CAN SPIDERS (ARGIOPE AURANTIA) INDIRECTLY AFFECT THE FITNESS OF ORANGE CONEFLOWERS (RUDBECKIA FULGIDA) BY LIMITING POLLINATOR VISITATION?

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CAN SPIDERS (ARGIOPE AURANTIA) INDIRECTLY AFFECT THE FITNESS OF ORANGE CONEFLOWERS (RUDBECKIA FULGIDA) BY LIMITING POLLINATOR VISITATION?

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ABSTRACT

The purpose of this research was to test for potential antagonist-mediated effects of orb-web building spiders (*Argiope aurantia*) on the pollinator visitation rate due to the presence of an orb-web building spider on the visitation time of pollinating insects to the Orange Coneflower (*Rudbeckia fulgida*). Orb-web building spiders have not been thoroughly studied in predator-pollinator-plant systems, and understanding their role may shed some light on the ecology of multi-species interactions. To test for indirect effects of orb-web building spiders on insect visitation to plants, a small-scale manipulative experiment was conducted at a 6x6m, off-road, grassy patch during August of 2007 about 30 meters northeast of the University of Akron Field Station at the Bath Nature Preserve (41° 10'53" N; 81° 39'05" W) in Bath, OH. Pollinator visitation to evenly spaced *R. fulgida* plants was recorded on 11 weather-permitting days during the hours of 0900 and 1600. Three replicates of three treatments groups (spider with web, web, and control) were set up in mesh covered hardware cloth frames, and each frame with the associated treatment was randomly assigned next to individual potted plants. A univariate ANOVA did not support the effect of the presence of *A. aurantia* on pollinators' visitation time to *R. fulgida*. The inconclusive results could indicate a lack of treatment effects due to the design of the experiment, and/or the ecology of the system.

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CHAPTER I

INTRODUCTION

Multi-species interactions:

Interactions between three or more trophic levels have not been as thoroughly studied as two level systems, yet may be important for the evolution and ecology of ecological communities (Strauss and Irwin 2004). These complex interactions across trophic groups are commonly known as multi-trophic or multi-species interactions (Tscharntke and Hawkins 2002) and will hereafter be referred to as multi-species interactions. Multi-species interactions may include both below and aboveground constituents in terrestrial (Schmitz et al. 2000), aquatic (Hill and Lodge 1995), and mixed systems (Knight et al. 2005a). The consideration of multi-species interactions may have implications for biological control in agricultural systems (Tscharntke and Hawkins 2002). The knowledge of multi-species interactions is limited for both agricultural systems and natural systems; empirical studies examining interactions at multiple functional levels are needed to understand the complexities of these systems (Tscharntke and Hawkins 2002).

Top-down ecology:

One specific type of multi-species interaction is a top-down interaction (Tscharntke and Hawkins 2002). Hairston, Smith, and Slobodkin (1960) mentioned the

idea of the top-down regulation of populations at lower trophic levels by predation from higher levels. Their paper discussed how predators might be regulating plant populations through the reduction of herbivore populations (Hairston et al. 1960). A classic top-down example found sea urchin populations to decline due to predation by sea otters, triggering a dramatic increase in the diversity and biomass of sea kelp (Duggins 1980). The increase was attributed to sea otters regulating populations of urchins that over-grazed the kelp beds in the absence of predators (Duggins 1980). Since the introduction of this idea, top-down effects have been found in a multitude of systems, including aquatic (Hill and Lodge 1995) terrestrial (Schmitz et al. 2000, Pace et al. 1999), and mixed aquatic and terrestrial systems (Knight et al. 2005a). It should be kept in mind that "top-down" ecology generally encompasses trophic-centered, population-level dynamics in a unidirectional cascade down trophic levels, but may include behavioral dynamics as well (Reader et al. 2006). Furthermore, top-down effects are not thought to be prominent in all ecological systems (Strong 1992) such as complex forest ecosystems (Gruner 2004), but may play an important role in community-level ecology through predation effects on mutualists, which may limit plant reproduction such as seed output (Knight et al. 2005a).

It should be known that examples of bottom-up effects have been found, but certain types of effects such as trait-mediated indirect effects have not been thoroughly studied in these systems (Werner and Peacor 2003). Bottom-up effects can be characterized as the effects of plant-centered traits and ecosystem qualities that may affect the population dynamics at higher trophic levels (White 1978, from Knight et al. 2006). Some of these density and population altering effects have been attributed to plant

resource limitations, chemical compounds that may deter herbivores from grazing, and active responses of prey such as predator avoidance (see Power 1992, White 1978).

Top-down versus bottom-up regulation of populations is debated, but both types of effects have been found to be significant (Hunter et al. 1997, Gripenberg and Roslin 2007). A suggested shift in paradigm moves from searching for the presence of an effect to quantifying the magnitude of these effects in trophic webs (Hunter and Price 1992) while taking spatial context into account (Gripenberg and Roslin 2007).

CHAPTER II

BACKGROUND AND NEED

Predator-Pollinator-Plant interactions:

The effects of predation on pollinators has not been extensively studied in terrestrial multi-species ecology (Suttle 2003), but may have an important role in terrestrial interactions among predators, pollinators and plants. For instance, predation on pollinators by bee wolves (Dukas 2005) and lizards (Munoz and Arroyo 2004) can affect fruit set (Dukas 2005) and seed output (Munoz and Arroyo 2004) by reducing pollinator densities (Dukas 2005) and pollinator visitation rates (Munoz and Arroyo 2004). The strength of these types of top-down effects of predators on plants through interactions with pollinators can be substantial. A meta-analysis of 21 studies examining visitation rate and reproductive success in multi-species studies specifically involving interactions among predators, pollinators and plants found a mean decrease of 40.5% in visitation rate and 25% in reproductive success due to the presence of predators (Knight et al. 2006). These strong, indirect effects of predators on plants through interactions with pollinators can have important implications for multi-species level ecology, and will be discussed below.

Some widely known natural predators of insect pollinators are: birds, reptiles, and other invertebrates. One particular group of invertebrates known to prey upon pollinating insects is the spiders (Olive 1980; Bilsing 1920; Nyffeler and et al. 1987; Morse 1986;

Dukas and Morse 2003; Tahir et al. 2009; personal observation). There is an estimate of over 50,000 species of spiders in the world, many of which prey on pollinating insects (Turnbull 1973). Spiders are a group of almost exclusively carnivorous predators with the exception of one recently described herbivorous species (Meehan 2009). This fact of functional exclusivity makes them an ideal group to examine multi-species level effects. Spiders have been found to affect the visitation of plants by pollinating insects by altering insect behavior (Dukas and Morse 2003; Suttle 2003; Robertson and Maguire 2005; Goncalves-Souza et al. 2008; Reader et al. 2006). Because pollinator visitation may be linked to plant fitness (Vasquez et al. 2005), these ubiquitous predators may be an important, yet overlooked, group of organisms guiding community-level interactions.

Community-level effects triggered by spiders may enhance plant species diversity characteristics such as species evenness by suppressing dominant species and allowing for less competitive species to persist (Schmitz 2003). Multi-species interactions involving orb-web building spiders could be a potentially crucial system contributing to the ecology and evolution of community-level interactions (Strauss and Irwin 2004) and ecosystem function (Schmitz 2008). Studying the effects of spider predation is also important to understand their role as biological control agents (Greenstone 1999). Although spiders are not viewed as the optimal group for biological control in all systems (Riechert and Lockley 1984), some orb-weaving spiders have been found to have the potential to reduce insect populations and were suggested for possible use as biological control agents in rice fields (Tahir et al. 2009). Because spiders are oftentimes generalists and their predation is not exclusive to herbivores, the effects of orb-web

building spiders on plant mutualists may be an important aspect of multi-species dynamics requiring consideration (Knight et al. 2006). In addition to direct spider predation, trait-mediated indirect effects caused by predators may also affect multi-species dynamics (Werner and Peacor 2003). Empirically examining these effects can help define community relationships and experimentally quantify the magnitude of these relationships (Bradley 1983).

There are three major components of this spider-pollinator-plant system: the link from pollinators to plants, the link from spiders to pollinators, and the indirect link from spiders to plants. These three components will be briefly described below. The indirect link from orb-web building spiders to plants has not been thoroughly examined and is the topic of this research.

Link from pollinators to plants:

Pollination is necessary for the reproduction of a vast number of plants, especially those that are solely dependent on animal vectors for the dispersal of pollen (Wilcock and Neiland 2002). The number of grains deposited on female reproductive parts is a function of the number of grains picked up by pollinators during their time spent foraging (Harder and Thomson 1989). Pollinator visitation is thus a necessary component of plant reproduction in certain systems. If plants are pollen limited, a reduced amount of pollen deposited to receptive flowers may reduce the number of seeds produced (Ashman et al. 2004) and may ultimately affect long-term plant fitness (Burd 1994).

Pollen limitation:

One way by which the reduction of pollinator populations may affect plant fitness is by decreasing the reproductive ability of pollinator dependent plants through inadequate pollen delivery (Haig and Westoby 1988). This decrease in seed or fruit production due to inadequate pollen receipt is known as pollen limitation (Knight et al. 2005). Pollen limitation is the underlying mechanism behind the reduction of plant fitness due to reduced pollinator visitation (Haig and Westoby 1988). Also, pollen limitation has the potential to affect community interactions by affecting competition through reductions in the number of seedlings of dominant species (Ashman et al. 2004). Pollen limitation may also affect the ecology and evolution of plants through trade-offs in investment between pollinator attraction and female reproductive investment (Haig and Westoby 1988). When pollen limited, plants may respond by increasing the amount of energy and resources to attract pollinators. Thus, pollen limitation may be exacerbated if predators such as spiders affect the abundance and/or behavior of pollinating insects, and cause a reduction in pollinator visitation.

Pollen limitation is not uncommon, and was documented in at least 159 out of 258 species of flowering plants studied (Burd 1994). Some consequences of pollen limitation include a lower percent fruit set and lower number of seeds per fruit (Burd 1994). There is potential for pollen limitation to be acute, and a meta-analysis of supplemental pollination studies from 1980 to 2003 found seed production to increase by an average of 42% with pollen supplementation (Ashman et al. 2004). Because an estimated 35% of global food production relies at least in part on pollinators, knowing as much as possible about community interactions involving pollinators can be very beneficial (Klein et al.

2007). If orb-web building spiders significantly impact the visitation of pollinating insects to pollen-limited plants, they may be an important but overlooked aspect of pollination ecology.

Linking arachnids and pollination:

Understanding how the presence of spiders may affect the population abundance or behavior of pollinators requires a dive into the research on insect vision in regards to web and spider avoidance. In the next section, insect vision will be briefly described followed by the perception of a spider's body and web by pollinating insects.

***Insect Vision*:**

Understanding the visual capabilities of pollinators can help determine if pollinators are able to detect spiders and their webs. Hymenopterans vary little in the types of color receptors they possess (Briscoe and Chittka 2001). With the exception of ants, they have three types of photoreceptors: UV, blue and green (Briscoe and Chittka 2001). Hymenopterans see objects at long ranges by motion detection and contour discrimination, which are mediated by green photoreceptors (Lehrer 1991). At close ranges, bees switch to chromatic contrast detection using green, blue and UV receptor cells (Giurfa and Lehrer 2001 from Hoese et al. 2006). Some bees have been shown to detect objects that are 1cm in diameter at close ranges of 14 cm (Chittka and Raine 2006). Due to their physiological capabilities, I feel that pollinators may be able to detect the presence of large colorful orb-weaving spiders such as *Argiope aurantia.*

Insect perception of spiders: There are different hypotheses about the function of the coloration of spiders (Craig and Ebert 1994; Hoese et al. 2006). Spider coloration is thought to both attract prey (Craig and Ebert 1994, Hauber 2002, Tso et al. 2006;

reviewed in Thery and Casas 2009), and camouflage spiders from predators (Hoese et al. 2006). One study found that yellow striped *Argiope keyserlingi* (Araneidae) captured more prey than *A. keyserlingi* captured with their stripes blackened out with a marker (Hoese et al. 2006). It was suggested that the difference in prey capture was from crypsis due to the disruptive coloration (Stevens and Merilaita 2009) of the spiders' color contrasting yellow bands (Hoese et al. 2006). Other studies also suggest that spiders may lure prey with their yellow body coloration (Fan et al. 2009). At close distances, the visibility of the color contrasting markings on spiders' abdomens may be apparent to bees (Chiao et al. 2009). The behavioral response of bees after detection of orb-web building spiders is not entirely known, however, and it is possible that bees may elicit various responses at different ranges. In general, I feel spiders attempt to appear cryptic opposed to visually appealing to prey, in order to avoid detection by predators. However, I do not feel they are able to avoid detection in all circumstances due to the visual capabilities of risk-avoiding prey.

