families with the greatest numbers of visitors were Chironomidae, Sciaridae, Cecidomyiidae, and Phoridae (order Diptera), and Platygasteridae, Formicidae, and Cynipidae (order Hymenoptera) (Table 5). Forty-five of the 106 families had sufficient numbers to use Chi-square analysis to test for random distribution among color, and among odor treatments (Table 5). Within families, 19 were not equally distributed for color and odor ($p < 0.01$), nine were not equally distributed for color only ($p < 0.01$), and ten were not equally distributed for odor only ($p < 0.01$) (Table 6). For the families not equally distributed across colors, the greatest average numbers of individuals were found on yellow flowers (47%), while

Table 3: Abundance of insects within orders collected on artificial flowers of varying color and odor. Orders in bold print had sufficient numbers to analyze with Chi-square to test for distribution among color and odor treatments. P-values indicate orders with insects numbers not equally distributed among color or among odor treatment.

Order	Color Treatment				Odor Treatment		
	Red	White	Yellow	p-value	Scent	No Scent	p-value
Araneae	95	83	105	ns	126	157	ns
Coleoptera	135	148	123	ns	317	89	p < 0.0001
Collembola	282	222	211	p = 0.0022	300	415	p < 0.0001
Diptera	4999	5460	8486	p < 0.0001	9017	9928	p < 0.0001
Hemiptera	16	18	14	ns	26	22	ns
Homoptera	319	283	600	p < 0.0001	623	579	ns
Hymenoptera	820	776	1299	p < 0.0001	1673	1222	p < 0.0001
Isoptera	4	5	6	ns	10	5	ns
Thysanoptera	25	12	21	ns	28	30	ns
Blattodea	1	0	0	—	1	0	—
Ephemeroptera	0	1	1	—	2	0	—
Lepidoptera	2	1	1	—	0	4	—
Odonata	0	1	0	—	0	1	—
Psocoptera	0	0	1	—	1	0	—
Strepsiptera	0	3	0	—	1	2	—
Trichoptera	0	2	2	—	3	1	—

(a)



(b)

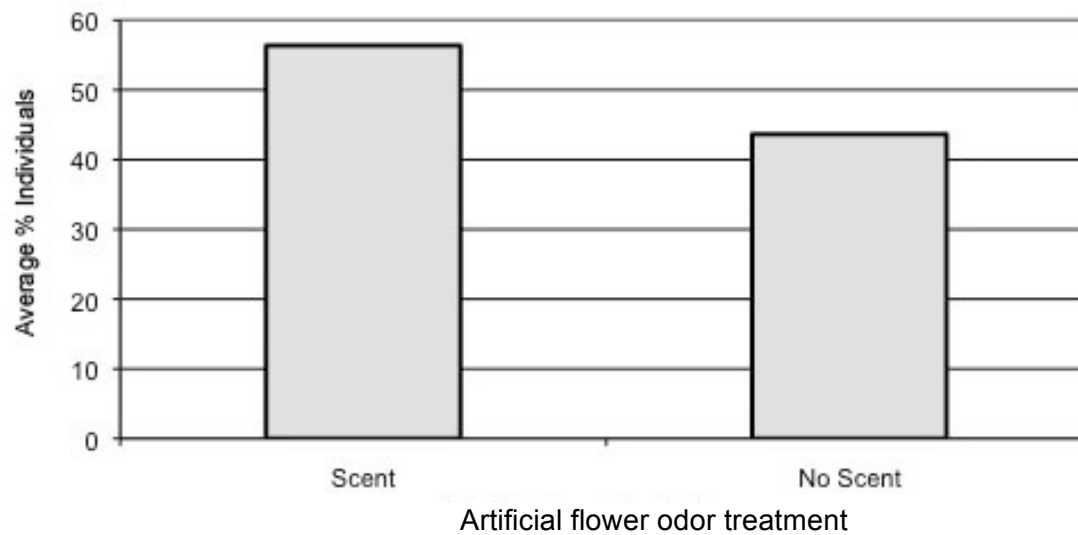


Figure 5: Average of the percent of individuals on artificial flower color (a) and odor (b) for orders of insects not equally distributed among colors or among odors ($p < 0.05$). An average of the percent individuals was calculated by adding the percent of individuals found on a particular treatment (e.g., red), dividing by the number of orders not equally distributed (for color in this case), multiplying by 100.

Table 4: Orders of insects analyzed for random distributions among the six color by odor treatment combinations. Orders in bold were not randomly distributed for at least one of the color by odor combination ($p < 0.05$).

Order	Red		White		Yellow	
	No Scent	Scent	No Scent	Scent	No Scent	Scent
Araneae	61	34	44	39	52	53
Coleoptera	28	107	31	117	30	93
Collembola	182	100	122	100	111	100
Diptera	2433	2566	3113	2347	4382	4104
Hemiptera	8	8	12	6	2	12
Homoptera	137	182	137	146	305	295
Hymenoptera	321	499	289	487	612	687
Thysanoptera	10	15	6	6	14	7

Table 5: Aundance of insects within families collected on artificial flowers of different color and odor treatments. Families in bold print had sufficient numbers to analyze with Chi-square to test for random distribution among color and odor treatments. Families of potential pollinators are represented with P and P?.

Order/Family	Potential Pollinators	Color Treatment			Odor Treatment	
		Red	White	Yellow	Scent	No Scent
<u>Araneae</u>						
Pholcidae		94	81	103	122	156
Salticidae		1	2	2	5	1
<u>Blattodea</u>						
Blattidae		1	0	0	1	0
<u>Coleoptera</u>						
Bostrichidae		22	7	8	29	8
Cantharidae	P	10	17	14	28	13
Carabidae		0	1	0	1	0
Cerambycidae	P	30	16	13	55	4
Chrysomelidae	P	2	16	5	20	3
Cleridae	P?	0	1	0	1	0
Coccinellidae		7	11	11	18	11
Curculionidae	P?	2	10	3	4	12
Dermestidae		0	1	0	1	0
Elateridae	P	13	12	18	26	15
Erotylidae		30	30	25	82	3
Histeridae		2	2	1	4	1
Monommidae		0	0	1	1	0
Mordellidae	P	0	1	0	1	0
Nitidulidae		3	1	1	3	2
Pselaphidae		0	2	0	2	0
Ptiliidae		1	0	0	0	1
Scarabaeidae		0	1	1	2	0
Staphylinidae		7	14	14	28	7
Tenebrionidae		1	0	0	1	0
Throscidae		3	0	3	5	1
<u>Collembola</u>						
Entomobryidae		270	215	192	274	403
Sminthuridae		12	7	19	26	12
<u>Diptera</u>						
Agromyzidae		0	1	5	1	5
Anthomyiidae	P?	0	1	0	1	0
Anthomyzidae	P?	3	1	1	5	0
Bibionidae		0	1	0	1	0
Calliphoridae		0	3	4	5	2
Cecidomyiidae		1130	1305	1177	1776	1836
Chironomidae		1542	1685	4464	3003	4688
Conopidae	P	0	1	1	0	2
Dolichopodidae		4	12	5	18	3
Drosophilidae	P?	278	193	135	562	44
Empididae	P	3	27	152	115	67
Fanniidae		1	32	41	66	8

Table 5: Continued

Order/Family	Potential Pollinators	Color Treatment			Odor Treatment	
		Red	White	Yellow	Scent	No Scent
<u>Diptera (continued)</u>						
Milichiidae		1	0	2	3	0
Muscidae		10	6	6	21	1
Mycetophilidae	P	94	111	135	253	87
Otitidae		1	3	1	5	0
Phoridae		325	449	540	724	590
Pipunculidae		0	0	2	2	0
Platypezidae		0	1	3	4	0
Psychodidae		59	48	59	77	89
Rhagionidae		0	0	1	1	0
Sarcophagidae		0	0	1	1	0
Scatopsidae		5	4	13	10	12
Sciaridae		1484	1539	1699	2272	2450
Sepsidae		0	3	0	2	1
Syrphidae		0	4	8	7	5
Tachinidae		0	1	0	1	0
Tephritidae	P	20	2	0	15	7
Tipulidae		19	27	31	44	33
Ephemeroptera						
Heptageniidae		0	1	0	1	0
unknown		0	0	1	1	0
<u>Hemiptera</u>						
Berytidae		1	0	0	0	1
Cercopidae		0	0	2	1	1
Corimelaenidae		13	16	11	24	16
Lygaeidae		1	0	0	0	1
Thyreocoridae		2	5	4	5	6
Tinigidae		1	1	1	0	3
<u>Homoptera</u>						
Aleyrodidae		0	2	2	2	2
Aphididae		245	228	275	420	328
Cicadellidae		62	49	279	172	218
Cixiidae		1	0	3	4	0
Membracidae		6	2	27	18	17
Psyllidae		5	3	14	8	14
<u>Hymenoptera</u>						
Andrenidae		0	0	2	2	0
Anthophoridae		4	10	44	22	36
Bethylidae		0	0	1	0	1
Braconidae		24	10	58	63	29
Ceraphronidae		24	31	38	44	49
Chalcididae		27	32	34	33	60
Cynipidae		129	142	281	245	307
Diapriidae		3	0	1	3	1
Eulophidae		31	60	70	118	43
Eupelmidae		0	1	0	0	1

Table 5: Continued

Order/Family	Potential Pollinators	Color Treatment			Odor Treatment	
		Red	White	Yellow	Scent	No Scent
<u>Hymenoptera (continued)</u>						
Eurytomidae		0	0	1	1	0
Formicidae		219	209	219	532	115
Halictidae		2	0	1	3	0
Ichneumonidae		40	84	114	153	85
Megachilidae		0	2	0	2	0
Megaspillidae		5	11	23	20	19
Mutillidae		0	0	10	10	0
Mymaridae		0	0	1	0	1
Perilampidae	P?	0	5	4	8	1
Platygastridae		291	148	311	323	427
Pompilidae		0	0	1	1	0
Pteromalidae		16	22	44	52	30
Scoliidae	P	0	1	0	1	0
Serphidae		0	1	1	2	0
Sphecidae		1	0	7	5	3
Tenthredinidae		3	6	29	25	13
Trichogrammatidae		0	1	2	3	0
Vespidae		1	0	2	2	1
<u>Isoptera</u>						
Kalotermitidae		4	5	6	10	5
<u>Lepidoptera</u>						
Crambidae		0	0	1	0	1
Gelechiidae		2	0	0	0	2
micro Lep		0	1	0	0	1
<u>Odonata</u>						
unknown		0	1	0	0	1
<u>Psocoptera</u>						
unknown		0	0	1	1	0
<u>Strepsiptera</u>						
Halictophagidae		0	3	0	1	2
<u>Thysanoptera</u>						
Thripidae		16	7	21	23	21
<u>Trichoptera</u>						
unknown		0	2	2	3	1
Totals		6669	7010	10,870	12,448	12,101

Table 6: Families of insects analyzed for color and odor treatment by Chi-square test. ns = those equally distributed ($p > 0.05$) and p-values represent those not equally distributed ($p < 0.05$) among treatments.

