INTRAGUILD PREDATION AMONG COCCINELLIDAE AND *LYSIPHLEBUS TESTACEIPES* IN AN OKLAHOMA WINTER WHEAT SYSTEM

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CHAPTER I

INTRODUCTION

In the Southern Great Plains of the United States, winter wheat (*Triticum aestivum* L.) is grown for grain production, forage production or a combination of both (Krenzer et al. 1999). In 2007, over 6 million acres of wheat were planted in Oklahoma (National Agricultural Statistics Service 2008). Whether these 6 million acres are for forage or grain production, wheat in Oklahoma is attacked by a number of insect pests.

The greenbug, (*Schizaphis graminum* Rondani), is the most important insect pest of winter wheat in Oklahoma and is often the limiting factor in profitable winter wheat production (Starks and Burton 1977, Webster 1995, Kindler et al. 2002). When greenbugs reach high numbers in fall or more likely late winter to early spring they can inhibit plant growth or kill plants reducing yields and net returns (Royer et al. 1997, Kindler et al. 2002). In Oklahoma, damage from greenbugs results in losses from $0.5 million to $135 million in winter wheat (Webster 1995). In 1976, a severe greenbug outbreak resulted in costs of over $80 million in applications of insecticides and losses in yield for farmers in Oklahoma (Starks and Burton 1977).

Winter wheat fields are also inhabited by a number of natural enemies which prey on or parasitize aphid pests. One group of highly effective organisms in the greenbug natural enemy complex are the hymenopteran parasitoids of which the braconid *Lysiphlebus testaceipes* (Cresson) is the most prevalent in the Southern Great Plains (Jackson et al. 1970, Walker et al. 1973, Archer et al. 1974, Summy et al. 1979, Giles et al. 2003, Jones et al. 2005). Another group of predators are the Coccinellidae. *Hippodamia convergens* Guérin-Méneville, a native ladybeetle in North America is well known for its role in aphid control. Both parasitoid and predator populations can exert

pressure on greenbugs resulting in varying degrees of biological control, such that the greenbug populations can be maintained below economic injury levels (Giles et al. 2003).

However, there is competition between predators and parasitoids for greenbugs. Organisms that share a common prey/host are known as a guild. When the predator *H. convergens* consumes a greenbug parasitized by the parasitoid *L. testaceipes*, a dynamic happens known as intraguild predation, where one organism feeds on another within the same predatory guild (Brodeur and Rosenheim 2000). Through intense intraguild predation, predators can reduce the impact of other guild members and trigger or worsen prey outbreaks indirectly and potentially allow for an increase in the amount of herbivore damage to plants (Rosenheim et al. 1993, Snyder and Ives 2001, Snyder and Ives 2003). Therefore it is necessary to study these dynamics and their effects as they take place in Oklahoma winter wheat fields.

The studies described in this thesis were conducted to examine the rate at which this kind of intraguild predation occurs in winter wheat fields and to investigate if parasitized aphids are a suitable food source for the development and survival of *H. convergens*.

Objectives

I. Determine the detectability of *L. testaceipes* mitochondrial DNA within the gut of *H. convergens* following intraguild predation.

II. Examine occurrence and frequency of predation of parasitized aphids by *H. convergens* during the winter wheat growing season.

III. Determine the suitability of greenbugs parasitized by *L. testaceipes* at early, late, and mummified stages for the development and survival of third and fourth instar *H. convergens*.

Explanation of Thesis Format

This general introduction is followed by a literature review (chapter II), then chapters III and IV, which are devoted to individual papers to be published, and a general summary (chapter V). Lists of references are provided for citations in the literature reviews and papers to be published. In paper I (chapter III) the detectability of *L. testaceipes* DNA within the gut of *H. convergens* is determined and the occurrence and frequency of this kind of predation is documented in winter wheat and alfalfa fields. The second paper (chapter IV) determines the suitability of greenbugs parasitized by *L. testaceipes* at different stages of parasitism for the development and survival of third and fourth instar *H. convergens*. These papers follow the general guidelines of the Entomological Society of America for submission to scientific journals.

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CHAPTER II

REVIEW OF LITERATURE

Oklahoma Winter Wheat Production

In the Southern Great Plains of the United States, winter wheat (*Triticum aestivum* L.) is grown for grain production, forage production or a combination of both (Krenzer et al. 1999). In 2007, over 6 million acres of wheat were planted in Oklahoma, up 7 percent from 2006 according to the United States Department of Agriculture (National Agricultural Statistics Service 2008). Of those 6 million acres, 4.3 million were harvested for grain which is an increase of 26 percent from the previous year. Overall, approximately 55% of those 6 – 7 million acres of wheat are grazed (Thompson 1990).

Insect Pests of Wheat

Oklahoma winter wheat is attacked by many herbivorous insects including aphids (Homoptera: Aphididae) consisting of the greenbug (*Schizaphis graminum* Rondani), Russian wheat aphid (*Diuraphis noxia* Kurdjumov), bird cherry-oat aphid (*Rhopalosiphum padi* Linnaeus), rice root aphid (*Rhopalosiphum rufiabdominalis* Sasaki), corn leaf aphid (*Rhopalosiphum maidis* Fitch), and the English grain aphid (*Sitobion avenae* Fabricius). Wheat pests from other taxa include armyworms (*Pseudaletia unipuncta* Hayworth and *Spodoptera frugiperda* J. E. Smith), cutworms (*Euxoa auxilaris* Grote and *Agrotis spp.*), mites (*Petrobia lateens* Muller, *Aceria tosichella* Keifer) and white grubs (*Cyclocephala spp.* and *Phylophaga spp.*)(Royer et al. 1998).

Aphids can be found on wheat from emergence to heading. Because aphids have the capability of reproducing very quickly they can injure wheat plants during a very small window of time (Starks and Burton 1977, Royer et al. 1997). Among aphids,

greenbugs appear to be the most important pest of winter wheat in Oklahoma and often limit profitable production (Starks and Burton 1977, Webster 1995, Kindler et al. 2002).

Greenbug

The greenbug was first reported as a pest in wheat in 1882 (Pfadt 1962) and in the last century sorghum has also become a preferred host (Beregovoy et al. 1988). Both of these crops are regularly infested in Oklahoma. Greenbugs infest a number of graminaceous crops throughout the United States, and feed on over 70 species of grasses; many of these non-cultivated plants serve as secondary hosts when winter wheat and other cereal crops are not present (Michels 1986). Greenbugs reproduce so rapidly that they can reach very high population levels in a short time (Starks and Burton 1977). Outbreaks occur almost every year in Oklahoma and statewide infestations happen every 5-10 years (Starks and Burton 1977, Giles et al. 2000, Giles et al. 2003, Jones et al. 2005).

Greenbug Biology

Greenbugs are small, light green aphids with a darker green dorsal line, black tipped cornicles, legs, and antennae, and black eyes (Wadley 1931). Greenbugs feed on phloem sap by inserting their stylets and proboscis designed for piercing/sucking into the plant tissue. This results in chlorosis and can eventually lead to the death of the plant (Burton 1986). Female greenbugs mainly reproduce asexually. Parthenogenesis results in viviparity or the birth of live young. Nymphs develop through four stages above a developmental threshold of 5.86℃ taking 7d to reach adulthood at 20℃ (Wadley 1931, Walgenbach et al. 1988, Metcalf and Metcalf 1993). Wadley (1931) recorded reproductive rates of 3.5 nymphs per day by parthenogenetic females and Webster and

Starks (1987) observed a mean of six nymphs per day by biotype E greenbugs on 'TAM 105' wheat at 26-28℃.

Greenbugs overwinter primarily as parthenogenetic females in the Southern Great Plains (Webster and Phillips 1918, Wadley 1931). However, non-parthenogenetic females and alate males may be produced in response to increased scotophase, the dark segment of a light-dark cycle (Mittler and Gordner 1991). After mating, oviparous females in the northern U.S. deposit eggs that overwinter from which apterous parthenogenetic females hatch in the spring (Dixon 1985, Miyazaki 1987).

Economic Status of the Greenbug

In Oklahoma, damage from greenbugs results in losses from $0.5 million to $135 million in winter wheat (Webster 1995). In 1976, a severe outbreak occurred that resulted in costs of over $80 million to farmers in Oklahoma from applications of insecticides and losses in yield (Starks and Burton 1977).

Kieckhefer et al. (1994) estimated reduced grain production at 41 kg/ha per 100 aphid feeding days (the number of greenbugs per plant / the number of days that they feed on the plant). Burton and Burd (1993) found a significant dry root weight loss for 10 greenbugs per tiller on 'TAM 101' wheat after 14 days of feeding. A loss of 14.5 kg/ha in yield for each greenbug per tiller during years with near average precipitation was recorded by Kindler et al. (2002) and 34.3 kg/ha under severe drought conditions.