Because there is evidence that spiders can attract prey, and prey can avoid spiders; it could be meaningful to conduct an empirical study to determine if the presence spiders can affect the visitation rate to nearby plants in spite of the differing opinions on prey attraction. A direct study of insect visitation to *Rudbeckia fulgida* plants near *Argiope aurantia* webs and spiders has not been done, and the visitation time of pollinators to nearby plants with and without *Argiope aurantia* and their webs is examined in this research.

Perception of webs: The silk of orb-web building spiders may cause indirect effects on plants by affecting visitation by pollinating insects. Orb-weaving spiders spin

sticky droplets on their capture threads, which may be visible by insects at close ranges (Craig and Freeman 1991). Orb-web building spiders are also known to build structures called stabilimenta into the center of their webs (T. Blackledge, personal communication). The functions of stabilimenta are not well known but they are hypothesized to attract insects (Craig and Benard 1990, Tso 1998 but see Zschokke 2002) as well as deter predators (Blackledge and Wenzel 1999, Jaffe et al. 2006 but see Seah and Li 2001). Stabilimenta may cause insect avoidance behavior because one study found webs with stabilimenta to intercept 34% less insects than webs without (Blackledge and Wenzel 1999). Contrastingly, prey is sometimes captured more frequently at webs with stabilimenta than without (Craig and Bernard 1990), although starved spiders do not build larger stabilimenta even though they would need to attract prey more than well-fed spiders (Blackledge 1998). This suggests that prey could be attracted to webs due to some other aspect of the webs that is associated with the body condition of spiders rather than the stabilimenta (Blackledge 1998). A more likely scenario may be the one described by Blackledge (1998), where stabilmenta were found to be cryptic to insect prey. The functions of stabilimenta are debated (Zschokke 2002), and more comprehensive reviews regarding the functions of stabilimenta have been written (Bruce 2006, Herberstein et al. 2000). In concurrence with Blackledge and Wenzel (1999), I feel that webs would have to appear both cryptic to prey and aposematic to predators, but depending on the situation, insects may be able to take advantage of any visual effects directed toward predators in order to avoid becoming prey.

Controversy aside, it is not well known whether any deterring or luring effects of stabilimenta extend to the visitation of insects to nearby plants. This study will examine

the transfer of any indirect effects of orb-webs, and their associated stabilimenta, by documenting insect pollinator visitation time to nearby plants.

Indirect link from arachnids to plants:

As previously discussed, spiders may affect pollinator abundance and/or behavior and may subsequently affect plant reproduction (reviewed in Knight et al. 2006). The effect of spiders on the visitation of pollinating insects to plants was empirically tested as early as 1986, when crab spiders were placed on the umbels of milkweeds, and predation rate was quantified (Morse 1986). This opened a door to the exploration of the effects of arachnid predators on pollinators. More studies have followed, and a few studies have found pollinators to avoid plants due to the presence of spiders (Louda 1982, Dukas 2001, Dukas and Morse 2003, Suttle 2003, Robertson and Maguire 2005, Goncalves-Souza et al. 2008, Reader et al. 2006). Explanations for the avoidance behaviors were: predation (Louda 1982), presence of dead bees and dead spiders (Dukas 2001), anti-predatory behavior (Dukas and Morse 2003), and recognition and avoidance of spiders' raptorial forelimbs (Goncalves-Souza et al. 2008). One of these studies found bees were significantly, "less likely to inspect and accept a flower or inflorescence if it harbored a [crab] spider" (Reader et al., 2006, p. 933). This effect was noted only in the honeybee (*Apis mellifera*) and not all species of hymenoptera included in the study (Reader et al. 2006). A similarity in the studies finding avoidance behaviors is that effects were predominately caused by behaviorally mediated indirect effects (Miller and Kerfoot 1987). Cascading indirect effects of predators may affect plant-pollinator interactions more than previously thought (Goncalves-Souza et al. 2008, Munoz and Arroyo 2004, Schmitz et al. 2000), possibly due to these indirect effects.

Types of indirect effects:

In order to understand the effect of spiders on plants, an examination of indirect effects is helpful. Trophic linkage (Knight et al. 2005), behaviorally mediated (Goncalves-Souza et al. 2008, Reader et al. 2006, Dukas and Morse 2003, Dukas 2001, Schmitz et al. 1997), and chemical response indirect effects of predators on plants (Hlivko and Rypstra 2003, Uma and Weiss 2010), as well as neutral or no effects (Morse 1986, Wilkinson et al 1991, Dukas and Morse 2005) may be occurring individually or simultaneously (Miller and Kerfoot 1987). However, identifying the individual effects of each of these interactions is beyond the scope of the study and would require more detailed empirically based studies to be conducted.

Summary of Spider-Pollinator-Plant interactions:

The research on indirect interactions may seem abundant, but overall, not much empirical research has been done on these complex multi-species interactions involving spiders, pollinators, and plants. Contrasting results have been found in multi-species interaction research, so more research must be done in order to understand each individual system. Many past studies dealt with the capture of insects and attraction of prey by anthophilous (flower-dwelling) spiders foraging directly on flowers. The present study examines any indirect effects of attraction or deterrence of pollinators by orb-web building spiders. Because orb-web building spiders are very abundant, and are known to capture pollinating insects, the effect on nearby plants is a potentially vital area of research.

CHAPTER III

RESEARCH QUESTIONS AND PREDICTIONS

The main question being addressed in this study is, "Can the black-and-yellow garden spider (*Argiope aurantia* Lucas: Araneidae) indirectly affect the pollinator visitation rate of the orange coneflower (*Rudbeckia fulgida* Aiton: Compositae)?" This question is important because interactions among multiple species may drive ecological and evolutionary interactions through a plethora of multi-species plant-pollinator interactions including, but not limited to: plant-mutualist-mutualist, plant-herbivore-pollinator, and plant-pollinator-seed predator interactions (see Strauss and Irwin 2004 for a review). Despite the complexities, the importance of considering predators in plant-pollinator interactions is necessary if not crucial (Suttle 2003).

Based on other studies that have found anti-predator responses by insects (Dukas and Morse 2003; Goncalves-Souza et al. 2008; Brechbuhl 2010, Reader et al. 2006), and the assortment of potential avenues for negative indirect effects, I predicted the presence of orb-web building spiders to negatively affect the pollinator visitation rate to orange coneflowers. This was expected because I feel it is reasonable to assume that trophic linkage effects, behaviorally mediated indirect effects, and chemical response effects of spiders and/or their webs would deter pollinating insects from visiting nearby plants.

CHAPTER IV

LIMITATIONS OF PAST AND CURRENT RESEARCH

Trade-offs have been found between a plant's attraction of beneficial plant-mutualists and harmful seed predators which are thought to shape floral characteristics (Cariveau et al. 2004). Thus, the attraction of insects could play a part in shaping the evolution of plant traits. Orb-web building spiders could indirectly affect plant traits as well, if they have significant effects on the visitation of pollinating insects. However, few if any studies examine the effects of orb-web building spiders on pollination to nearby plants. This research will help to fill in this gap by examining the effects of spiders that utilize a different type of foraging mode than anthophilous spiders, on the visitation of pollinating insects to *R. fulgida*.

Detritovores, parasites, herbivores, and interactions involving predators of predators have all been found to affect multi-species communities (Tschartnke and Hawkins 2002), but were not included in the study due to limited resources for data collection.

Finally, examples of mutualistic relationships between spiders and plants have been found (Whitney 2004). The presence of spiders was found to reduce seed predation while spiders benefited from higher prey capture rates (Whitney 2004). The current study only examined indirect effects of the presence of spiders on the pollinator visitation time to plants, and not any benefits to spiders due to plant presence.

CHAPTER V

MATERIALS AND METHODS

Study site:

The experiment was conducted at a 6x6m, off-road, grassy patch during August of 2007 about 30 meters northeast of the University of Akron Field Station at the Bath Nature Preserve (41° 10'53" N; 81° 39'05" W) in Bath, OH. The research area was chosen because of its relative isolation from human impact and disturbances, and its accessibility for research needs.

Study organisms:

Argiope aurantia:

A lot of previous research on multi-species effects of spider-pollinator-plant relationships has been done with anthophilous spiders such as crab spiders (Thomisidae), whose foraging range overlaps with their prey. The current research examines the effects of an orb-weaving spider (*Argiope aurantia)*. Spiders, in general, are a great model species to study trophic interactions because of their predatory nature. Spiders are almost strictly predatory carnivores with the exception of one recently described herbivorous species (Meehan et al. 2009). This allows any direct effects on plants due to omnivory to be ignored. *Argiope aurantia* were selected because they spin orb-webs, have a high relative abundance, and are easy to capture and work with (personal observation).

Argiope aurantia (Araneidae, Lucas) can be found throughout the United States (Kaston 1978). Mature females can be found during the months of July through mid to late October (Levi 1968 from Howell and Ellender 1984).

In Mississippi, spiders with webs attached to tall composite plants or shrubs caught more hymenopteran prey (McReynolds 2000). It was also found that spiders captured more hymenopteran prey in open fields than forest edges (McReynolds 2000). For these reasons, a study site hosting composite plants in a grassy field habitat was chosen to maximize the potential for pollinator visits. *A. aurantia* is a generalist predator whose diet changes over time (Howell and Ellender 1984). The reasons may include changes in web placement and habitat changes (Howell and Ellender 1984). Because spiders are generalists, bees and other pollinators are oftentimes part of their diet (Bilsing 1920, Nyffeler et al. 1987). In one older study, honeybees were found in 14% of the webs out of 1,250 spiders' webs (Bilsing 1920). This high predation on pollinating insect prey makes them an ideal subject for the study. The study spiders were fed to satiation each day, or as needed, and remained alive throughout the experiment. The study spiders were collected from nearby fields, and at the end of the study, all *A. aurantia* and their egg sacs were returned to the fields.

Rudbeckia fulgida:

The orange coneflower (*Rudbeckia fulgida*) is both a natural and a garden plant that may provide structure for the building of orb-webs by *Argiope*. *R. fulgida* blooms from mid-summer through October (Palmer et al. 2009), which overlaps with the presence of mature female *Argiope*. They are drought and disease resistant which makes them a common ornamental that are easy to care for (Palmer et al. 2009). Some varieties

reproduce by self-pollination and apomixis, but they have been deemed a highly self-incompatible group with certain varieties being completely self-incompatible (Palmer et al. 2009). A particular variety (*Rubeckia fulgida ait. var. sullivantii*) is a mostly self-incompatible variety and is listed by the US Forest Service as a Regional Forester's Sensitive species (Scott and Molano-Flores 2007). *R. fulgida* can be found on the east coast from Pennsylvania to Georgia and Florida and as far west as Louisiana and Arkansas (Torrey and Grey 1969). Their overlapping range with *Argiope aurantia* makes them ideal to study. *R. fulgida* are not found on the Bath Nature Preserve, but a similar species, *R. hirta,* may be found at a nearby patch on the preserve (R. Mitchell, personal communication).

Although the *R. fulgida* in the study were not tested for pollen limitation, the taxa to which it belongs has been deemed highly self-incompatible, and certain varieties were found to be completely self-incompatible (Palmer et al. 2009). Regardless, it is likely that other pollen limited plants fall within the expansive natural range of *A. aurantia*.