Order	Family	Color p-value	Odor p-value
Araneae	<i>Pholcidae</i>	ns	$p = 0.0414$
Coleoptera	<i>Bostrichidae</i>	$p = 0.0033$	$p = 0.0006$
	<i>Cantharidae</i>	ns	$p = 0.0191$
	<i>Cerambycidae</i>	$p = 0.0153$	$p < 0.0001$
	<i>Chrysomelidae</i>	$p = 0.0008$	$p = 0.0004$
	<i>Coccinellidae</i>	ns	ns
	<i>Curculionidae</i>	$p = 0.0386$	$p = 0.0455$
	<i>Elateridae</i>	ns	ns
	<i>Erotylidae</i>	ns	$p < 0.0001$
	<i>Staphylinidae</i>	ns	$p = 0.0004$
	Collembola	<i>Entomobryidae</i>	$p = 0.0008$
<i>Sminthuridae</i>		ns	$p = 0.0231$
Diptera	<i>Cecidomyiidae</i>	$p = 0.0011$	ns
	<i>Chironomidae</i>	$p < 0.0001$	$p < 0.0001$
	<i>Dolichopodidae</i>	ns	$p = 0.0011$
	<i>Drosophilidae</i>	$p < 0.0001$	$p < 0.0001$
	<i>Empididae</i>	$p < 0.0001$	$p = 0.0004$
	<i>Fanniidae</i>	$p < 0.0001$	$p < 0.0001$
	<i>Muscidae</i>	ns	$p < 0.0001$
	<i>Mycetophilidae</i>	$p = 0.0237$	$p < 0.0001$
	<i>Phoridae</i>	$p < 0.0001$	$p = 0.0002$
	<i>Psychodidae</i>	ns	ns
	<i>Scatopsidae</i>	$p = 0.0366$	ns
	<i>Sciaridae</i>	$p = 0.0004$	$p = 0.0096$
	<i>Tephritidae</i>	$p < 0.0001$	ns
	<i>Tipulidae</i>	ns	ns
Hemiptera	<i>Corimelaenidae</i>	ns	ns
Homoptera	<i>Aphididae</i>	ns	$p = 0.0008$
	<i>Cicadellidae</i>	$p < 0.0001$	$p = 0.0198$
	<i>Membracidae</i>	$p < 0.0001$	ns
	<i>Psyllidae</i>	$p = 0.0094$	ns
Hymenoptera	<i>Anthophoridae</i>	$p < 0.0001$	ns
	<i>Braconidae</i>	$p < 0.0001$	$p = 0.0004$
	<i>Ceraphronidae</i>	ns	ns
	<i>Chalcididae</i>	ns	$p = 0.0051$
	<i>Cynipidae</i>	$p < 0.0001$	$p = 0.0083$
	<i>Eulophidae</i>	$p = 0.0005$	$p < 0.0001$
	<i>Formicidae</i>	ns	$p < 0.0001$
	<i>Ichneumonidae</i>	$p < 0.0001$	$p < 0.0001$
	<i>Megaspillidae</i>	$p = 0.0016$	ns
	<i>Platygastridae</i>	$p < 0.0001$	$p = 0.0001$
<i>Pteromalidae</i>	$p = 0.0004$	$p = 0.0151$	
<i>Tenthredinidae</i>	$p < 0.0001$	ns	
Isoptera	<i>Kalotermitidae</i>	ns	ns
Thysanoptera	<i>Thripidae</i>	$p = 0.0322$	ns

average numbers were similar on red and white flowers (26 and 27% respectively) (Figure 6a). For the families not equally distributed across odors, the greatest average numbers of individuals were found on scented flowers (65%) (Figure 6b). Of the 17 families analyzed using the Cochran-Mantel-Haenszel Chi-square test, 32% were not equally distributed across color by scent combinations (Table 7). Patterns among families were difficult to assess and differences in color by scent really depended on which insect visitor was investigated.

It is interesting to note that families within an order that varied in visitation among color, odor, and color by scent combinations did not necessarily follow the pattern of visitation that was found for the order it belonged. For example, the order Coleoptera was not randomly distributed only among odors, but some families within this order were not randomly distributed for color along with odor treatments. However, consistent with order-level results, all families were not randomly distributed for color only (Table 8).

Results of the MDS-ordination for color and order (Figures 7a and 7b) do not show any clear patterns between the three color treatments or two odor treatments (stress = 0.17 for both analyses). However, ANOSIM indicated a difference between color treatments (Global R = 0.024, SL = 2.7%) but not for odor treatments (Global R = -0.026, SL = 91.5%) (Table 9). Pairwise tests indicate no difference in visitors between red and white (SL = 71.9%), but there is a difference in visitors between red and yellow (SL = 1.3%), and white and yellow (SL = 3.2%).

Discussion:

Insect Visitors on Real Plants

Numbers of insects visiting *T. cuneatum* and *T. grandiflorum* were low but consistent with other studies reporting visitation to *Trillium spp.* to be infrequent and

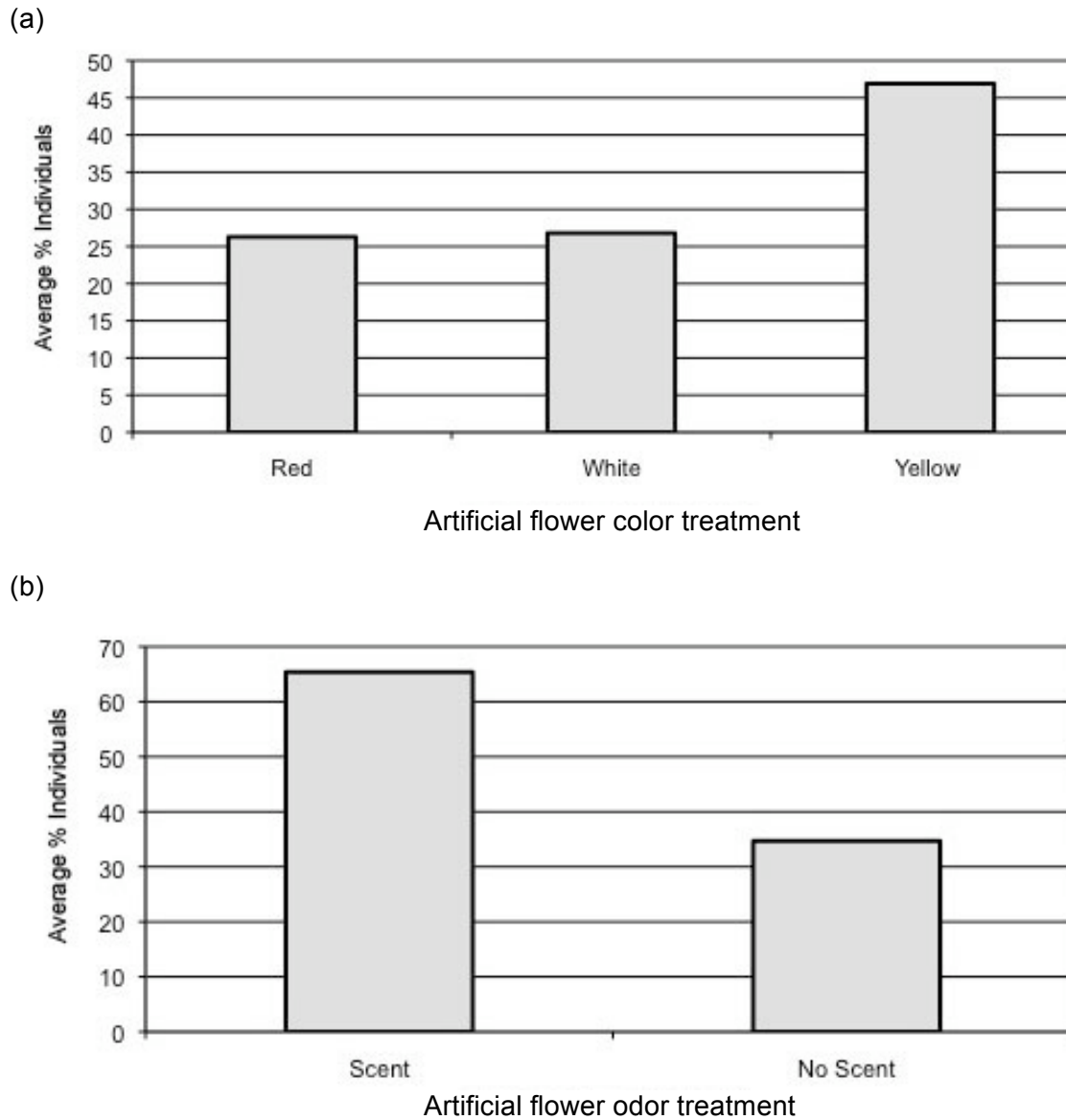


Figure 6: Average of the percent of individuals on artificial flower color (a) and odor (b) for families of insects not equally distributed among colors or among odors ($p < 0.05$). An average of the percent individuals was calculated by adding the percent of individuals found on a particular treatment (e.g., red), dividing by the number of orders not equally distributed (for color in this case), multiplying by 100.