Natural Enemies of the Greenbug

Winter wheat fields are also inhabited by a number of natural enemies which prey on herbivore pests. Elliott et al. (2006) conducted a D-VAC sampling study on arthropod predators in Oklahoma winter wheat fields and found lady beetles (Coccinellidae),

predatory Heteroptera (Nabidae and Anthocoridae), lacewings (Chrysopidae), spiders (Araneae), ground beetles (Carabidae), and rove beetles (Staphylinidae). Many of these predators feed on greenbugs through multiple life stages. Additionally the greenbug natural enemy complex includes a group of highly effective Hymenopteran parasitoids. Field surveys of greenbug parasitoids were conducted in wheat and sorghum fields in the southern Great Plains, yielding mostly the braconid *Lysiphlebus testaceipes* Cresson. The braconid *Diaertiella rapae* McIntosh, the aphelinid *Aphelinus varipes* Forester, and *A. nigritus* Howard were less abundant (Jackson et al. 1970, Walker et al. 1973, Archer et al. 1974, Summy et al. 1979, Giles et al. 2003).

Coccinellidae

The common red or orange-colored lady beetles make up only a part of the 4000 species of the world-wide Coccinellidae (Korschefsky 1931, Hagen 1962). In America north of Mexico there are at least 64 genera and 453 species of aphidophagous Coccinellidae (Gordon 1985). They prey primarily on aphids in wheat fields throughout the Great Plains, playing an important role in aphid control (Kring et al. 1985, Rice and Wilde 1988, Elliott et al. 1997, Jones 2001). Those species commonly encountered in Oklahoma wheat fields are *Hippodamia convergens* Guerin-Meinville, *Hippodamia sinuata* Muslant, *Coccinella septempunctata* L. and *Coleomegilla maculata* De Geer (Teetes et al 1973, Michels et al 1997). Of these, the most frequently encountered species is *H. convergens* (Elliott et al. 2006) which plays an important role in greenbug control (Teetes et al. 1973).

Hippodamia convergens

Hippodamia convergens has an orange color often with 0-13 black spots on its elytra. They are somewhat elongate in shape ranging in length from 4-7 mm, having two converging white lines on the pronotum (Sloderbeck et al. 1996). It is often sold by private companies for the purpose of aphid control (Hoffman and Frodsham 1993, Sloderbeck et al. 1996). They feed on a variety of aphid species and opportunistically on eggs, mites, mealybugs, and scales (Hoffman and Frodsham 1993, Carr 1955). Adults can consume upward of 30-60 greenbugs per day and larvae eat 20-100 per day depending on ladybeetle instar (Hunter and Glenn 1909, Clausen 1915, Fenton and Dahms 1951, Daniels 1965). Development from egg to adult can take two to three weeks with adults living for weeks or months depending on prey availability, time of year, and location (Hoffman and Frodsham 1993). One or two generations are completed a year with the adults overwintering in protected sites and migrating during the warming temperatures of spring (Sloderbeck et al. 1996). *H. convergens* third and fourth stage larval and pupal developmental times in other feeding studies vary, due in part to differences in temperature and prey and water availability (Michels et al. 1991, Phoofolo et al. 2007, Royer et al. 2008). Development times were 3.0-3.2 d for 3rd instar larvae, 3.0-6.0 d for 4th instar larvae, and 3.9-9.0 d for pupae. Those developmental times where daily water was available occur at the higher end of the ranges as suggested by Michaud (2005).

Lysiphlebus testaceipes

Lysiphlebus testaceipes is a solitary endoparasitoid found in the Nearctic, Neotropical, and Oceanic ecozones as well as the Palearctic due to planned releases (Mackauer and Stary 1967, Krombein et al. 1979). *Lysiphlebus testaceipes* females

oviposit in all life stages of the greenbug (Webster and Phillips 1912). Once oviposition takes place, roughly 2 days pass before the egg hatches and larvae develop through four instars. During development larvae consume first the hemolymph and later the internal organs of the host (Quicke 1997). During the fourth instar the larva moves around inside the host creating the characteristic 'mummy' which is bulbous and tan in appearance (Royer 1998, Jones et al. 2005). Prior to pupation the larva chews a ventral opening in the exoskeleton of the host through which it attaches the mummy with silk to a substrate. The larva then develops through pupation and emerges as an adult wasp through a dorsal circular cut hatch (Hardee et al. 1990, Knutson et al. 1993).

Webster (1909) noted the importance of *L. testaceipes* in keeping aphid populations in check in America. The effectiveness of *L. testaceipes* is due in part to the fact that it attacks aphids in several genera (Flint and Dreistat 1995, Hoffman and Frodsham 1993), is adaptable to warm and cool climatic conditions (Elliott et al. 1999, Jones et al. 2007), can continue to attack aphids during cold winter months (Jones et al. 2007), has a high attack and reproductive rate (Jones 2001, Giles et al. 2003), sterilizes the aphids that it attacks (Spencer 1926, Hight et al. 1972, Eikenbary and Rogers 1974), and dislodges aphids from the plant as it forages (Losey and Denno 1998). *Lysiphlebus testaceipes* has been observed to keep greenbug populations below Economic Injury Levels in wheat (Pergande 1902, Spencer 1926, Sekhar 1957, Wood and Chada 1969, Eikenbary and Rogers 1974, Krombein et al. 1979, Salto et al. 1983, Jones 2001, Giles et al. 2003) and has the potential for quickly reducing outbreak densities of greenbugs and other small grain aphids (Patrick and Boring 1990, Jones 2001, Giles et al. 2003, Jones et al. 2003, Royer et al. 2005).

Intraguild Predation (IGP)

Organisms of the same trophic level which share a common prey/host are considered a guild of predators and when those guild members feed on each other it is known as intraguild predation (Brodeur and Rosenheim 2000). This kind of action by a guild member provides energy to the intraguild predator and may reduce competition for resources, also potentially reducing the risk of being preyed upon (Polis et al. 1989, Polis and Holt 1992).

Intraguild interactions are prevalent in many systems (Brodeur and Rosenheim 2000). IGP can affect other trophic levels indirectly when the effect of one organism preying on another guild member releases an extraguild herbivore from upper trophic level pressure (Spiller and Schoener 1990, Diehl 1993, Brodeur and Rosenheim 2000). Through intense intraguild predation, predators can trigger or worsen prey outbreaks indirectly and potentially increase the amount of herbivore damage to plants (Rosenheim et al. 1993, Snyder and Ives 2001, Snyder and Ives 2003). However, it has been shown in some systems that despite high levels of intraguild predation the per capita mortality caused by parasitism was not affected and the combination of coccinellid and parasitic wasps together drove down aphid populations nearly to extinction at a local level (Hoelmer et al. 1993, Heinze et al. 1994, Rosenheim et al. 1997, Colfer and Rosenheim 2001)

In Oklahoma winter wheat and sorghum fields, the aphidophagous guild that utilizes cereal aphids primarily consists of the parasitoid *L. testaceipes* and various species of Coccinellidae. In this system, coccinellid larvae and adults act as the intraguild predators and the parasitoids as intraguild prey; wasps are within their aphid

host in egg, larval, or pupal form upon predation. Adult wasps do not reciprocate predatory behavior. At first glance this dynamic appears to be solely detrimental to the parasitoid population, but a few studies suggest that parasitoid mummies can have deleterious effects on coccinellid development (Takizawa et al. 2000, Royer et al. 2008). This consumption of parasitized aphids by coccinellids is observed in wheat and sorghum fields; however, the frequency of feeding and the impact on coccinellid development and survival have not been fully quantified (Colfer and Rosenheim 2001, Brodeur and Rosenheim 2000, Meyhofer 2001, Meyhofer and Hindayana 2000, Lebusa 2004, Royer et al. 2008).

Preference for Parasitized Aphids

Studies suggest that generalist coccinellid predators do not discriminate between aphids that are parasitized versus those that are not (Hagen and van den Bosch 1968, Stary 1970, Royer et al 2008). Colfer and Rosenheim (2001) conducted cage studies with the parasitoid *L. testaceipes* and adults of the convergent lady beetle, *H. convergens*, and showed that the beetles commonly fed on both unparasitized and mummified aphids (earlier stages of parasitism were not part of the experiment). During a 24 hour period, *H. convergens* consumed an average of 22 mummies and 32 unparasitized aphids. A conclusion drawn from this these results was that the beetles preferred unparasitized aphids; however the amount of handling time necessary for the predator to consume a mummy versus a non-mummy was not taken into account. In a study by Lebusa (2004), handling time (time spent consuming the organism) for mummies doubled for *H. convergens* and was also significantly greater for *Coccinella septempunctata* compared to the handling time for unparasitized aphids. In the same study, *C. septempunctata* showed

no preference for either the unparasitized or parasitized greenbugs, but *H. convergens* did not always completely consume the mummified greenbugs (Lebusa 2004, Royer et al. 2008).