Treatment Set-Up:

Treatment Groups:

Three treatments groups were selected for this study in an attempt to disentangle any overlapping effects of the spiders and their webs. The first treatment group consisted of a spider on its web, the second had a web only, and the third was a no spider, no web control. From here on, the treatments will be referred to as spider, web, and control respectively. No trophic linkage effects would be expected in the web or control treatment due to the lack of a predatory spider, and no behaviorally mediated effects due to the presence of a spider or web in the control.

Plant Placement:

Twenty plants were purchased from Petitti's Garden Inc. in Akron, OH. The plants selected were similar in height, approximately 0.5m. They were housed in a nursery under similar conditions so any differential environmental conditions were minimal. The plants were transported to an off road, grassy patch near the University of Akron Field Station located at Bath Nature Preserve in Bath, Ohio. The bulk of the plants were kept in an area approximately 6 meters away from the edge of the study site. The plants were kept at a nearby location in an attempt to attract pollinators to the study site. A small grain and extent of one 6X6m study site was used so pollinators did not have to relearn patch location.

With regard to preferential selection of individual plants by pollinators, one study found that plants with a larger floral display size (number of flowering heads on a plant) attracted more regular and occasional visiting bees than those with a lesser size (Makino et al. 2007). The same study found more occasionally visiting bees in areas with higher local flower densities (number of flowers on neighboring plants) (Makino et al. 2007). Although plants were not standardized for number of composite flower heads, plant height, or floral display, they were purchased from a nursery and appeared to have relatively similar floral characteristics. In addition, randomization and replication of treatments was used to account for variation among plants. The randomization also helped account for any bias caused by any potential effect of observer presence on pollination time (Cahill et al. 2001). After the plants began to mature and had flowering heads, they were taken in groups of 9 to be placed at the study site. Plants were placed 1.5 meters apart in an attempt to reduce dependence on neighboring treatments (Fig. 1).

1 **Spider (D)** Plant	2 **Web** Plant	3 Plant **Control**
4 Plant **Web**	5 Plant **Spider (V)**	6 **Control** Plant
7 Plant **Spider (V)**	8 Plant **Web**	9 Plant **Control**

Figure 1: An example of the experimental design of the 6x6m study plot. At the beginning of each day, treatments were randomly assigned to a randomly selected side of the plants shown by the juxtaposition of the treatment (Spider, Web, or Control) and the plant. The D or V corresponds to whether the dorsal or ventral side of the spider was randomly selected to face the plant. A distance of ~1.5 meters separated the plants. The numbers in the top left corners represent the order of observation from 1 to 9.

Frame construction:

Adult specimens of *Argiope aurantia* were captured from areas surrounding the field station and transferred to constructed frames. Constructed frames were used so the positions of the spiders could be easily manipulated. The frames were constructed by forming hardware cloth into cylinders. The hardware cloth cylinders were covered with green mesh cloth along the perimeter to prevent spiders from escaping through gaps. When the study was not being conducted, the open sides were covered with plastic wrap attached by rubber bands (T. Blackledge, personal communication) to prevent spiders from escaping. Neither the sizes of the frames nor the webs were measured, but the frames were estimated to be approximately 0.3 meters in diameter. It is not unusual for

Argiope to spin webs of more than 0.75m, in diameter (Bilsing 1920), so the size selected was not unusual. All the webs contained linear stabilimenta structures because adult *A. aurantia* almost always spin a linear design (T. Blackledge, personal communication). Standardizing the presence of stabilimenta within webs prevented the presence or lack of structures from causing an effect on visitation within or between treatments.

Frame placement:

A single designated treatment frame was attached to two wooden poles by metal wire, and was staked next to one randomly selected plant from the nine plants within the plot (Fig. 1). This was done for three frames of each treatment group. The frames were arbitrarily positioned approximately 25 centimeters away from the plant, at the approximate height of the approximate center of all composite flowers. The height of the frame and distance from the center were not measured and may have varied slightly between plants. The frames were placed on one of 4 randomly selected sides of the plants in order to control for any directional effect of approaching or retreating insects (Rao et al. 2008). Whether the spiders' dorsal or ventral side was facing the plants was randomized as well. The position of the spider on the web was not tracked throughout the study and the possibility of the spider shuttling, or switching from one side of the web to the other was possible (Wells 1938). However, the spider usually returns to the original side of the web within seconds or minutes (Wells 1938), and the orientation of the spider has been found to have no effect on prey capture success (Herberstein and Heiling 2001). Nevertheless, this helped to prevent any confounding effect of the body coloration attracting pollinators (Craig and Ebert 1994; Hauber 2002, Tso et al. 2006). The dorsal or ventral positioning was done by horizontally rotating the frame 180 degrees. The only

differences between sides of the webs were the angle of the stabilimenta structures and any other asymmetries (T. Blackledge, personal communication). Web attributes such as mesh width and diameter were not measured, and webs were sometimes used for more than one day depending on availability.

Visitation Time Collection:

All data were collected during August of 2007 during rainless weather conditions. The data were collected using an instantaneous sampling method during 5-minute observation sessions divided into 15-second intervals with 20 sampling points per session (Martin and Bateson 1993). The observation sessions took place during 11 days at various hours (time-points) between 9a.m. and 4p.m., hereafter referred to as time-points (See Fig. 2). Most observations began within the first 15 minutes of the time-point, but two started after and were omitted from the analysis due to an overlap into the next time-point.

Time of Day	9a.m.	10a.m.	11a.m.	12p.m.	1p.m.	2p.m.	3p.m.	4p.m.
Days of Observation	2	4	7	8	7	8	11	2

Figure 2: Observation sessions during various time-points. The figure shows the number of days when data was collected for each time-point.

There were a total of 49 5-minute observation sessions across the 11 days. Each of the 9 plants, with a randomly assigned replicate, was observed in rotation (See Fig. 1). On the rare occasion that a 5-minute observation was interrupted, the observation was restarted after all other replicates were recorded. If a 15-second interval was interrupted, the observation was restarted as well. There was a total observation time of

approximately 4 hours per treatment, for a grand total of approximately 12 hours of observation time across all days.

Pollinator visitation was observed through binoculars from at least 1.5m away to minimize disturbance. The number of insects present during each 15-second interval was hand tallied on waterproof paper. The recording time was not continuous and short gaps of time existed between 15-second intervals to allow time to record data. Pollinating insects that landed on any of the composite flower heads of the plant were counted. Not all sides of the composite flower heads could be seen at once, so there was a tendency to underestimate pollinator visitation. Data was recorded as presence/absence but when more than one insect was present; an additional "presence" mark was recorded. Some insects were only present for part of the 15-second interval, while others were present for all 15 seconds, but both were counted as one visit. For example, an insect could leave and come back 4 times, but because there was no more than one insect present on the plant at one time, only one visit would be recorded for the 15-second interval. Comparatively, another could come at the beginning of the interval and stay for the entire 15-second interval, but it would also be recorded as one visit. The instantaneous sampling method was selected because of the difficultly of tracking individual insects' pollination times when multiple insects were present.

The number of insects present during an interval was multiplied by 15 to obtain an estimate of the number of seconds of pollinator visitation time. For example, if there were 2 tallies for visitation during the first 15 seconds of the observation, then 30 seconds of pollination time was counted. The number of seconds of pollinator visitation time was converted to minutes for analysis.

The type of pollinator was recorded to Order as best as possible. The marks recorded for pollinators were: Small, black bee (possibly *Ceratina* or *Lasioglossum*), other Hymenoptera (possibly Halictidae), Bumblebee (Apidae), Lepidoptera (possibly Nymphalidae or Hesperiidae), and Diptera (Syrphidae). Syrphid flies were initially tallied as Hymenoptera but were corrected to Diptera upon realization of the error. Insect lengths were not measured, but based on visual observation the small black bees and other Hymenoptera categories mostly consisted of small pollinators under 1 cm long.

CHAPTER VI

RESULTS

Descriptive data:

Pollinators:

Small Hymenopterans (possibly Ceratina, Lasioglossus, and/or Halictidae sp.: ~81%) made up the majority of visits. Butterflies (possibly Hesperiidae: 13.8%) were regular visitors in the beginning, but dwindled off and appeared as occasional visitors later in the study. Syrphid flies (Syrphidae: 1.6%), and bumblebees (Apidae: 2.8%) were occasional visitors. Visitors such as spiders, a caterpillar, and a wasp were rarely seen on composite flower heads (<1%).

Visitation Time Analysis:

In order to search for net indirect effects of orb-web building spiders on pollinator visitation to plants, empirical data was collected for the month of August of 2007 during 5-minute observational periods. These 5-minute observations were taken during various days and various time-points within each day. The mean pollination times (minutes/5-minute observational period) of the three plant replicates during 5-minute observational periods were compared across all days (two-way ANOVA: day and treatment; n=11), all time-points (two-way ANOVA: time-point and treatment; n=8) and all 5-minute observations (one-way ANOVA; n=48). To test for an overall treatment effect for the

month of August, data needed to be pooled across all days. Spider webs may have different reflectance properties during different times within days (Craig 1988), which could affect visitation, so a comparison of data across time-points was also necessary. For these data to be pooled, two-way ANOVAs were run for comparison of data across days, and across time-points to examine any significant differences in pollinator visitation times. If no patterns across days or time-points were apparent, then the data were presumed to be safe for a pooled overall analysis.

Visitation time across days:

The data for the three replicates of each treatment were averaged across time-points to get an estimate of mean visitation time (min) per 5-minute observation for each day (n=11; Fig. 3). One day with only one 5-minute time-point observation was thrown out when comparing data across days because obtaining an average was not possible with one data point. Two hours of data were omitted because the time-point overlapped into the next hour. For example, one observation hour began at 11:30am and ran until 12:30pm. Because the overlap was over 15 minutes, the data were eliminated from analyses. The sampling units were averages for each of the 3 replicates for 11 days and 3 treatments for a total of 99 samples for analysis. The means for each treatment group were compared using a two-way ANOVA with day and treatment as factors using SPSS version 19.

Visitation time across time-points:

The data for the three replicates per treatment were averaged across all days to get an estimate of mean visitation time per 5-minute observation for each time-point (n=8; Fig. 4). Again, two hours of data were thrown out because the observation period overlapped into the next hour. This allowed for a more precise estimate of visitation time for each time point due to difficulty in sorting data that fell between time-points. Some overlap still occurred with observations running into the next time point up to 15 minutes. The sampling units were averages for each of the 3 replicates for 8 time-points and 3 treatments for a total of 72 samples for analysis. The means for each treatment group were compared across time points using a two-way ANOVA with time-point and treatment as factors using SPSS version 19.

Visitation time across 5-minute observations during August:

The data for the three replicates per treatment were averaged for each 5-minute observation period to get an estimate of mean visitation time per 5-minute observation (n=48, Fig. 5). The sampling units were 48 averages of the three replicates for 5-minute observations, and 3 treatments for a total of 144 samples for the overall analysis. The means for average visitation time across all 5-minute observations were compared using a one-way ANOVA with visitation time per 5-minute observation as the dependent variable and treatment as the independent categorical variable using SPSS version 19.

Day:

A two-way ANOVA for day X treatment was run. The data were averaged for each day and graphically compared across days and treatments (Fig. 3). The degrees of freedom were 10 for day, 2 for treatment and 66 for the error term. There was no

significant effect of day (F(10,66)=1.602; p=0.126), treatment across days (F(2, 66)=0.473 p-value=0.625), or interaction (p>0.05).