Table 7: Families of insects analyzed for color by odor combination. Families in bold had at least one color by odor combination where odor was not randomly distributed within the color treatment ($p < 0.05$).

Order	Family	Red		White		Yellow	
		No Scent	Scent	No Scent	Scent	No Scent	Scent
Araneae	<i>Pholcidae</i>	61	33	43	38	52	51
Coleoptera	<i>Bostrichidae</i>	5	17	0	7	3	5
	<i>Cantharidae</i>	1	9	5	12	7	7
	<i>Cerambycidae</i>	0	30	0	16	4	9
	<i>Elateridae</i>	6	7	5	7	6	12
	<i>Erotylidae</i>	3	27	0	30	0	25
Collembola	<i>Staphylinidae</i>	3	4	4	10	0	14
	<i>Entomobryidae</i>	178	92	119	96	106	86
	<i>Sminthuridae</i>	4	8	3	4	5	14
Diptera	<i>Cecidomyiidae</i>	536	594	730	575	570	607
	<i>Chironomidae</i>	906	636	1277	408	2505	1959
	<i>Drosophilidae</i>	10	268	18	175	16	119
	<i>Empididae</i>	0	3	12	15	55	97
	<i>Fanniidae</i>	1	0	3	29	4	37
Diptera	<i>Muscidae</i>	0	10	1	5	0	6
	<i>Mycetophilidae</i>	20	74	35	76	32	103
	<i>Phoridae</i>	159	166	197	252	234	306
	<i>Psychodidae</i>	30	29	33	15	26	33
	<i>Sciaridae</i>	757	727	786	753	907	792
Hemiptera	<i>Tipulidae</i>	8	11	10	17	15	16
	<i>Corimelaenidae</i>	5	8	11	5	0	11
Homoptera	<i>Aphididae</i>	100	145	106	122	122	153
	<i>Cicadellidae</i>	30	32	26	23	162	117
	<i>Membracidae</i>	5	1	1	1	11	16

Table 7: Continued

Order	Family	Red		White		Yellow	
		No Scent	Scent	No Scent	Scent	No Scent	Scent
Hymenoptera	<i>Anthophoridae</i>	2	2	3	7	31	13
	<i>Braconidae</i>	7	17	2	8	20	38
	<i>Ceraphronidae</i>	15	9	13	18	21	17
	<i>Chalcididae</i>	13	14	24	8	23	11
	<i>Cynipidae</i>	71	58	87	55	149	132
	<i>Eulophidae</i>	7	24	15	45	21	49
	<i>Formicidae</i>	33	186	41	168	41	178
	<i>Ichneumonidae</i>	9	31	16	68	60	54
	<i>Megaspilidae</i>	4	1	7	4	8	15
	<i>Platygastridae</i>	151	140	73	75	203	108
Thysanoptera	<i>Pteromalidae</i>	7	9	4	18	19	25
	<i>Tenthredinidae</i>	1	2	2	4	10	19
	<i>Thripidae</i>	1	15	6	1	14	7

Table 8: Comparison of patterns of attraction for color and odor between families within orders. Families in bold are those that differ from the order level results. A check mark indicates where orders and families were not randomly distributed for color and/or odor treatments.

Order	Family	Color	Odor
Coleoptera			✓
	Bostrichidae	✓	✓
	<i>Cantharidae</i>		✓
	Cerambycidae	✓	✓
	Chrysomelidae	✓	✓
	Coccinellidae		
	Curculionidae	✓	✓
	Elateridae		
	<i>Erotylidae</i>		✓
	<i>Staphylinidae</i>		✓
Collembola		✓	✓
	<i>Entomobryidae</i>	✓	✓
	Sminthuridae		✓
Diptera		✓	✓
	Cecidomyiidae	✓	
	<i>Chironomidae</i>	✓	✓
	Dolichopodidae		✓
	<i>Drosophilidae</i>	✓	✓
	<i>Empididae</i>	✓	✓
	<i>Fanniidae</i>	✓	✓
	Muscidae		✓
	<i>Mycetophilidae</i>	✓	✓
	<i>Phoridae</i>	✓	✓
	Psychodidae		
	Scatopsidae	✓	
	<i>Sciaridae</i>	✓	✓
	Tephritidae	✓	
	Tipulidae		
Homoptera		✓	
	Aphididae		✓
	Cicadellidae	✓	✓
	<i>Membracidae</i>	✓	
	<i>Psyllidae</i>	✓	

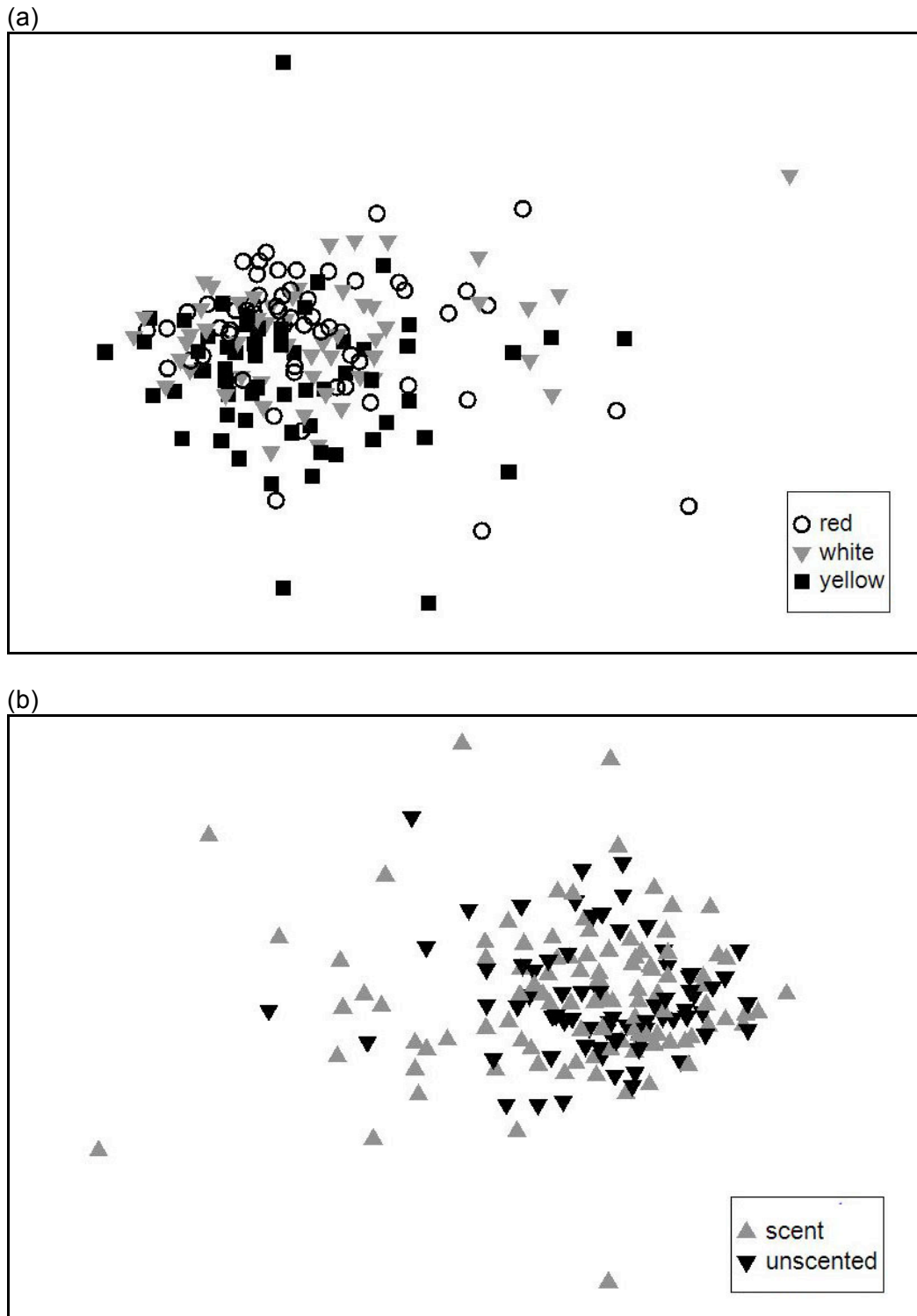


Figure 7: MDS ordination of the three color treatments (a), (stress = 0.17), and the two odor treatments (b), (stress = 0.17) based on $\sqrt{}$ transformed abundances of families and Bray-Curtis similarities. Each data point is an individual flower and points close together are more similar than points that are far apart.

Table 9: Pairwise comparisons of color and odor treatments using \sqrt{t} transformed data in ANOSIM.