Suitability of Parasitized Aphids

Predaceous coccinellids feed on a wide array of prey and are dependant on prey availability for survival and development (Phoofolo et al. 2007). Lebusa (2004) showed that a mixed diet consisting equally of aphids parasitized by *L. testaceipes* and healthy greenbugs had adverse effects on the development and survival of *C. septempunctata* and *H. convergens* larvae, causing longer development time and resulting in smaller adult size. Third and fourth instar coccinellid larvae that were fed solely mummies took even longer to develop and did not survive through pupation. Takizawa et al. (2000) evaluated the effects of parasitized aphids in the diets of fourth instar *C. septempunctata*, *Harmonia axyridis*, and *Propylea japonica*. They found that the survival rate of *C. septempunctata* larvae feeding on mummies was significantly lower than that of larvae which fed on non-parasitized aphids or aphids in earlier stages of parasitism; however, there were no differences for the other coccinellid species. Weight gain was greater for larvae that did not feed on mummies and development time was longer for those that did. However, the study did not take into account handling time regarding aphids, parasitized aphids, or mummies and also provided an unlimited amount of food each day making it difficult to quantitatively evaluate the suitability of specific prey items and may have only reflected the differences in amount of prey consumed (Royer et al. 2008). Limiting prey helps isolate suitability and simulates field conditions; 4mg/day in the case of coccinellid larvae is enough to survive and develop on and allows for evaluation of diet suitability and

eliminates the possible confounding effects of satiation (Giles et al. 2001, 2002, 2003, Phoofolo et al. 2007).

Examining the suitability of greenbugs parasitized by *L. testaceipes* at various stages for the development of coccinellid larvae provides insights on the intraguild predation dynamics that take place in the field. Studies on the interactions between aphidophagous parasitoids and predators are also needed to increase our understanding of the combined effects of this predator/parasitoid complex. Royer et al. (2008) quantitatively described the suitability of mummified greenbugs as a food source. However, because greenbugs in all stages of parasitism are preyed upon, greater resolution is needed as to when parasitized greenbugs are not suitable. That is, at which stage of parasitism do greenbugs become less suitable prey?

Guild Dynamics

Aphids in a wheat system are attacked by both predators and parasitoids. Wasps in the family Aphidiidae are endoparasitoids and as a result of the feeding habits of aphidophagous predators, the developing larvae of the wasps inside the aphids are consumed (Lebusa 2004). This places negative pressure on the parasitoid population since they become the intraguild prey in this situation; however, the impact that this type of IGP has on the predators has not been fully studied.

Consumption of immature parasitoids is not limited only to mummies, but predation of parasitized aphids also takes place while the host is still living. In either instance, this IGP results in the death of the parasitoid (Colfer and Rosenheim 2001, Rosenheim et al. 1995, Sunderland et al. 1997). Several studies have shown that predation on aphid mummies by coccinellids can be intense. In walnut orchards,

predation of walnut aphid mummies reduced survivorship of *Trioxys pallidus* by 80% (Nowierski 1979). In potato fields, Couture (1997) observed an increase in the predation of *Praon simulans* and *Aedes nigripes* mummies in which 72.5% and 95.0%, respectively, of the mummies were consumed before harvest. Brodeur and Rosenheim (2000), found that all stages of parasitoids were killed by a large number of different natural enemy species and that IGP serves as a key factor in regulating aphid parasitoid densities and their potential for exerting biological control. They proposed that this view could have a great influence on our understanding of the structure of aphid communities and the efficacy of parasitoids in biological control. However, the effects of IGP have not been fully explored in relation to the potentially negative impact on predatory coccinellids (Royer et al. 2008).

Parasitoid wasps manipulate host physiology and biochemistry to create an environment conducive to the development of the immature parasitoid (Beckage and Gelman 2004). Endoparasitic wasps or koinobionts are absolutely dependent on the nutrients provided by their host (Askew and Shaw 1986, Godfray 1994, Quicke 1997). Those parasitoids that specialize on aphids can utilize all of their host's developmental stages including the first instar (Cloutier and Mackauer 1979, Mackauer et al. 1997, Cloutier et al. 2000). Some of the known products parasitoids use to regulate their host environment include venoms, polydnaviruses (PDVs), and teratocytes (Beckage and Gelman 2004). Polydnaviruses replicate only in the calyx cells of Ichneumonidae and Braconidae and are injected into the host along with the egg at oviposition. The viruses play an essential role in subduing the host's immune response (Kroemer and Webb 2004, Espagne et al. 2004, Turnbull and Webb 2002, Kaeslin et al. 2005). Venoms are injected

at oviposition and act in conjunction with the polydnaviruses (Stoltz 1993, Soller and Lanzrein 1996, Zhang et al. 2004). The injection of venom by *Aphidius ervi* Haliday, an endoparasitoid of the pea aphid, *Acyrthosiphon pisum* Harris plays a major role in the castration process of the aphid significantly improving the nutritional suitability of the host (Digilio et al. 1998). Teratocytes are cells from the serosal membrane of the egg and are involved in arresting host development in some systems (Pennacchio et al. 1995) and aid in breaking down fat bodies in other systems (Nakamatsu et al. 2002). Although not documented, each of these factors likely occurs within *L. testaceipes* hosts.

These factors may reduce the suitability of parasitized aphids for Coccinellidae, however, the specific impact of venoms, polydnaviruses, and teratocytes on beetle survival and development have not been studied. The initial studies that documented reduced survivorship and delayed development of coccinellids that fed on parasitized aphids suggest that factors associated with parasitism may influence the dynamics of the predator portion of this guild. Perhaps this altered suitability is a parasitoid defense mechanism that under natural conditions prevents coccinellids from eliminating the local wasp population. This concept is new to the study of IGP dynamics and may help to explain guild dynamics in agricultural systems.

Detection of Predation

Predator gut analysis is a potentially useful way to determine predation rates and has been conducted with dissection, serological assay, and now the preferred method, polymerase chain reaction (PCR) (Greenstone and Shufran 2003, Hoogendorn and Heimpel 2001, Chen et al. 2002, Weathersbee et al. 2004, Sheppard et al. 2005). Identification of the gut contents of insect predators can be used to help understand

trophic relationships and predator-prey dynamics (Hoogendoorn and Heimpel 2001) and molecular gut analysis has been used to identify key aphid predators, both insects and spiders (Chen et al. 2002, Greenstone and Shufran 2003). Primers have been designed and tested for the identification of the immature parasitoid, *L. testaceipes*, in cereal aphids (Jones et al. 2005). The 16S rDNA gene can be used as a marker to detect parasitoid presence in a host (Chen et al. 2002). This potentially allows for the detection of *L. testaceipes* after having been consumed in its immature stage by predatory coccinellids and will be useful in determining the frequency of such occasions in field studies.

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CHAPTER III

DETECTABILITY OF *LYSIPHLEBUS TESTACEIPES* DNA IN THE GUT OF *HIPPODAMIA CONVERGENS*

Introduction

Winter wheat and sorghum fields in the southern Great Plains are inhabited by a number of natural enemies which help suppress greenbug pest populations. One group of highly effective organisms in the greenbug natural enemy complex are the Hymenopteran parasitoids of which the braconid *Lysiphlebus testaceipes* Cresson is the most prevalent (Jackson et al. 1970, Walker et al. 1973, Archer et al. 1974, Summy et al. 1979, Giles et al. 2003, Jones 2005). Another group is the coccinellid predators, of which the native species, *Hippodamia convergens* Guérin-Méneville, is often the most prevalent in Oklahoma winter wheat (Elliott 2006). Both the parasitoid and predator populations can exert pressure on greenbugs resulting in varying degrees of biological control, such that the greenbug pest populations are maintained below economic injury levels (Jones et al. 2001).

However, conflict does occur between these two natural enemies of the greenbug. Both organisms are considered to be in the same trophic level because they share a common prey/host; when organisms within such a guild feed on each other it is known as intraguild predation (IGP) (Brodeur and Rosenheim 2000). When present together in wheat and sorghum fields, *H. convergens* larvae and adults act as the intraguild predators and parasitoids as the intraguild prey because immature stages of developing *L. testaceipes* are within their greenbug host when being preyed upon. Adult wasps are not able to reciprocate predatory behavior. Consumption of a guild member provides energy to the intraguild predator and may reduce interspecific competition for resources (Polis et al. 1989, Polis and Holt 1992). Intraguild interactions are prevalent in many systems and recognized to be important (Brodeur and Rosenheim 2000). IGP can affect other trophic

levels indirectly when the effect of one organism preying on another guild member releases the extraguild herbivore from upper trophic level pressure (Spiller and Schoener 1990, Diehl 1993, Brodeur and Rosenheim 2000). Through intense intraguild predation, predators can trigger or worsen herbivorous pest outbreaks indirectly and potentially increase the amount of damage to plants (Rosenheim et al. 1993, Snyder and Ives 2001, Snyder and Ives 2003).

In a review conducted by Brodeur and Rosenheim (2000), it was found that all stages of parasitoids are killed by a large number of natural enemy species and that IGP can serve as a key factor in regulating aphid parasitoid densities and their potential for exerting biological control. They proposed that this view could have a great influence on our understanding of the structure of aphid communities and the efficacy of parasitoids in biological control. Given the possible effects that these intraguild predation dynamics can have on all of the populations involved it is necessary to determine the frequency of such occasions in the field.