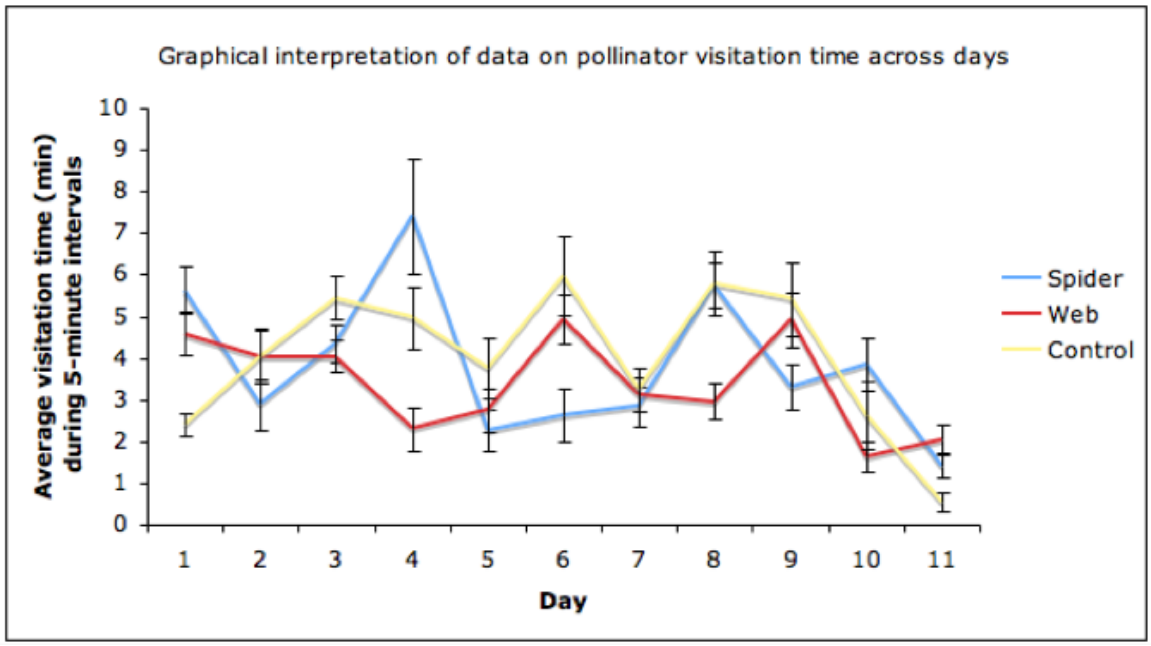

Figure 3: Graphical interpretation of data on pollinator visitation time across days. The three treatment groups are spider, web and control. Averages of the three replicates for each treatment group during each 5-minute observation were taken. These mean visitation rates were averaged across all 5-minute observations and for each day to get a daily visitation time per treatment group during a 5-minute observation. There were no statistically significant differences between groups (p-value=0.625; n=66; error bars are ±1 SE).

Time-points:

A two-way ANOVA across time points was run. The data were averaged by days and graphically compared across time-points (Fig. 4). The degrees of freedom were 7 for hour and 2 for treatment and 48 for the error term. There was a significant effect when

looking at visitation time across time points (F(7, 48)=3.591; p=0.003). A post-hoc Tukey analysis revealed a significant difference between the hours of 9a.m. and 12p.m. (p-value=.003), 9a.m. and 1p.m. (p-value .030), and 9a.m. and 2p.m. (p-value=.007). No other statistically significant effects were found across treatments (F(2, 48)=0.570; p=.569) or interaction (p>0.05).

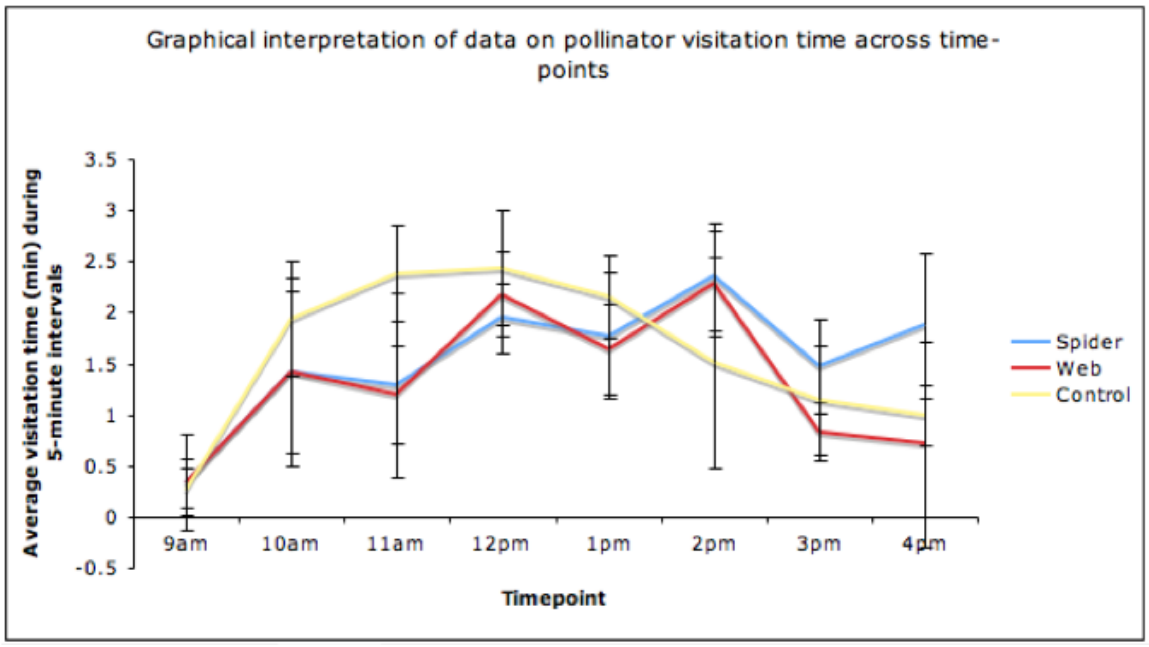

Figure 4: Graphical interpretation of data on pollinator visitation time across time-points. The three treatment groups are spider, web and control. Averages of the three replicates for each treatment group during each 5-minute observation were taken. These mean visitation rates were averaged across all 5-minute observations and for each time-point to get an hourly visitation time per treatment group during a 5-minute observation. There were no statistically significant differences between groups (p-value=0.569; n=48; error bars are ±1 SE).

5-minute observations:

A one-way ANOVA across all 5-minutes observations was run. The 9a.m. data were omitted, and because no other significant differences were found across days or time-points, the remaining data were pooled for analysis. Data for mean visitation time (min) for 5-minute observations was averaged across all time-points for each day. These mean visitation times for 5-minute observations were averaged for each treatment and compared graphically (see Fig. 5). No significant effects were found across treatments ($F(2, 141)=0.495$; $p=0.611$).

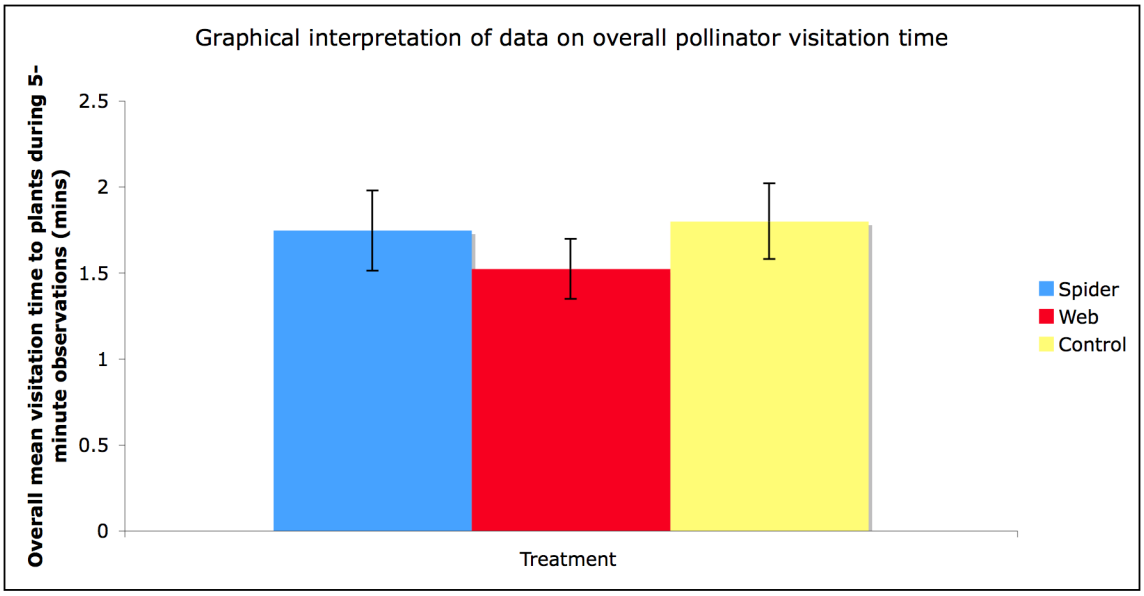

Figure 5: Graphical interpretation of data on overall pollinator visitation time. The three treatment groups are spider, web and control. Averages of the three replicates for each treatment group during each 5-minute observation were taken. These mean visitation rates were averaged across all 5-minute observations taken during all time-points and all days to get an overall average visitation time per treatment group during a 5-minute observation. There were no statistically significant differences between groups (p-value 0.611; n=141; error bars are ±1 SE).

Source	df	Mean Square	F	P-value	Observed Power[b]
Time-point	7	3.263	3.591	0.003	0.949
Treatment (Time-point)	2	.518	0.570	0.569	0.139
Time-point * Treatment	14	.491	0.540	0.896	0.278
Day	10	1.958	1.602	0.126	0.724
Treatment (Day)	2	0.578	0.473	0.625	0.124
Day * Treatment	20	1.009	0.825	0.675	0.545

Table 1: ANOVA table – No treatment effects or interaction effects across days or time-points were statistically significant. There was a significant effect when looking at visitation time across time-points ($F(7, 48)=3.591$; p-value=0.003). A post-hoc Tukey analysis revealed a significant difference between the hours of 9a.m. and 12p.m. (p-value=.003), 9a.m. and 1p.m. (p-value .030), and 9a.m. and 2p.m. (p-value=.007). No other statistically significant effects were found across treatments ($F(2, 48)=0.570$; p-value=0.569) or interaction ($p>0.05$).

Power analysis:

When taking the average of the replicates for each treatment and comparing data across all days, all time-points, and for all 5-minute observations (n=48), there were no significant differences between treatments. A power analysis using G*Power 3.1.2 (Faul et al. 2009) revealed a power of: $1-\beta=0.132$, based on an effect size of $\eta^2=0.007$. Based on an a priori power analysis, a sample size of: n=1371 would be required for any detection of an effect with a power sensitivity of $1-\beta=0.8$ (Faul et al. 2009).

A power analysis revealed a low power ($1-\beta=0.132$) of the test and a high probability of making a Type II error. Based on this analysis, there was a high

probability of making an incorrect assertion of no effect. Therefore, the test was concluded to be inconclusive rather than accepting the null hypothesis (Toft and Shea 1983).

CHAPTER VII

DISCUSSION

Understanding the effects of orb-web building spiders on plant visitation by pollinators allows for a more comprehensive understanding of multi-species interactions. As previously mentioned, communities and assemblages that involve arachnid predators, pollinators, and plants have not been studied in great detail. Over an evolutionary scale of time, complex interactions between pollinators and plants may shape floral traits (Cariveau 2004) and plant fitness (Suttle 2003). Understanding these interactions could ultimately contribute to the conservation of the diversity of organisms. Flower-dwelling spiders have been previously found to trigger behavioral changes by pollinating insects and affect pollinator visitation (Dukas and Morse 2003; Brechbuhl et al. 2010), which could ultimately affect plant fitness (Suttle 2003). Past research, however, has not thoroughly examined the effects of orb-web building spiders on pollinator visitation behavior. This study helped to extend spider-pollinator-plant research to encompass orb-web building spiders with a different foraging mode than anthophilous spiders, and focused on the indirect effects of an orb-web building spider (*A. aurantia*) on the visitation time of pollinating insects to a plant's (*R. fulgida*) composite flowers.

Pollinator visitation across days:

The visitation time of pollinators did not appear to show any statistically significant pattern across days (see Figure 2). The variation in pollinator visitation time appeared to be consistent until the end of the month when the flowers died and pollinator visitation tapered off. The lack of any significant differences suggests that pollinators may not vary their pollination behavior much between treatment groups from day to day. This allowed for the data of 5-minute observations across all 11 days to be pooled for an overall analysis of pollinator visitation.