Treatment	R	p-value
Color	0.024	0.027
red - white		0.719
red - yellow		0.013
white - yellow		0.032
Odor	-0.026	0.915

much lower than other spring blooming ephemerals (Motten 1986, Zomlefer 1996, Irwin 2000, Gonzales *et al.* 2006). It is typical of flowers with low insect visitation to show evidence of floral longevity and extended receptivity in the absence of pollination (Willson and Schemske 1980, Motten 1986, Lundemo and Totland 2007, Berjano *et al.* 2009). Berjano *et al.* (2009) found that floral longevity was related to insect numbers, finding that *Aristolochia baetica* L. had shorter floral longevity when the percent of flowers with pollinators was greater. The floral longevity of *Trillium* is reported to be longer than other spring blooming flowers and last up to three weeks (Motten 1986, Knight 2003). With insect visitation being low the longevity of trillium flowers could increase the chances of plants being visited by more than one insect necessary for pollination (Motten 1986, Schemske *et al.* 1978, Berjano *et al.* 2009).

Even though the two trillium species varied in color and scent, there was overlap in the overall numbers, orders, and families of insect visitors captured on the two flower species suggesting the insects were not responding to the that red versus white color cues or the scented versus unscented odor cues (Table 2). Thien *et al.* (1993) found the flowers of *Illicium floridanum* Ellis to be pollinated by the numerous insects with generalist foraging patterns that emerged from the leaf litter in early spring and that the emergence of flowers and insects was correlated; as the number of blooming flowers increased, the number of insects increased. This is similar to the results of my study where the insect families captured on the *Trillium* flowers were those which emerge from leaf litter beginning in early spring when weather warms (Borror and White 1970), therefore landing on the plants blooming in the area at that time. Many flower species in temperate habitats attract generalist insect visitors, especially when similar rewards are offered (Waser *et al.* 1996). The flowers of *Trillium grandiflorum* offer insect visitors with rewards of nectar and pollen, while the flowers of *T. cuneatum* offer insects with rewards

of pollen, therefore there is some overlap in floral rewards offered therefore, the lack of differences in insect visitors to the two *Trillium* species indicates that the insects are generalist insect foragers, visiting flowers of different floral cues that blooming during their lifecycle.

Families of small dipteran insects were the most abundant on both species and although they are characteristic of *T. cuneatum* (Zomlefer 1996, Gonzales *et al.* 2006), *T. grandiflorum* is primarily associated with hymenopteran visitors (*Bombus* spp.) (Irwin 2000, Knight 2003, Gonzales *et al.* 2006) and to a lesser extent dipterans (Irwin 2000). The abundance of Hymenoptera varies widely between habitats and in space and time (Triplehorn and Johnson 2005) therefore, it is likely that few Hymenoptera were present in the area during the duration of this study.

Diptera are generally an unspecialized group of visitors (Faegri and van der Pijl 1979, Lundemo and Totland 2007) and often one of the most abundant visitors (Galen and Kevan 1980, Kevan and Baker 1983, Eberling and Olesen 1999) that seem to switch between species (Galen and Kevan 1980). Multiple studies show small weakly flying Diptera such as *Sciaridae* and *Mycetophilidae* to be pollinators of species with a variety of colors and fetid odors such as *Stelis aemula* Schltr. (pale green to reddish, unpleasant odor), *Aristolochia californica* Torr. (green with purple stripes, unpleasant odor), *Listeria cordata* (L.) R. Br. (purple green, unpleasant odor), *Scoliopus bigelovii* Torrey (pale yellow or green with dark purple stripes, unpleasant odor), and *Trillium erectum* L. (wine-red, unpleasant odor) (Stebbins 1971, Mesler *et al.* 1980, Case and Case 1997, Irwin 2000). Although *T. grandiflorum* is unscented, insects were not discriminating against odor cues and visited both scented and unscented flowers.

Insect species richness and composition is largely determined by the vegetative characteristics of the area (Jonas and Joern 2007, Kato *et al.* 2008) and since the study

plots for *T. cuneatum* and *T. grandiflorum* were within a continuous stretch of mixed deciduous forest, insect species should overlap. Plants that offer similar sources of food can attract similar visitors (Bosch *et al.* 1997), therefore, despite differences in color and odor cues *T. cuneatum* and *T. grandiflorum* apparently offered similar rewards to the same potential pool of pollinators. Although floral cues such as color and odor are important for insects to find flowers, insects visiting *T. cuneatum* and *T. grandiflorum* are not specializing on a particular color or odor cue.

The generalist insect visitors found on blooming plants of both *Trillium* species were also found on non-blooming plants. Insect visitors were collected in greater abundance on non-blooming *T. cuneatum* than blooming plants. However, for *T. grandiflorum* abundance of insects collected were randomly distributed among blooming and non-blooming plants, but the assemblage of insect visitors was greater on blooming plants. From this study it is not clear why insects are landing on both blooming and non-blooming plants. However, some of the insects collected on both species of *Trillium* visit flowers because they feed on vegetation while some insects are parasitic and prey on other insects. Of those that are potential pollinators, many are generalist insect visitors and might land on anything in the area. In addition, the structure of blooming plants for the two *Trillium* species varies. The flower of *T. cuneatum* has erect twisted petals that are positioned on top of the large leaves (Case and Case 1997) therefore, insect visitors might use the leaves as a landing pad as it makes its way to the flower. The flower of *T. grandiflorum* is pedicelate and hangs below the leaves of the plant therefore, insect visitors might fly up and directly land on the flower instead of using the leaves as a landing pad. In my study site *Trillium* might be attracting a generalist population of insect visitors without a fixed preference for floral cues. These insects are those that happen to be in the area and emerge from the leaf litter beginning in the early spring and

are active during cool, foggy, or rainy weather conditions found during this time (Mesler *et al.* 1980).

Insect Visitors on Artificial Flowers

Insects collected in my study visited multiple flowers of varying floral cues, their degree of generalization can be seen along a gradient where color and odor cues did not matter to some insect families while for other insect families color and/or odor did attract more visitors (Table 5). For example, 15.5 % of families were randomly found on all color and odor treatments. In contrast, 20 % families indicated preferences in color only, 22.2 % families indicated preferences for odor only, and 42.2 % families indicated preferences for both color and odor. The differences in visitation to color and odor treatments varied in importance between each family collected. Families such as Coccinellidae (Coleoptera), Psychodidae (Diptera), and Ceraphronidae (Hymenoptera) are generalized, randomly visiting color and odor treatments. In contrast, families such as Cerambycidae (Coleoptera), Chironomidae (Diptera), and Platygastriidae (Hymenoptera) were more specialized, with greater abundance on specific color by odor treatment combinations. Although some families appeared to prefer particular color and/or odor treatments, all treatments were visited in high numbers indicating that even when insects visit floral cues in greater abundance there is still a degree of generalization. Therefore, generalized to specialized plant-insect interactions are not opposite ideas but a continuum with exact specialization rare (Waser *et al.* 1996, Gómez and Zamora 2006, Minckley and Roulston 2006, Gómez *et al.* 2007).

Visitation does not mean pollination (Hingston and McQuillan 2000, Waser *et al.* 1996) and greater visitation can lead to increased chances in pollen clogging the stigmatic receptors or transfer of incompatible pollen (Roy and Raguso 1997). This is

important and indicates that fewer visitors that pollinate effectively will increase plant fitness and effect pollination relating to an optimal level of pollinator diversity.

Diptera are one of the largest orders of insects and are found almost everywhere. However, abundance and time of emergence differs greatly (Triplehorn and Johnson 2005). Some of the dipteran families were found in low numbers even though the dull red, brown (Hingston and McQuillan 2000, Miller *et al.* 2011), or yellow (Weiss 2001) floral cues presented by artificial flowers are ones that dipterans are said to visit. The dipteran families found in low numbers might affect pollination but their abundance in my study site is low for at least the time period of my study.

Most of the Hymenoptera families recorded on real and artificial flowers were parasitoid wasps and prey on insect larvae in the orders of Diptera, Lepidoptera, Coleoptera and Hymenoptera (Triplehorn and Johnson 2005). Although some adult parasitoid wasps are known to visit open flowers for food sources of nectar or pollen or in search of prey, their importance as pollinators is unknown (Jervis *et al.* 1993, Tooker and Hanks 2000). In my study these Hymenoptera might have been present due to the abundance of potential Diptera prey (Triplehorn and Johnson 2005). However, ants (Formicidae) are common inhabitants of many ecological systems and are known seed dispersers of *Trillium*, and are often found feeding on the eliasomes of seeds (Kalisz *et al.* 1999, Junker *et al.* 2007).

Although Coleoptera visited *T. cuneatum*, this order has not been reported to be a visitor in the literature. However, the literature does report small Coleoptera families to visit and pollinate *Calycanthus* and possibly *Asimina* (Grant 1950, Willson and Schemske 1980, Knight 2003), which like *T. cuneatum* have wine-red flowers with a sweat to yeasty smell. Since *Calycanthus* and *Asimina* are offering similar floral rewards, orders such as Coleoptera could be viable pollinators of *T. cuneatum*. The

families within Coleoptera found in sufficient numbers to analyze on the artificial flowers are associated with flowers (Tripplehorn and Johnson 2005) while the families in low numbers were those typically found on the ground in the leaf litter feeding on fungi, fungi spores, dung, or are found on trees (Triplehorn and Johnson 2005).

Families of insects belonging to the orders Lepidoptera, Hemiptera, Homoptera, Psocoptera, and Thysanoptera varied in abundance but all families in these orders typically are nectar feeders (Table 5). The finding of some of these families in abundance on artificial flowers might be random and not associated with pollination. Families belonging to the orders Isoptera, Blattodea, Ephemeroptera, Odonata, Strepsiptera, and Trichoptera are associated with moist habitats and are often found near streams and are either parasitic or feed on other insects (Triplehorn and Johnson 2005). However, Blattodea (cockroaches) does feed on decaying tree bark (Triplehorn and Johnson 2005). Finding these insects families most likely was by chance, and while flying through the study site they randomly landed on an artificial flower.