Predator gut analysis is a potentially useful way to determine predation rates and has been conducted with dissection, serological assay, and now the preferred method, polymerase chain reaction (Greenstone and Shufran 2003, Hoogendorn and Heimpel 2001, Chen et al. 2000, Weathersbee III et al. 2004, Sheppard et al. 2005). Identification of the gut contents of insect predators can be used to help understand trophic relationships and predator-prey dynamics (Hoogendoorn and Heimpel 2001) and molecular gut analysis has been used to identify key aphid predators, both insects and spiders (Chen et al. 2000, Greenstone and Shufran 2003). Primers have been designed and tested for identification of the immature parasitoid, *L. testaceipes*, in small grain

cereal aphids (Jones et al. 2005). The 16S rDNA gene fragment sequenced by Chen et al. (2002) for common small grain aphid parasitoids in the southern Great Plains with specificity for *L. testaceipes* can be used as a marker of parasitoid presence in a host. This potentially allows for the detection of *L. testaceipes* after having been consumed in its immature stage by a coccinellid in the field. It is important to show how long this event is detectable by PCR. Half-life of detectability (the amount of time after which only half of the meal can be detected; Greenstone and Hunt 1993) of the DNA of a single aphid was determined for *H. convergens*, *C. septempunctata*, *C. plorabunda*, and others (Greenstone 1983, Ragsdale et al. 1981, Harwood et al. 2001, Chen et al. 2000). These detectability half-lives are useful when documenting the role of predators and may aid in designing sampling plans that document feeding cycles (Greenstone and Shufran 2003, Chen et al. 2000). Similarly, half-life data for detection of *L. testaceipes* in coccinellid guts would be useful in evaluating levels of intraguild predation in field situations.

Our objectives with this research were to 1) determine the detectability of *L. testaceipes* DNA within 3rd and 4th instar *H. convergens*; and 2) determine the level of detectable predation of *L. testaceipes* by Coccinellidae collected from Oklahoma winter wheat fields using PCR markers.

Materials and Methods

Greenbug Colony

Biotype 'E' greenbugs, *Schizaphis graminum*, were obtained from the USDA's Agricultural Research Station in Stillwater, OK and used to start a colony. The greenbugs were kept on 'OK101' wheat grown in a 1:1 mixture of potting soil and fritted

clay. Colonies were kept inside double-walled fine mesh cages within a greenhouse (22-28℃).

***Lysiphlebus testaceipes* Colony**

Adult *Lysiphlebus testaceipes* were identified (by taxonomic key) and isolated from specimens collected in multiple locations in Oklahoma and Texas. Five colonies were maintained in double mesh-walled cages containing pots of grain sorghum ('SG-925') infested with greenbugs from a lab colony. Each cage consisted of a colony that originated from a different location; periodically wasps from separate cages were transferred among cages to maintain the genetic diversity of each colony and improve vitality. The double layers of mesh (2.5 cm apart) on the walls of each cage prevented parasitism from outside sources and provided adequate airflow to the colony; each of the five cages also had a cloth sleeve for access. New pots of sorghum plants infested with greenbugs were added every 3-4 days to maintain a steady supply of parasitoids for feeding trials and water was made available to the adult parasitoids by spraying plants with a typical spray bottle. Colonies were maintained at 24℃ and a photoperiod of 12:12 (L:D) h. Newly formed mummies were collected daily for feeding trials.

Rearing of Coccinellidae

Adult *Hippodamia convergens* were collected from multiple field locations in Oklahoma or purchased (Rincon-Vitova Insectaries, Inc.). Mating pairs were isolated into half-pint cardboard containers covered with a fine nylon mesh, fed an unlimited diet of pea aphids (*Acyrthosiphon pisum* Harris), reared on faba beans '*Victa faba*' and maintained in an environmental chamber at 24℃ and a photoperiod of 16:8 (L:D) h. The diet was coupled with a supplement of a honey-wheat-yeast mixture obtained from

'Planet Natural' (Bozeman, MT) to promote egg production and a regular supply of water (saturated ball of cotton). When egg-laying initiated, males were moved to other containers to prevent female disruption and egg consumption. Upon eclosion, individual larvae were isolated into 5ml glass vials topped with cotton, supplied daily with water through use of a small saturated cotton ball and fed 4mg of greenbugs per day until initiation of experimental trials.

Detectability of *Lysiphlebus testaceipes* DNA

Individual *H. convergens* (3^{rd} and 4^{th} instars) were placed into glass vials and fed a single greenbug parasitized by *L. testaceipes* (mummy stage) at the beginning of a 30 minute period. After which, the coccinellids were kept in a growth chamber at 24℃ for a pre-determined time of digestion; 0h, 4h, 8h, 12h, and 16h (Chen et al. 2000, Greenstone and Shufran 2003). Seven to eleven 3^{rd} and nine to eleven 4^{th} instar larvae per time group were assessed, resulting in a total of 89 beetles. Negative controls consisted of 5 larvae that had not consumed *L. testaceipes*; positive controls consisted of five adult wasps identified as *L. testaceipes* which were then ground together for PCR to test the accuracy of the primers. Those beetles that did not feed in the 30 minute period were discarded from the experiment.

DNA Extraction

Upon completion of the digestion time each *H. convergens* larva that had consumed *L. testaceipes* was placed in a cryovial and frozen in liquid nitrogen. Each whole larva was thawed on ice and transferred into 100 µl of lysis buffer (2M Tris, 0.5M EDTA (pH 8.0), 5M NaCl, dd H_2O, and 10% SDS (pH 7.2)) in a well on a glass spot-plate and homogenized by hand with the tip of a sterile microcentrifuge tube. The

solution was pipetted into another sterile microcentrifuge tube to which 150 µl TE buffer (pH 8.0) and 200 µl phenol-chloroform (1:1) were added. The homogenate was briefly vortexed and spun for 6 min/13,000 rpm; supernatant (200 µl) was drawn off and the DNA was precipitated with an equal amount of isopropanol, spun 10 min/13,000 rpm. Isopropanol was discarded before adding 1 ml ethanol (70%), spun 2 min/13,000 rpm, ethanol was then discarded, and the sample was vacuum dried for 10 min; DNA was resuspended in 40 µl dd H_2O.

Primer Design

Primers were developed (Integrated DNA Technologies) on the conserved part of the 16S rDNA gene to detect the presence of *L. testaceipes* (GenBank accession nos. for *L. testaceipes* AF289139-AF289142; Chen et al. 2002) DNA from the coccinellid. These primers were designated Ltesta F (5'-GGC TGC AGT ATC AAT AAC TGT AC-3') and Ltesta R (5'-AAA TTC TAA AGG GTC TTC TCG TC -3'); the Ltesta F and Ltesta R primers have a predicted amplicon size of 223-bp. Ltesta F and R primers were designed with an annealing temperature of 52℃ and for a smaller PCR product opposed to the primers used by Jones et al. (2005) for *L. testaceipes* which had a larger 299bp product and low Tm and annealing temperature. Preliminary tests revealed that the primers used by Jones et al. (2005) in conjunction with protocol used for PCR did not amplify *L. testaceipes* DNA from the gut of *H. convergens*. These new primers (Ltesta F and R) amplify a smaller fragment and will improve detection of parasitoid DNA in the gut of the ladybeetle (Agusti et al. 1999, Zaidi et al. 1999).

PCR Reaction

To lessen interference from the greater amount of *H. convergens* DNA within the sample and improve the probability of the primers annealing successfully with the *L. testaceipes* DNA strands, serial dilutions of sample DNA were performed at 1 x 10^{-1} and 1 x 10^{-2} concentrations. PCR reactions (15 µl) contained 4 µl of the DNA sample (1 x 10^{-1} concentration) along with 7.5 µl GoTaq® Green Master Mix 2X (Taq DNA polymerase, reaction buffer (pH 8.5), 400µM dATP, 400µM dGTP, 400µM dCTP, 400µM dTTP, 3mM $MgCl_2$, yellow and blue loading dyes) (Promega, Madison, WI), 22 picomoles of each Ltesta F and Ltesta R primer, and ddH_2O.

PCR Program

The PCR program in the thermocycler consisted of 2 min denaturing at 94℃, followed by 40 amplification cycles of 15 sec denaturing at 94℃, 20 sec annealing at 52℃, and 20 sec extending at 72℃, this was followed by a final 5 min extension at 72℃. After amplification, PCR products were separated by electrophoresis in a 2% agarose gel stained with ethidium bromide, and photographed under UV light. A 1Kb Plus DNA ladder (Invitrogen Corporation, Carlsbad, CA) was used as the standard.