Pollinator visitation across time-points:

The visitation time of pollinators showed a statistically significant difference between the 9a.m. and 12p.m., 9a.m. and 1p.m.; and 9a.m. and 2p.m. data. However, there were only two data points for the 9a.m. data, so the 9a.m. data were omitted from the pooled overall analysis (Table 1). The sample sizes for each time point varied, but only the 9a.m. data showed a significant difference across various time-points within days. The remaining data for 5-minute observations did not show any other statistical patterns, so were pooled across all time-points.

Pollinator visitation across all 5-minute observations in August:

After omitting the 9a.m. data and pooling all visitation data across days and time-points, a remaining total of 48 five-minute observations for each replicate of each treatment group during the month of August were included in the analysis. No

statistically significant treatment effects during 5-minute observations were found. This indicates there was no significant effect of the presence of *A. aurantia* on pollinator visitation time to *R. fulgida.*

Summary of findings:

Overall, there were no significantly detectable effects of the presence of *A. aurantia* and/or their webs on the visitation time of pollinating insects to *R. fulgida*. The analysis of visitation over days and time-points suggests that the variation in pollinator visitation to *R. fulgida* was consistent over the entire month and was not affected by the presence of *A. aurantia*. The results, however, were inconclusive and the lack of an effect could not be ascertained due to low statistical power. Two possible reasons for a lack of an effect are: the lack of significance due to the ecology of the system, and/or limitations in the design of the study. The ecological explanations will be described first and then design limitations.

Ecological Explanations:

The hypothesis that the presence of spiders may have indirect effects on *R. fulgida* due to any direct or indirect effects on the visitation of pollinating insects could neither be supported nor fully determined to be non-existent due to inconclusive results. Any behavioral, chemical, or trophic linkage indirect effects due to the presence of *A. aurantia* on the pollinators' visitation times to adjacent *R. fulgida* plants may have been non-existent or not strong enough to detect. The data did not show any statistically significant treatment effects. Under the assumption that a type II error did not exist, the

data does not support the idea of significant indirect effects of orb-web building spiders on pollinator visitation time to plants, and rather supports studies that have not found strong indirect effects of spiders on pollinator visitation and/or plant fitness (Wilkinson et al. 1991; Morse 1986; Dukas and Morse 2003; Dukas and Morse 2005; but see Goncalves-Souza 2008).

There are at least three possible, but not necessarily mutually exclusive pathways to explain why pollinating insects did not show any discrimination between the three treatment groups. The first is that pollinators recognized and avoided the spiders and/or webs but still approached the plants. The second is that pollinators did not detect the presence of *A. aurantia* and approached the plants without ever recognizing or avoiding a spider and/or web. The third is that insects were captured by spiders and/or avoided the plants due to the presence of *A. aurantia*, but the naturally high variation in pollinator visitation time masked any small indirect trophic linkage, behaviorally mediated, or chemical response effects. After the three situations listed above are discussed, the species in the current system will be evaluated and discussed.

Pollinators encountered, but avoided spiders and/or webs and still approached individual plants:

Insects have been found to recognize webs at close ranges and respond by continuing through existing holes in the webs (Craig 1986). Pollinators may also have become entrapped in the webs temporarily, yet escaped and continued on to forage on *R. fulgida*. One study found that about 78% of all prey to escape from orb-webs within 100

seconds (Nentwig 1982). Although not documented, the majority of insects may have escaped from the spiderless webs and continued on to the plants, and spiders may not have been motivated to catch prey due to satiety from hand feedings; pollination time would not have differed much between the control and other treatments.

Bees have also been found to be capable of visually recognizing orb-weaving spiders' color patterns (Chiao et al. 2009). Bees may use color contrast to recognize objects at close ranges when there is a large subtending angle relative to their focal point (Giurfa et al. 1996). This translates to bees being able to detect an object that is 5cm in diameter from a distance of about 45cm (Girufa et al. 1996). If the distance of detection scales to the size of an object, bees could recognize the presence of a nearby adult female *Argiope aurantia*. For example, if the subtending angle remains above 15° then an insect would be able to detect an adult female *A. aurantia* which is about 1.9 to 2.8 cm long (Kaston 1978) from a distance of roughly 17 to 25.2 cm away. The distance of the spider from the flower was less than 25cm (estimate) away from the *R. fulgida* plants, so the nearby juxtaposition may have been sufficient for a bee to detect and avoid the spider using chromatic contrast recognition alone. Both stingless bees (Guedot et al. 2007) and honeybees (Giurfa and Lehrer 2001) use color contrast to detect objects. Although honeybees did not visit the site, they have been found to switch from achromatic to chromatic object detection at larger subtending angles (Giurfa and Lehrer 2001). Because some stingless bees, honeybees, and hymenopterans in general share the same receptors (Briscoe and Chittka 2001) and share other similarities in vision such as color

contrast for object detection, it is possible that the bees in the study needed to be quite close to spiders before they could detect their presence and then proceed around to the neighboring flower heads. This may be likely, because some insects have been known to approach webs at very close distances and even touch webs before fleeing in another direction (Craig 1986).

Pollinators did not detect the presence of* Argiope aurantia*:

The second, more parsimonious explanation for a lack of effect is that the pollinating insects did not discriminate between plants next to different treatments because they did not detect any presence of nearby *A. aurantia*. Orb-webs and their associated stabilimenta may appear cryptic and achromatic to pollinators (Blackledge 1998), possibly because of a low UV reflectance (Zschokke 2002). Because insects are known to use green photoreceptors for contrast with the background to detect objects (Lehrer 1991), the achromatic reflectance could have rendered the webs and associated stabilmenta structures invisible to the insects' eyes. Insects may also not have detected the spiders' bodies, which appear colorful to humans, but may appear cryptic to pollinating bees (Hoese et al. 2006). The coloration of *A. aurantia* spiders may have evolved to break up the outlines of their bodies against the background vegetation through camouflaging body patterns (Hoese et al. 2006) as a means to reduce predation.

Another aspect of the lack of awareness of *A. aurantia* could be the attraction of the *R. fulgida* plants overpowering any visual recognition of nearby spiders. *Rudbeckia* plants have been photographed with a technique created to visualize how a composite

flower would appear to a foraging honeybee (McCrea and Levy 1983). The resulting photographs revealed a three ringed bull's-eye design with UV reflecting ray tips, but UV absorbing disks and ray bases (McCrea and Levy 1983). This specific pattern in conjunction with a nectar or pollen reward could be highly adaptive to the attraction of foraging insects, which could drown out any visual cues of the spiders and/or their webs. The UV reflectance of *Argiope bruennichi* (Araneae) has even been found to be negative (Zschokke 2002) which may make them appear less brilliant to pollinators and cause UV reflecting flowers to appear even more noticeable relative to the spider.

The visual capabilities of solitary bees have not been thoroughly studied, but the small solitary bees in the study could have lacked the capabilities to detect the spiders due to the size of their compound eyes in relation to the distance of the spiders from the nearby plants. If the spiders were essentially invisible to pollinating insects and prey capture was stochastic, unpredictable, and infrequent, the results of the current findings would be expected.

Masked indirect effects due to high variation in pollinator visitation time: The third explanation for why pollinating insects did not show any discrimination between the three treatment groups could be due to the high within treatment variation in visitation being very high relative to the between treatment variation which resulted in a high F-value and no statistical detection of an effect (see Table 1). There are many sources of variation that could have affected the internal validity of the study including: floral

display, additional predators, time and/or day, data collection methods, and web variation, all of which will be explained below.

Experimental Design Limitations

As stated previously, the results of the study were inconclusive, so no positive assertion of a lack of an effect could be stated. It is possible that an effect could be more closely isolated with an improvement of the internal and external validity of the experimental design. The internal and external design limitations will be discussed in order and then improvements for future studies will be suggested.

Internal validity:

Whole plant response variable: In a review on multi-species interactions involving plant-animal relationships, the selection of an appropriate plant response variable is underscored (Strauss and Irwin 2004). Furthermore, it has been cautioned that different plant response variables may indicate different magnitudes of effect (Schmitz et al. 2000). In this study, the response variable measured was pollinator visitation time to all composite flower heads on plants during 5-minute observations. One source of the high variation in pollination time could be attributed to the varying number of flowering heads on each plant. The experiment was centered on the visitation to all flowering heads on an entire plant rather than the visitation to an individual flowering head. The treatments were randomly assigned which gave all treatments an equal probability of being adjacent to a plant with any number of flowering heads, which controlled for the variation, but a difference in number of flowers open per flowering head, and number of

flowering heads per plant may have caused an inflated measure of variation. Because the floral display of a plant can affect the visitation of pollinators (Makino et al. 2007), standardizing the number of flowering heads may have reduced the amount of variation within treatments. In addition, the effects of differing floral displays may have affected the visitation of insects because bees were found to spend more time visiting large inflorescences over small ones (Ishii 2006). A different response variable that may have reduced variation in visitation time would be pollinator visitation time per floral unit after standardizing for number of floral units per plant. This allows the differing number of composite flower heads per plant to be standardized, and any variation due to differing pollinator attraction effects to be reduced.

Plant fitness response variable: Because seed set or other direct measure of plant fitness was not included, visitation time was used as a proxy of plant fitness (Vasquez and Morris 2005). A meta-analysis has shown that the frequency of pollinator interactions may be an appropriate proxy for plant fitness, but caution must be taken when making generalizations from the analysis (Vasquez and Morris 2005). For example, a study on a similar species, *R. hirta*, showed that reduced pollinator visitation rates were not the cause of reduction in reproductive output (Hamback 2001). A more robust measure of a plant fitness response variable such as second generation plant fitness (Knight et al. 2005) may allow for a less ambiguous examination of plant fitness. Measuring seed set may not have been appropriate for the present study because *R. fulgida* has been documented to be apomictic (Palmer 2009). The use of a pollinator

dependent species that does not reproduce by cloning or apomixis would circumvent this issue to allow for the inclusion of a fitness variable.

The dynamics of pollinator visitation should be examined when considering the addition of a direct measure of plant fitness. The amount of pollen removed by pollinators may be a function of pollination time that increases at a decelerating rate (Harder and Thomson 1989). Also, the overall time spent by pollinators may have surpassed the maximum visitation time needed to remove all pollen from the flowers. For example, more visits would not necessarily mean higher seed production if the pollination/provisioning limitations curve described by Haig and Westoby (1988) was at or above equilibrium. The equilibrium point falls at the intersection where any additional increase in pollination effort would not result in additional reproductive benefits through seed production (Haig and Westoby 1988). In addition, any insects caught by spiders may not always affect pollination efficiency unless the number of pollen carrying insects is limited. Unless insect visitation is below a critical limit, any deterring effects of spiders may not make a difference; different pollinators could functionally replace displaced individuals (Tscharntke and Hawkins 2002). A test on the pollen limitation of the study plants would be required to elucidate this issue, and a pollen analysis on the insect visitors could provide helpful supplementary information (Bosch et al. 2009). Nevertheless, a clear line between pollen and resource limitation may not exist and care must be taken when defining plants as solely resource or pollen limited (Campbell and

Halama 1993). In short, before including an additional plant fitness response variable, more research must be done on the pollination dynamics of *R. fulgida*.

Height of web placement: The height of the web varied between treatments and was not measured. If web height was recorded, the data could be used as a covariate in a statistical analysis. *A. trifasciata* was found to forage higher up in the vegetation than *A. aurantia* (Brown 1981). The height of the webs could have been manipulated to be better suited to the natural placement of *A. aurantia* webs on the reserve, which may have yielded different results. Also, standardizing the height of each frame may have reduced any potential variability due to web placement.

Lurking crab spiders: Two crab spiders were found, one with a prey item, during two separate observations. The effect of lurking crab spiders on visitation time on a particular treatment was reduced by randomization, but may have affected the variation in pollination time in conjunction with *A. aurantia*. In order to prevent this in future studies, plants could be standardized to all have crab spiders present, or plants could be checked and any spiders could be removed periodically and before the start of the data collection process.

