THE EFFECTS OF INTRAGUILD PREDATION OF

LYSIPHLEBUS TESTACEIPES BY *CHRYSOPERLA*

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TABLE OF CONTENTS

LIST OF TABLES

Table	Page

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Oklahoma, the second largest producer of winter wheat (*Triticum aestivum* L.) in the United States, produced more than 120 million bushels in 2010 (USDA National Agricultural Statistics Service 2012). Price trends have increased in the last decade from $2.57/bushel in 2000 to $7.45 in 2011 (USDA National Agricultural Statistics Service 2012). With the rise in prices and an increase in production, pest outbreaks can pose a significant threat to net profits and trigger preventative pesticide treatments (insecticides, herbicides and fungicides).

First introduced into the United States in 1882, the greenbug (*Schizaphis graminum* Rondani, Hemiptera: Aphididae) reached outbreak proportions on small grain crops by 1901 (Jackson et al. 1970). The greenbug continues to be the most important and abundant winter wheat pest in the Southern Great Plains (Kring and Gilstrap 1983, Kindler et al. 2002). Greenbugs have piercing-sucking mouthparts with which they remove plant fluids; this feeding reduces the number of fertile plant tillers (i.e. leaves and stems) and seeds per plant, and ultimately grain yields (Burton et al. 1985, Kindler et al. 2002).

Cost effective management tools for greenbug population control involve resistant cultivars, insecticide treatments, and biological control. Unfortunately, the use of resistant cultivars is decreasing (Burton et al. 1985, Kindler et al. 2002) and insecticide resistance to some insecticides is common throughout the Southern Plains (Shufran et al. 1997, Wilde et al. 2001). Alternatively, biological control involving greenbug natural enemies has proven reliable in winter wheat systems (Jones 2001, Giles et al. 2003, Brewer and Elliot 2004).

In the Southern Great Plains, the most common natural enemies of the greenbug in winter wheat include an endoparasitoid wasp (*Lysiphlebus testaceipes* Cresson, Hymenoptera: Braconidae), ladybeetles (Coleoptera: Coccinellidae), and lacewings (Neuroptera: Chrysopidae) (Fenton and Fisher 1940, Jones 2001, Elliott et al. 2006, Donelson and Giles 2012). These organisms are considered to be in the same 'guild' because they share and compete for the same resource, the greenbug; and when one guild member consumes another it is termed intraguild predation (Polis and Holt 1992).

Several studies have examined the outcomes of intraguild predation. In particular, studies on the interactions between ladybeetle larvae and *L. testaceipes* have demonstrated negative outcomes for Coccinellidae that feed on parasitized greenbugs (Lebusa 2004, Mullins 2008, Royer et al. 2008). While green lacewings commonly occur throughout the Southern Great Plains (Donelson and Giles 2012), little is known about green lacewing biology in this region of the United States. Furthermore, no studies have documented interactions between guild members *L. testaceipes* and common *Chrysoperla* species. The studies described in this thesis were conducted to examine the effects of intraguild predation on *Chrysoperla rufilabris* Burmeister larvae to better understand how these predators function in winter wheat systems and ultimately contribute to aphid suppression.

Objectives

I.   Document the feeding capabilities and attack times for *C. rufilabris* larvae when provided with mummified greenbugs (parasitized greenbugs containing the prepupal/pupal stage of *L. testaceipes*).

II.   Determine the suitability of parasitized greenbugs for *C. rufilabris* preimaginal development, survival, and adult body weight.

Explanation of Thesis Format

This thesis describes the results of two experiments. The first experiment describes the feeding capability and attack time by *C. rufilabris* larvae when presented with *L. testaceipes* parasitized greenbugs. The second experiment quantifies the effects of *L. testaceipes* parasitized greenbugs as a food source on *C. rufilabris* larval survival, development, and weight gain. This general introduction is followed by a literature review (Chapter II), materials and methods (Chapter III), results (Chapter IV), discussion (Chapter V), and concludes with a brief summary of findings (Chapter VI). References are provided after each chapter.

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CHAPTER II

REVIEW OF LITERATURE

Winter Wheat and the Greenbug

Winter wheat, *Triticum aestivum* L., is a major commodity used for forage and grain and is primarily grown in the Central and Southern Great Plains. Oklahoma is the second largest winter wheat producing state in the country (second to Kansas); in 2010, a total of 120,900,000 bushels was harvested in Oklahoma (USDA National Agricultural Statistics Service 2012). In this unique region of the Southern Plains, winter wheat can be planted as a forage-only, grain-only, or a dual-purpose crop (Hossain et al. 2004). In a recent survey, Hossain et al. (2004) documented an equal percentage of Oklahoma winter wheat fields being used for dual-purpose and grain-only (39%), while only 22% was used as forage-only. In 2011, a total of 3.4 million acres of grain-only wheat was harvested in Oklahoma, despite a severe drought (National Agricultural Statistics Service, 2011). With the increase in price trends (from $2.57/bushel in 2000 to $7.45 in 2011), wheat production and profits are on the rise (USDA National Agricultural Statistics Service 2012). Unfortunately, this increase in wheat production may also lead to pest outbreaks and subsequent preventative pesticide treatments, as well as herbicide and fungicide treatments. Environmental damage from excessive chemical treatments in winter wheat is yet unknown, but alternative practices, especially for pest control, need to be considered in order to decrease chemical inputs into the environment, and sustain profitable winter wheat production in Oklahoma.

As with all cereal crops, there are several major arthropod pests that are capable of causing serious crop injury and yield reduction. Frequent winter wheat pests include: multiple aphid species, Lepidopteran caterpillars, mites, Hessian fly (*Mayetiola destructor* Say, Diptera: Cecidomyiidae), and grasshoppers (Royer et al. 1998). Arguably, the most important pest in winter wheat in the Southern Plains is the greenbug, *Schizaphis graminum* Rondani (Hemiptera: Aphididae). Kring and Gilstrap (1983) found the greenbug to be the most abundant aphid species in Texas winter wheat fields.

The greenbug was first introduced into the United States in 1882 and reached outbreak proportions throughout the country by 1901 on small grain crops, including winter wheat (Jackson et al. 1970). This aphid damages wheat plants by inserting its proboscis into the plant and removing fluids from the phloem; this causes wheat tillers (i.e. leaves and stems) to turn brown and die. Greenbugs can negatively impact crop productivity by reducing the number of fertile tillers and seeds per plant and subsequent grain yields (Burton et al. 1985, Kindler et al. 2002). Between 1934 and 1939 Oklahoma regularly had high populations of greenbug that nearly devastated small grain crops (Fenton and Fisher 1940). Since first being introduced, greenbug damage had resulted in a net loss of 61 million bushels of grain by 1970 (Jackson et al. 1970). More recent studies in the Southern Plains indicate that greenbugs continue to reach economically damaging levels in winter wheat and limit profitable production of this important crop (Burton et al. 1985, Kindler et al. 2002, Giles et al. 2003). Based on data from replicated field experiments, Kindler et al. (2003) predicted a reduction in winter wheat yield of 0.22bu/acre for every greenbug per tiller during normal precipitation years, whereas, during a severe drought the reduction in yield increased to 0.51bu/acre.

Clearly, greenbugs remain a threat to winter wheat production in the Southern Plains and their potential for damage justifies the use of cost effective management tools. One such tool involves greenbug resistant wheat cultivars. Some cultivars of wheat are naturally resistant to greenbug attacks and have been shown to reduce greenbug reproduction and development

(Painter 1951, Lazar et al. 1995, Michels et al. 1997). Unfortunately, it has been documented that greenbugs can overcome a plant's resistance. Kindler et al. (2002) found that yield loss caused by greenbugs occurred regardless of wheat cultivar or initial density of greenbugs per tiller, but less damage occurred on naturally resistant plants. Similarly, Burton et al. (1985) showed that resistant winter wheat cultivars can be colonized and damaged by greenbugs albeit to a lesser degree than susceptible wheat cultivars. In fact, resistant cultivars can only tolerate 50% more greenbug damage than susceptible cultivars (Burton et al. 1985). In sorghum, Huang (2011) found 21 greenbug resistance genes in a worldwide sorghum germplasm database, and these genes are likely to provide the foundation of future resistance efforts associated with greenbug management.