Based on my results insect visitors at both the order and family level will visit multiple floral colors, but yellow flowers attracted the greatest number of individuals while white and red flowers attracted similar but fewer numbers of individuals. The lack of difference between white and red is consistent with my real flower results and the preference for yellow is consistent with the literature where yellow flowers have more visits than white or pink-red flowers (McCall and Primack 1992, Roy and Raguso 1997). The yellow color might contrast more with the background color of brown leaf litter early in the season and green vegetation later in the season and thus more easily signal insect visitors (Robertson 1928, Faegri and van der Pijl 1979, Menzel and Shmida 1993, Salzmann *et al.* 2007, Wright and Schiestl 2009, Miller *et al.* 2011). Studies have also shown that generalist insect visitors such as the dipterans found on real trillium flowers,

will often pick novel floral colors when that color is one that has previously been learned (Schaefer *et al.* 2004). Miller *et al.* (2009) summarized colors of the earliest spring blooming flowers as white, purples, light pinks and wine-red with more yellow flowers blooming later in the season. In my study site, the earliest blooming trillium and non-trillium species formed clumps of wine-red, white-pink, and white with yellow showing up slightly later in the season but overlapping with the other colors. The clumps of yellow in my study design could be acting as a novel floral color.

For most families, overall abundance of visits to scented and non-scented flowers were almost the same resulting in no difference in attraction within families analyzed overall in MDS and ANOSIM. However, when each individual order or family was analyzed, more of those that were not equally distributed among odors had greater visitation on scented flowers. Olfactory cues can elicit both a positive or negative response in insect visitation and in either case odor is influencing visitation structure through attraction, repellence, or associative learning (Junker *et al.* 2010). My results suggest that scented flowers increased visitation through attraction because for orders and families that varied among odor treatments, visitation was greater on scented flowers. For example, in my study the order Hymenoptera visited all colors and both odor treatments, but had higher visitation on scented flowers, indicating that scented flowers would more easily attract potential pollinator species in this order through attraction.

My results for color, odor, and the combination showed that floral cues are important to attract insect visitors and in some cases color and/or odor cues increased visitation (Table 5). However, the relative importance of the cue depends on the insect investigated. For example, Cecidomyiidae (Diptera) randomly visited color and odor treatments therefore, color and odor cues did not increase visitation of one color or scent

over the others. In contrast, Drosophilidae (Diptera) visited red flowers and scented flowers of all colors more frequently than other treatment combinations. Many studies show that floral cues are multisensory but combinations that were important and the value of one cue over another varies among orders and families within orders (McCall and Primack 1992, Roy and Raguso 1997, Wright and Schiestl 2009). For example in the order Lepidoptera, Raguso and Willis (2002) found the hawkmoth *Manduca sexta* L. prioritized odor over color and would not visit a flower if the visual cue was correct but lacked its olfactory cue. Omura and Honda (2005) found the butterfly *Vanessa indica* Herbst appeared to prioritize color over odor but the addition of an odor cue increased probability of floral visits. These studies are consistent with my results where visitors in the same order were using different floral cues or a combination of cues. For example, in the order Diptera, Mycetophilidae and Drosophilidae visited all floral colors but visited in higher numbers if the flower was scented. For Empididae, color appeared to be more important although more insects were collected from the scented flowers of yellow color. For insects that were found in too few numbers for analysis, it is difficult to determine if these insects are repelled by the floral cues studied or if these insects are just not abundant in this particular site because of the large variation in insect composition that can occur between and within habitats (McCall and Primack 1992, Hingston and McQuillan 2000, Gómez *et al.* 2007, Kato *et al.* 2008) or vary in space and time (Bosch *et al.* 1997, Hingston and McQuillan 2000).

An interesting result of my study was that some families of insects within a given order varied from the color and odor preference found at the order level (Table 8). This indicates that identification to at least the family level is important in pollination studies. Many pollination syndromes are described only at the level of order or above (Waser *et al.* 1996), but my findings show families do not always follow the same pattern of

attraction found at the order level. Syndromes might better predict insect visitors to flowers if they were described at the family level instead of at the order level. For example, reviews by Hingston and McQuillan (2000) and Miller *et al.* (2011) say that flowers pollinated by dipterans are dull red or brown in color and odorless, while a review by Menzel and Shmida (1993) said that flowers visited by dipterans were primarily yellow. However, at the family level the preference of floral color varies. Weiss (2001) reviewed studies that show syrphids, calliphorids, tephritids, and anthomyiids seem to prefer yellow flowers, while bombyliids tend to visit pink, blue, or violet flowers (Proctor *et al.* 1996). At the order level, my results show Diptera visited yellow and non-scented artificial flowers in greater abundance, while visits to red and white flowers were similar. While Chironomidae and Sciaridae (Diptera) in my study visited yellow flowers and non-scented flowers in greater abundance, this is not the case for all families. For example, Cecidomyiidae visited white flowers and non-scented flowers in greater abundance than red or yellow flowers, and Drosophilidae visited red flowers and scented flowers in greater abundance than white or yellow flowers. Therefore, not all families of insects will follow the general description of color or odor preferences shown at the order level.

In addition, *Trillium* plants and artificial flowers were in a clumped distribution increasing chances for intraspecific visitation important for pollen transfer. Many studies report insects to move short distances between flowers and show this movement to be constant, where insects visit several flowers of the same species before switching to another species (Schemske *et al.* 1978, Mesler *et al.* 1980, Chittka and Menzel 1992, Salzmann *et al.* 2007). Most artificial flowers in my study contained a relatively high diversity of insects indicating that the insects captured are generalists, visiting flowers such as *Claytonia virginica*, *Sanguinaria canadensis*, *Erythronium americanum* with similar rewards (nectar and pollen) that are available in the area. Many insects visited a

variety of my artificial flowers and in most cases did not visit just one color or one odor combination. Although one color or odor treatment may have been visited in greater numbers, other color or odor treatments were still visited. This may have been to reduce competition for a food source, the similar rewards that these different flowers offered, or the generalist nature of many insect species (Bosch *et al.* 1997, Waser *et al.* 1996).

The greater numbers of insects on artificial flowers compared to the real flowers indicates a large pool of potential pollinators, even though not all were landing on real flowers. Gómez *et al.* (2007) found that even for generalized plant and insect populations, an intermediate level of pollinator diversity was optimum because reproductive success of plants was greater. Their finding suggests that the quality of pollen distributed during insect foraging is greater when insect richness is intermediate because too high of a diversity of pollinators may increase delivery of geitonogamous pollen (Gómez *et al.* 2007). In my study area insect species diversity was high (106 families) however, there was not evenness among the families collected. More than 50% of families were found in low numbers (less than 15 individuals), indicating the balance of families found in large and small abundances might have represented an intermediate level of pollinator richness potentially creating an optimal level of pollinator diversity.

Even though there was no reward present, the floral cues of the artificial flowers were the same as those represented by trillium and other spring blooming plants in the area that had rewards. This indicates insect visitors could make choices based on floral cues. Although a greater number of families of insects were found on artificial flowers compared to the real trillium flowers, many families were found in low numbers. This is consistent with the literature where Gómez *et al.* (2007) found high species richness in each population but found few abundant species and many scarce species. The artificial

flowers could have received greater numbers of visitors because of the longer sampling period, and they did not vary in color or decrease in odor as the “flower” got older. The artificial flowers mimicked newly blooming flowers indicating a floral reward would be available and not already taken by another insect visitor (Waser *et al.* 1996, Chittka and Raine 2006).

Both artificial and real flowers had several families in low numbers indicating their floral cues might not be attractive to or could be repelling families found in low numbers (Junker and Blüthgen 2008). Alternatively, the feeding habits and food sources of insects in low numbers might differ from the study and these insects might not visit flowers for sources of food (Triplehorn and Johnson 2005, Gómez *et al.* 2007). In addition, pollinator assemblages and abundance vary greatly within and between habitats and in space and time and some insect families are naturally low in abundance (McCall and Primack 1992, Bosch *et al.* 1997, Hingston and McQuillan 2000, Triplehorn and Johnson 2005, Gómez *et al.* 2007, Kato *et al.* 2008).

In contrast to the insects in low abundance, dipteran species followed by hymenopteran and coleopteran families were the most abundant insect visitors on real and artificial flowers. Insects such as small *Diptera* spp. are seldom studied and are often discredited as viable pollinators because they carry low amounts of pollen, often switch between plant species (Galen and Kevan 1980, Irwin 2000), and thus are viewed as inefficient or ineffective pollinators (Kendall and Solomon 1973, Grace and Nelson 1981, Lundemo and Totland 2007). However, other studies have shown that these insects can be successful pollinators of flowers (e.g, Stebbins 1971, Mesler *et al.* 1980, Irwin 2000). It was also reported that flowers using dipteran species as pollinators rely on floral longevity (Motten 1986, Irwin 2000, Gonzales *et al.* 2006) because plants need to be visited by more than one insect for pollination to occur (Thien *et al.* 1983, Motten

1986, Berjano *et al.* 2009). Successful pollination of *Trillium* in my study area by Diptera is likely because both plant species have long-lived flowers and insect abundance in the study area is high for a portion of the insects captured. The artificial flowers also represented floral longevity mimicking newly blooming plants by maintaining floral color and odor throughout the study period (5 weeks) indicating they would continue to attract insects found to visit flowers in the area.