Predation of *Lysiphlebus testaceipes* Field Study

To document the level of detectable predation of *L. testaceipes* by coccinellids in field situations it was necessary to identify and sample fields that had parasitoids, greenbug hosts, and coccinellids. Through initial scouting efforts, three suitable wheat fields were identified near Chickasha, OK. Coccinellids were collected by vacuum sampling within a one meter diameter ring at four locations in each field every 14 days from 24February-24March (2006) (Elliott et al. 2006). Within each field, 100 tillers were randomly collected along two transects that stretched the distance of the field to estimate

greenbug intensity and percent parasitism. From the infested tillers collected, greenbugs were dissected to determine percent parasitism and adults were collected throughout the growing season with sticky traps to identify which parasitoid species were present.

Larval and adult coccinellids (≈25-50 per date and location) from suction samples were immersed in 100% ethanol and put into an ice-filled Styrofoam box. After returning from the field, individual specimens destined for PCR were quickly placed into fresh 100% ethanol within 1.5 ml microcentrifuge tubes and frozen at -4°C for short term storage for Hymenopteran DNA preservation and extraction (Quicke et al. 1998, Dillon et al. 1996, Post et al. 1993). Individuals were then ground (see previous protocol) and placed onto Whatman FTA cards for longer term storage (18 months). Two washes with distilled water (5 min each) with brief vortexing in between were found to be sufficient to amplify *L. testaceipes* DNA from the card with PCR. Aforementioned PCR materials and procedures (excluding DNA extraction and serial dilutions) with Ltesta F and R primers were used to detect the presence or absence of *L. testaceipes* DNA within the coccinellid sample.

Results and Discussion

Detectability Half-Life

Using Ltesta F and R primers *L. testaceipes* DNA was successfully amplified after consumption by the predator *H. convergens*. These primers yield a 223bp product designed to amplify smaller DNA fragments which have been shown to last for longer periods of time during digestion (Chen et al. 2000, Hoogendoorn and Heimpel 2001). Another set of primers was used, Ltepu F and R (Tm: 44.1℃ and 43.9℃), developed by

Jones et al. (2005); in only one instance out of 38 initial observations was amplification of parasitoid DNA successful with an annealing temperature of 42℃.

With the new Ltesta F and R primers, all 3rd and 4th instar *H. convergens* larvae fed one parasitized greenbug (mummy stage) tested positive by PCR for *L. testaceipes* DNA at 0h, 4h, 8h, 12h, and 16h (*n*=15, 17, 15, 20, and 20 respectively) post-feeding. Because of this the detectability half-life of *L. testaceipes* DNA within the gut of *H. convergens* was not determined; however, it does show that an intraguild predation event of this nature is detectable the same day of its occurrence which is useful knowledge for future sampling and predation studies. It has been suggested by Chen et al. (2000) that the mere proportion of predators shown positive with target DNA does not necessarily indicate the importance of a predator taxon. Detectability half-lives were considered a more reliable tool given the predators need to consume more frequently as a particular prey type is digested more quickly. *Lysiphlebus testaceipes* DNA half-life detectability is at least twice as long (being at least beyond 16h) as the half-life for *Rhopalosiphum maidis* (corn leaf aphid) at 8.78h as recorded by Chen et al. (2000), suggesting that *L. testaceipes* in pupal form may not be as easily digested by larval *H. convergens*; however, the amount of DNA in *R. maidis* and *L. testaceipes* may vary and knowing this would be necessary for comparison.

Prior to serial dilutions of the sample DNA, positive bands were obtained less than half of the time for *L. testaceipes* using the Ltesta F and R primers (Table 3.1). Diluting the sample enabled PCR to amplify the small amounts of *L. testaceipes* DNA every time among a larger amount of predator DNA. For example, samples at 0h, 4h, 8h, 12h, and 16h post feeding times in Figure 3.1 showed no bands prior to dilution (Lanes 2-

6), the same samples at 1 x 10^{-1} concentration (Lanes 7-11) and 1 x 10^{-2} concentration (Lanes 12-16) amplified *L. testaceipes* DNA. All unfed *H. convergens* used for negative controls were negative and Ltesta F and R primers were successful with positive controls.

Table 3.1 Proportion of *H. convergens* positive for *L. testaceipes* DNA Using Ltesta F and R Primers.

H. convergens	*n*	Proportion of *H. convergens* positive for *L. testaceipes* DNA	
		Without Sample Dilution	1 x 10^{-1} Concentration
3rd Instars			
0h	8	0.50	1.0
4h	9	0.33	1.0
8h	7	0.71	1.0
12h	9	0.44	1.0
16h	9	0.22	1.0
4th Instars			
0h	10	0.20	1.0
4h	8	0.25	1.0
8h	9	0.22	1.0
12h	9	0.44	1.0
16h	11	0.82	1.0
Total (3rd + 4th)			
0h	18	0.33	1.0
4h	17	0.29	1.0
8h	16	0.44	1.0
12h	18	0.44	1.0
16h	20	0.55	1.0

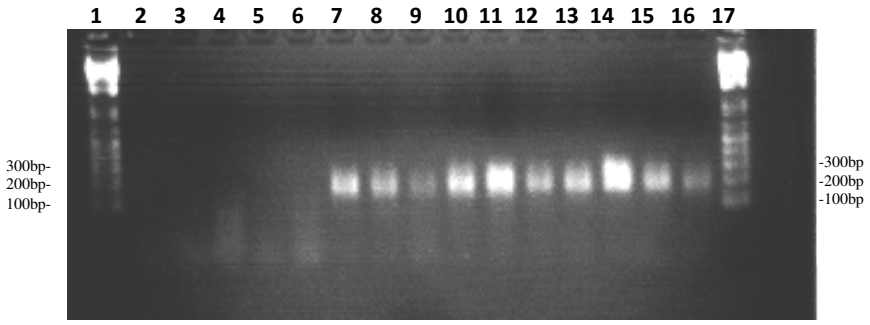

Figure 3.1. PCR amplification of *Lysiphlebus testaceipes* DNA after being consumed by *Hippodamia convergens*. Lanes 2-6: 0h, 4h, 8h, 12h, 16h without dilution (10^0); Lanes 7-11: 0h, 4h, 8h, 12h, 16h at 10^{-1} concentration; Lanes 12-16: 0h, 4h, 8h, 12h, 16h at 10^{-2} concentration.

Detection of IGP in the Field

Coccinellids collected from winter wheat fields tested positive for *L. testaceipes* DNA (Figure 3.2). In fact, the majority of individual larvae and adults of *H. convergens*

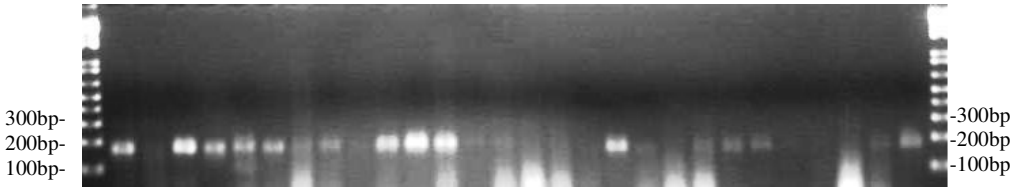

Figure 3.2. PCR amplification of *Lysiphlebus testaceipes* DNA within coccinellids from a winter wheat field.

and *C. septempunctata* had *L. testaceipes* DNA amplified from them (Table 3.3). In 3 fields of winter wheat sampled on 24 February, 10 March, and 24 March the proportion of intraguild predation events by coccinellids ranged from 0.667 (n=44) to 0.871 (n=31).

In all 3 winter wheat fields intraguild predation of *L. testaceipes* occurred as sticky traps yielded ≥ 99.5% *L. testaceipes* among adult parasitoids (M.W.P., unpublished data) and as percent parasitism increased from 75% (24 February) to 100%

(24 March); aphid populations were dropping from 1.1-2.9 aphids per tiller to 0-0.4 aphids per tiller (Table 3.2). During the same period coccinellid larval density dropped from 8.0 (±2.0) - 9.5 (±2.3) individuals (per m^2) to 0 - 0.8 (±0.5) individuals (per m^2); adult coccinellid density steadily increased at the same time from 0 - 0.3 (±0.3) to 0.8 (±0.5) - 1.3 (±0.5) for *C. septempunctata* and from 0 - 0.5 (±0.3) to 2.3 (±0.75) - 5.3 (±2.7) for *H. convergens*.

These results clearly indicate two important findings relative to documenting intraguild predation of *L. testaceipes* by Coccinellidae in Oklahoma winter wheat fields. First, any intraguild predation event appears to be detectable on the day that event occurred; *L. testaceipes* DNA was still present for successful amplification up to 16h post feeding. Second, when *L. testaceipes*, aphids, and coccinellids occur simultaneously in winter wheat fields, a high percentage of ladybeetles consume parasitized aphids. The findings justify the use of PCR as a tool to document intraguild predation, and may lead to more quantitative evaluations on the dynamics and consequences of this common ecological phenomenon.

Table 3.2 Coccinellid larvae and adult density, aphid intensity, and percent parasitism for 3 winter wheat fields.