Traditionally, wheat producers have relied on curative insecticidal treatments to reduce greenbug numbers; however, there are a limited number of insecticides available to treat greenbug populations (Royer and Giles 2010). Many producers are quick to apply an insecticide whenever a greenbug is encountered, but applications applied late in the growing season do little to save yield (Wratten et al. 1990). While there is documentation showing insecticides effectively inhibit greenbug feeding and reproduction (Costa et al. 2010), greenbug resistance to insecticides is common (Shufran et al. 1997, Wilde et al. 2001). Wilde et al. (2001) discovered greenbugs that are resistant to 4 different insecticides exist in low population numbers throughout Oklahoma and Kansas. The presence of resistant greenbug populations in the Southern Great Plains is clearly a threat to sustainable winter wheat production.

Biological control, primarily through conservation of natural enemies that feed on pest species, has proven effective in winter wheat systems for greenbug management (Jones 2001, Giles et al. 2003, Brewer and Elliott 2004). Using replicated field cages, Jones (2001) documented greenbug suppression by *Lysiphlebus testaceipes* Cresson, an endoparasitoid wasp (Hymenoptera: Braconidae). When cages had a parasitism rate of >1.5%, greenbugs were unable to reach economic thresholds on both susceptible and resistant wheat cultivars (Jones 2001).

Greenbug suppression by *L. testaceipes* is predictable enough that a binomial sequential sampling plan, Glance 'n Go, was established to provide producers with a quick and reliable sampling method to determine if greenbug numbers warrant an insecticide application (Giles et al. 2003). Ladybeetles (Coleoptera: Coccinellidae) are also capable of maintaining low greenbug populations in winter wheat and sorghum (Kring et al. 1985, Jones 2001). Once optimal temperatures are reached, ladybeetles are effective at regulating greenbug populations in sorghum (Kring et al. 1985). Aside from Glance 'n Go, producers seldom utilize biological control for greenbug management in winter wheat.

With the rapid loss of available insecticides labeled for use in agricultural crops (Food Quality Protection Act 1996) and the occurrence of insecticide-resistant greenbugs (Shufran et al. 1997, Wilde et al. 2001), it is imperative that greenbug management in the future incorporates more sustainable practices, such as biological control, to ensure continued production of winter wheat in the Southern Great Plains.

Lysiphlebus testaceipes and Greenbugs

Parasitoids, most in the orders Hymenoptera and Diptera, are effective natural enemies utilized in biological control programs throughout the world (Van Driseche and Bellows 1996). While not much is known about Hymenopteran parasitoid mating and courtship, oviposition has been studied and detailed for many parasitoid species (Matthews 1974). Before oviposition occurs, however, the female parasitoid must locate its host using semiochemicals, usually plant chemicals that are emitted when tissue damage occurs from herbivore feeding (Matthews 1974). Once a suitable host is found, female parasitoids deposit eggs in or on a host. Parasitoid larvae then develop using the host as food and protection, and the host is eventually killed prior to adult emergence (Godfray 1994, Jervis et al. 2008).

Parasitoids can be divided into several categories based on how quickly the host is killed, where oviposition occurs and how many parasitoids are produced per host. Idiobionts immediately arrest host development or cause instant death, whereas koinobionts delay host death

9

by slowing down development (Pennacchio and Strand 2006, Jervis et al. 2008, Asgari and Rivers 2011). Ectoparasitoids lay their eggs on the surface of or around a host, and those that oviposit eggs directly into a host's hemocoel are endoparasitoids (Pennacchio and Strand 2006). Using the host as protection for preimaginal development, endoparasitoids do not have to exert extra energy on creating thick egg chorions which prevent terrestrial eggs from desiccation, or egg yolk to feed developing embryos (Pennacchio and Strand 2006). Endoparasitoids are thus able to have a higher fecundity compared to ectoparasitoids because more energy can be directed to producing a higher number of eggs (Pennacchio and Strand 2006).

Hymenopteran parasitoids can interfere with or entirely disrupt their host's immune response by injecting toxins, venom, and/or DNA into the host's body during oviposition (Fleming 1992, Strand and Pech 1995, Beckage and Gelman 2004). These injected "additives" typically produce host behavioral changes, such as reduced feeding and/or delayed reproduction (Fleming 1992, Strand and Pech 1995, Beckage and Gelman 2004). Polydnaviruses are symbiotic viruses transmitted by a female parasitoid during oviposition; once in the host, the viruses synthesize and release immunosuppressant enzymes disrupting the host's immune defenses (Beckage and Gelman 2004, Pennacchio and Strand 2006).

In the Southern Plains, winter wheat systems are relatively complex and support a variety of greenbug natural enemies (Kring and Gilstrap 1983, Jones 2001, Brewer and Elliott 2004, Elliott et al. 2006). The Braconid wasp, *L. testaceipes*, is a solitary endoparasitoid koinobiont that parasitizes many aphid species and is the most common parasitoid wasp of cereal aphids in the Southern Great Plains (Fenton and Fisher 1940, Jackson et al. 1970, Hight et al. 1972, Archer et al. 1974, Arnold 1981, Kring and Gilstrap 1983, Rice and Wilde 1988, Fernandes et al. 1998, Jones 2001, Giles et al. 2003, Brewer and Elliott 2004, Mullins 2008, Mullins et al. 2011). Females oviposit eggs regardless of the host's developmental stage (Hight et al. 1972). *Lysiphlebus testaceipes* prohibits younger aphid hosts from maturing and eventually reproducing, and decreases the fecundity of aphids attacked at the adult stage (Hight et al. 1972, Jones et al.

2005). In fact, greenbugs are no longer able to reproduce five days after being parasitized by *L. testaceipes* (Hight et al. 1972).

Depending on temperature, the typical life cycle of *L. testaceipes* requires 7-9 days for a wasp egg to mature into a pupa (Hight et al. 1972, Royer et al. 2001). During this final stage inside its host the aphid is referred to as a 'mummy' because of its swollen exoskeleton (Colfer and Rosenheim 2001). Between 10-13 days after oviposition, the adult wasp emerges from the back of the aphid and leaves behind the mummy case.

Parasitoid wasps, primarily *L. testaceipes*, help regulate aphid populations in the Southern Great Plains in a variety of cropping systems (Fenton and Fisher 1940, Archer et al. 1974, Kring and Gilstrap 1983, Jones 2001, Giles et al. 2003, Elliott et al. 2006). Indeed, *L. testaceipes* has been observed to prevent greenbug populations from exceeding the economic thresholds in wheat and sorghum systems (Fernandes et al. 1998, Jones 2001). The Glance 'n Go sampling method takes into account the number of mummies present in order to delay insecticide treatment; for example, if there are > 4 mummies in 15 randomly sampled wheat tillers then chemical treatment is not necessary regardless of the number of greenbugs on the sampled plants (Giles et al. 2003, Royer et al. 2004).

Lacewings

Insect predators are capable of fully consuming prey and, unlike parasitoids, typically require more than one prey item to complete development and reproduction (Van Driesche and Bellows 1996, Norris et al. 2003). In Oklahoma winter wheat, the most abundant predators are Coccinellidae, Chrysopidae, Carabidae, Nabidae, Staphylinidae, and Araneae (Elliott et al. 2006).

The main focus of greenbug predators has been on Coccinellidae (Jones 2001, Lebusa 2004, Mullins 2008, Phoofolo et al. 2007 and 2008, Royer et al. 2008); however, little is known about the biology of green lacewings (Neuroptera: Chrysopidae) in Oklahoma winter wheat systems. Lacewings are common insect predators throughout the world and are used as biological control agents in row crops, orchards, greenhouses, and home gardens because of their voracious

larval appetites and tolerance to many pesticides (Afzal and Khan 1978, Canard et al. 1984,

Hagley 1989, Mizel and Schiffhauer 1990, Nordlund et al. 1991, Romeis et al. 2004).