External validity:

Sample size: Based on the small to negligible effect size of $\eta^2=0.007$, a very large sample size (n=1371) would be needed to detect any treatment effects. With the current methods, a large sample size of n=1371 would exceed the amount of time available for data collection by one person, if all 5-minute observations during the month

of August were taken into account. Assuming data are collected while the plants were flowering during 8 consecutive hours and all 31 days in August, there would only be 248 possible 5-minute observations. The design would call for about 9-10 times as many 5-minute observations to be added to the pooled analysis to reach the predicted sample size. Within the limitations and availability of resources for rigorous data collection, garnering a sample size of n=1371 would not have been possible. Collecting visitation data during 5-minute observations at multiple patches or increasing the number of replicates in the study could attain this high sample size, but stochastic variation between sites would also have to be considered.

Spatial Scale: Scale is thought to be a central issue in ecology (Levin 1992), and knowing more about the dynamics of the scale of the study could prove to be important. In a study on the effects of scale on different pollinator groups, Steffan-Dewenter et al. (2002) concluded, "that only analyses of multiple spatial scales may detect the importance of landscape context for pollinator communities" (Steffan-Dwenter et al. 2002, p. 1421). Metapopulation dynamics and spatial processes may affect multi-species interactions and should therefore be taken into consideration (Tscharntke and Hawkins 2002). For instance, one study on the pollination of *R. fulgida* found a lower diversity of insect pollinators on smaller patches, possibly because of a low-density floral display (Scott and Molano-Flores 2007). Because small isolated plant populations may be prone to extinction due to reduced pollinator visitation rate (Kunin 1997) and/or population size

(Wilcock and Neiland 2002), more research on the effect of spiders on various pollinating insect species at various scales is needed.

The scale of the study was a 6 x 6m plot. When considering the entire range of *A. aurantia* and *R. fulgida*, the grain and extent of scale of the study can be considered very fine. Previous studies found avoidance effects due to the presence of spiders at the scale of the patch rather than individual plants (Dukas and Morse 2003). The three spiders could have acted as artificially facultative group foragers, which would have eliminated insects from the entire patch once they were caught by any one of the three spiders (Craig 1991). A different approach would be to conduct the study over a greater area or at multiple scales, and search for any emerging patterns (Wiens 1989). In a future study, the randomized design could still be used, but a larger spatially scaled plot with the same design could be conducted at the patch level. Single treatments could be designated to whole 9 plant plots. Each of these treatments could be randomly assigned to a larger 18 X18 m landscape plot. For example, there would be three 6 X6 m spider plots, three 6 X6 m web plots, and three 6 X6 m control plots randomly assigned within a 18 X18 m landscape patch. This would potentially allow for a more natural setting with a more diverse assemblage of pollinators. Conducting the study at this suggested scale, with individual treatments assigned to whole patches, would allow for a detection of any effect of pollinators avoiding entire patches due to the presence of spiders. In addition, this would allow for a better "snapshot" in space by increasing the extent of the study (Gotelli and Ellison 2004).

Temporal scale: Conducting the study over multiple months and/or years may help in understanding the system better. One multi-species study found significant results during some, but not all years of research (Carter and Rypstra 1995). The lack of an effect during one year of the three-year study was speculated to be due to a threshold of herbivore activity below which predators could not have an effect (Carter and Rypstra 1995). Although these specific implications may not apply to the current system due to large dissimilarities between herbivore and pollinator systems, it is possible the lack of any effect is due to low numbers of pollinators for the year or other year-specific characteristics. If the low abundance and/or diversity of larger pollinators are atypical for the year, the results may not be generalized across years. Furthermore, conducting the study over multiple years would allow for more data to be pooled in order to attain a sample size large enough to detect an effect.

Consideration of alternative species:

The spider-pollinator-plant combination studied could be the reason an effect was not found. Studying a different assemblage of organisms may have yielded different results.

Spider species: Web spiders were found to have a higher capture success of small flying insects such as *Drosophila* than spiders with different foraging tactics (Rypstra 1982). Similarly, different genera of orb-web building spider may have varying capture success for flying pollinators as well. For example, spiders from the genus *Araneus* were

found to catch more pollinating insects than *Argiope* (Olive 1980). Similarly, *A. trifasciata* tended to take smaller winged prey (Brown 1981), which made up the majority of visits to *R. fulgida* in the current study. Although no strong effect was found for *A. aurantia,* a different species such as *A. trifasciata* may have been more adapted to prey on the pollinating insects in the study, causing a different effect. Furthermore, it has been suggested that the effects of multiple predators should be considered in the context of predator-prey relationships (Sih et al. 1998).

The widely studied crab spiders are known to affect pollinator visitation rates (Dukas 2001, Dukas and Morse 2003, Reader et al. 2006). An orb-web building spider does not forage directly on a host plant and may affect visitation differently than an anthophilous spider that utilizes the same foraging grounds as pollinators. Crab spiders and orb-web building spiders may capture different types of pollinators at different rates (Olive 1980; Morse 1986), so there may be additive trophic linkage, or behaviorally mediated effects when multiple predators are taken into consideration. Riechert and Lockley (1984) state that multiple species of spiders must be taken into consideration when dealing with the suppression of arthropods to low densities because individual spider species do not necessarily respond to numerical prey increases in a density dependent fashion (Riechert and Lockley 1984). Spiders could cause prey to respond in different ways, so observing additional predators that would be found in a natural setting could offer insight to multi-species interactions. Although not common, an examination of the literature on multiple predator effects on prey found risk-enhancing effects in

certain situations (Schmitz 2007). A stationary predator such as a crab spider could potentially have risk-enhancing effects conjointly with orb-web building spiders, which could be a potentially interesting avenue of research. More generally, smaller invertebrate predators such as spiders may not support top-down regulation as other medium sized predators, such as lizards do (Spiller and Schoener 1988). Spiders may not be great organisms with which to study top-down effects in general, because invertebrate predators have been stated to have less dramatic cascading effects than vertebrate carnivores (Schmitz et al. 2000).

Pollinator species: Other studies finding an effect of spiders on pollinator visitation included *Apis mellifera* as a pollinator (Dukas 2001; Reader et al. 2006; Dukas and Morse 2005). In the current study, *Apis mellifera* was not observed visiting the plot. These bees are an introduced species, and the results of past studies may only be an artifact of an introduced species not being able to adapt to web encounters and other novel situations from an evolutionary perspective (Heiling and Herberstein 2004). If they have not evolved ways in which to interact with species that have had millions of years to interact with each other, results contrary to what may be expected of endemic species may arise (Chiao et al. 2009). Solitary bees are even thought to exhibit stronger antipredatory responses than social bees (Clark and Dukas 1994). This was attributed to the reproduction of solitary bees being contingent upon returning to their nest to lay eggs at the end of a day whereas a social bee can incrementally contribute to the reproduction of the colony during each foraging trip throughout the course of the day (Clark and

Dukas 1994). A study considering individual species of pollinators and a larger diversity of pollinators may help to shed more light onto species-specific behavioral reactions to the presence of spiders.

A study that concentrated on the foraging behavior and diet of a related species of spider (*A. trifasciata*) found that prey items <3mm were insignificant in the diet of larger individuals (Olive 1980). This could mean a trophic linkage effect of larger orb-weavers on small pollinators may not exist. In the current study, small prey items were seen suspended in the webs (personal observation), but the number of prey and escape frequency was not documented. One study found that about 78% of all prey escape within 100 seconds (Nentwig 1982). Nentwig's (1982) study used a different species of spider and was conducted at a different geographic location, so measuring the pollinator escape rate from *A. aurantia* webs may be helpful in future studies. Because almost all the visitors were in this small size category, and a trend for *A. aurantia* to take larger winged prey than *A. trifasciata* was found (Brown 1981), a different species of spider that preferentially forages on smaller prey could have yielded different results. Furthermore, stingless bees have been found to intercept webs with spiders at a lower frequency than webs without spiders while foraging from nearby hives (Rao et al. 2008). Even though solitary bees and other social bees may share similar vision characteristics, solitary bees may differ morphologically, and the research on visual perception of objects by honeybees and stingless bees may not apply. However, if vision capabilities of all bees are generalized and solitary bees recognize spiders in the same way as honeybees and

stingless bees, solitary bees may have recognized yet avoided the *A. aurantia* and their webs, and continued on to the nearby plant to collect pollen and nectar.

As mentioned previously, a way to attract a more diverse group of pollinators would be to expand the grain and extent of the study and to increase plant density. One multi-species study found bumblebees to preferentially visit larger patches over smaller ones (Schmalhofer 2001). While the current study was being conducted, one bumblebee was noted to fly into a web, escape, and then fly over an entire spider enclosure as if exhibiting learning behavior (personal observation). Another bumblebee was captured by a spider and may have decreased the average pollination time to the plant associated with that treatment during that specific observation under certain circumstances (personal observation). Although in a vastly different location and setting, and not directly researched, one experimenter noticed only occasional pollinator visits in smaller plots compared to regular visits to plots with 50 or greater plants (Agren 1996). As a consequence, a different response may have been noted if more bumblebees were present and trophic-linkage effects were common. Trophic linkage and avoidance incidents were not documented for any of the smaller pollinators in the study; however, this could be attributed to the difficulty in observing smaller insects. Lastly, pollinators could have avoided the site entirely due to the type of flower and past predation experience. Bumblebees may generalize their avoidance behavior of particular color of flower based on past simulated predation attempts (Ings and Chittka 2009). Of all the bumblebees

observed, not a single one visited plants next to a control treatment, but the data were too scarce to analyze.

The overall lack of pollinator diversity may have been a consequence of limited data collection. The types of pollinators were clumped into very general categories. This may not have been the best way to approach the study, because pollinators were found to vary in their pollination efficiency (Schemske and Horvitz 1984). An analysis of the pollination efficiency of different pollinators of *R. fulgida* could guide the observation of the effectiveness of certain species of pollinators over others.

Plant species: The UV reflectance of *R. fulgida* was not measured, but knowledge of the visual perception of flowers by bees may be helpful in understanding the attraction effects on pollinating insects. Some spiders have been found to reflect UV light (Heiling et al. 2005). In contrast, some flowers such as those of *Leucanthemum vulgare* were found to have a largely negative UV reflectance (Zschokke 2002). This contrast may be the underlying reason for pollinator avoidance of plants that host crab spiders if their coloration does not match the host plant (Thery and Casas 2002). In one study that found predator avoidance effects of crab spiders, *Leucanthemum vulgare* was the plant species studied (Suttle 2003). Using a different species of plant or spider that has a different UV reflectance may have also yielded different results.

A plant species better suited to the study would be one whose reproduction is dependent on plant mutualists (Knight et al. 2005, Knight et al. 2006). But, it is also suggested that research needs to be done to understand how threatened plants compensate

for pollen limitation by various means such as agamospermy and vegetative reproduction for their long-term survival (Wilcock and Neiland 2002). Pollen limitation may affect small populations because higher seed production per flower, and total seed number per plant have been found in larger populations than smaller ones (Agren 1996). This may be important when researching small populations of certain rare plant species (Wilcock and Neiland 2002). A self incompatible, short-lived, seed limited species that is dependent on pollinators, and does not have clonal growth would be a better candidate for future studies (Knight et al. 2005, Knight et al. 2006). Also, a plant in a fluctuating pollen environment may be more acutely affected by pollen limitation during times of low pollen dispersal (Haig and Westoby 1988). Nevertheless, the general effect of the spiders and webs on pollination visitation time could still be helpful in understanding multi-species interactions.

Other improvements:

An interesting component that may be helpful for future studies would be conducting a multi-scale study in a controlled environment by setting the treatments in the path of trained pollinators that approach from long distances, and documenting the incidences of insects that are captured, escape, continue to plants near treatments, travel to nearby plants, or retreat, similar to the methods used by Craig (1994). Also, conducting the study with spiders of different body conditions could remove any effect of a change in foraging tactics due to the satiety of the spiders (Blackledge and Zvenbergen 2007). Werner and Peacor (2003) suggest that future studies examining indirect effects

should focus on mechanisms causing trait-mediated indirect effects rather than search for the presence or absence of an effect. The current study did not examine any mechanisms for an effect, but a carefully designed study could be conducted to test hypotheses about the functions of spider coloration or web characteristics in situations where an avoidance effect is evident.