Summary

The results of my study are consistent with the literature where floral cues are important in the attraction of some insect visitors (Brody 1997, Berjano *et al.* 2009) and the relative importance of these cues depends on the insect visitor investigated (Roy and Raguso 1997, Wright and Schiestl 2009). My results show many insect visitors in my study site are small species that are difficult to observe in the field in contrast to much of the pollination literature that examined effects of floral cues on bees (Chittka and Thomson 1997, Chittka and Waser 1997, Chittka and Raine 2006). More studies of the behavior and pollination potential of these small weakly flying insects are needed. My study also indicated that generalization of plant species and insect visitors is a common characteristic and is best understood along a gradient of generalized to specialized (Waser *et al.* 1996, Gómez *et al.* 2007). Plants have to interact with the pollinators present, and often the pollinator of the highest quality is not the most abundant (Aigner 2001). Kearns (2001) also makes a case that small insects might be important when the most effective pollinators are not present. The ability to attract a generalist pollinator assemblage might be especially important where visitation is affected by varying weather conditions (McCall and Primack 1992, Lundemo and Totland 2007) and

advantageous in the event of environmental change (Hingston and McQuillan 2000) and in human altered ecosystems (Jules 1998, Johnson and Steiner 2000).

The results of my study have generated new questions related to the plant-pollinator interactions of *Trillium* and other early spring blooming flowers in the community. Many of the insect visitors of *T. cuneatum* and *T. grandiflorum* were small weakly flying dipterans. A follow up study would be to investigate their pollen loads and movements to determine if they are transferring pollen between flowers. In addition, because insect abundance varies in time and space more information is needed regarding within site variation of insect abundance and composition. Finally, a comparison of insect visitors of *T. cuneatum* and *T. grandiflorum* in other habitats is needed to determine if insect visitors of trillium vary between habitats, or if they are attracting a specific set of pollinators.

CHAPTER 4

LITERATURE CITED

- Aigner, P.A. 2001. Optimality modeling and fitness trade-offs: when should plants become pollinator specialists? *Oikos* 95: 177-184.
- Aker, C.L. and D. Udovic. 1981. Oviposition and pollination behavior of the yucca moth, *Tegiticula maculata* (Lepidoptera: *Proxidiidae*), and its relation to the reproductive biology of *Yucca whipplei* (Agavaceae). *Oecologia* 49: 96-101.
- Baker, H.G. 1963. Evolutionary mechanisms in pollination biology. *Science* 139: 877-883.
- Baker, H.G. and P.D.J. Hurd. 1968. Intrafloral ecology. *Annual Review of Entomology* 13: 385-414.
- Berjano, R., P.L. Ortiz, M. Arista, and S. Talavera. 2009. Pollinators, flowering phenology and floral longevity in two Mediterranean *Aristolochia* species, with a review of flower visitor records for the genus. *Plant Biology* 11: 6-16.
- Blum, J.C., A.L. Chang, M. Liljeström, M.E. Schenk, M.K. Steinberg, and G.M. Ruiz. 2007. The non-native solitary ascidian *Ciona intestinalis* (L.) depresses species richness. *Journal of Experimental Marine Biology and Ecology* 342: 5-14.
- Borror, D.J. and R.E. White. (eds). 1970. *Peterson Field Guides: Insects*. Houghton Mifflin Company. New York, NY.
- Bosch, J., J. Retana, and X. Cerdá. 1997. Flowering phenology, floral traits and pollinator composition in a herbaceous Mediterranean plant community. *Oecologia* 109: 583-591.
- Brody, A.K. 1997. Effects of pollinators, herbivores, and seed predators on flowering phenology. *Ecology* 78: 1624-1631.
- Burns, P.G. 2005. Impulsive bees forage better: the advantage of quick, sometimes inaccurate foraging decisions. *Animal Behavior* 70: e1-e5.
- Case, F.J. and R.B. Case. 1997. *Trilliums*. Timber Press. Portland, Oregon.
- Chapman, R.R. 1998. *The Insects: Structure and Functions*. Cambridge University Press. Cambridge
- Chittka, L. and R. Menzel. 1992. The evolutionary adaptation of flower colours and the insect pollinators' colour vision. *Journal of Comparative Physiology A* 171: 171-181.

- Chittka, L. and J.D. Thomson. 1997. Sensori-motor learning and its relevance for task specialization in bumble bees. *Behavioral Ecology and Sociobiology* 41: 385-398.
- Chittka, L. and N.M. Waser. 1997. Why red flowers are not invisible to bees. *Israel Journal of Plant Sciences* 45: 169-183.
- Chittka, L. and J.D. Thomson. 2001. *Cognitive Ecology of Pollination: Animal Behavior and Floral Evolution*. Cambridge University Press. Cambridge.
- Chittka, L. and N.E. Raine. 2006. Recognition of flowers by pollinators. *Current Opinion in Plant Biology* 9: 428-435.
- Clarke, K.R. and R.M. Warwick. 2001. *Change in marine communities: an approach to statistical analysis and interpretation*, 2nd ed. PRIMER-E, Plymouth, UK.
- Clarke, K.R. and R.N. Gorley. 2006. *PRIMER v6: User Manual/Tutorial*. PRIMER-E Ltd. Plymouth, UK.
- Crepet, W.L. 1983. The role of insect pollination in the evolution of the angiosperms. Pages 29-50 *in* Real L, (ed.). *Pollination Biology*. Academic Press, Orlando, Florida.
- Cunningham, J.P., C.J. Moore, M.P. Zalucki, and B.W. Cribb. 2006. Insect odour perception: recognition of odour components by flower foraging moths. *Proceeding of The Royal Society B* 273: 2035-2040.
- Davis, M.A. 1981. The effect of pollinators, predators, and energy constraints on the floral ecology and evolution of *Trillium erectum*. *Oecologia* 48: 400-406.
- Dieringer, G., L. Cabrera, R.M. Lara, L. Loya, and P. Reyes-Castillo. 1999. Beetle pollination and floral thermogenicity in *Magnolia tampulipana* (Magnoliaceae). *International Journal of Plant Sciences* 160: 64-71.
- Dobson, H.E.M. 1987. Role of flower and pollen aromas in host-plant recognition by solitary bees. *Oecologia* 72: 618-623.
- Dobson, H.E.M. 1994. *Floral volatiles in insect biology*. *Insect-Plant Interactions*. CRC Press. Boca Rafton, FL.
- Elberling, H. and J.M. Olesen. 1999. The Structure of a high latitude plant-flower visitor system: The dominance of flies. *Ecography* 22: 314-323.
- Eriksson, O. and B. Bremer. 1992. Pollination systems, dispersal modes, life forms, and diversification rates in angiosperm families. *Evolution* 46: 258-266.
- Faegri, K. and L. van der Pijl L. 1979. *The Principles of Pollination Ecology*, 3rd ed. Pergamon: Oxford.

- Fenster, C.B., W.S. Armbruster, P. Wilson, M.R. Dudash, and J.D. Thomson. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics* 35: 375-403.
- Galen, C. 1985. Regulation of seed-set in *Polemonium viscosum*: Floral scents, pollination, and resources. *Ecology* 66: 792-797.
- Galen, C. and P.G. Kevan. 1980. Scent and color, floral polymorphisms and pollination biology in *Polemonium viscosum* Nutt.. *American Midland Naturalist* 104: 281-289.
- Galizia, C.G. and R. Menzel. 2000. Probing the olfactory code. *Nature Neuroscience* 3: 853-854.
- Gegear, R.J. and T.M. Lavery. 2001. The effect of variation among floral traits on the flower constancy of pollinators. Pages 1-20. *in* Chittka, L. and J.D. Thomson (eds.). *Cognitive Ecology of Pollination: Animal Behavior and Floral Evolution*. Cambridge University Press: Cambridge.
- Goodrich, K.R., M.L. Zjhra, C.A. Ley, and R.A. Raguso. 2006. When flowers smell fermented: the chemistry and ontogeny of yeasty floral scent in Pawpaw (*Asimina Triloba*: Annonaceae). *International Journal of Plant Sciences* 167: 33-46.
- Goodrich, K.R., and R.A. Raguso. 2009. The olfactory component of floral display in *Asimina* and *Deeringothamnus* (Annonaceae). *New Phytologist* 183: 457-469.
- Gómez, J.M., and R. Zamora. 2006. Ecological factors that promote the evolution of generalization in pollination systems, Pages 145-166. *in* Waser, N.M. and J. Olerton (eds). *Plant-Pollinator Interactions from Specialization to Generalization*. The University of Chicago Press: Chicago.
- Gómez, J.M., J. Bosch, F. Perfectti, J. Fernández, and M. Abdelaziz. 2007. Pollinator diversity affects plant reproduction and recruitment: the tradeoffs of generalization. *Oecologia* 153: 597-605.
- Gonzales, E., J.L. Hamrick, P.E. Smouse and R.J Dyers. 2006. Pollen-mediated gene dispersal within continuous and fragmented population of a forest understorey species, *Trillium cuneatum*. *Molecular Ecology* 15: 2047-2058.
- Gonzales, E., J.L. Hamrick, and P.E. Smouse. 2008. Comparison of clonal diversity in mountain and piedmont population of *Trillium cuneatum* (Melanthiaceae-Trilliaceae), a forest understory species. *American Journal of Botany* 95: 1254-1261.
- Goulson, D., and N.P. Wright. 1998. Flower constancy in the hoverflies *Episyrphus balteatus* (Degeer) and *Syrphus ribesii* (L.) (*Syrphidae*). *Behavioral Ecology* 9: 213-219.