Chrysoperla species are among the most abundant predator groups throughout North America

inhabiting many agricultural systems such as cotton, corn, alfalfa, wheat, soybean, and citrus and

apple orchards (Afazal and Khan 1978, Tassan et al. 1979, Elkarmi et al. 1987, Hagley 1989,

Romeis et al. 2004, Woolfolk and Inglis 2004, Freier et al. 2007). During a 2011 study, green

lacewings were the most abundant predator group on sticky traps in winter wheat systems; in fact,

lacewings outnumbered ladybeetles in Oklahoma wheat fields 5:1 (Donelson and Giles 2012).

The most abundant green lacewings found in North America are *C. rufilabris* Burmeister

and *C. carnea* Stephens. *Chrysoperla rufilabris* is found as far north as Ontario and as far south

as northeast and central Mexico, and range from the east coast of the United States to as far west

as Minnesota, Kansas, and Texas (Tauber 1974). The closely related species, *C. carnea*, is a

Holoarctic species that has been recorded throughout the United States. Tauber and Tauber

(1983) found that *C. rufilabris* is better adapted to moist climates, whereas *C. carnea* is more

suited for drier areas.

Chrysoperla eggs are small green ovals that are laid in clusters or singly (Canard et al.

1984). Eggs typically have a silk stalk that not only serves as protection from predation but also

cannibalism (Ruzicka 1997). When larvae begin to hatch, they remain near their egg shell for a

period of time, ranging from several minutes to a couple days, and refrain from eating (Canard et

al. 1984). Larvae are caterpillar-like in shape and undergo two larval molts, resulting in a total of

three larval instars (Afzal and Khan 1978, Canard et al. 1984). Tauber and Tauber (1983) found

that low relative humidity (<35%) can lengthen larval development and decrease survivorship.

Malpighian tubules, located in the hindgut, produce silk that is used to spin a pupal cocoon

(Canard et al. 1984). Ru et al. (1976) found that *C. rufilabris* pupation was also dependent on

relative humidity and other abiotic factors such as temperature. Adults are bright green with large

membranous wings, and can survive for 3 months (Canard et al. 1984). *Chrysoperla rufilabris*

12

females are able to lay significantly more eggs (23 eggs/day) than *C. carnea* (13 eggs/day) and can continually lay eggs for 15-20 days (Ru et al. 1976, Elkarmi et al. 1987).

Lacewing larvae feed via extra-oral digestion (Cohen 1995). Their mandibles are hollowed tubes that allow for digestive fluid to be injected into a prey item from the lacewing's midgut (Canard et al. 1984). The digestive fluid breaks down the prey's solid internal materials which are then taken up through the mandibles for further digestion within the digestive tract. *Chrysoperla* adults feed on pollen, nectar and honeydews (Afzal and Khan 1978, Canard et al. 1984). However, these foods contain few nutrients necessary for the adults to survive and reproduce (Woolfolk et al. 2004). Nutrient rich yeasts, bacteria and fungi are obtained from the natural environment; the symbiotic yeasts are then stored within the esophageal diverticulum, whereas bacteria and fungi are considered nonresidents and pass through the digestive track entirely (Hagan et al. 1970; Woolfolk and Inglis 2004, Woolfolk et al. 2004). Larvae and young adults (<24hr old) do not contain yeasts within their digestive tracks (Hagan et al. 1970, Woolfolk and Inglis 2004).

The quantity and quality of larval diet plays a significant role in lacewing development, survival and reproduction (Hydorn and Whitcomb 1979, Canard et al. 1984, Greenberg et al. 1994, Balasubramani and Swamiappan 1994 and 1998, Chen and Liu 2001, Atlihan et al. 2004). Hydorn and Whitcomb (1979) showed that *C. rufilabris* larvae had the highest survival rate (75%), a longer duration of ovipositioning (59.3 days), and higher fecundity (188.7 eggs) when fed an aphid diet (*Myzus persicae* Sulzer, *Acyrthosiphon pisum* Harris, *Aphis spiraecola* Patch, *A. rhamni* Fonscolombe, and *A. craccivora* Koch) compared to a diet of citrus mite, *Tetranychus tumidus* Banks (Acarina: Tetranychidae). Chen and Liu (2001) evaluated the suitability of three aphid species: *Aphis gossypii* Glover, *M. persicae*, and *Lipaphis erysimi* Kaltenbach on *C. rufilabris* larvae. Development was significantly different among treatments with larvae developing quickest on a diet of *A. gossypii*, whereas larvae on the *L. erysimi* diet did not survive to adult emergence (Chen and Liu 2001). Atlihan et al. (2004) showed that varying aphid

densities can significantly increase developmental rates for *C. carnea* when fed *Hyalopterus pruni* Geoffroy. Larvae that were fed >10 aphids/day developed significantly faster during the third instar and the total preimaginal stage; however, even at low prey densities (<10/day), larvae were still able to complete their life cycle (Atlihan et al. 2004).

The suitability of aphid prey can also be influenced by differences in host plants, i.e. tritrophic interactions (Messina et al. 1995, Legaspi et al. 1996, Balasubramani and Swamiappan 1998, Clark and Messina 1998, Giles et al. 2000). Giles et al. (2000) documented a host plant-related qualitative difference in aphid diets for *C. rufilabris* larvae. Pea aphids reared on alfalfa, *Medicago sativa* L., had a higher myristic acid and total fatty acid content than pea aphids reared on faba bean, *Vicia faba* L.; and this resulted in quicker development for larvae supplied with alfalfa-reared pea aphids compared with those supplied pea aphids reared on faba bean (Giles et al. 2000).

Intraguild Predation

A phenomenon termed intraguild predation occurs when one guild member consumes another guild member (Polis and Holt 1992). Most intraguild predation studies have focused on interactions between predators competing for the same prey resource (Rosenheim et al. 1993, Finke and Denno 2003, Holt and Huxel 2007); however, intraguild predation can occur between predators and parasitoids. Many studies have shown the negative effects intraguild predation has on specialist natural enemies (Rosenheim et al. 1993, Snyder and Ives 2001 and 2003, Finke and Denno 2003). Finke and Denno (2003) state that intraguild predation decreases the pressure of top-down interactions and thus the lower-trophic level organism may no longer be under control. Snyder and Ives (2001) examined the interactions between pea aphids (*A. pisum*), carabid beetles, and the Braconid parasitoid *Aphidius ervi* Haliday. They found that the carabid beetles decreased the parasitoid population and thus reduced the control the wasps had over the pea aphids in an alfalfa system; however they did not examine how other predators interacted within that system and thus provided additional herbivore suppression (Snyder and Ives 2001).

Some predators are able to differentiate, or show preference for, unparasitized aphids from parasitized aphids. Colfer and Rosenheim (2001) evaluated interactions among the cotton aphid *A. gossypii*, the convergent ladybeetle *Hippodamia convergens* Guérin-Méneville, and *L. testaceipes*. During a preference study, *H. convergens* was more likely to consume an unparasitized aphid than a parasitized one and this combined mortality resulted in pest reductions (Colfer and Rosenheim 2001). When ladybeetles were coupled with *L. testaceipes* in field cages, cotton aphids were significantly reduced and maintained at low population levels (Colfer and Rosenheim 2001). Because ladybeetles showed a preference for unparasitized aphids, wasps were able to escape intraguild predation (in the parasitized aphids) and suppress aphid populations.

Recently, there has been an increase in intraguild predation research involving Hymenopteran parasitoids, ladybeetles, and aphids. Takizawa et al. (2000) demonstrated that the seven-spotted ladybeetle, *Coccinella septempunctata* L., had a significantly lower survival rate when fed mummies (*A. craccivora* parasitized by *Aphidius colemani* Viereck). It was suggested that parasitized greenbugs and nonparasitized greenbugs have different nutritional qualities, and a diet of parasitized greenbugs is suboptimal for the ladybeetle (Takizawa et al. 2000). Using *L. testaceipes*, Lebusa (2004) found that *H. convergens* and *C. septempunctata* first and second larval instars were incapable of consuming late-stage mummified greenbugs; however, no preference could be detected for older ladybeetle instars. They also showed that a diet of parasitized greenbugs slowed development for third and fourth instars and caused a decrease in adult weight (Lebusa 2004, Royer et al. 2008). Another experiment using *L. testaceipes* showed early and late stages of parasitism (prior to mummification) also negatively affected development of *H. convergens* third and fourth instars (Mullins 2008). These recent life history studies between Coccinellidae and parasitoids have revealed that intraguild predation may overall be more detrimental to the predator because parasitized aphids are a lower quality food source.