Suggested future study:

Due to the lack of an effect and inconclusive results, a more finely tuned study would be required before any definitive conclusions could be made regarding the effect of *A. aurantia* on the visitation time of pollinating insects *to R. fulgida* plants. After the careful consideration of multiple aspects governing the validity of multi-species studies involving arachnid predators, pollinators and their associated mutualist plants, the following properties of a future study are suggested: An examination at multiple scales both spatially and temporally; an inclusion of multiple predators and any risk-enhancing effects; a highly controlled environment with less environmental stochasticity and unpredictability; an examination of mechanisms; and, a fitness component. These 5 factors along with a higher sample size would allow for a better understanding of such complex systems and would allow for the comparison of multiple additional factors.

CHAPTER VIII

CONCLUSIONS

Although multiple studies have found attraction and repulsion effects of webs and spiders on prey, the indirect effects, although inconclusive, did not appear to extend to nearby plants in this particular system. The ecological implications of this is that multi-species level effects may not be strong in all spider-pollinator-plant communities, and even strong effects of predators on pollinator avoidance behavior may not extend down to the plant level. Possibly due to the complexity of the deterministic settings of ecological studies and/or experimental design issues, the current study did not have any definitive results. The results of the study lean towards the findings that spiders do not strongly affect the visitation times to plants by pollinating insects (Morse 1986). In accordance with Morse (1986), the findings also suggest that plant attraction may exert a stronger selective pressure on pollinating insects than spider predators, however, the importance of behaviorally mediated indirect effects of spiders (Goncalves-Souza et al. 2008, Dukas and Morse 2003) should not be discounted. I do not feel that the inconclusive results of the study can definitively reject the idea that the presence of spiders does not affect the pollinator visitation rate because of the many variables that may have affected the

variation in the study. Thus, a more finely tuned study would need to be designed in order to more accurately examine any effect.

Different effects on plant mutualists by spiders may be occurring at different ecological regions (Knight 2006). For example, a very similar, previously mentioned study found different results in the tropics (Goncalves and Souza, 2008). If one variable changes, such as the plant being studied or the species of spider, then very different results could occur. The results of the current study may only apply to specific semi-controlled occurrences with similar habitat characteristics.

Although no statistical differences between treatments were found, some things can be learned from the study. In a review by Schmitz et al. (2000) regarding the cascading effects of predators, it was noted that not all studies finding unexpected results proceed to publication. It is possible that more studies have found neutral effects, but have not been published because of what is known as the, "file drawer problem" (Rosenthal 1979) as mentioned by Schmitz et al. (2000). These data may be helpful for a broader understanding of these types of multi-species interactions by increasing the amount of empirical data on the visitation of pollinators to plants in the presence of orb-web building spiders. Based on the high number of factors that may have inflated the amount of variation in visitation time, I feel that the inconclusive results are primarily artifacts of the research design, but may have ecological implications. Knowing that this type of study yielded few results could help in designing future studies to look at different aspects that may have arisen from the current study.

LITERATURE CITED

Agren J. "Population Size, Pollinator Limitation, and Seed Set in the Self-Incompatible Herb Lythrum Salicaria." Ecology 77.6 (1996): 1779-1790.

Ashman TL, Knight TM, Steets JA, Amarasekare P, Burd M, Campbell D, Dudash MR, Johnston MO, Mazer SJ, Mitchell RJ, Morgan MT, and Wilson WG. "Pollen Limitation of Plant Reproduction: Ecological and Evolutionary Causes and Consequences." Ecology 85.9 (2004): 2408-2421.

Bilsing SW. "Quantitative Studies in the Food of Spiders." The Ohio Journal of Science 20.7 (1920): 215-260.

Blackledge TA. "Signal conflicts in spiders driven by predators and prey." Proceedings of the Royal Society B: Biological Sciences 265 (1998): 1991-1996.

Blackledge TA and Wenzel JW. "Do stabilimenta in orb webs attract prey or defend spiders?" Behavioral Ecology 10.4 (1999): 372-376.

Blackledge TA and Zevenbergen JM. "Condition-dependent spider web architecture in the western black widow, *Latrodectus hesperus*." Animal Behaviour 73(2007): 855-864.

Bosch J, Gonzalez A, Rodrigo A, and Navarro D. "Plant-pollinator networks: adding the pollinator's perspective." Ecology Letters 12 (2007): 409-429 2009.

Bradley RA. "Complex Food Webs and Manipulative Experiments in Ecology." Oikos 41.1(1983): 150-152.

Brechbuhl R, Kropf C, and Bacher S. "Impact of flower-dwelling crab spiders on plant-pollinator mutualisms." Basic and Applied Ecology 11 (2010): 76-82.

Briscoe AD and Chittka L. "The Evolution of Color Vision in Insects." Annual Reviews Entomology 46 (2001): 471-510.

Brown KM. "Foraging Ecology and Niche Partitioning in Orb-Weaving Spiders." Oecologia 50 (1981): 380-385.

Bruce MJ. "Silk decorations: controversy and consensus." Journal of Zoology 269 2006): 89-97.

Burd M. "Bateman's Principle and Plant Reproduction: The Role of Pollen Limitation in Fruit and Seed Set. Botanical Review 60.1 (1994): 83-139.

Cahill JF, Castelli JP, Casper BB. "The Herbivory Uncertainty Principle: Visiting Plants Can Alter Herbivory." Ecology 82.2 (2001): 307-312.

Campbell DR and Halama KJ. "Resource and Pollen Limitations to Lifetime Seed Production in a Natural Plant Population." Ecology 74.4 (1993): 1043-1051.

Cariveau D, Irwin RE, Brody AK, Garcia-Mayeya LS, and von der Ohe A. "Direct and indirect effects of pollinators and seed predators to selection on plant and floral traits." Oikos 104 (2004): 15-26.

Carter PE and Rypstra AL. "Top-Down Effects in Soybean Agroecosystems: Spider Density Affects Herbivore Damage. Oikos 72.3(1995): 433-439.

Chiao CC, Wu WY, Chen SH, Yang EC. "Visualization of the spatial and spectral signals of orb-weaving spiders, Nephila pilipes, through the eyes of a honeybee." The Journal of Experimental Biology 212 (2009): 2269-2278.

Chittka L and Raine NE. "Recognition of flowers by pollinators." Current Opinion in Plant Biology 9 (2006): 428-435.

Clark CW and Dukas R. "Balancing Foraging and Antipredator Demands: An Advantage of Sociality." The American Naturalist 144.3 (1994): 542-548.

Craig CL. "Orb-web visibility: the influence of insect flight behaviour and visual and physiology on the evolution of web designs within the Araneoidea." Animal Behaviour 34 (1986): 54-68.

Craig CL. "Insect Perception of Spider Orb Webs in Three Light Habitats." Functional Ecology 2.3 (1988): 277-282.

Craig CL, Benard G. "Insect Attraction to Ultraviolet-Reflecting Spider Webs and Web Decorations." Ecology 71.2 (1990): 616-623.

Craig CL. "Physical Contraints on Group Foraging and Social Evolution: Observations on Web-Spinning Spiders." Functional Ecology 5.5 (1991): 649-654.

Craig CL and Freeman CR. "Effects of predator visibility on prey encounter: a case study on aerial web weaving spiders." Behavioral Ecology and Sociobiology 29 (1991): 249-254.

Craig CL and Ebert K. "Colour and pattern in predator-prey interactions: the bright body colours and patters of a tropical orb-spinning spider attract flower-seeking prey." Functional Ecology 8 (1994): 616-620.

Duggins D. "Kelp beds and sea otters: an experimental approach." Ecology 6.3 (1980): 447-453.

Dukas R. "Effects of perceived danger on flower choice by bees." Ecology Letters 4 (2001): 327-333.

Dukas R, Morse D. "Crab spiders affect flower visitation by bees." Oikos 101 (2003): 157-163.

Dukas R. "Bumble bee predators reduce pollinator density and plant fitness." Ecology 86.6 (2005): 1401-1406.

Dukas R, Morse D. "Crab spiders show mixed effects on flower-visiting bees and no effect on plant fitness components." Ecoscience 12.2 (2005): 244-247.

Fan CM, Yang EC, Tso IM. "Hunting efficiency and predation risk shapes the color-associated foraging traits of a predator." Behavioral Ecology 20 (2009): 808-816.

Faul F, Erdfelder E, Buchner A, and Lang AG. "Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses." Behavioral Research Methods 41.4 (2009): 1149-1160.

Giurfa M., Vorobyvev M, Kevan P, Menzel R. "Detection of coloured stimuli by honeybees: minimum visual angles and receptor specific contrasts. Journal of Comparative Physiology 178 (1996): 699-709.

Giurfa, M and Lehrer, M. "Honeybee vision and floral displays: from detection to close-up reconition." p. 61-82 from Cognitive Ecology of Pollination: Animal Behaviour and Floral Evolution. Cambridge University Press: UK 2001.

Goncalves-Souza T, Omena P, Souza J, Romero G. "Trait-Mediated Effects on Flowers: Artificial Spiders Deceive Pollinators and Decrease Plant Fitness." Ecology 89.9 (2008): 2407-2413.

Gotelli NJ and Ellison AM. A Primer of Ecological Statistics Sinauer Associates, Inc.: Sunderland, MA 2004.

Greenstone MH. "Spider Predation: How and Why We Study It." Journal of Arachnology 27.1 (1999): 333-342.

Gripenberg S, Roslin T. "Up or down in space? Uniting the bottom-up versus top-down paradigm and spatial ecology." Oikos 116 (2007): 181-188.

Gruner DS. "Attenuation of Top-Down and Bottom-Up Forces in a Complex Terrestrial Community." Ecology 85.11 (2004): 3010-3022.

Guedot C, Bosch J, and Kemp J. "Effect of Three-dimension and Color Contrast on Nest Localization Performance of Two Solitary Bees (Hymenoptera: Megachilidae)." Journal of the Kansas Entomological Society 80.2 (2007): 90-104.

Haig D and Westoby M. "On limits to Seed Production." The American Naturalist 131.5 (1988): 757-759.

Hairston NG, Smith FE, Slobodkin LB. "Community Structure, Population Control, and Competition." American Naturalist 94.879 (1960): 421-424.

Hamback PA. "Direct and indirect effects of herbivory: Feeding by spittlebugs affects pollinator visitation rates and seedset of Rudbeckia hirta[1]." Ecoscience 8.1 (2001): 45-50.

Harder LD and Thomson JD. "Evolutionary options for maximizing pollen dispersal of animal pollinated plants." American Naturalist 133 (1989): 323-344.

Hauber ME. "Conspicuous colouration attracts prey to a stationary predator." Ecological Entomology 27 (2002): 686-691.

Heiling AM and Herberstein ME. "Predator-prey coevolution: Australian native bees avoid their spider predators." Proceedings of The Royal Society B: Biological Sciences 271 (2004): S196-S198.

Heiling AM, Cheng K, Chittka L, Goeth A, Herberstein M. "The role of UV in crab spider signals: effects on perception by prey and predators." The Journal of Experimental Biology 208 (2005): 3925-3931.

Herberstein ME, Craig CL, Coddington JA, Elgar MA. "The functional significance of silk decorations of orb-web spiders: a critical review of the empirical evidence." Biological Reviews 75 (2000): 649-669.

Herberstein ME and Heiling AM. "Positioning at the hub: does it matter on which side of the web orb-web spiders sit?" Journal of Zoology, London 255 (2001): 157-163.

Hill AM and Lodge DM. "Multi-Trophic-Level Impact of Sublethal Interactions between Bass and Omnivorous Crayfish." Journal of the North American Benthological Society 14.2 (1995): 306-314.

Hlivko J, Rypstra A. "Spiders Reduce Herbivory: Nonlethal Effects of Spiders on the Consumption of Soybean Leaves by Beetle Pest." Annals of the Entomological Society of America 96.6 (2003): 914-919.

Hoese JF, Law EAJ, Rao D, Herberstein ME. "Distinctive yellow bands on a sit-and-wait predator: prey attractant or camouflage?" Behavior 143 (2006): 763-781.

Howell F, Ellender R. "Observations on growth and diet of *Argiope aurantia* Lucas (Araneidae) in a successional habitat." The Journal of Arachnology 12 (1984): 29-36.