- Grace, J., and M. Nelson. 1981. Insect and their pollen loads at a hybrid *Heracleum* site. *New Phytologist* 87: 978-988.
- Grant, V. 1949. Pollination systems as isolating mechanisms in angiosperms. *Evolution* 3: 82-97.
- Grant, V. 1950. The pollination of *Calycanthus occidentalis*. *American Journal of Botany* 37: 294-297.
- Grant, V. 1952. Isolation and hybridization between *Aquilegia formosa* and *A. pubescens*. *El Aliso* 2: 341-359.
- GraphPad QuickCalcs. 2005. GraphPad Software, Inc, La Jolla, CA.
<http://www.graphpad.com>
- Guerenstein, P.G., M.G. Lorenzo, J.A. Nuñez, and C.R. Lazzari. 1995. Bakers-yeast, an attractant for baiting traps for chagas-disease vectors. *Experientia* 51: 834-837.
- Healy, S.D. and T.A. Hurley. 2001. Foraging and spatial learning in hummingbirds. Pages 127-147. in Chittka, L. and J.D. Thomson (eds.). *Cognitive Ecology of Pollination: Animal Behavior and Floral Evolution*. Cambridge University Press: Cambridge.
- Hegland, S.J., Ø. Totland. 2005. Relationships between species' floral traits and pollinator visitation in a temperate grassland. *Oecologia* 145: 586-594.
- Heinrich, B., P.R. Mudge, P.G. Deringis. 1977. Laboratory analysis of flower constancy in foraging bumblebees: *Bombus ternarius* and *B. terricola*. *Behaviour Ecology Sociobiology* 2: 247-265.
- Henning, J.A., Y.S. Peng, L.R. Teuber. 1992. Honey bee (*Hymenoptera: Apidae*) behavioral response to primary alfalfa (*Rosales, Fabaceae*) floral volatiles. *Journal of Economic Entomology* 85: 233-239.
- Herrera, C.M. 1996. Floral traits and plant adaptation to insect pollinators: a devil's advocate approach. Pages 65-87. in Lloyd, D.G. and S.C.H. Barrett (eds). *Floral biology: Studies on Floral Evolution in Animal Pollinated Plants*. Chapman and Hall: New York.
- Hill, L.M. 2005. The genus *Trillium* (Liliaceae) in Virginia. *Castanea* 70: 13-19.
- Hingston, A.B., and P.B. McQuillan. 2000. Are pollination syndromes useful predictors of floral visitors in Tasmania? *Austral Ecology* 25: 600-609.
- Honnay, O., and B. Bossuyt. 2005. Prolonged clonal growth: escape route or route to extinction? *Oikos* 108: 427-432.
- Horvitz, C.C., and Schemske, D.W. 1990. Spatiotemporal variation in insect mutualists of a neotropical herb. *Ecology* 71: 1085-1097.

- Irwin, R.E. 2000. Morphological variation and female reproductive success in two sympatric *Trillium* species: evidence for phenotypic selection in *Trillium erectum* and *Trillium grandiflorum* (Liliaceae). *American Journal of Botany* 82: 205-214.
- Janzen, D. 1979. How to be a fig. *Annual Review of Ecology and Systematics* 10: 13-51.
- Jervis, M.A., N.A.C. Kidd, M.G. Fitton, T. Huddleston, and H.A. Dawah. 1993. Flower-visiting by hymenopteran parasitoids. *Journal of Natural History* 27: 67-105.
- Johnson, S.D. and K.E. Steiner. 2000. Generalization versus specialization in plant pollinator systems. *Trends in Ecology and Evolution* 15: 140-143.
- Jonas, J.L. and A. Joern. 2007. Grasshopper (Orthoptera: Acrididae) communities respond to fire, bison grazing and weather in North American tallgrass prairie: a long-term study. *Oecologia* 153: 699-711.
- Jules, E.S. 1998. Habitat fragmentation and demographic change for a common plant: trillium in old-growth forest. *Ecology* 79: 1645-1656.
- Junker, R., A.Y.C. Chung, and N. Blüthgen. 2007. Interaction between flowers, ants and pollinators: additional evidence for floral repellence against ants. *Ecological Research* 22: 665-670.
- Junker, R., and N. Blüthgen. 2008. Floral scents repel potentially nectar-thieving ants. *Evolutionary Ecology Research* 10: 295-308.
- Junker, R.R., N. Höcherl, and N. Blüthgen. 2010. Responses to olfactory signals reflect network structure of flower-visitor interactions. *Journal of Animal Ecology* 79: 818-823.
- Kalisz, S., F.M. Hanzawa, S.J. Tonsor, D.A. Thiede, and S. Voigt. 1999. Ant-mediated seed dispersal alters pattern of relatedness in a population of *Trillium grandiflorum*. *Ecology* 80: 2620-2634.
- Kato, M., Y. Kosaka, A. Kawakita, Y. Okuyama, C. Kobayashi, T. Phimmith, and D. Thongphan. 2008. Plant-pollinator interactions in the tropical monsoon forests in southeast Asia. *American Journal of Botany* 95: 1375-1394.
- Kay, Q.Q.N. 1976. Preferential pollination of yellow-flowered morphs of *Raphanus raphanistrum* by *Pieris* and *Eristalis* spp. *Nature* 261: 230-232.
- Kearns, C.A., and D.W. Inouye. 1994. Fly pollination of *Linum lewisii* (Linaceae). *American Journal of Botany* 81: 1091-1095.
- Kearns, C.A. 2001. North American dipteran pollinators: assessing their value and conservation status. *Conservation Ecology* 5: 5-14.
- Kendall, D.A., and M.E. Solomon. 1973. Quantities of pollen on the bodies of insects visiting apple blossoms. *Journal of Applied Ecology* 10: 627-634.

- Kevan, P.G. and H.G. Baker. 1983. Insects as flower visitors and pollinators. *Annual Review of Entomology* 28: 407-453.
- Kevan, P.G., M. Giurfa, and L. Chittka. 1996. Why are there so many and so few white flowers? *Trends in Plant Sciences* 1: 280-284.
- Knight, T.M. 2003. Floral density, pollen limitation, and reproductive success in *Trillium grandiflorum*. *Oecologia* 137: 557-563.
- Knudsen, J.T., S. Andersson, and P. Bergmann. 1999. Floral scent attraction in *Geonoma macrostachys*, an understory palm of the Amazonian rain forest. *Oikos* 85: 409-418.
- Kremen, C., and T. Ricketts. 2000. Global perspectives on pollination disruptions. *Conservation Biology* 14: 1226-1228.
- Kudo, G., H.S. Ishii, Y. Hirabayashi, and T.Y. Ida. 2007. A test of the effect of floral color change on pollination effectiveness using artificial inflorescences visited by bumblebees. *Oecologia* 154: 119-128.
- Kunze, J., and A. Gumbert. 2001. The combined effect of color and odor on flower choice behavior of bumble bees in flower mimicry systems. *Behavioral Ecology* 12: 447-456.
- Laloi, D., O. Bailez, M.M. Blight, B. Roger, M-H. Pham-Delegue, and L.J. Wadhams. 2000. Recognition of complex odors by restrained and free flying honeybees, *Apis mellifera*. *Journal of Chemical Ecology* 26: 2307-2319.
- Landolt, P.J., H.C. Reed, J.R. Aldrich, A.L. Antonelli, and C. Dickey. 2001. Social wasps (Hymenoptera: Vespidae) trapped with acetic acid and isobutanol. *Florida Entomologist* 83: 609-614.
- Lundemo, S., Ø. Totland. 2007. Within-population spatial variation in pollinator visitation rates, pollen limitation on seed set, and flower longevity in an alpine species. *Acta Oecologica* 32: 262-268.
- Majetic, C.J., R.A. Raguso, and T. Ashman. 2009. The sweet smell of success: floral scent affects pollinator attraction and seed fitness in *Hesperis matronalis*. *Functional Ecology* 23: 480-487.
- Mauseth, J.D. 1998. *Botany: An Introduction to Plant Biology*. Jones and Bartlett Publishers, Inc. Sundbury, MA.
- McCall, C., and R.B. Primack. 1992. Influence of flower characteristics, weather, time of day, and season of insect visitation rates in three plant communities. *American Journal of Botany* 79: 434-442.
- Menzel, R., and A. Shmida. 1993. The ecology of flower colors and the natural color vision of insect pollinators: the Israeli flora as a study case. *Biological Review* 68: 81-120.

- Mesler, M.R., J.D. Ackerman, and K.L. Lu. 1980. The effectiveness of fungus gnats as pollinators. *American Journal of Botany* 67: 564-567.
- Miller, R.S., S. Passoa, R.D. Waltz, and V. Mastro. 1993. Insect removal from sticky traps using a citrus oil solvent. *Entomological News* 104: 209-213.
- Miller, R., S.J. Owens, and B. Rorslett. 2011. Plants and colour: flowers and pollination. *Optics and Laser Technology* 43: 282-294.
- Minckley, R.L., and T.H. Roulston. 2006. Incidental mutualisms and pollen specialization among bees. Pages 69-98. *in* Waser, N.M. and J. Olferton. *Plant-pollinator Interactions from Specialization to Generalization*. The University of Chicago Press: Chicago.
- Mitchell, W.A. 1989. Informational constraints on optimally foraging hummingbirds. *Oikos* 55: 145-154.
- Mitchell, R.J., R.E. Irwin, R.J. Flanagan, and J.D. Karron. 2009. Ecology and evolution of plant-pollinator interactions. *Annals of Botany* 103: 1355-1363.
- Motten, A.F. 1986. Pollination ecology of the spring wildflower community of a temperate deciduous forest. *Ecological Monographs* 56: 21-42.
- Omura, H. and K. Honda. 2005. Priority of color over scent during flower visitation by adult *Vanessa indica* butterflies. *Oecologia* 142: 588-596.
- Orbison, L., and M. Ruiz. 1999. A guide to the Botanical Gardens at Asheville: wildflower blooming dates throughout the southeast. Asheville, NC. The Botanical Gardens at Asheville.
- Pellmyr, O. 2002. Pollination by animals. Pages 157-184. *in* Herrera, C.M., and O. Pellmyr (eds). *Plant-animal Interactions, An evolutionary Approach*. Blackwell Publishing.
- Pettersson, M.W. 1991. Pollination by a guild of fluctuating moth populations option for unspecialization in *Silene vulgaris*. *Journal of Ecology* 79: 591-604.
- Preacher, K. J. 2001. Calculation for the chi-square test: an interactive calculation tool for chi-square tests of goodness of fit and independence [Computer software]. Available from <http://quantpsy.org>.
- Proctor, M., P. Yeo, and A. Lack. 1996. *The natural history of pollination*. Timber Press: Portland, OR.
- Raguso, R.A. 2001. Floral scent, olfaction, and scent-driven foraging behavior. Pages 83-105. *in* Chittka, L., and J.D. Thomson (eds). *Cognitive Ecology of Pollination*. Cambridge University Press: Cambridge.