Chrysoperla species are often the most abundant predators in winter wheat systems of the Southern Plains (Fenton and Fisher 1940, Gaona et al. 2000, Donelson and Giles 2012); however,

no studies have documented the interactions between the greenbug-guild members *L. testaceipes* and lacewings. Studies investigating the effects of parasitized aphids on *Chrysoperla* biology will provide important information on how these predators interact with parasitoids, and ultimately, their contribution to aphid suppression in winter wheat systems in the Southern Great Plains.

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CHAPTER III

MATERIALS AND METHODS

Greenbug Colonies

Four colonies of Biotype 'E' greenbugs, *Schizaphis graminum*, were maintained using specimens obtained from the USDA Agricultural Research Service Laboratory located in Stillwater, Oklahoma. Susceptible wheat (*Triticum aestivum*) cultivars ('OK101' and 'Custer') were planted in a 50:50 mixture of fritted clay and potting soil with a 20:20:20 (N:P:K) fertilizer in 15cm diameter pots (Mullins 2008). Approximately 150 seeds were planted into each pot and when plants were approximately 6 inches tall (1 week after emergence), greenbugs were added by placing older infested wheat tillers on top of the new plants. All infested plants were kept in double-walled (2.5cm) fine mesh cages which prevented parasitism and aphids escaping through the mesh but allowed for airflow into the cages (Jones 2001, Mullins 2008). Cages were maintained in the laboratory at room temperature (~24°C) under florescent lighting (12:12 L:D). Plants damaged due to greenbug feeding were replaced two times per week and plants were watered as needed.

Pea Aphid Colonies

Pea aphids, *Acyrthosiphon pisum*, were reared on faba bean, *Vicia faba* (Cultivar 'Windsor'). All plants were grown in 15cm diameter plastic pots in a 50:50 mixture of fritted clay and potting soil with 20:20:20 (N:P:K) fertilizer. When plants were 11 days old (approximately 6 inches tall), pea aphids were added directly onto the leaves and infested pots were then placed in four separate single-walled mesh cages in the laboratory at room temperature under florescent lights (12:12 L:D). Six new plants were added weekly to each cage and old, damaged plants were

removed as needed. Pea aphids were harvested daily to prevent excessive plant injury and all plants were watered as needed.

Lysiphlebus testaceipes Colonies

The *L. testaceipes* colonies were initiated by obtaining parasitized aphids from an infested greenhouse in Stillwater, Oklahoma. Adult wasps were identified as *L. testaceipes* using a taxonomic key. Emerged parasitoid adults were aspirated and placed into double-walled (2.5cm) fine mesh cages that contained 3-4 pots (15cm diameter) of greenbug infested wheat (see Greenbug Colonies); each cage had a cloth sleeve for access. Cages were maintained at room temperature under florescent bulbs (12:12 L:D) in a separate laboratory to prevent contamination of aphid colonies (Jones 2001, Mullins 2008). In addition, parasitoid colonies were attended last in sequence to prevent contamination of aphid colonies.

One week old plants were infested with greenbugs 24 hours prior to being placed in parasitoid cages. Newly formed parasitoid mummies were removed as needed for experiments (see Experiments I and II) and wheat plants were replaced weekly. Damaged wheat tillers with mummies were cut and placed on new plants which allowed for retention of wasps that had not yet emerged from parasitized greenbugs, and were interchanged among cages to maintain parasitoid vitality (Mullins 2008). All plants were watered as needed which provided adequate moisture in the cages for adult parasitoids (Mullins 2008).

Experiment I. Feeding capability and attack time for *Chrysoperla rufilabris* larvae when provided *L. testaceipes* parasitized greenbugs

Lacewing eggs were purchased from Rincon-Vitova Insectaries Inc., California. Eggs were isolated into 30ml plastic containers with lids and observed every 12 hours for eclosion. Upon eclosion, larvae were provided unlimited pea aphids daily prior to feeding observation studies. Feeding capability evaluations were conducted during the first or third instar stage for *C. rufilabris*. First instars were 3 days old at the time of observations (just prior to molting), whereas observations on third instars were initiated at the beginning of the stadium. Prior to beginning

individual observations, larvae were starved for 24 hours. All larvae were maintained in table-top environmental chambers at 24°C, 16:8 L:D cycle, and 24% RH (See Experiment II for low relative humidity explanation). A total of 59 larvae were randomly assigned to three treatments for first and third instars (9-10 individuals/treatment). First (n=29) or third (n=30) instars were provided four 1 day old mummies, four 3 day old mummies, or four unparasitized greenbugs.

Observations of individual larval behavior occurred within a 5cm petri dish over 30 minutes using a handheld stopwatch. For each individual observation, the assigned food items were placed in the center of the petri dish followed by a randomly assigned individual lacewing larva. The number of attacks per food item, length of attack and consumption of any food items were recorded during each observation. "Consumption" of unparasitized greenbugs was recorded when feeding caused the greenbug to be shriveled and white. Consumption of the mummy food items was recorded when, upon dissection, the wasp larvae (1 day old mummy) or pupae (3 day old mummy), was shriveled in appearance (C. N. Jessie unpublished data).

Experiment II. Suitability of *L. testaceipes* parasitized greenbugs for *C. rufilabris* preimaginal development, survival and adult body weight

Lacewing eggs were purchased (Rincon-Vitova Insectaries Inc., California) 6 different times throughout 2011. Eggs were isolated into 30ml plastic containers with lids and maintained in table-top environmental chambers (24°C; 16:8 LD; 24% RH). The low RH used for this experiment is typical of the stressed conditions organisms face in Western Oklahoma during late winter and early spring when atmospheric saturation is low due to moderate-high temperatures and little precipitation (National Oceanic and Atmospheric Administration 2012). Upon eclosion, lacewing larvae were randomly assigned one of 4 daily diet treatments. The daily treatments were: 10 large, unparasitized greenbugs (2mg); 2 large, unparasitized pea aphids (2mg); 12 mummies (2mg); and 12 mummies (2mg) with a moistened cottonball ("mummy+water"). *Chrysoperla* larvae are capable of completing development at these low prey levels which allows for quantitative assessment of suitability differences among diet items (Legaspi et al. 1994, Giles

et al. 2000, Atlihan et al. 2004). Mummies used for this study were considered to be mid-stage (2 day old), that is, the mummy had taken form but the parasitoid larvae was not yet a fully formed adult wasp. Previous studies have evaluated early-stage (1 day old) or late-stage mummies (Lebusa 2004 and Mullins 2008, respectively).

There were a total of 60 larvae assigned to the greenbug (GB) treatment, 52 larvae assigned to the pea aphid (PA) treatment, 48 larvae assigned to the mummy (M) treatment, and 43 larvae assigned to the mummy+water (MW) treatment. An additional 43 larvae were individually fed 16mg of pea aphids per day and this diet treatment was considered an unlimited check (Giles et al. 2000) but was not included in the statistical analyses. Individual larvae were systematically checked every 24 hours. Old, uneaten prey items were removed daily and replaced with freshly collected prey. All lacewing molts and deaths were recorded daily. Upon adult emergence, a total of 32 lacewings from the 2mg diet treatments (GB = 8, PA = 11, M = 5, MW = 8) and 29 lacewings from the unlimited diet treatment were placed into an oven at 50°C for 4 days and individual dry weights were recorded using a digital microbalance. Voucher specimens from each diet treatment were placed in the Department of Entomology and Plant Pathology Museum at Oklahoma State University, Stillwater.