Field/Date	Mean number per m^2 (± SE)			Aphids Per Tiller	Proportion Parasitized
	Total Larvae	*C. septempunctata* Adults	*H. convergens* Adults		
FIELD 1					
24FEB	8.00 ± 2.0	0.25 ± 0.3	0.50 ± 0.3	2.86	0.79
10MAR	11.25 ± 1.9	1.00 ± 0.4	1.75 ± 1.0	0.16	1.00
24MAR	0.75 ± 0.5	1.25 ± 0.5	5.25 ± 2.7	0	1.00
FIELD 2					
24FEB	9.50 ± 2.3	0	0.25 ± 0.3	2.66	0.75
10MAR	6.00 ± 1.7	0.25 ± 0.3	1.50 ± 0.3	0.22	0.97
24MAR	0.75 ± 0.5	1.00 ± 0.7	2.25 ± 0.8	0.35	0.96
FIELD 3					
24FEB	8.00 ± 1.9	0	0	1.14	0.83
10MAR	1.50 ± 0.5	1.00 ± 0.6	2.00 ± 1.0	0.07	0.93
24MAR	0	0.75 ± 0.5	2.75 ± 0.5	0	1.00

Table 3.3 Proportion of intraguild predation events for Coccinellidae in 3 winter wheat fields.

Field/Date	Proportion of Intraguild Predation Events (# individuals tested)						
	C. septempunctata			*H. convergens*			Total (Species Combined)
	Larvae	Adult	Total	Larvae	Adult	Total	
FIELD 1							
24FEB	0.775 (40)	1.000 (8)	0.888 (48)	0.429 (7)	1.000 (2)	0.715 (9)	0.772
10MAR	1.000 (7)	0.714 (7)	0.857 (14)	1.000 (5)	0.833 (12)	0.917 (17)	0.871
24MAR	-	0.833 (24)	0.833 (24)	-	0.769 (13)	0.769 (13)	0.811
FIELD 2							
24FEB	0.577 (26)	0.900 (10)	0.739 (36)	0.714 (7)	1.000 (1)	0.857 (8)	0.667
10MAR	0.667 (6)	0.727 (11)	0.697 (17)	1.000 (1)	1.000 (3)	1.000 (4)	0.696
24MAR	-	0.579 (19)	0.579 (19)	-	1.000 (5)	1.000 (5)	0.667
FIELD 3							
24FEB	0.744 (39)	1.000 (11)	0.872 (50)	0.333 (6)	1.000 (1)	0.667 (7)	0.754
10MAR	1.000 (1)	0.792 (24)	0.896 (25)	-	1.000 (10)	1.000 (10)	0.857
24MAR	-	0.786 (14)	0.786 (14)	-	0.875 (8)	0.875 (8)	0.818

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CHAPTER IV

SUITABILITY OF GREENBUGS IN THREE STAGES OF PARASITISM BY *LYSIPHLEBUS TESTACEIPES* FOR THE DEVELOPMENT AND SURVIVAL OF *HIPPODAMIA CONVERGENS*

Introduction

Sorghum and winter wheat fields in the southern Great Plains are inhabited by a number of natural enemies which help suppress aphid pest populations primarily the greenbug *Schizaphis graminum* Rondani. One group of highly effective organisms in the aphid natural enemy complex are the Hymenopteran parasitoids of which the braconid *Lysiphlebus testaceipes* Cresson is the most prevalent (Jackson et al. 1970, Walker et al. 1973, Archer et al. 1974, Summy et al. 1979, Giles et al. 2003, Jones et al. 2005). Another group is the coccinellid predators; of which the native ladybeetle in North America, *Hippodamia convergens* Guérin-Méneville, is often the most abundant (Elliott 2006). Both parasitoid and predator populations can exert pressure on greenbugs resulting in varying degrees of biological control, such that the greenbug populations are maintained below Economic Injury Levels (Jones 2001).

Organisms in the same trophic level which share a common prey/host are considered a guild of predators and when predation happens within that guild it is known as intraguild predation (IGP) (Brodeur and Rosenheim 2000). When present together in wheat and sorghum fields, *H. convergens* larvae and adults are the intraguild predators and hymenopterous parasitoids the intraguild prey; immature stages of developing *L. testaceipes* are within their greenbug host when being preyed upon. Adult wasps are not able to reciprocate predatory behavior. This consumption of a guild member provides energy to the intraguild predator and may reduce competition for resources, as well as reduce the risk of being preyed upon when either predator population can feed on the other (Polis et al. 1989, Polis and Holt 1992). Intraguild interactions are prevalent in many systems and recognized to be functionally important (Brodeur and Rosenheim

2000). IGP can affect other trophic levels indirectly when the effect of one organism preying on another guild member releases the extraguild herbivore from upper trophic level pressure (Spiller and Schoener 1990, Diehl 1993, Brodeur and Rosenheim 2000). Through intense intraguild predation, predators can trigger or worsen herbivorous pest outbreaks indirectly and potentially increase the amount of damage to plants (Rosenheim et al. 1993, Snyder and Ives 2001, Snyder and Ives 2003).

Several studies have shown that predation on aphid mummies by coccinellids can be intense. In walnut orchards, predation of walnut aphid mummies reduced survivorship of the parasitic wasp, *Trioxys pallidus,* by 80% (Nowierski 1979). In potato fields, Couture (1997) observed an increase in the predation of Braconid *Praon simulans* and *Aphidius nigripes* mummies in which 72.5% and 95.0%, respectively, of the mummies were consumed before harvest. In a review conducted by Brodeur and Rosenheim (2000), it was found that all stages of parasitoids are killed by a large number of natural enemy species and that IGP can serve as a key factor in regulating aphid parasitoid densities and their potential for exerting biological control.

Simultaneously, coccinellids are dependant on prey suitability for proper development and survival (Hodek and Honek 1996, Phoofolo et al. 2007). Royer et al. (2008) showed that a mixed diet consisting equally of fully formed mummies (*L. testaceipes*) and healthy greenbugs had adverse effects on the development and survival of *C. septempunctata* and *H. convergens* larvae; causing longer development time and smaller adult size. Third and fourth instar coccinellid larvae that were fed fully formed mummies took even longer to develop and did not survive pupation. Takizawa et al. (2000) evaluated the effects of parasitized aphids in the diets of fourth instar *C.*

septempunctata, *Harmonia axyridis*, and *Propylea japonica*. They found that the survival rate for *C. septempunctata* larvae feeding on mummies was significantly lower and development time was longer than larvae which fed on unparasitized aphids or aphids in earlier stages of parasitism.

On average, 79% of coccinellids collected during early spring from 3 winter wheat fields had preyed on *L. testaceipes* (Chapter 3). Examining the suitability of greenbugs parasitized by *L. testaceipes* at various stages for the development of coccinellid larvae provides insight on the intraguild predation dynamics that take place in the field. Studies on the interactions between aphidophagous parasitoids and predators are needed also to increase our understanding of the combined effects that this predator/parasitoid complex has on aphid pest reduction. Royer et al. (2008) quantitatively described the suitability of fully mummified greenbugs as a food source; however, greenbugs at earlier stages of parasitism are preyed upon in field conditions and their suitability for Coccinellidae survival and development is unknown.

My objective with this research was to determine the suitability of greenbugs parasitized by *L. testaceipes* at early, late, and mummified stages for the development and survival of third and fourth instar *H. convergens*.

Materials and Methods

Initial Greenbug Colony

Biotype 'E' greenbugs, *S. graminum*, were obtained from the USDA Agricultural Research Service in Stillwater, OK and used to start a colony. The greenbugs were kept on 'OK101' wheat grown in a half and half mixture of potting soil and fritted clay. Colonies were kept inside double-walled fine mesh cages within a greenhouse (22-28℃).

As feeding by greenbugs damaged the plants fairly quickly, blades of wheat infested with greenbugs from older pots were transferred to fresh pots of wheat weekly.

***Lysiphlebus testaceipes* Colony**

Lysiphlebus testaceipes were isolated from specimens collected in wheat fields from Oklahoma and Texas and reared on grain sorghum ('SG-925') infested with biotype 'E' greenbugs. Each of 5 colonies consisted of wasps from different locations and I routinely introduced field collected wasps from different locations to increase genetic diversity in an effort to maintain vitality of colonies. The double layers of mesh 2.5 cm apart on the walls of each cage prevented parasitism from outside sources and provided adequate airflow to the colony; each of the five cages also had a cloth sleeve for access. New pots of sorghum infested with greenbugs were added every 3-4 days to maintain a steady supply of parasitoids for feeding trials and water was made available to the adult parasitoids by spraying plants with a typical spray bottle. Colonies were maintained at 24℃ and a photoperiod of 12:12 (L:D) h.