- Raguso, R.A. 2004a. Flowers as sensory billboards: progress towards an integrated understanding of floral advertisement. *Current Opinion in Plant Biology* 7: 434-440.
- Raguso, R.A. 2004b. Why are some floral nectars scented? *Ecology* 85: 1486-1494.
- Raguso, R.A. 2008. Start making scents: the challenge of integrating chemistry into pollination ecology. *Chemistry in Pollination* 128: 196-207.
- Raguso, R.A., and M.A. Willis. 2002. Synergy between visual and olfactory cues in nectar feeding by naive hawkmoths, *Manduca sexta*. *Animal Behaviour* 64: 685-695.
- Raguso, R.A. and M.A. Willis. 2005. Synergy between visual and olfactory cues in nectar feeding by wild hawkmoths, *Manduca sexta*. *Animal Behavior* 69: 407-418.
- Renner, S.S. 2006. Rewardless flowers in the angiosperms and the role of insect cognition in their evolution. Pages 123-144. *in* Waser, N.M. and J. Olferton (eds). *Plant-pollinator Interactions from Specialization to Generalization*. The University of Chicago Press: Chicago.
- Richardson, D.M. N. Allsopp, C.M. D'Antonio, S.J. Milton, and M. Rejmánek. 2000. Plant invasions-the role of mutualisms. *Biological Review* 75: 63-99.
- Robertson C. 1928. Flowers and insects: XXV. *Ecology* 9: 505-526.
- Roy, B.A., and R.A. Raguso. 1997. Olfactory versus visual cues in a floral mimicry system. *Oecologia* 109: 414-426.
- Sahley, C.T. 1996. Bat and hummingbird pollination of an autotetraploid columnar cactus, *Weberbauerocereus weberbaueri* (*Cactaceae*). *American Journal of Botany* 83: 1329-1336.
- Salzmann, C.C., A.M. Nardella, S. Cozzolino, and F.P. Schiestl. 2007. Variability in floral scent in rewarding and deceptive orchids: the signature of pollinator-imposed selection? *Annals of Botany* 100: 757-765.
- Salzmann, C.C., and F.P. Schiestl. 2007. Odour and colour polymorphism in the food-deceptive orchid *Dactylorhiza romana*. *Plant Systematics and Evolution* 267: 37-45.
- Schaefer, H.M., V. Schaefer, and D.J. Levey. 2004. How plant-animal interactions signal new insights in communication. *Trends in Ecology and Evolution* 19: 577-584.
- Schemske, D.W., M.F. Willson, M.N. Melampy, L.J. Miller, L. Verner, K.M. Schemske, and L.B. Best. 1978. Flowering ecology of some spring woodland herbs. *Ecology* 59: 351-366.
- Schiestl, F.P. 2010. The evolution of floral scent and insect chemical communication. *Ecology Letters* 13: 643-656

- Serota, C.A., and B.W. Smith. 1967. The cyto-ecology of four species of *Trillium* from Western North Carolina. *American Journal of Botany* 54: 169-181.
- Statistical Analysis System. 2008. SAS Version 9.2. Statistical Analysis System Institute, Cary, NC
- Stebbins, G.L. 1970. Adaptive radiation of reproductive characteristics in angiosperms. I: pollination mechanisms. *Annual Review of Ecology and Systematics* 1: 307-326.
- Stebbins, G.L. 1971. California pipe vine: a light trap for unwary flies. *California Native Plant Society Newsletter* 7: 4-5.
- Strauss SY. 1997. Floral characters link herbivores, pollinators, and plant fitness. *Ecology*. 78: 1640-1645.
- Thien, L.B., D.A. White, and L. Yatsu. 1983. The reproductive biology of a relict – *Illicium floridanum* Ellis. *American Journal of Botany* 70: 719-727.
- Thies, N., and R.A. Raguso. 2005. The effect of pollination on floral fragrance in thistles. *Journal of Chemical Ecology* 31: 2581-2600.
- Thies, N., M. Lerdau, and R.A. Raguso. 2007. The challenge of attracting pollinators while evading floral herbivores: patterns of fragrance emission in *Cirsium arvense* and *Cirsium repandum* (Asteraceae). *International Journal of Plant Sciences* 168: 587-601.
- Thomson, J. 2003. When is it mutualism? *The American Naturalist* 162: S1-S9.
- Tooker, J.F. and L.M. Hanks. 2000. Flowering plant hosts of adult hymenopteran parasitoids of central Illinois. *Conservation Biology and Biodiversity* 93: 580-588.
- Totland, Ø. 1994. Influence of climate, time of day and season, and flower density on insect flower visitation in Alpine Norway. *Arctic and Alpine Research* 26: 66-71.
- Triplehorn, C.A. and N.F. Johnson. 2005. Borror and DeLong's introduction to the study of insects, 7th ed. Brooks/Cole: Belmont, CA.
- USDA. 2009. US Department of Agriculture Plants Database. Accessed on April 15, 2011. <http://plants.usda.gov>
- Van den Berg, J., B. Torto, J.A. Pickett, L.E. Smart, L.J. Wadhams, and C.M. Woodcock. 2008. Influence of visual and olfactory cues on field trapping of the pollen beetle, *Astylus atromaculatus* (Col.: Melyridae). *Journal of Applied Entomology* 132: 490-496.
- Van der Pijl, L. 1961. Ecological aspects of flower evolution. II. Zoophilous flower classes. *Evolution* 15: 44-59.
- Waser, N.M. 1983. The adaptive nature of flower traits: ideas and evidence. Pages 241-285. in Real, L.A. (ed.). *Pollination Biology*. Academic Press: Orlando, FL.

- Waser, N.M. 1986. Flower constancy: definition, cause, and measurement. *American Naturalist* 127: 593-603.
- Waser, N.M. 2006. Specialization and generalization in plant-pollinator interactions: a historical perspective. Pages 3-17. *in* Waser, N.M. and J. Ollerton (eds). *Plant-pollinator Interactions from Specialization to Generalization*. The University of Chicago Press: Chicago.
- Waser, N.M., and M.V. Price. 1981. Pollinator choice and stabilizing selection for flower color in *Delphinium nelsonii*. *Evolution* 35: 376-390.
- Waser, N.M., L. Chittka, M.V. Price, N.M. Williams, and J. Ollerton. 1996. Generalization in pollination systems, and why it matters. *Ecology* 77: 1043-1060.
- Weiss, M.R. 2001. Vision and learning in beetles, flies, moths, and butterflies. Pages 171-190. *in* Chittka, L. and J.D. Thomson. *Cognitive Ecology of Pollination: Animal Behavior and Floral Evolution*. Cambridge University Press: Cambridge.
- Wells, P.H., and H. Wells. 1985. Ethological isolation of plants 2. Odour selection by honeybees. *Journal of Apicultural Research* 24: 86-92.
- Willmer, P.G., and G.N. Stone. 1997. How aggressive ant-guards assist seed-set in *Acacia* flowers. *Nature* 388: 165-167.
- Willson, M.F., and D.W. Schemske. 1980. Pollinator limitation, fruit production, and floral display in pawpaw (*Asimina triloba*). *Bulletin of the Torrey Botanical Club* 107: 401-408.
- Wilson, P., and M.C. Castellanos, J.N. Hogue, J.D. Thomson, and W.S. Armbruster. 2004. A multivariate search for pollination syndromes among penstemons. *Oikos* 104: 345-361.
- Winter, Y., and O. von Helversen. 2001. Bats as pollinators: foraging energetics and floral adaptations. Pages 148-170. *in* Chittka, L., and J.D. Thomson (eds). *Cognitive Ecology of Pollination: Animal Behavior and Floral Evolution*. Cambridge University Press: Cambridge.
- Woolley, C., C.F.G. Thomas, L. Hutchings, S. Goodacre, G.M. Hewitt, and S.P. Brooks. 2007. A novel trap to capture ballooning spiders. *Journal of Arachnology* 35: 307-312.
- Wright, G.A., and F.P. Schiestl. 2009. The evolution of floral scent: the influence of olfactory learning by insect pollinators on the honest signaling of floral rewards. *Functional Ecology* 23: 841-851.
- Wyatt, R. 1983. Pollinator-plant interaction and the evolution of breeding systems. Pages 51-95. *in* Real, L. (ed.), *Pollination Biology*. Academic Press.

Zjhra, M., R. Raguso, and A. Boyd. 2007. Components of floral attraction in a functionally specialized but ecologically generalized flower guild. National Science Foundation Ecology Grant Proposal.

Zomlefer, W.B. 1996. The Trilliaceae in the southeastern United States. Harvard Papers in Botany 9: 91-120.