Analyses were performed using SAS version 9.1 (SAS Institute 2003), and a significance level of $P = 0.05$ was used for all analyses. For feeding observations the number of attacks, length of attack (time in seconds), and number of prey items consumed over a 30 minute period were analyzed using ANOVA (PROC MIXED) followed by LSMEANS comparisons among treatments. For the suitability study, differences in developmental time and adult body weight among the 4 diet treatments were analyzed using ANOVA (PROC MIXED) with a Satterthwaite adjustment for degrees of freedom. LSMEANS comparisons were performed when diet treatments were significantly different. Lacewing purchase number (1-6) was included as a categorical blocking factor to account for potential differences in fitness among orders. Chi-square analysis (PROC FREQ) was used to compare acute and cumulative survival ratios (larval,

pupal, and larval+pupal) for all of the diet treatments. Acute survival is the ratio of larvae surviving within a life stage, whereas cumulative survival is the ratio of larvae surviving to each successive life stage.

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CHAPTER IV

RESULTS

Experiment I. Feeding capability and attack time for *Chrysoperla rufilabris* larvae when provided *Lysiphlebus testaceipes* parasitized greenbugs

First Instar C. rufilabris

Among diet treatments, handling times differed significantly for first instar *C. rufilabris* (Table 1; F = 10.98, df = 2, 26, p < 0.001). Larvae supplied with 1 day old mummies (1d M) or 3 day old mummies (3d M) spent significantly less time handling ("Attack Time") prey items (p = 0.002 and p < 0.001, respectively) than did larvae supplied with unparasitized greenbugs (GB). The number of attacks by first instars were significantly different among treatments (F = 4.25, df = 2, 26, p = 0.025), and larvae supplied the 1d M treatment had a significantly higher number of attacks than larvae supplied the greenbug treatment (p = 0.008). The number of prey items fully consumed by first instars did not differ among treatments (F = 1.24, df = 2, 26, p = 0.306). First instars supplied the greenbug treatment did not fully consume any prey items, whereas two larvae in the 1d M treatment consumed 1 prey item each, and one larva supplied the 3d M treatment consumed 1 prey item.

Third Instar C. rufilabris

Handling time for third instars (Table 1) did not differ significantly among diet treatments (F = 2.63, df = 2, 27, p = 0.090). In addition, there was no significant difference in the number of attacks among treatments for third instars (F = 0.39, df = 2, 27, p = 0.680), or the number of prey items consumed for third instars (F = 0.20, df = 2, 27, p = 0.821). Third instars in

the 3d M treatment consumed an average of 2.0 prey items, while larvae supplied the 1d M treatment and greenbug treatment consumed an average of 1.8 and 1.7 prey items, respectively.

Experiment II. Suitability of *L. testaceipes* parasitized greenbugs for *C. rufilabris* preimaginal development, survival and adult body weight

As previously described, the unlimited (16mg/day) pea aphid daily treatment (PAU) was included in the study as a separate control. And as expected, larvae supplied with the unlimited daily levels of pea aphids had the highest survivorship levels, the shortest developmental times and largest body weight (Tables 2 and 3; Figure 1). These findings indicate that the 2mg/day diet treatments were indeed limited and allowed for quantitative evaluation and comparisons of prey suitability.

Survivorship

Cumulative proportion survivorship differed among diet treatments at each successive developmental stage (Table 2; $\chi^2 > 10.8$, df = 3, p = 0.013). Among diet treatments statistically compared, larvae fed the limited (2mg/day) pea aphid (PA) diet had the highest overall cumulative survivorship (0.500), whereas those fed the mummy diet had the lowest overall cumulative survivorship (0.184). Larvae provided the mummy (M) diet had the lowest survival rate through each successive developmental stage, whereas larvae provided the pea aphid diet had the highest survival rate (Table 2).

Acute survivorship (within instar only) was only significant for first instars ($\chi^2 > 35.3$, df = 3, p < 0.001); there was no significant difference in acute survivorship for second instars ($\chi^2 = 5.15$, df = 3, p = 0.1614), third instars ($\chi^2 = 4.26$, df = 3, p = 0.2345), or pupae ($\chi^2 = 3.75$, df = 3, p = 0.29) among diet treatments.

A small proportion of adults were not able to successfully emerge from pupal casings (Table 2 "Success"). Interestingly, the highest level of mortality associated with this phenomenon occurred among larvae supplied with limited daily levels of pea aphids. When these proportions

were included as a measure of survivorship ("Success"), overall successful emergence as adults were similar among treatments ($\chi^2 = 2.15$, df = 3, p = 0.542).

Preimaginal Development

Developmental times were significantly different among the 4 diet treatments for first instar *C. rufilabris* (Table 3; F = 5.00, df = 3, 101, p = 0.003). First instars provided with greenbugs had a significantly shorter average developmental time compared with those fed pea aphids (p = 0.002) and mummies+water (MW) (p = 0.002); however, developmental times were similar among larvae provided with pea aphids, mummies+water, or mummies. While developmental times varied for second instars, there were no significant differences among the diet treatments (F = 2.50, df = 3, 83.9, p = 0.065). Similar to first instars, second instars fed the greenbug diet had the shortest development time. There were no significant differences in developmental times among diet treatments for third instars (F = 1.41, df = 3, 68.3, p = 0.248). Third instars fed the mummy diet went into pupation more quickly and larvae fed the mummy+water diet took the longest to begin pupation. There were no significant differences in overall larval developmental times among the 4 diet treatments (F = 1.03, df = 3, 69, p = 0.384), but interestingly, larvae fed the mummy+water diet took the longest to develop. There were significant differences in pupal developmental times among diet treatments (F = 4.10, df = 3, 67, p = 0.010). Larvae fed the mummy+water diet were in pupation for a significantly shorter period than larvae fed the greenbug diet and pea aphid diet (p = 0.004 and p = 0.002, respectively); however, larvae provided with greenbug and pea aphid diets did not differ significantly. The total time for preimaginal (larval+pupal) development was not significantly different among diet treatments (F = 1.01, df = 3, 67, p = 0.395). Larvae developed quickest when fed the mummy+water diet; whereas larvae fed the pea aphid diet developed the slowest.

Adult Body Weight

Average adult dry weights ranged from 1.28 - 1.45mg for the limited daily diet treatments, and 2.86mg for the unlimited daily pea aphid treatment. In general, larvae weighed

more when fed the limited pea aphid diet, whereas, larvae fed the mummy diet weighed the least. However, there were no significant differences in adult body weights among limited diet treatments (Figure 1; $F = 1.12$, $df = 3, 28$, $p = 0.357$). Females and males were combined in analyses as *C. rufilabris* sex ratios pertaining to low levels of daily prey have been documented as insignificant (Giles et al. 2000).

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(Homoptera: Aphididae) reared on alfalfa and faba bean. Environmental Entomology 29:304-311.

Table 1. Attack time, number of attacks and number of prey consumed (mean ± SE) for *C. rufilabris* larvae when provided one of 3 diet treatments[a] during a 30min observation.

Instar	*n*	Treatment	Attack Time (sec)	# of Attacks	# Fully Consumed
First[b]	10	GB	1501.5 ± 180.3a[c]	1.4 ± 1.3a	0.0a
	9	1d M	557.8 ± 190.0b	7.0 ± 1.4b	0.2 ± 0.1a
	10	3d M	376.4 ± 180.3b	4.6 ± 1.3ab	0.1 ± 0.1a
Third	10	GB	1345.6 ± 120.2a	2.8 ± 0.6a	1.7 ± 0.3a
	10	1d M	976.2 ± 120.2a	3.5 ± 0.6a	1.8 ± 0.3a
	10	3d M	1269.1 ± 120.2a	2.9 ± 0.6a	2.0 ± 0.3a

[a]Treatments: GB = received four unparasitized greenbugs; 1d M = received four 1 day-old mummies; 3d M = received four 3 day-old mummies.

[b]First and third instars analyzed separately.

[c]Means within a column and within an instar followed by the same letter are not significantly different.

Table 2. Effects of diet treatments[a] on *C. rufilabris* larval and pupal cumulative[b] survivorship at 24°C, 16:8 (L:D), and 24% RH.