Rearing of Coccinellidae

Adult *Hippodamia convergens* were either collected from different field locations in Oklahoma or purchased (Rincon-Vitova Insectaries, Inc.). Coccinellidae mating pairs were isolated into half-pint cardboard containers covered with a fine nylon mesh, fed an unlimited diet of pea aphids (*Acyrthosiphon pisum*, reared on faba beans) and maintained in an environmental chamber at 24℃ and a photoperiod of 16:8 (L:D) h.. The diet was coupled with a supplement of a honey-wheat-yeast mixture obtained from 'Planet Natural' in order to promote egg production and a regular supply of water (saturated ball of cotton). Egg producing pairs were numbered and their offspring tracked in order to

account for the differences that may result from parental lines (Giles et al. 2005). When egg-laying initiated, males were moved to other containers to prevent female disruption and egg consumption. Mating pairs and larvae were kept in an environmental chamber at 24℃ and a photoperiod of 16:8 (L:D) h.

Suitability of Parasitized Greenbugs for Coccinellid Development and Survival

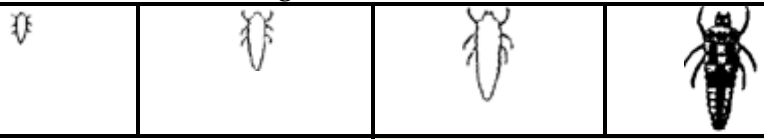
The suitability of parasitized aphids as a food source for third and fourth instar *H. convergens* was investigated at each of three stages of parasitism by *L. testaceipes*. Upon eclosion, coccinellid larvae were separated individually into 5mL glass vials topped with cotton. First and second instars were provided daily with a small, water-soaked ball of cotton and 4mg of greenbugs from the wheat colony; each vial was cleaned of any remaining greenbugs (dead or alive) each day. Larvae were observed daily for molting, development time, and mortality. Upon molting into the third instar, larvae were then provided with one of four (includes control) daily diet treatments. The control diet consisted of greenbugs from wheat while the remaining diets consisted of greenbugs reared on wheat but transferred to sorghum after parasitism for the allotted time necessary to reach the right stage required for the diet treatment (2-9 days). This was necessary due to results found in preliminary studies showing that wheat grown in the conetainers for this length of time with greenbugs often died before harvesting the parasitized aphids. All coccinellid larvae were supplied with 4mg of parasitized greenbugs per day to limit prey (simulating field conditions) and to isolate suitability. Four mg/day for *H. convergens* 3rd and 4th instars is enough to survive and develop on and allows for separating out diet suitability and eliminating the possible confounding effects of satiation (Giles et al. 2003, Phoofolo et al. 2007). The four diets were

greenbugs only, early stages of parasitism, late stages of parasitism or pre-pupal, and mummy or pupal; each diet treatment was initiated to cohorts of *H. convergens* at the beginning of the 3rd or 4th instar (See Figure 4.1). From 18-47 larvae were reared per diet treatment/initiation stage resulting in a total of 167 larvae evaluated. Larvae were observed daily for mortality, molting, or pupation; weight, time of pupation, and successful pupation were also documented.

To provide the daily diet treatments, individual sorghum plants were grown to a height of 22 cm in a 5 cm diameter by 20 cm tall conetainer and covered with a 5 cm diameter by 30 cm tall clear acetate tube that fit securely around the top of the conetainer. Two 5 cm holes in each side of the tube were covered with fine mesh that allowed for air movement and the top was also covered with a fine mesh secured by a rubber band; this allowed for access when adding wasps and greenbugs. Greenbugs from the wheat colony were added to the sorghum plants, and allowed to settle before adding wasps that had been aspirated from one of the parasitoid colonies. Preliminary studies showed that placing 200 greenbugs on two sorghum plants per conetainer and introducing 8-12 parasitoids (at least half females) for a twelve hour period resulted in 80-100% parasitism. All wasps were aspirated from the sorghum container after twelve hours and added back to the parasitoid colonies. Groups of parasitized greenbugs were kept on the plants for the required number of days (see below) before being fed to the coccinellid larvae as part of the specified experimental diet. Following oviposition, groups of parasitized aphids were reared (24°C and 12:12 (L:D) h) to achieve cohorts of the three parasitized diet treatments: early (all stages of parasitism before pre-pupae), late (pre-pupae), and mummy (pupal). In the pre-pupal stage the parasite larva is visible within

the host and the initial stage of mummification is evident, however the early stages of parasitism are not visibly evident. Preliminary studies and sample dissections ensured appropriate age classes of parasitoids were used for diet treatment cohorts. *Lysiphlebus testaceipes* 'early' larval stages occurred anywhere from 2 - 4 days after parasitism, pre-pupae stages anywhere from 5 – 6 days after parasitism and mummification at 7 – 9 days; at 26°C, development from egg to adult takes an average of 8.86 days (Royer et al. 2001). Parasitized greenbugs at various stages of development were collected and temporarily maintained (1-3 days) within a growth chamber at 6°C to arrest development (Elliott et al. 1999, Royer et al. 2001, Jones 2005).

Figure 4.1. Diet treatments; initiated at the beginning of either 3rd or 4th *H. convergens* larval stages. 1) Unparasitized greenbug 2) Early parasitoid larva 3) Late parasitoid larva 4) Parasitoid pupa. Immature stages of *Lysiphlebus* residing in the host are shown in drawings 2-4 (Stary 1970).

	***H. convergens* Larval Instars 1-4**			
				
Treatment Group	**Pre-Treatment Diet**		**Diet Treatment**	
Control	 1			
3rd Early			 2	
4th Early				
3rd Late			 3	
4th Late				
3rd Mummy			 4	
4th Mummy				

Data Analysis

All analyses were performed using SAS version 9.1 (SAS Institute 2003). A significance level of 0.05 was used for all statistical analyses. Ratios of larval survival (3^{rd} and 4^{th}), pupal survival, and larval + pupal survival were compared to the control treatment using Chi-square analyses (PROC FREQ). Larval and pupal developmental times (days) and adult weights (mg) of *H. convergens* were compared among diet treatments by analysis of variance (PROC MIXED). The Mixed Procedure was used because it does not assume equal variances among treatments and it supplies ANOVA with both random and fixed effects. Parental line and sex were removed as random variables before treatments were compared.

Results and Discussion

Before the fourth instar, survival for *H. convergens* was not significantly different among treatments; however, survival of fourth instars was reduced for the 'late' parasitized diet treatment initiated at the third and fourth instar and the 'mummy' treatment initiated at the fourth instar (Table 4.1). Overall larval survivorship was reduced for the same three mentioned (Late 3 and 4, Mummy 4) along with the 'early' parasitized diet treatment initiated at the third instar, which had accrued enough larval mortality cumulatively to become significant. Pupal survivorship was not significantly different among treatments whereas total survivorship (3^{rd}+4^{th}+Pup) was lower for the 'late' parasitized diet treatment initiated at the third and fourth instar as well as for the 'mummy' treatment initiated at the fourth instar. With the exception of individuals supplied with mummies starting at the 3^{rd} instar, parasitoid diet treatments showed greater overall mortality.

There were no significant differences in developmental times or adult weights (Table 4.2); however total developmental times were noticeably longer for the 'mummy' diet treatments initiated at both the third and fourth instars (Table 4.3). Additionally, individuals supplied with mummies at the 3rd or 4th instar had markedly lower weights, despite no overall significance among diet treatments (Tables 4.2, 4.3).

In general, similar to Royer et al. (2008) *H. convergens* exhibits reduced survivorship when supplied with a diet of parasitized greenbugs early in their life cycle. It is unclear why a relatively high number of larvae survived on a diet of mummies initiated at the 3rd instar. With this exception in mind, results show that the negative effect of a diet of parasitized greenbugs on *H. convergens* is not limited to the mummy stage of parasitism. *Lysiphlebus testaceipes* in the 'late' stage of parasitism appears to have an important impact on *H. convergens* survivorship when it is in the 'early' stage of parasitism. This could be the result of typical endoparasitoid feeding, which results in less and less of the actual host over time beginning with the hemolymph and moving to the internal organs (Quicke 1997). Possibly, parasitoid development would gradually reduce the nutrients available to a ladybeetle. The potential negative impacts on survivorship could also stem from the biochemical and physiological tools used by the immature wasp to regulate its host; with the effects of these tools increasing over the course of the internal parasites process of maturation (Beckage and Gelman 2004). Either of these suggestions would be consistent with the negative chronic effects observed in this study and those observed by Royer et al. (2008).

Intraguild predation of this nature does occur in field situations (Chapter 3) when all three organisms are present, as observed by the relatively high proportion of IGP

events in winter wheat fields. Parasitized greenbugs appear to be a sub-optimal diet for coccinellids significantly reducing survivorship, delaying development, and reducing the size of emerging adults, each of which may influence the intraguild predation dynamics taking place in the field setting. How do pervasive sub-optimal diets and a reduction in survivability affect Coccinellidae at the population level? Perhaps this altered suitability is an indirect parasitoid defense mechanism that under natural conditions reduces competition and prevents coccinellids from eliminating the local wasp population. This concept is new to the study of IGP dynamics and may help to explain guild dynamics in agricultural systems.

Table 4.1. Effects of diet treatments[a] on *H. convergens* larval and pupal survivorship at 24°C.