Hunter M, Varley G, Gradwell G. "Estimating the relative roles of top-down and bottom-up forces on insect herbivore populations: A classic study revisited." Proceedings of the National Academy of Science. 94 (1997): 9176-9181.

Hunter M, Price P. "Playing Chutes and Ladders: Heterogeneity and the Relative Roles of Bottom-Up and Top-Down Forces in Natural Communities." Ecology 73.2 (1992): 724-732.

Ings TC and Chittka L. "Speed-Accuracy Tradeoffs and False Alarms in Bee Responses to Cryptic Predators." Current Biology 18 (2008): 1520-1524.

Ishii H.S. "Floral display size influences subsequent plant choice by bumble bees." Functional Ecology 20 (2006): 233-238.

Jaffe R, Eberhard W, De Angelo C, Eusse D, Guiterrez A, Quijas S, Rodriguez A, and Rodriguez M. "Caution, Webs in the Way! Possible Functions of Silk Stabilimenta in *Gasteracantha Cancriformis* (Araneae, Araneidae)." The Journal of Arachnology 34 (2006): 448-455.

Kaston BJ. How to Know The Spiders. 3rd Edition Wm. C. Brown Company Publishers, Dubuque, IA: 1978.

Klein AM, Vaissiere B, Cane J, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T. "Importance of pollinators in a changing landscapes for world crops." Proceedings of The Royal Society B: Biological Sciences 274.1608 (2007): 303-313.

Knight T, McCoy M, Chase J, McCoy KA, Holt RD. "Trophic cascades across ecosystems." Nature 437.6 (2005a): 880-883.

Knight TM, Steets JA, Vamosi JC, Mazer SJ, Burd M, Campbell DR, Dudash MR, Johnston MO, Mitchell RJ, Ashman T. "Pollen Limitation of Plant Reproduction: Pattern and Process. Annual Review of Ecology, Evolution, and Systematics. 36 (2005): 467-497.

Knight TM, Chase JM, Hillebrand H, Holt RD. "Predation on mutualists can reduce the strength of trophic cascades." Ecology Letters 9 (2006):1173-1178.

Kunin WE. "Population Size and Density Effects in Pollination: Pollinator Foraging and Plant Reproductive Success in Experimental Arrays of *Brassica Kaber*.' Journal of Ecology 85.2 (1997): 225-234.

Lehrer M. "Locomotion Does More Than Bring the Bee to New Places." The Behaviour and physiology of bees. C.A.B. International, UK: 1991: 185-202.

Levi HW. "The spider genera Gea and Argiope in America (Araneae: Araneidae). Bulletin of the Museum of Comparative Zoology. 136: 319-352.

Levin SA. "The Problem of Pattern and Scale in Ecology: The Robert H. MacArthur Award Lecture. Ecology 73.6 (1992): 1943-1967.

Louda SM. "Inflorescence Spiders: A Cost/Benefit Analysis for the Host Plant, Haplopappus venetus Blake (Asteraceae). Oecologia 55 (1982): 185-191.

Makino, T, Ohashi K, Sakai S. "How do floral display size and the density of surrounding flowers influence the likelihood of bumble bee revisitation to a plant?" Functional Ecology 21 (2007): 87-95.

Martin P, and Bateson P. Measuring behaviour: An introductory guide 2nd edition Cambridge University Press, UK: 1993.

McCrea KD, and Levy M. "Photographic Visualization of Floral Colors as Perceived by Honeybee Pollinators." American Journal of Botany 70.3 (1983): 369-375

McReynolds CN. "The Impact of Habitat Features on Web Features and Prey Captures of *Argiope Aurantia* (Aranea, Araneidae) The Journal of Arachnology 28 (2000): 169-179

Meehan CJ, Olson EJ, Reudink MW, Kyser TK, Curry RL. "Herbivory in a spider through exploitation of an ant-plant mutualism." Current Biology 19.19 (2009): R892-R893

Miller TE and Kerfoot CW. "Redefining indirect effects." In Predation: Direct and Indirect Impacts on Aquatic Communities, eds. W.C. Kerfoot and A. Shi, p33-37. University of New England Press, Hanover, NH: 1987

Morse D. "Predatory Risk to Insects Foraging at Flowers." Oikos 46.2 (1986): 223-228.

Munoz AA and Arroyo MTK. "Negative Impacts of a Vertebrate Predator on Insect Pollinator Visitation and Seed Output in *Chuquiraga oppositifolia*, a High Andean Shrub." Oecologia 138.1 (2004): 66-73.

Nentwig W. "Why Do Only Certain Insects Escape from a Spider's Web?" Oecologia 53 (1982):412-417.

Nyffeler M, Dean D.A., Sterling W.L. "Feeding Ecology of the Orb-Weaving Spider *Argiope* a*urantia* [Araneae : Araneidae] in a Cotton Agroecosystem." Entomophaga 32.4 (1987): 367-375.

Olive CW. "Foraging Specialization in Orb-Weaving Spiders." Ecology 61.5 (1980): 1133-1144.

Pace ML, Cole JJ, Carpenter SR, Kitchell JF. "Trophic cascades revealed in diverse ecosystems." Trends in Ecology and Evolution (TREE) 14.12 (1999): 483-488.

Palmer IE, Ranney TG, Lynch NP and Bir RE. "Crossability, Cytogenetics, and Reproductive Pathways in *Rudbeckia* Subgenus *Rudbeckia*." HortScience 44.1 (2009): 44-48.

Power M. "Top-Down and Bottom-Up Forces in Food Webs: Do Plants Have Primacy?" Ecology 73.3 (1992): 733-746.

Rao D, Cheng K, Herberstein M. "Stingless bee response to spider webs is dependent on the context of encounter." Behavioral Ecology Sociobiology 63.2 (2008): 209-216.

Reader T, Higginson A, Barnard C, and Gilbert FS. "The effects of predation risk from crab spiders on bee foraging behavior." Behavioral Ecology 17.6 (2006): 933-939.

Riechert SE, Lockley T. "Spiders as Biological Control Agents." Annual Reviews Entomology 29 (1984): 299-320.

Robertson I and Maguire D. "Crab spiders deter insect visitations to slickspot peppergrass flowers." Oikos 109 (2005): 577-582.

Rosenthal R. "The "File Drawer Problem" and Tolerance for Null Results." Psychological Bulletin 86.3 (1979): 638-641.

Rypstra AL. "Building a Better Insect Trap; An Experimental Investigation of Prey Capture in a Variety of Spider Webs. Oecologia 52.1 (1982): 31-36.

Schemske DW and Horvitz CC. "Variation among Floral Visitors in Pollination Ability: A Precondition for Mutualism." Science, New Series 225.4661 (1984): 519-521.

Schmalhofer VR. "Tritrophic interactions in a pollination system: impacts of species composition and size of flower patches on the hunting success of a flower-dwelling spider." Oecologia 129 (2001): 292-303

Schmitz OJ, Beckerman AP, O'Brien KM. "Behaviorally Mediated Trophic Cascades: Effects of Predation Risk on Food Web Interactions. Ecology 78.5 (1997): 1388-1399.

Schmitz OJ, Hamback PA, and Beckerman AP. "Trophic Cascades in Terrestrial Systems: A Review of the Effects of Carnivore Removals on Plants." The American Naturalist 155.2 (2000): 141-153.

Schmitz OJ. "Top predator control of plant biodiversity and productivity in an old-field ecosystem. Ecology Letters 6 (2003): 156-163.

Schmitz OJ. "Predator Diversity and Trophic Interactions." Ecology 88.10 (2007): 2415-2426.

Schmitz OJ. "Effects of Predator Hunting Mode on Grassland Ecosystem Function." Science 319 (2008): 952-954.

Scott L, Molano-Flores B 2007. "Reproductive ecology of *Rudbeckia fulgida* Ait. Var. *sullivantii* (C.L. Boynt and Beadle) Cronq. (Asteraceae) in northeastern Illinois." Journal of the Torrey Botanical Society 134.3 (2007): 362-368.

Seah WK and Li D. "Stabilimenta attract unwelcome predators to orb-webs." Proceedings to the Royal Society B: Biological Sciences 268 (2001): 1553-1558.

Sih A, Englund G and Wooster D. "Emergent impacts of multiple predators on prey." Trends in Ecology and Evolution 13.9 (1998): 350-355.

Spiller DA and Schoener TW. "An Experimental Study of the Effect of Lizards On Web-Spider Communities." Ecological Monographs 58.2 (1988): 57-77.

Steffan-Dewenter I, Munzenberg U, Burger C, Thies C, and Tscharntke T. "Scale-Dependent Effects of Landscape Context on Three Pollinator Gulids." Ecology 83.5 (2002):1421-1432.

Stevens M and Merilaita S. "Defining disruptive coloration and distinguishing its functions." Philosophical Transactions of The Royal Society Biological Sciences 364.1516 (2009): 481-488.

Strauss SY, and Irwin RE. "Ecological and Evolutionary Consequences of Multi-species Plant-Animal Interactions." Annual Review of Ecology, Evolution, and Systematics 35 (2004): 435-466.

Strong DR. "Are Trophic Cascades All Wet? Differentiation and Donor-Control in Speciose Ecosystems." Ecology 73.3 (1992): 747-754.

Suttle K. "Pollinators as mediators of top-down effects on plants." Ecology Letters 6 (2003): 688-694.

Tahir HM, Butt A, and Sherawat SM. "Foraging strategies and diet composition of two orb web spiders in rice ecosystems." The Journal of Arachnology 37 (2009): 357-362.

Thery M and Casas J. "Predator and prey views of spider camouflage." Nature 415.10 (2002): 133.

Thery M and Casas J. "The multiple disguises of spiders: web colour and decorations, body color and movement." Philosophical Transacdtions of The Royal Society B: Biological Sciences 364 (2009): 471-480.

Toft CA and Shea PJ. "Detecting community-wide patterns: Estimating power strengthens statistical inference." The American Naturalist 122.5 (1983): 618-625

Torrey J and Gray A. A Flora of North America: Volume II. Hafner Publishing Company, NY and London: 1969

Tscharntke T, Hawkins B. Multitrophic Level Interactions. Cambridge University Press, UK: 2002.

Tso I, Liao C, Huang R, and Yang E. "Function of being colorful in web spiders: attracting prey or camouflaging oneself?" Behavioral Ecology 17 (2006): 606-613.

Tso I. "Stabilimentum-Decorated Webs Spun by Cyclosa conica (Aranea, Araneidae) Trapped More Insects than Undecorated Webs." Journal of Arachnology 26.1 (1998): 101-105.

Turnbull AL. "Ecology of the True Spiders (Aranemorphae)." Annual Reviews Entomology 18 (1973): 305-348.

Uma DB and Weiss MR. "Chemical Mediation of Prey Recognition by Spider-Hunting Wasps." Ethology 116.1 (2010): 85-95.

Vasquez DP, Morris WF, Jordano P. "Interaction frequency as a surrogate for the total effect of animal mutualists on plants." Ecology Letters 8 (2005): 1088-1094.

Wells FL. ""Shuttling" In *Argiope aurantia*." Psyche (1938): 62-71.

Werner EE and Peacor SD. "A Review of Trait-Mediated Indirect Interaction in Ecological Communities." Ecology 84.5 (2003): 1083-1100.

White TCR. "The Importance of a Relative Shortage of Food in Animal Ecology." Oecologia 33.1 (1978): 71-86.

Whitney K. "Experimental evidence that both parties benefit in a facultative plant-spider mutualism." Ecology 85.6 (2004): 1642-1650.

Wiens J A. "Spatial scaling in ecology." Functional Ecology 3 (1989): 385-397.

Wilcock C, Neiland R. "Pollination Failure in plants: why it happens and when it matters." TRENDS in Plant Science 7.6 (2002): 270-277.

Wilkinson K, Westmoreland D, Westmoreland G. "Effects of Spider Predation on Insect Visitation and Pollination of Queen Anne's Lace." The American Midland Naturalist 125.2 (1991): 364-367.

Zschokke S. "Ultraviolet Reflectance of Spiders and Their Webs." The Journal of Arachnology 30 (2002): 246-254.