Treatment	n	Proportion[c] Surviving				
		1st Instar	2nd Instar	3rd Instar	Pupal	Success
GB	60	0.667a	0.633a	0.483a	0.417a	0.267a
PA	52	0.750a	0.654a	0.519a	0.500a	0.308a
M	48	0.208b	0.184b	0.184b	0.184b	0.158a
MW	43	0.465c	0.395c	0.372ab	0.302ab	0.233a
PAU[d]	42	0.905	0.881	0.810	0.738	0.690
	χ^2	35.3	26.2	12.2	10.8	2.8
	df	3	3	3	3	3
	P	<0.0001	<0.0001	0.0068	0.0127	0.422

[a]Treatments: GB = received 2mg/day of greenbugs; PA = received 2mg/day of pea aphids; M = received 2mg/day of mummies; MW = received 2mg/day of mummies plus a moist cottonball.

[b]Cumulative survivorship is the proportion of larvae surviving to each successive stage.

[c]Proportions followed by the same letter are not significantly different.

[d]PAU was not used in statistical analysis.

Table 3. Effects of diet treatments[a] on *C. rufilabris* larval and pupal developmental times (±SE) at 24°C, 16:8 (L:D), and 24% RH.

Diet Treatment	n	Developmental Time (days)					
		1st Instar	2nd Instar	3rd Instar	Larval	Pupal	Larval + Pupal
GB	60	3.9 ± 0.3ab	3.5 ± 0.2a	10.2 ± 0.8a	17.6 ± 0.1a	12.2 ± 0.1a	29.2 ± 0.7a
PA	52	4.7 ± 0.3b	4.0 ± 0.2a	8.9 ± 0.9a	17.3 ± 0.8a	12.2 ± 0.1a	30.3 ± 0.7a
M	48	4.4 ± 0.4ab	4.4 ± 0.5a	7.9 ± 1.5a	16.9 ± 1.4a	11.9 ± 0.3ab	28.7 ± 1.3a
MW	43	4.9 ± 0.3b	4.4 ± 0.3a	10.3 ± 1.1a	19.3 ± 1.0a	11.5 ± 0.2b	28.5 ± 1.0a
PAU[c]	43	4.1 ± 0.7	3.1 ± 0.4	4.3 ± 0.8	11.5 ± 1.0	12.1 ± 0.4	23.5 ± 1.0

[a]Treatments: GB = received 2mg/day of greenbugs; PA = received 2mg/day of pea aphids; M = received 2mg/day of mummies; MW = received 2mg/day of mummies plus a moist cottonball; PAU = received 16mg/day of pea aphids.

[b]Developmental times (LSMEANS) of a diet treatment within a column followed by the same letter are not significantly different.

[c]PAU was not used in statistical analysis.

Figure 1. Dry adult body weights (±SE) for each diet treatment (GB n=8; PA n=11; M n=5; MW n=8; PAU n=42).
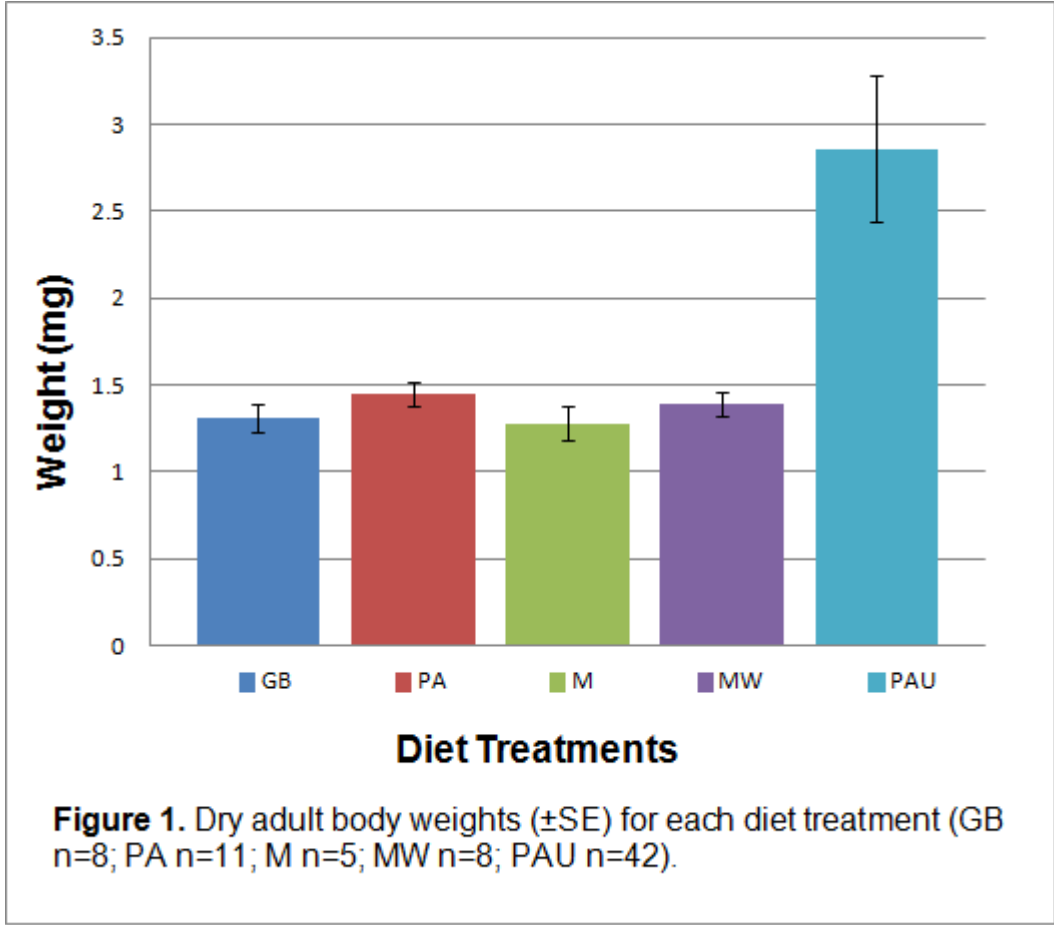
CHAPTER V

DISCUSSION

By the end of the growing season (April-June), winter wheat (*Triticum aestivum*) in the

Southern Great Plains is reguilarly inhabited by large populations of greenbug (*Schizaphis*

graminum) natural enemies, including parasitoids, ladybeetles, and lacewings (Giles et al. 2003,

Elliott et al. 2006, Phoofolo et al. 2010, K. L. Giles unpublished data). However, evidence is

unclear as to the timing of when predator and parasitoids initially interact in winter wheat

systems. Rice and Wilde (1988) documented that ladybeetles are negatively impacted by cold

winter weather and *Lysiphlebus testaceipes* is often absent, therefore, these organisms rarely

interact. In Oklahoma, however, *L. testaceipes* is present in winter wheat fields throughout the

growing season and will continue to parasitize greenbugs, even at low atmospheric temperatures

(Jones 2001, Giles et al. 2003, Jones et al. 2003). Predators (ladybeetles and lacewings) in the

Southern Plains are present in winter wheat during the late summer-fall but more often achieve

high populations during the mid-to-late spring when higher populations of parasitoids are actively

foraging (Agnew et al. 1981, Phoofolo et al. 2010, Mullins et al. 2011, K. L. Giles unpublished

data). With the co-occurrence of these natural enemies in Oklahoma, intraguild predation is likely

to occur. Indeed, Mullins et al. (2011) used PCR techniques to document that ladybeetles (both

adults and larvae) collected from winter wheat fields during early spring were in fact consuming

L. testaceipes parasitized greenbugs; up to 100% of ladybeetles collected had parasitoid DNA

within their gut tracks. Recent studies describing ladybeetle-parasitoid life history interactions

provide evidence that intraguild predation can negatively affect both natural enemies (Takizawa

et al. 2000, Lebusa 2004, Mullins 2008, Royer et al. 2008). However, lacewings are often the

most common predators in these systems, but nothing is known about their interactions with *L. testaceipes*.

Despite being the only grain crop during winter months in the Southern Plains, winter wheat grows during stressed conditions for most natural enemies. The low atmospheric moisture is unsuitable for organisms, such as lacewings, that require high relative humidities for optimal survival and development (Tauber and Tauber 1983). Food supply is also limited because greenbug populations have a characteristic "boom/bust" cycle dependent on host plant quality, natural enemy interactions and weather conditions (Dixon 1977, Phoofolo et al. 2009). These common stressed conditions likely increase competition for greenbug resources and subsequently the frequency of intraguild predation events. The experiments described in this thesis were designed to mimic the stressed conditions common in the Southern Great Plains region with low relative humidities and low prey densities and to define intraguild interactions between *Chrysoperla rufilabris* and *L. testaceipes*.