Treatment	*n*	Proportion Surviving					
		3rd Instar	4th Instar	Larval (3rd+4th)	Pupal	Total (3rd+4th +Pup)	Percent Female
Control	47	1	0.809	0.809	0.974	0.787	51.4
Early 3	18	0.944	0.588	0.556	1	0.556	50.0
Early 4	21	1	0.762	0.762	0.938	0.714	46.7
Late 3	20	0.950	0.368	0.35	0.714	0.25	60.0
Late 4	22	1	0.409	0.409	1	0.409	0
Mummy 3	19	1	0.789	0.789	0.933	0.737	100
Mummy 4	20	1	0.5	0.5	0.8	0.4	75.0
	χ^2	6.9	22.0	23.2	10.6	26.1	
	df	6	6	6	6	6	
	P	0.331	0.001	0.001	0.101	< 0.001	

[a]Treatments: Control = received 4 mg/day of greenbugs; Early 3, Late 3, and Mummy 3 = received 4 mg/day of greenbugs in early, late, and mummy stages of parasitism, respectively, beginning at the 3rd ladybeetle instar; Early 4, Late 4, and Mummy 4 = received 4 mg/day of greenbugs in early, late, and mummy stages of parasitism, respectively, beginning at the 4th ladybeetle instar. Sex ratios were not analyzed due to low survivorship.

[b]Underlined values are significantly different than control ($P = 0.05$) for 2×2 χ^2 tests.

Table 4.2. ANOVA results (Mixed Procedure, SAS) for developmental times (days) and adult weight (mg) of *H. convergens* supplied with diet treatments[a] of greenbugs in different stages of parasitism by *L. testaceipes*.

	Tests of Fixed Effects				
Response Variable	Source of Variation	Num df	Den df	F	*P*
Developmental Time					
Third Instar	Diet Treatment	6	82	1.64	0.1457
Fourth Instar	Diet Treatment	6	82	1.33	0.2543
Larval (3^{rd}+4^{th})	Diet Treatment	6	82	0.70	0.6496
Pupal	Diet Treatment	6	82	1.04	0.4064
Total (3^{rd}+4^{th}+Pup)	Diet Treatment	6	82	0.59	0.7379
Adult Weight (g)	Diet Treatment	6	82	2.19	0.0517

[a]Treatments: Control = received 4 mg/day of greenbugs; Early 3, Late 3, and Mummy 3 = received 4 mg/day of greenbugs in early, late, and mummy stages of parasitism, respectively, beginning at the 3rd ladybeetle instar; Early 4, Late 4, and Mummy 4 = received 4 mg/day of greenbugs.

Table 4.3. Effects of diet treatments[a] on *H. convergens* larval and pupal developmental times (±SE) and adult weight (±SE) at 24°C.

Treatment	*n*	Developmental Time (days)					Combined Adult Weight (mg)
		3^{rd} Instar	4^{th} Instar	Larval (3^{rd}+4^{th})	Pupal	Total (3^{rd} + 4^{th} + Pup)	
Control	47	2.5 ± 0.1	5.5 ± 0.3	8.0 ± 0.3	5.0 ± 0.1	13.1 ± 0.3	10.2 ± 0.7
Early 3	18	2.2 ± 0.1	6.0 ± 0.5	8.2 ± 0.5	5.1 ± 0.2	13.3 ± 0.5	9.9 ± 0.9
Early 4	21	2.3 ± 0.1	5.5 ± 0.4	7.9 ± 0.4	5.2 ± 0.1	13.1 ± 0.5	9.4 ± 0.8
Late 3	20	2.4 ± 0.2	5.6 ± 0.6	7.9 ± 0.6	5.2 ± 0.2	13.1 ± 0.7	10.0 ± 1.0
Late 4	22	2.7 ± 0.2	4.9 ± 0.5	7.5 ± 0.5	5.5 ± 0.2	13.0 ± 0.5	10.4 ± 0.9
Mummy 3	19	2.4 ± 0.1	6.1 ± 0.5	8.6 ± 0.5	5.1 ± 0.2	13.7 ± 0.5	9.1 ± 0.8
Mummy 4	20	2.3 ± 0.2	6.4 ± 0.5	8.8 ± 0.5	5.1 ± 0.2	13.9 ± 0.6	8.2 ± 0.9

[a]Treatments: Control = received 4 mg/day of greenbugs; Early 3, Late 3, and Mummy 3 = received 4 mg/day of greenbugs in early, late, and mummy stages of parasitism, respectively, beginning at the 3^{rd} ladybeetle instar; Early 4, Late 4, and Mummy 4 = received 4 mg/day of greenbugs in early, late, and mummy stages of parasitism, respectively, beginning at the 4^{th} ladybeetle instar.

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CHAPTER V

SUMMARY AND CONCLUSIONS

In 2007, over 6 million acres of wheat were planted in Oklahoma (USDA-NASS 2008). Whether these 6 million acres are for forage or grain production they are attacked by a number of insect pests; especially the greenbug *Schizaphis graminum* Rondani which is often the limiting factor in profitable winter wheat production (Starks and Burton 1977, Webster 1995, Kindler et al. 2002). The wasp, *Lysiphlebus testaceipes*, an endoparasitoid of greenbugs and the ladybeetle predator *Hippodamia convergens* constitute part of an aphidophagous guild responsible for maintaining aphid pest population numbers below Economic Injury Levels (Jones 2001). These ladybeetles will consume *L. testaceipes* within their greenbug hosts (Royer 2008). The studies described in this thesis were conducted to examine the detectability of intraguild predation and the rate at which this kind of intraguild predation occurs in winter wheat fields, and to investigate if and at what stage parasitized aphids are a suitable food source for the development and survival of third and fourth instar *Hippodamia convergens* larvae.

It was possible to detect *L. testaceipes* mitochondrial DNA within the gut of the predatorial ladybeetle *H. convergens* with PCR following intraguild predation. Detection was possible up to 16 hours post-feeding 100% of the time for both 3rd and 4th instar larvae. This places the detectability half-life beyond 16 hours using the Ltesta F and R primers and will prove useful in designing sampling plans regarding feeding cycles. The majority of coccinellids sampled from wheat fields at several dates through the spring season tested positive for *L. testaceipes* DNA and thus, intraguild predation. In general, feeding trials showed reduced survivability for 3rd and 4th instar *H. convergens* larvae when feeding on greenbugs in all stages of parasitism by *L. testaceipes*.

It appears that the prevalence of *L. testaceipes* influences coccinellid populations by forcing them to consume a suboptimal diet that affects growth, development and survivability. These insights may help lead to a greater understanding of IGP dynamics taking place in agricultural systems and perhaps others and aid in defining the structure and dynamics of aphid-predator-parasitoid communities.

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VITA

Christopher Blake Mullins

Candidate for the Degree of

Master of Science

Thesis: INTRAGUILD PREDATION AMONG COCCINELLIDAE AND *LYSIPHLEBUS TESTACEIPES* IN AN OKLAHOMA WINTER WHEAT SYSTEM

Major Field: Entomology and Plant Pathology

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Title of Study: INTRAGUILD PREDATION AMONG COCCINELLIDAE AND *LYSIPHLEBUS TESTACEIPES* IN AN OKLAHOMA WINTER WHEAT SYSTEM

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Major Field: Entomology and Plant Pathology

Scope and Method of Study: In 2007, over 6 million acres of wheat were planted in Oklahoma. Whether these 6 million acres are for forage or grain production they are attacked by a number of insect pests; especially the greenbug *Schizaphis graminum* Rondani which is often the limiting factor in profitable winter wheat production. The endoparasitic wasp, *Lysiphlebus testaceipes* Cresson, along with the ladybeetle predator *Hippodamia convergens* Guérin-Méneville constitute part of an aphidophagous guild responsible for maintaining greenbug, Schizaphis graminum Rondani, populations below economic injury levels. These ladybeetles will consume *L. testaceipes* within their aphid hosts, a dynamic known as intraguild predation. The studies described in this thesis were conducted to examine the occurrence and frequency of this kind of intraguild predation in winter wheat fields and to investigate if and at what stage parasitized aphids are a suitable food source for the development and survival of *H. convergens*.

Findings and Conclusions: *Lysiphlebus testaceipes* mitochondrial DNA within the gut of the ladybeetle *Hippodamia convergens* was detectable with PCR following intraguild predation of the immature parasitoid within its greenbug host. Detection was possible up to 16h post-feeding 100% of the time using Ltesta F & R primers. Three fields of winter wheat were sampled on 24 February, 10 March, and 24 March (2006) for intraguild predation of *L. testaceipes* by coccinellids; proportion of intraguild predation events of this nature ranged from 0.667 to 0.871. Both 3rd and 4th instar *H. convergens* larvae were fed 4mg/day of greenbugs in 3 different stages of parasitism by *L. testaceipes* to determine the suitability of that stage as a food source. Results showed reduced survivability for both 3rd and 4th instar *H. convergens* larvae when feeding on greenbugs in all stages of parasitism by *L. testaceipes* with chronic effects having greater negative impacts.

ADVISER'S APPROVAL: Kristopher L. Giles, Ph.D.