Lacewing Larval Feeding Capability and Attack Time

Unlike third instars, first instars of *C. rufilabris* had higher number of attacks and a shorter handling (attack) time when supplied with mummies (1 day and 3 day old mummies: 1d M and 3d M) compared to greenbugs (Table 1). The shorter handling time of *C. rufilabris* suggests that first instars were rejecting 1d M and 3d M by attacking the prey items multiple times and only handling the prey items for short periods. Therefore, first instar *C. rufilabris* larvae appeared to prefer unparasitized greenbugs over mummified greenbugs. Similarly, Nordlund and Morrison (1990) showed that for preferential prey, such as Lepidopteran larvae, *C. rufilabris* larvae had a longer handling time compared to prey items that were not preferred (Lepidopteran eggs). Alternatively, the hard mummy exoskeleton could be inhibiting the small mandibles of first instar *C. rufilabris* from penetrating the mummy in order to feed. Lebusa (2004) found that first instar ladybeetles were unable to penetrate the hardened mummy exoskeleton and thus were incapable of feeding on mummies. However, despite the apparent

rejection of mummies, some *C. rufilabris* first instars were capable of consuming 1d M and 3d M (Table 1). These contrasting results demonstrate a significant difference in feeding capabilities between lacewing and ladybeetle larvae.

Chrysoperla rufilabris third instars showed no significant difference in handling times among the treatments, although handling times with greenbug prey were slightly longer than the handling times of 1d M and 3d M. Meyhöfer and Klug (2002) documented similar results with *C. carnea* third instars and the aphid *Aphis fabae* Scopoli; however, *C. carnea* spent an average of 10min per mummy (*A. fabae* parasitized by *L. fabarum* Marshall) whereas *C. rufilabris* had a handling time of 5min per mummified greenbug (although aphid species and/or parasitoid species could account for these differences). Lacewing handling times differ from ladybeetle larvae which take a significantly longer time to handle mummies than unparasitized greenbugs (Lebusa 2004, Royer et al. 2008). Regardless of stadia, compared with ladybeetle larvae, lacewing larvae have larger sized mandibles and are likely able to manipulate and consume prey more quickly (Michaud and Grant 2003).

Lacewing Larval Survivorship and Development

Cumulative survivorship levels (the proportion of larvae surviving to each successive life stage) were significantly different among the diet treatments for the larval stages and the pupal stage, but ultimately, the proportion of larvae surviving to adulthood was not significantly different among the diet treatments (Table 2). For each diet treatment, the vulnerable first larval stage had the highest mortality level suggesting that under stressed conditions this stage is critical for *C. rufilabris* larval survivorship. Considering the feeding capabilities of first instar *C. rufilabris* larvae on mummies, it is no surprise that the first larval stage experienced a significant drop in survivorship, especially for those larvae supplied mummy and mummy+water diets. Comparing survival between larvae supplied the limited 2mg/day pea aphid diet and 16mg/day unlimited pea aphid diet, larvae were twice as likely to survive on the unlimited diet (69% versus 30% survivorship). This indicates, relative to the current study, quantity of diet, not "quality" of

43

diet, may play the most important role in *C. rufilabris* survival. In fact, many studies have documented the significance of diet quantity as it pertains to *Chrysoperla* survival. (Canard et al. 1984, Giles et al. 2000). Despite significant differences in larval survivorship, the proportion of successfully emerged adults was similar for all limited diet treatments, indicating that quality of diet was less of a factor in *C. rufilabris* surviving to adulthood.

Low survival among the 2mg/day diet treatments could also be a result of an interaction between prey quantity and low relative humidity (Tauber and Tauber 1983). Pappas et al. (2008) suggest that lacewing larvae could overcome low relative humidities by feeding on aphids or nectar and thus increasing their water content. Michaud (2005) suggested that ladybeetle rearing include a water treatment especially when ladybeetles are fed a non-aphid diet and maintained at low relative humidities. Therefore, dietary water, or the lack thereof, could play a significant role in lacewing larval survival when exposed to suboptimal relative humidities. In this study, as expected, higher (nonsignificant) levels of survivorship occurred when *C. rufilabris* larvae were supplied with mummies+water compared with larvae supplied mummies only.

Among diet treatments, developmental times were significantly different for *C. rufilabris* during the first larval stage and the pupal stage. Overall, preimaginal (larval+pupal) developmental times were similar among the limited diet treatments, but the quickest developmental times occurred among larvae supplied with either mummies+water or mummies alone (Table 3). Since mummies were proposed to be a less suitable food source for predators (Royer et al. 2008, Mullins et al. 2011), these results were unexpected and appear to differ from many studies involving *C. rufilabris* as well as *C. carnea* that evaluated prey consumption and suitability (Hydorn and Whitcomb 1979, Canard et al. 1984, Giles et al. 2000, Atlihan et al. 2004). Typically, when supplied with an inadequate quantity and/or quality of prey, lacewing larvae take longer to develop. Atlihan et al. (2004) supplied varying prey densities to *C. carnea* and documented that when larvae were supplied <20 aphids/day, developmental time was increased significantly. Similarly, Giles et al. (2000) documented a significantly longer

developmental time for *C. rufilabris* larvae when supplied with low daily prey levels
(<8.2mg/day), but also demonstrated slower developmental rates when "lower-quality" prey were
consumed. Relative to the current study, perhaps the combined stressed conditions of low relative
humidity and low daily prey levels are more of a hindrance to *C. rufilabris* preimaginal
development than prey quality. Tauber and Tauber (1983) documented *C. rufilabris* larvae
(reared on *ad libitum* grain moth eggs) took 28.4 days to complete their life cycle when reared at
35% relative humidity, whereas larvae reared at the optimal 75% relative humidity had a
significantly shorter life cycle (24.2 days).

Initiation of pupation for holometabolous insects may be influenced by an interaction
between prey resource availability and probabilities of survival (Shafiei et al. 2001, Phoofolo et
al. 2009). For example, dung beetles (*Onthophagus taurus* Schreber, Coleoptera: Scarabaeidae)
have been documented to undergo premature pupation when food is scarce (Shafiei et al. 2001).
Premature pupation did not occur during this study; compared with *C. rufilabris* larvae supplied
the unlimited pea aphid diet (16mg/day), larvae in the 2mg/day diet treatments took 3-6 days
longer to initiate pupation (Table 3). The factors that trigger pupation for lacewings are complex
and involve abiotic factors (Ru et al. 1976, Canard et al. 1984), but prolonged larval stages may
simply be related to limited daily food intake. Alternatively, *C. rufilabris* third instars in the
2mg/day diet treatments may have delayed pupation (compared to larvae supplied with the
16mg/day diet treatment) until a critical weight was reached. Little is known about this pupation
trigger for lacewings (Canard et al. 1984), however, Phoofolo et al. (2009) documented that
fourth larval stage ladybeetles must achieve a critical weight in order to initiate pupation and
successfully eclose. Because lacewings are generalist predators and are able to utilize a variety of
prey, this pupation strategy can increase the probability of survival even when primary aphid prey
exhibit boom/bust cycles (Canard et al. 1984). Clearly, multi-factorial studies evaluating prey
level, relative humidity, and prey suitability are required to evaluate the primary factors that drive
developmental time and initiation of pupation.

Evolutionary Implications

Larval diet has a significant impact on adult body size, reproduction and subsequently fitness (Hydorn and Whitcomb 1979, Canard et al. 1984, Atlihan et al. 2004). Ovarian development is dependent upon nutrient reserves acquired during the larval stage and therefore an inadequate larval diet can delay ovarian development (Canard et al. 1984). Atlihan et al. (2004) documented that *C. carnea* fecundity increased with increasing daily larval prey levels. For *C. rufilabris*, no significant differences were detected among adult body weights for the 2mg/day diet treatments (Figure 1) suggesting that mummified aphids had no effect on adult body weight and potentially fitness. Larvae supplied unlimited pea aphids had a much higher adult body weight which further indicates that daily prey level was the primary factor influencing adult weight for *C. rufilabris*.

Similarities for survival, larval+pupal development and *C. rufilabris* adult body weights among the limited diet treatments suggests that parasitized greenbugs are a relatively suitable prey resource, and winter wheat systems in the Southern Great Plains appear to be suitable source habitats for lacewings. In particular, populations of *Chrysoperla* would be expected to increase rapidly in winter wheat fields when unparasitized aphids were present; ideally, gravid female lacewings would arrive to winter wheat fields prior to parasitoids. This would allow first instar lacewings to easily consume unparasitized aphids which are not as frequently rejected as mummified aphids. During this time, initial aphid populations would be small but still growing. Early lacewing instars do not consume as many aphids as older instars (Balasubramani and Swamiappan 1994, Atlihan et al. 2004) thus the occurrence of young lacewing larvae and small aphid populations is ideal. By the lacewing's second instar, aphid populations would be larger and therefore more accommodating for the increased larval appetites. As parasitoids arrive, the proportion of mummified prey increases and thus intraguild predation is more likely to occur (Mullins et al. 2011). Older lacewing larvae are more adept at capturing, feeding and surviving on

a diet of mummified aphids and the potential negative outcomes associated with intraguild predation on *L. testaceipes* (Mullins 2008, Royer et al. 2008) would be avoided.

The results of this thesis suggest that generalist lacewings are more adapted to consuming, developing and surviving on mummified greenbugs compared with ladybeetles; and therefore lacewings are less likely to be negatively impacted during intraguild interactions with *L. testaceipes* in winter wheat. When ladybeetles feed on parasitized greenbugs, larval development and weight gain is significantly slower and survival is substantially reduced (Takizawa et al. 2000, Lebusa 2004, Mullins 2008, Royer et al. 2008). In fact, when reared on an exclusive late-stage mummy diet, ladybeetles (*Coccinella septempunctata* and *Hippodamia convergens*) fail to complete pupation (Lebusa 2004, Royer et al. 2008). Compared with larvae supplied with unparasitized aphids only, larval *C. rufilabris* supplied with mummies exhibited reduced survivorship, however, overall survivorship was similar among the limited diet treatments because of low levels of pupal mortality on the mummy diet (Table 2). In addition, *C. rufilabris* had a shorter preimaginal development when supplied a mummy diet suggesting that generalist *Chrysoperla* species are adapted at surviving in agricultural crops where parasitism and intraguild predation is prevalent.

Biological Control

Predatory lacewings are highly effective natural enemies and are successful biological control agents (Hassan et al. 1985, Hagley 1989, Nordlund et al. 1991, Breene et al. 1992). Lacewings are not only capable of feeding on and regulating pest populations, but are also tolerant to many pesticides including pyrethroids and Bt toxins (Mizel and Schiffhauer 1990, Romeis et al. 2004, Li et al. 2008). It is unclear how *Chrysoperla* species interact with other natural enemies (ladybeetles and *L. testaceipes*) in field situations, and how such interactions affect overall natural enemy suppression of aphid populations in winter wheat systems.

Biological control in agricultural systems has proven most effective when multiple natural enemies act together in regulating aphid populations (Colfer and Rosenheim 2001, Jones 2001, Snyder and Ives 2003, Brewer and Elliott 2004, Gardiner and Landis 2007). Parasitoid wasps are effective at relatively low aphid intensities and are able to prevent small aphid populations from increasing exponentially (Jones 2001, Giles et al. 2003). Unfortunately, intraguild predation of parasitoids can decrease parasitoid populations, especially since *Chrysoperla* species are capable of consuming adult parasitoids (Meyhöfer and Klug 2002, C. N. Jessie personal observation). Because *Chrysoperla* species are so abundant in the Southern Plains, high levels of intraguild predation could potentially lead to a reduction in overall natural enemy suppression of cereal aphids in winter wheat during early spring. Future field studies evaluating the combined effects of lacewings and parasitoids on aphid population density would provide insight into the ecological impact of intraguild predation.

Conclusions and Future Research

Chrysoperla rufilabris larvae are capable of feeding and developing on a diet of mummified greenbugs. The results of this thesis show that ultimately there is no significant difference in survival, developmental time and adult body weights when larvae are reared on mummified greenbugs compared to unparasitized greenbugs; however, the first instar stage appears to be the most vulnerable. Documenting the impact of a parasitized aphid diet on lacewing reproduction and fecundity would shed more light on how intraguild interactions shape the evolution of these species in winter wheat systems.

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CHAPTER VI

SUMMARY OF FINDINGS

Oklahoma is the second largest winter wheat producer in the United States; in 2010 over 120 million bushels were harvested (USDA National Agricultural Statistics Service 2012). Greenbugs (*Schizaphis graminum*) are the most important winter wheat pest and are capable of reaching economically damaging levels in a short amount of time resulting in significant grain yield losses (Kring and Gilstrap 1983, Kindler et al. 2002). Natural enemies in winter wheat have proven effective at regulating greenbug populations (Jones 2001, Giles et al. 2003, Brewer and Elliott 2004); however, intraguild predation among natural enemies can occur (Mullins et al. 2011). Intraguild predation results when one natural enemy consumes another (Polis and Holt 1992). The studies described in this thesis were conducted to document the feeding capabilities and handling times of larval *Chrysoperla rufilabris* supplied with mummified greenbugs (parasitized by *Lysiphlebus testaceipes*), and determine the suitability of parasitized greenbugs for lacewing larval development, survival and adult body weights.

First and third *C. rufilabris* instars are capable of consuming mummified greenbugs; and while first instars show a significant difference in handling times between mummified greenbugs and unparasitized greenbugs, third instars showed no difference. Reduced handling times by first instars indicates reduced preference and/or inability to easily feed on mummified aphids. Cumulative survival was significantly different among diet treatments for all three larval stages

and the pupal stage; however, because of low mortality on a diet of mummified aphids overall preimaginal survival did not differ between mummified greenbugs and unparasitized greenbugs. Overall, developmental times for *C. rufilabris* were similar between mummified greenbug and unparasitized greenbug diet treatments. Prey differences had little effect on lacewing development and survivorship and thus *C. rufilabris* would be predicted to persist in winter wheat systems in the Southern Great Plains where intraguild predation levels are high. Based on the results from this thesis, it appears that *C. rufilabris* is better adapted to prey on parasitized aphids compared with ladybeetles that inhabit the same agricultural systems (Takizawa et al. 2000, Lebusa 2005, Mullins 2008, Royer et al. 2008).

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Scope and Method of Study: Winter wheat (*Triticum aestivum* L.) is a major commodity grown in the Southern Great Plains. In fact, Oklahoma is the second largest winter wheat producer in the United States. With the increase in wheat production and the subsequent rise in prices, pest outbreaks can seriously destroy crops. The most common and destructive pest in winter wheat is the greenbug (*Schizaphis graminum* Rondani). Fortunately, the greenbug has several natural enemies including the endoparasitoid wasp *Lysiphlebus testaceipes* Cresson and predatory lacewing larvae. These organisms comprise a 'guild' of greenbug natural enemies. When one guild member consumes another guild member it is termed intraguild predation. The studies conducted in this thesis examine how *Chrysoperla rufilabris* Burmeister biology is affected when involved in intraguild predation interactions with *L. testaceipes*. Studies involved documenting intraguild predation effects on lacewing larval feeding capability and handling time, as well as larval development, survival and adult body weight.

Findings and Conclusions: First and third instar *C. rufilabris* were capable of attacking and consuming mummified greenbugs during 30min feeding observations. Handling times for first instars differed significantly for larvae supplied mummies versus greenbugs; however there were no differences for third instars. Lacewing larvae were reared on 2mg/day diets: unparasitized greenbugs, pea aphids, mummies, mummies plus a moist cottonball. Survivorship was greatly reduced in all diet treatments compared to an unlimited diet (16mg/day) of pea aphids; however, within the limited (2mg/day) diet treatments, larvae supplied either of the mummy diets had lower survival for all larval instars and the pupal stage. Development was significantly shorter for larvae supplied the greenbug diet in the first instar and pupal stage. However, there were no differences in total preimaginal survival and development among the diet treatments.

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